DR AMARA E EZEAMAMA (Orcid ID : 0000-0003-2164-8802)

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In-utero and Peripartum Antiretroviral Exposure as Predictor of Cognition in 6 to 10 years old HIV exposed Ugandan Children - A Prospective Cohort Study

AE Ezeamama [1], SK Zalwango [2], A Sikorskii [1], R Tuke [1], PM Musoke [3,4], B Giordani [5], MJ Boivin [6,7]

[1] Department of Psychiatry, College of Osteopathic Medicine, Michigan State University, East Lansing, Michigan, USA

[2] Directorate of Public Health and Environment, Kampala Capital City Authority, Kampala, Uganda

[3] Department of Paediatrics and Child Health, School of Medicine, College of Health Sciences, Makerere University, Kampala, Uganda

[4] Makerere University-Johns Hopkins University Research Collaboration, Kampala, Uganda

[5] Departments of Psychiatry, Neurology, and Psychology, University of Michigan, Ann Arbor, Michigan, USA.

[6] Departments of Psychiatry and Neurology & Ophthamology, College of Osteopathic Medicine, Michigan State University, East Lansing, Michigan, USA

[7] Department of Psychiatry, University of Michigan, Ann Arbor, Michigan, USA

Corresponding author: Amara E. Ezeamama

Address: 909 Wilson Road, 322B West Fee Hall; East Lansing, MI 48824.
Tel: (517) 353-1244; *Email*: ezeamama@msu.edu

ABSTRACT

Objective: To quantify association between *in-utero*/peripartum antiretroviral (IPA) exposure and cognition– i.e., executive function (EF) and socioemotional adjustment (SEA), in school-aged Ugandan children perinatally HIV-infected (CPHIV, n=100) and children HIV-exposed uninfected (CHEU, n=101).

Methods: Children were enrolled at 6-10 years old and followed for 12 months from March 2017 through December 2018. Caregiver-reported Child EF and SEA competencies were assessed using validated questionnaires at baseline, six and twelve months. IPA type - combination antiretroviral-therapy (cART), intrapartum Nevirapine±Zidovudine (sdNVP±AZT), Nevirapine+Zidovudine+Lemavudine (sdNVP+AZT+3TC) or no IPA (reference), was verified via medical records. IPA-related standardized mean differences (SMDs) with corresponding 95% confidence intervals (CIs) in cognitive competencies were estimated from regression models with adjustment for caregiver sociodemographic and contextual factors. Models were fit separately for CPHIV and CHEU.

Results: Among CPHIV children, cART (SMD= -0.82, 95%CI: -1.37 to -0.28) and sdNVP±AZT (SMD= -0.41, 95%CI: -0.81 to -0.00) vs. no IPA predicted lower executive dysfunction over 12 months. Intrapartum sdNVP+AZT+3TC vs. no IPA predicted executive dysfunction (SMD=0.80, 95%CI:0.30 to 1.31), SEA problems (SMD=0.63 to 0.76, 95%CI:0.00 to 1.24) and lower adaptive skills (SMD= -0.36, , 95%CI:-0.75 to 0.02) over 12 months among CHEU. Further adjustment for contextual factors attenuated associations though most remained of moderate clinical importance (|SMD|>0.33).

Conclusion: Among CPHIV children, cART and sdNVP±AZT IPA-exposure on average predicted lower executive dysfunction 6-10 years later. However, peripartum sdNVP+AZT+3TC predicted executive and SEA dysfunction among CHEU 6-10 years later. These data underscore the need for more research on long-term effects of *in-utero* ART to inform development of appropriate interventions to mitigate cognitive sequelae.

Key words: Maternal ART, *in-utero* ART, cognitive function, HIV-exposed children, Executive Function, Socio-emotional Adjustment

Running Title: Early antiretroviral therapy & long-term behavioral dysfunction

Word count: Abstract: **245**, Manuscript: 3574 Tables: 3, Figures: 2

1.0. INTRODUCTION

With expansion of antiretroviral therapy (ART), HIV Mother-to-child-transmission (MTCT) has plummeted by as much as 50% while the population of perinatally HIV-exposed uninfected (CHEU) children has doubled since 1990.[1] . Maternal ART is effective for prevention of MTCT (PMTCT) of HIV[2] but this exposure comes with several known neurodevelopmental risks- low birthweight,[3] metabolic dysregulations,[4] mitochondrial toxicity[5-8] and neurotoxicity.[9,10] For children born to HIV-infected women, this risk will necessarily continue making higher neurodevelopmental risk a price paid to avert the more adverse outcome of HIV transmission[11,12] Given this reality, there is corresponding imperative of long-term surveillance of neurodevelopmental outcomes among *in-utero*/peripartum ART (IPA) exposed children to determine extent of impairments and to inform interventions research to mitigate them.

Globally, most children with IPA exposure live in sub-Saharan Africa, but specific long-term studies in this setting are few, evidence from available studies are inconsistent and investigations in school-aged children are limited.[13-15] WHO PMTCT guidelines, coverage and scope of IPA exposure among children born to HIV-positive pregnant women varied across HIV-treatment eras[16] with possible implications for neurodevelopmental outcomes. For example, a metanalysis of preschool children found worse neurodevelopmental outcomes for CPHIV and CHEU vs. HIV-unexposed uninfected (HUU) preschoolers and cognitive outcomes for CHEU was worse with IPA exposure.[17] Among American children enrolled in the surveillance monitoring of ART toxicities (SMARTT), an elevated risk of language impairment was noted in CHEU with combination ART (cART) based IPA vs. CPHIV at three and five years of life[18] although cognitive and academic outcomes at 5-13 years were similar between groups.[19] Among Thai and Cambodian children, both CPHIV and CHEU had worse cognition relative to HUU regardless of the timing of ART initiation in pregnancy.[20] Of the studies in Africa, no ART related differences in cognition was evident among pre-school age children[13,15] or among 6-8 years Cameroonian children in a cross-sectional study.[14] Longitudinal studies of IPA-related change in cognition among African HIV-exposed children during school and adolescence are lacking.

Hence, we report on the longer-term association between IPA exposure and deficits in socio-emotional adjustment and executive dysfunction by 6–10 years old among HIV-exposed children of HIV-positive women followed for 12 months. We hypothesized that IPA would be associated with worse

cognition by early school-aged years. However, associations between IPA and cognition may vary according to type of early ART regimen and the HIV status of the exposed child.

2.0. MATERIALS AND METHODS

2.1. Participants, Study Context and Design

Children HIV-unexposed and uninfected (CHUU, n=97), CHEU (n=100) and CPHIV (n=101) 6-10 years old at enrolment born in a formal healthcare setting and their adult primary caregivers were enrolled as part of a larger study of functional survival with perinatal HIV exposure among 306 children between 6 – 10 years old from Uganda.[21] Children were enrolled on a first come first served basis from Kawaala Health Center (KHC), in Kampala, Uganda, between March 15, 2017 and September 15, 2018. Of these, eight children lacking cognition measures were excluded. CPHIV were enrolled from current patients at KHC. CHEU were identified through medical records of HIV-infected adult women cared for at KHC. In addition, Early Infant Diagnosis (EID) registry was used to identify age-eligible CHEU who remained HIV-free until discharge from the EID program. Age-eligible CHUU were recruited from KHC's Outpatient Department upon presentation for healthcare services and from the social networks – family, friends and neighbors – of already enrolled caregiver-child pairs. Cognitive performance measures in CHUU was used as a reference for standardizing by age and sex the cognitive performance of HIV-exposed children. Because this analysis is specifically focused on investigating the extent to which cognitive trajectory among HIV-exposed children differs based on in-utero/peripartum ART (IPA) exposure type/history, CHUU for whom IPA exposure did not apply were excluded. During the study period, HIV prevalence in Kawaala District was 11% compared to national prevalence of 7.3%.

2.2. Eligibility/Exclusion Criteria

Caregiver eligibility included being 18 years or older and affirmative response to being primary caregiver for study eligible child for a period of at least 6 months prior to enrolment. Eligibility criteria for children included: age 6-10 years old at enrolment and availability of health records with objective data regarding HIV status of biological mother, mode of birth, full or preterm birth, birthweight, participation in PMTCT services, any/type of ART exposure (if applicable) and HIV status of index child at birth or by date of discharge from the Early Infant Diagnosis program (if CHEU). Children born in non-clinic settings for whom antenatal register/delivery medical records could not be found were ineligible to participate as IPA exposure status, the HIV-status of child and their birth mother could not be reliably ascertained.

2.3 Statement of Ethical Approval: The study protocol was approved by the research ethics review committees of Michigan State University (IRB Protocol#: 16-828), Makerere University College of Health Sciences, School of Medicine (Protocol REC REF# 2017-017) and the Uganda National Council for Science and

Technology (Protocol #: SS4378). All caregivers gave written informed consent and children provided assent for study participation.

2.4 ***Outcome Definition:*** Two domains of cognition: socio-emotional adjustment (SEA) and executive function (EF) were defined per caregiver responses to 175 questions in the Behavioral Assessment System for Children (BASC-3)[22] and the shortened 24-item version of Behavior Rated Inventory of Executive Function (BRIEF)[23] at intake, 6 and 12 months. At all study assessments, responding caregivers must have cared for dependent child for at least six months. For vast majority(>95%) of children, the same respondent caregiver- usually the biological parent or grand-parent, completed all timepoints. Questions were administered by trained interviewers (n=3) according to a common manual of operations. To maximize interaction rapport, whenever possible, the same interviewer was matched to a caregiver during follow-up. Snacks were provided before each interview session to mitigate the distracting effect of hunger on responses. In the absence of locally normed scores, cognitive measures were internally age and sex standardized to performance among CHUU children: i.e., $Z\text{-}Score_{it}=(Score_{it} - mean_{t0(CHUU)}) /(standard\ deviation_{t0(CHUU)})$.[24]

Four composite SEA scales were defined using information scored according to BASC-3 manual: externalizing problems composite (EPC), internalizing problems composite (IPC), behavioral symptoms index (BSI) and adaptive skills index (ASI). Higher scores on the EPC, IPC and BSI indicate worse SEA. On the other hand, higher scores in the ASI composite indicate higher SEA. Two EF domains and the corresponding subscales within each domain were defined per manufacturer instructions: a) meta-cognition domain – a composite of working memory, task initiation, planning, monitoring and material organization subscales, and b) the behavioral regulation domain – a composite of inhibition, flexibility and emotional control subscales. In addition, a global executive component score (GEC) was defined as the sum of scores in the meta-cognition and behavioral regulation domains of BRIEF.

Validation and Psychometric Properties of Tools: Respective tools were adapted for cultural context, forward and back translated to Luganda as we have previously described.[24] In a small sample (n=30 caregivers) at enrolment, each tool was administered twice 14 to 21 days apart to same respondent by different interviewers to determine the internal consistency among individual questions and test-retest reliability[25] estimated using the %INTRACC macro.[26] The internal consistency reliability for the BRIEF global executive composite (Cronbach's α=0.89), meta-cognition index (Cronbach's α =0.88) and behavioral regulation index (Cronbach's α=0.68) domains were excellent and acceptable. Test-retest reliability for caregiver responses to questions about EF in their dependent children was excellent (intra-class correlation (ICC)=0.84 – 0.90).

All BASC SEA subscales demonstrated excellent internal consistency (Cronbach's α: 0.83 to 0.91). Test-retest reliability values in the good to excellent range (ICC: 0.51 to 0.79) confirms caregivers stably reported relevant behaviors or symptoms independent children over 14 to 21 days interval.

2.6 Perinatal HIV Status*:* Perinatal HIV status was established by 18 months via DNA PCR. Current HIV-status of CHEU was confirmed via HIV-rapid diagnostic test at enrolment.

2.6.1 In-utero/*Peripartum ART (IPA) *exposure: Several landmark ART studies that directly informed standard of ART care have been implemented in Uganda.[27-32] These efforts in coordination with Uganda Ministy of Health and global partners contributed to systemic strengthening of HIV care delivery and harmonization of HIV data collection systems that permit completed and objective determination of IPA exposure in all HIV-exposed children based on routinely recorded medical data. As a function of rapid changes in HIV-care and treatment, IPA exposure (if any) and IPA regimen was determined year of birth and pregnant woman's CD4 cell count. For pregnant HIV-positive women not yet ARV eligible per prevailing CD4 guidelines, three options applied depending on year of pregnancy: a) pregnant women received single dose nevirapine (sdNVP) only at onset of labor and new born infant received same at birth; b) zidovudine (AZT) to pregnant woman was begun at 28 weeks or shortly thereafter, continued at same dose in labor with addition of sdNVP at labor onset. Their infant received sdNVP at birth with one week of AZT, c) pregnant woman received AZT plus Laminvudine (3TC) starting at 36 weeks and maintained through one week post-partum with infant receiving one week of AZT+3TC.[33] All IPA exposure was abstracted from the antenatal register, ART card or EID register and classified according to maternal exposure status as: No IPA (reference), intrapartum prophylactic sdNVP± AZT, intrapartum prophylactic sdNVP+AZT +3TC and cART (i.e. ≥two antiretroviral drug classes).

2.6.2 Other Covariates

a. ***Caregiver demographic and behavioral factors:*** Information on alcohol use (ever vs. never drinker), marital status and perceived social standing using standardized questionnaires.[24,34]

b. ***Caregiving Environment****:* Caregiver's socioeconomic status was defined as: presence or absence of income and years of formal education. Maternal functioning was defined as proxy for caregiving quality using the Barkin's Index[35] and psychosocial stress was measured using the perceived stress scale.[36]

c. ***Child characteristics:*** Biological sex (male vs. female), chronological age (in years) are adjusted for in all analyses. Prematurity (i.e. <37 weeks gestation at birth), five-minutes APGAR score <7, and low birthweight (≤2500g) were abstracted from medical records. Height-for-age at enrollment was calculated.

d. ***Current cART regimen, & CD4-nadir****:* These three variables are defined for CPHIV only. Current cART regimen was determined as part of health assessment at enrollment and classified: protease inhibitor (including Lopinavir/Ritonivir/Kaletra), nucleoside reverse transcriptase inhibitor (i.e. nevirapine or efavirenz) based cART or cART naïve – if child is HIV-infected and not on treatment. Lastly, the lowest CD4-cell count of enrolled children since the beginning of HIV-care at KHC was established via medical records.

2.7. Statistical Analysis

Separate analyses were conducted for CHEU and CPHIV because CPHIV required current ART for HIV management. When ART was started for CPHIV depended on treatment guidelines, severity of illness/immune deficiency – factors that did not apply to CHEU. Caregiver demographic and behavioral factors, caregiving environment, child characteristics, pertinent HIV-treatment related factors (if applicable) and baseline cognitive performance were summarized via means and SD's for continuous variables and frequencies and percentages for categorical variables. Differences by exposure to ART regimen (any ART versus none) were evaluated using *t* tests for continuous variables and X^2 tests for categorical variables**.**

Multivariable linear mixed effects models were implemented to estimate early ART exposure-related standardized mean differences (SMD) in three repeated measures of cognitive outcomes (SEA, EF) over 12 months using SAS PROC MIXED.[37] In all models, confounders – child's age, sex, relationship with caregiver, caregivers' age, sex, caregiver depression and socio-economic status, were adjusted for in light of subject matter knowledge – an approach grounded in causal theory that assures robust non-biased inference[38-42] by recognizing child perinatal risk factors as causal path variables influenced by maternal IPA.[3-10] Among CPHIV, the multivariable models were sequentially adjusted for current ART regimen, timing of ART initiation, and CD4 nadir. Random effect of the caregiver was included in all models to account for nesting of children within households. Time was entered as a class variable to model potentially non-linear patterns. A time by early ART exposure interaction was included to assess potentially changing effect of ART exposure over the course of 12 months. The least square (LS) means according to the ART exposure were output from the LME models, and differences among them were tested at each time point. When there was no appreciable time trend, the LS means were averaged over time within the LME model, and differences by the main effect of ART exposure were tested. Since cognitive outcomes are age- and sex-standardized, estimated SMD is comparable to Cohen's effect size. |SMD| of ≥0.50 are universally recognized as being of clinical importance for patient reported outcomes.[43] However, there has been precedent for judging |SMD|≥0.33 as clinically important for the outcome of quality of life or wellbeing – a patient reported outcome similar to proxy reported cognition of dependent children in this study.[44] Like QOL, attainment of cognitive potential is of central lifelong importance for achieving the human potential

of developing children. Therefore, |SMD|<0.33, 0.33≤|SMD|< 0.50 and |SMD|≥0.50 are respectively considered to be of small, moderate, and large clinical importance in this study per existing precedent for QOL.[44] All hypotheses tests were two-sided at .05 level of significance.

3.0. RESULTS

A total of 201 HIV-affected children were enrolled and followed for 12 months. During the *In-utero* or peripartum period, most (n=102, 50.7%) had no ART exposure, 20.9% (n=42) were exposed to sdNVP±AZT, 10.9% (n=22) were exposed to sdNVP+AZT+3TC and 17.4% (n=35) were exposed to cART. Most caregiver and child demographic, clinical and HIV-treatment related factors (if CPHIV) were similar for children with and without IPA exposure. However, CHEU with IPA exposure were more likely than CHEU peers without IPA exposure, to have a female caregiver(98.1% vs 86.6%) and a caregiver in highest quintile of functioning (27.3% vs 11.1%). Likewise, a greater proportion of CPHIV with IPA relative to no IPA exposure had biological parent caregivers (88.6% vs. 64.9%), and higher ASI score [mean (SD): 0.34(1.0) vs. -0.24 (0.77)]. **(Table 1)**

3.1 IPA Related difference in SEA six to ten years later among CHEU

Among CHEU, a consistent trend of worse performance in all cognitive outcomes by 6-10 years old was evident for sdNVP+AZT+3TC vs. no IPA exposure. Adjusted for caregiver demographic factors, sdNVP +AZT+3TC vs. no IPA predicted moderate decline in ASI (SMD = -0.36, 95%CI:[-0.75,0.02]), moderate to large increase in BSI, IPC and EPC domains of SEA (SMD =0.63 to 0.76, 95%CI: [0.00,1.44]) and large increase in executive dysfunction (SMD = 0.80, 95%CI:[0.30,1.31]). With further adjustment for caregiving quality and caregiver depression, sdNVP +AZT+3TC vs. no IPA associated cognitive deficit was attenuated and of small (ASI: SMD= -0.30, 95%CI:[-0.67, 0.08]) to moderate clinical importance for BSI, IPC and EPC domains of SEA (SMD =0.40 to 0.52, 95%CI:[-0.17, 1.19]) and for global executive dysfunction (SMD=0.54, 95%CI:[0.06, 1.03]). However, IPA exposures including cART or peripartum sdNVP± AZT vs. no IPA exposure were not related to cognitive performance and associations were of small clinical importance (i.e. |SMD|<0.28). **(Table 2)**

3.2 IPA related SEA and EF Dysfunction six to ten years later among CPHIV

Among CPHIV, peripartum cART (SMD= -0.82, 95%CI:[-1.37,-0.28]) and intrapartum sdNVP±AZT (SMD= -0.41, 95%CI:[-0.81,-0.00]) vs. no IPA predicted moderate to large reductions in executive dysfunction 6-10 years later. For all other SEA outcomes, CPHIV with cART or sdNVP±AZT IPA vs. peers without IPA exposure had comparable cognitive outcomes 6-10 years later even after adjusting for caregiving context and HIV-specific factors. **(Table 3)**

3.3 IPA-relationship to SEA varied over 12 months period for ASI, but not for Global EF

Among HIV-exposed children followed from 6-10 years old, IPA-related 12 months change in ASI was stable among CHEU (time*early ART, p-value=0.22) but not among CPHIV(time*early ART, p-value<0.01, Figure 1). Among CPHIV, sdNVP±AZT vs. no IPA exposure associated ASI advantage at enrollment (SMD=0.80, 95%CI: [0.21,1.39]) attenuated an advantage of small clinical importance by month 6 (SMD = 0.19, 95%CI:[-0.34,0.72]) by month 6 was associated with ASI disadvantage of small clinical importance (SMD = -0.25, 95%CI:[-0.73,0.27]) (Figure 1). Although the relative magnitude of specific IPA vs. no IPA related change in global executive dysfunction varied by HIV status, the direction was consistent over 12 months regardless of HIV status (time*early ART, p≥0.502) . Regardless of HIV status peripartum sdNVP+AZT+3TC was associated with greater executive dysfunction 6 to 10 years later whereas peripartum cART predicted lower executive dysfunction over the same 6 to 10 year period in comparison to peers without IPA exposure. **(Figure 2)**

4.0. DISCUSSION

In this cohort of HIV-exposed Ugandan children born between 2008 and 2012, most received no IPA intervention for PMTCT of HIV. Of note, the proportion of HIV-positive women who received ART for PMTCT in Uganda increased from 9% in 2004 to 33% by end of 2007.[45] When IPA intervention occurred, maternal exposure was dependent of being CD4 eligible and when applicable varied in type and duration according to year of pregnancy.[33] For their infant, it often included intrapartum administration of sdNVP±AZT±3TC to newborns within 72 hours of delivery and continued for one week. A small number of children born to pregnant women on ART for their own health, had the benefit of optimal intervention (cART). These low IPA exposure rates and the low prevalence of optimal IPA regimen is a direct reflection of prevailing standard of HIV care in the study locale during era of children's birth because HIV-positive pregnant women had to meet minimum immune suppression thresholds to be provided cART.[45] Independent of caregiver factors – age, sex, socioeconomic circumstances, depression, quality of caregiving, psychosocial stress, and child's current ART regimen (if CPHIV), early life IPA-regimen related differences in neurodevelopmental trajectory were evident by six to ten years later. With few exceptions, many of the observed peripartum IPA-related differences in SEA and EF at baseline assessment were sustained over 12 months of follow-up. However, while the IPA regimen was associated with cognition, the direction of association and the specific domains affected varied according to perinatal HIV status. Overall, these findings are consistent with our *a priori* hypothesis that IPA exposure during critical developmental windows is associated with cognitive performance in the long-term. Results underscore the need for investigations along the developmental life-course continuum as associations maybe dynamic within extended developmental windows.[46]

In-utero or Peripartum sdNVP±AZT and relationship to Cognitive at 6-10 years of life

In CPHIV and CHEU alike, our data suggest there is no association between prophylactic peripartum *sdNVP±AZT* exposure and behavioral dysfunction subscales of SEA 6-10 years later. Among CHEU, this exposure was also not associated global EF competencies, while CPHIV demonstrated a different pattern of association for global executive function and adaptive subscales of SEA. Specifically, among CPHIV, an initial positive association of large clinical importance of between peripartum *sdNVP±AZT* and ASI at enrolment was down modulated to small clinical importance six months later. By study end peripartum *sdNVP±AZT* was observed to have an adverse association with ASI of modest clinical importance among CPHIV. This unstable association of *sdNVP±AZT* with adaptive skills over 12 months beginning from age 6-10 years – if true, may suggest that sustaining the cognitive benefit of a salutary exposure in long-term requires intentional efforts to optimize current biopsychosocial circumstances. Our team has recently reported on the importance of high quality nutrition[47,48] and stress[49,50] in relationship to cognition in this sample. With respect to stress, we found that SEA, QOL and psychosocial adjustment of CPHIV is comparable to CHEU and HUU peers in Uganda among children of caregivers that reported low psychosocial stress. However, the same outcomes for CPHIV are substantially worse when primary caregiver reported high levels of psychosocial adversity.[49,50] In other words, in highly vulnerable CPHIV the imperative of providing consistent and predictable social support, high quality nutrition and minimizing psychosocial adversity is high to maintain any neurodevelopmental benefits of potentially healthy peripartum exposures. However, it is also possible that fluctuations in IPA relationship to adaptive skills over the 12 months prospective observation period represents inherent measurement error in the outcome determination driven by differences in how caregivers interpreted adaptive skills assessment questions for their dependent children.

These absent or unclear relationship of prophylactic *sdNVP±AZT* exposure with SEA outcomes contrasts with evidence of sustained protective association of large clinical importance for this *sdNVP±AZT* in relationship to EF six to ten years later among CPHIV. On the one hand, the lack of any or consistent association between peripartum *sdNVP±AZT* among CPHIV and CHEU is not surprising given that *sdNVP±AZT* is in hindsight a sub-optimal intervention that may not have sufficiently normalized the *in-utero* environment of HIV exposed children. Our findings among CHEU aligns with observations in the Pediatric AIDS Clinical Trial Group at 3.2 to 5.6 years of life.[51] It is also consistent with previously reported similarity in cognition as assessed by the Mullen scale of early development for Zambian CHEU exposed to zidovudine vs. CHEU peers exposed to cART at two years old[15] and with similarity in cognitive and language outcomes for CHEU compared to HUU at 15-36 months of life.[13] Our data suggests that this direction of association is sustained in later childhood years.

In-utero or Peripartum sdNVP±AZT+3TC and relationship to Cognition at 6-10 years of life

We show internally consistent evidence that behavioral and executive dysfunction was consistently higher in CHEU exposed to sdNVP+ AZT+3TC vs. CHEU peers without IPA exposure. This finding is similar to reports of peripartum efavirenz+AZT+3TC -exposure associated higher risk of socioemotional and adverse developmental outcomes among CHEU from Malawi at 24 months[52] and suggestive evidence of peripartum AZT exposure associated higher risk of hyperactivity by 3 years of life in a small sample of CHEU from Venezuela.[53] Studies have implicated nucleoside reverse transcriptase inhibitors (NRTI's) such as AZT and 3TC in CNS neuronal mitochondrial toxicity (NMT)[54] with mitochondrial dysfunction particularly for IPA exposures in the third trimester.[55] Complimentary evidence from studies of mice subjected to AZT+3TC combination treatment have demonstrated the greatest mitochondrial DNA damage[56] resulting in adverse somatic/sensorimotor development and adverse behavioral and social interaction relative to mice exposed to respective agents separately.[57] Therefore, exposure to combined AZT+3TC regimen in sensitive developmental window has plausible potential for inducing the kinds of behavioral dysfunctions that impair the ability to CHEU to appropriately engage cognitive processes necessary to cope with social stress, self-monitor in a variety of goal-oriented, social and academic contexts.[58,59]

We note that this observation among CHEU contrasts with absence of any evidence that peripartum *sdNVP±AZT+3TC* compared to no IPA was associated with cognitive outcomes by 6-10 years of life among CPHIV. This discrepancy of association by perinatal HIV status may in part reflect the huge differences in physiologic status between CHEU and CPHIV who require cART for life. The neurodevelopmental trajectory of these two groups of children maybe fundamentally different and confounded by chronic HIV-related morbidity which limits the ability to detect association between cognition assessed at 6-10 years old and this more distal peripartum cART exposure.

In-utero or Peripartum cART and relationship to Cognitive at 6-10 years of life

In line with our study thesis, peripartum cART exposure predicted sustained and gains of large clinical importance by early school-age years among CPHIV. This finding is also internally consistent with a pattern of cART related modest to moderate reductions in BSI, IPC and EPC domains of SEA in CPHIV. These cART-associated positive trends in executive function and multiple SEA subscales reflects the long-term cognitive sparing benefit of having an immunologically stable intrauterine environment with relatively lower viremia that is directly linked to maternal cART in pregnancy.[4] This finding is similar to the neuroprotective association reported for certain cART regimen among 1-12 years old American children in the SMARTT cohort.[4] However, future specifically designed investigations will be important to confirm and clarify the observed encouraging cART-related long-term cognitive benefit among CPHIV children.

Among CHEU, cognitive outcomes by 6-10 years of life was comparable for those with peripartum cART exposure relative to peers without any IPA exposure. Boivin and colleagues have recently reported no difference in cognition for CHEU with i*n-utero* cART exposure sustained through breastfeeding cessation vs. similarly aged HUU children at 48 months.[60] Yet another study found a lower frequency of adaptive behavior with higher frequency of adverse neurodevelopmental outcomes for CHEU exposed to various IPA compared to HUU children.[46] While those investigations are similar in empirical intent to the present study, there are important differences that make direct comparison tenuous. Unlike Boivin and colleagues[60] for example, our study base is by design is constrained to understanding the long-term neurodevelopmental trajectory of children born to HIV-infected women who differed by type and quality of IPA exposure in sensitive developmental windows. Future studies where HIV-exposed children with and without IPA exposures are compared to HUU children will be important to confirm whether cART related cognitive sparing noted for CHEU in this study mitigates disparity in cognitive outcome relative to HUU children.

Limitations and Strengths

In this study, proxy-reported neurodevelopmental outcomes were used. Proxy reported outcome measures are possitively associated-though not equivalent to, child self-report of same outcomes or performance based measures of cognition. Further, in some IPA categories, the number of children were small (e.g Among CPHIV ten each received cART or 3TC inclusive IPA) possibly limiting statistical power in multivariable models. Additional limitations lie in the absence of randomization which precludes elimination of residual confounding by design and the fact that this analysis did not distinguish between timing of cART exposure in pregnancy. The latter means this study provides no insight regarding gestational timing of IPA and severity of long-term neurodevelopmental trajectory . In spite of these limitations, we have used longitudinal design with repeated assessment of cognitive endpoints, robust control for confounding covariates and ascertained IPA exposures from objective medical records, which limits primary exposure misclassification. These are important strengths that should increase confidence in reported findings. Additional strength lies in our use of sensitive cognitive competencies such as executive function and SEA that emerge progresively through early childhood and late adolescence[61,62] via complex interaction between children and their environments.[63] This study therefore provides insight of children's functional adjustment to their respective environmental contexts and has implications for devising strategies to optimize learning and scholastic achievement,[64] impulse control, chronic disease self-management, and other adaptive skills for social success.[65] Lastly, in light of the small sample size of exposed children within certain IPA strata – e.g. cART, standardized mean differences or effect size rather than risk differences were calculated as measure of effect to provide much

needed information on clinical importance of respective associations . Larger future studies will be important to confirm these findings.

In summary, among children exposed to HIV and various ART regimen during the peripartum period, we demonstrate that all IPA regimen do not have equal long-term cognitive effects.[66] Among CPHIV, cART and sdNVP±AZT IPA-exposure on average predicted lower executive dysfunction 6-10 years later. However, peripartum sdNVP+AZT+3TC predicted executive and SEA dysfunction among CHEU 6-10 years later. These data underscore the imperative of longitudinal studies across the developmental continuum to understand ART related long-term cognitive sequalae in HIV-affected children. Given the high burden of cognitive impairment in this population, empirically informed strategies to identify at-risk CHEU for remedial interventions are needed to support long-term functional survival for growing global population of CHEU.

Data Sharing

Data will be made available only upon request and subject to terms of a data use and collaboration agreement.

Author Contributions: Conceptualization, A.E,E.; formal analysis, A.E.E., A.S.; data curation, A.E.E., RT; writing—original draft preparation, A.E.E., RT, AS.; writing—review and editing, A.E.E., R.T., A.S., S.Z., P.M., BG, M.J.B.; project administration, A.E.E., S.K.Z., P.M. ; funding acquisition, A.E.E.

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Table 1: Socio-demographic description of adult caregivers and clinical description of perinatally exposed or infected children of HIV-infected women from Uganda from birth through 6-10 years of life

	CHEU (n=100)		P-value ¥	CPHIV (n=101)		P-value ¥
Caregiver Socio-demographics	***In utero / Peripartum ARV* Exposed (n=55)**	***No In utero / Peripartum ARV* Exposure (n=45)**		***In utero / Peripartum ARV* Exposed (n=44)**	***No In utero / Peripartum ARV* Exposure (n=57)**	
% Female Sex	54 (98.1)	39 (86.6)	0.025	38 (90.5)	51 (91.1)	0.920
Biological parent	52 (94.6)	40 (88.9)	0.300	39 (88.6)	37 (64.9)	0.006
Caregiver Depressed	26 (48.2)	17 (37.8)	0.300	12 (28.6)	10 (17.9)	0.208
Has own income	40 (74.1)	32 (71.1)	0.742	31 (75.6)	39 (70.9)	0.608
Caregiver age (years, mean, SD)	35.3(7.8)	36.6 (8.4)	0.432	32.2 (6.2)	35.5 (9.9)	0.062
Highest CG Quality (5th Quintile)	15 (27.3)	5 (11.1)	0.044	11 (25.0)	9 (15.7)	0.249
Child Demographic/Clinical Characteristics						
Female Child	32 (59.3)	22 (40.7)	0.354	21 (43.8)	27 (56.3)	0.971
	Mean (SD)	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**	
Child Current Age (in years,)	7.5 (1.4)	7.5 (1.4)	0.775	7.8 (1.6)	7.9 (1.4)	0.570
Height for age z-score (mean, SD)	0.54 (1.3)	0.41 (1.17)	0.921	-0.05 (1.1)	-0.06 (1.19)	0.944
5 minutes Apgar Score	8.0 (2.0)	7.4 (2.9)	0.186	7.9 (2.4)	7.9 (1.8)	0.951
Birth weight (in kg)	3.53 (0.76)	3.43 (0.50)	0.480	3.36 (0.53)	3.27 (0.48)	0.415
Caregiver Reported Cognitive Score**						
Adaptive Skills Index (ASI)	0.05 (1.04)	0.06 (0.98)	0.961	0.34 (1.0)	-0.24 (0.77)	0.002
Behavioral Symptoms Index (BSI)	0.16 (1.01)	-0.08 (1.02)	0.246	-0.06 (1.03)	-0.05 (0.86)	0.940
Internalizing Problems Composite (IPC))	0.18 (0.97)	-0.09 (1.05)	0.191	0.07 (1.04)	-0.03 (0.91)	0.606
Externalizing Problems Composite (EPC)	0.23 (1.07)	-0.05 (1.09)	0.191	0.03 (1.02)	-0.10 (0.87)	0.491
Global Executive Composite (GEC)	0.07 (1.13)	-0.12 (1.04)	0.395	-0.23 (0.91)	0.04 (0.94)	0.146
cART Treatment Related Factors (if HIV+)						

Current Child cART Status/Regimen						
NNRTI Regimen (Nevirapine/Efavirenz)	(··)	(··)	(··)	25 (56.8)	38 (71.7)	
Protease Inhibitor (Lopinavir/Ritonivir/Kaletra)	(··)	(··)	(··)	18 (40.9)	13 (24.5)	0.222
cART naïve	(··)	(··)	(··)	1(2.3)	2 (3.8)	
Currently Virologically suppressed	(··)	(··)	(··)	22 (64.7)	30 (58.8)	0.586
Child ever had AIDS defining condition	(··)	(··)	(··)	8 (18.8)	10 (19.2)	0.896
CD4 measures	(··)	(··)	(··)	Mean (SD)	Mean (SD)	
CD4 nadir (cells/uL)	(··)	(··)	(··)	799.6 (505)	766 (606)	0.773
Current CD4 (cells/uL)	(··)	(··)	(··)	1316 (635)	1236(676)	0.556

*: includes Any ART exposure includes children identified as ART exposed but regimen was not specified in medical records (i.e. 1 CPHIV and 7 CHEU). **: ASI includes child proficiency in the following five areas: adaptability, social skills, leadership, activities of daily living and functional communication. BSI: captures extent of child having following problematic behaviors: attention problems, atypicality, withdrawal, depression, hyperactivity, aggression; IPC includes display of behaviors consistent with: depression, anxiety, somatization; EPC: captures children's display of following problematic behaviors: hyperactivity, aggression, conduct problems; GEC: a global measure of caregiver rating of dependent child for behaviors reflective of deficits in executive function. Integrates all eight executive function subscales: inhibition, shift, emotional control, initiation, working memory, planning organization, materials organization and monitoring. **ɣ:** P-value for difference in proportion (via chi-square tests) or difference in means (via t-tests) for children with and without any in utero/peripartum ART exposure.

Table 2: Early life antiretroviral exposure as determinant of socio-emotional adjustment and executive Function over 12 months' period in 6-10 year old perinatally HIV-exposed uninfected Ugandan children with or without maternal antiretroviral therapy

		Adaptive Skills Index$_{\varphi}$					
		Unadjusted Comparisons		***Multivariable Model 1***		***Multivariable Model 2***	
Early ART	N	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	45	0.13 ± 0.11	Ref	0.06 ± 0.09	Ref	0.11 ± 0.11	Ref
sdNVP/sdNVP+AZT	18	0.48± 0.25	0.35(-0.20, 0.90)	0.44± 0.24	0.38(-0.20, 0.95)	0.35± 0.23	0.23(-0.29, 0.75)
sdNVP +AZT+ 3TC	12	-0.20 ± 0.13	-0.34 (-0.69, 0.02)	-0.29 ± 0.15	-0.36 (-0.75, 0.02)	-0.18± 0.16	-0.30 (-0.67, 0.08)
cART	25	0.31± 0.12	0.18 (-0.16, 0.51)	0.24± 0.13	0.18 (-0.17, 0.48)	0.23± 0.13	0.11 (-0.21, 0.43)
		Behavioral Symptoms Index$_{\varphi}$					
Early ART	N	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	45	-0.11 ± 0.12	Ref	-0.11 ± 0.12	Ref	-0.22 ± 0.11	Ref
sdNVP/sdNVP+AZT	18	-0.39 ± 0.16	-0.29 (-0.71, 0.13)	-0.27 ± 0.16	-0.17 (-0.56, 0.22)	-0.09± 0.17	0.07 (-0.40, 0.55)
sdNVP +AZT+ 3TC	12	0.63 ± 0.31	**0.73 (0.04, 1.41)**	0.66 ± 0.27	**0.76 (0.08, 1.44)**	0.29± 0.28	0.52 (-0.16, 1.19)
cART	25	-0.05 ± 0.15	0.05(-0.38,0.48)	-0.09 ± 0.19	0.01(-0.45,0.48)	-0.17 ± 0.17	0.06 (-0.44,0.38)
		Internalizing Problems Composite$_{\varphi}$					
Early ART	N	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	45	-0.10 ± 0.13	Ref	-0.08 ± 0.11	Ref	-0.19± 0.10	Ref
sdNVP/sdNVP+AZT	18	-0.29± 0.15	-0.19(-0.59, 0.20)	-0.15 ± 0.15	-0.07(-0.44, 0.31)	0.00 ± 0.16	0.19 (-0.19, 0.56)
sdNVP +AZT+ 3TC	12	0.53 ± 0.29	0.63 (-0.01, 1.27)	0.55 ± 0.26	**0.63 (0.00, 1.27)**	0.22 ± 0.27	0.40 (-0.12, 0.96)
cART	25	-0.00 ± 0.16	0.10 (-0.33, 0.52)	-0.03 ± 0.19	0.06 (-0.40, 0.51)	-0.10± 0.18	0.08 (-0.39, 0.43)
		Externalizing Problems Composite$_{\varphi}$					
Early ART	N	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	45	-0.02 ± 0.15	Ref	0.01 ± 0.13	Ref	-0.10± 0.12	**Ref**
sdNVP/sdNVP+AZT	18	-0.17 ± 0.15	-0.15(-0.55, 0.26)	-0.05 ± 0.17	-0.07(-0.49, 0.35)	0.12± 0.19	0.22(-0.23, 0.66)
sdNVP +AZT+ 3TC	12	0.59 ± 0.30	0.61 (-0.07, 1.29)	0.69 ± 0.25	**0.68 (0.06, 1.30)**	0.35 ± 0.26	0.45 (-0.17, 1.07)

cART	25	0.08 ± 0.17	0.10 (-0.36, 0.55)	0.08 ± 0.20	0.07 (-0.41,0.55)	0.02 ± 0.17	0.11 (-0.31,0.54)
		Global Executive Composite Deficits $_{\varphi}$					
Early ART	N	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	45	0.06± 0.14	Ref	-0.01± 0.12	Ref	-0.13± 0.11	Ref
sdNVP/sdNVP+AZT	18	-0.32 ± 0.22	-0.38 (-0.90, 0.14)	-0.39 ± 0.23	-0.37 (-0.91, 0.16)	-0.18 ± 0.19	-0.05 (-0.51, 0.41)
sdNVP +AZT+ 3TC	12	0.81 ± 0.21	**0.81 (0.39, 1.23)**	0.79 ± 0.20	**0.80 (0.30, 1.31)**	0.41± 0.19	**0.54 (0.06, 1.03)**
cART	25	-0.25 ± 0.20	-0.31 (-0.81,0.19)	-0.38 ± 0.23	-0.33 (-0.87,0.20)	-0.42 ± 0.22	-0.28 (-0.79,0.23)

Estimates shown are time averaged peripartum ART regimen vs. no peripartum ART related standardized mean difference (SMD) in respective socioemotional adjustment outcomes. Least square means (LSMs) and corresponding standard errors (SEs) are also shown from corresponding models. SMD values reflect clinically meaningful change in age/sex standardized outcomes interpreted according to the Cohen criteria as: Small to modest: ES<|0.33|, Moderate: |0.33|≤ES<|0.50| and Strong/Large: ES≥|0.50|. ‡All estimates are calculated from repeated measures linear mixed models adjusted for: time, ART regimen, ART regimen*time. Multivariable model 1 is adjusted for caregiver demographic factors (age, sex, education, parent vs. non-parent relationship with child). Multivariable Model 2 is adjusted for all variables in model 1 plus caregiving quality and caregiver depression. $_{\varphi}$: *Early ART*time, p>0.10*

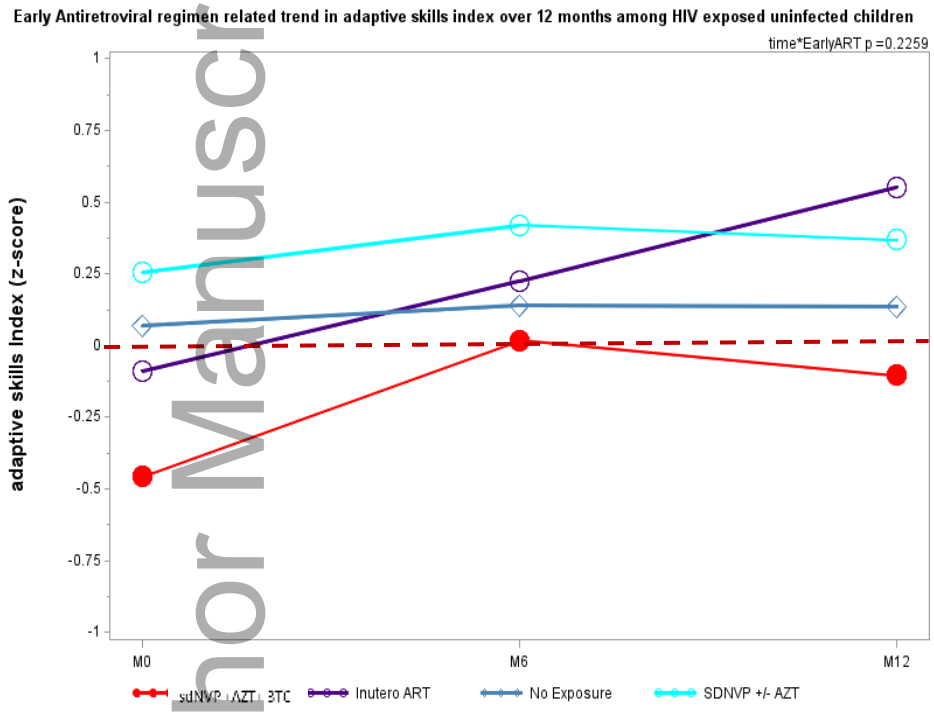
Table 3: Early Life Antiretroviral Exposure in relationship to time-averaged performance in socioemotional adjustment outcomes over 12 months' period in 6-10 year old Perinatally HIV-infected Ugandan Children with or without maternal antiretroviral therapy

		Behavioral Symptoms Index$_{\psi}$					
		Unadjusted Comparison		***Multivariable Model 1***		***Multivariable Model 2***	
Early ART$^{\pm}$	N	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	57	-0.13± 0.10	Ref	-0.12± 0.12	Ref	-0.12± 0.12	Ref
sdNVP/sdNVP+AZT	24	-0.30 ± 0.20	-0.17 (-0.71, 0.36)	-0.20 ± 0.20	-0.05 (-0.57, 0.47)	-0.22 ± 0.23	-0.10 (-0.69, 0.50)
sdNVP +AZT+ 3TC	10	-0.04 ± 0.21	0.09(-0.49, 0.67)	-0.23 ± 0.23	-0.08(-0.64, 0.48)	-0.08 ± 0.21	0.05(-0.53, 0.62)
cART	10	-0.29± 0.15	-0.16 (-0.61, 0.28)	-0.52 ± 0.20	-0.38 (-0.91, 0.16)	-0.53 ± 0.22	-0.41 (-1.08, 0.27)
		Internalizing Problems Composite$_{\psi}$					
Early ART$^{\pm}$	N	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	57	-0.08± 0.09	Ref	-0.08± 0.09	Ref	-0.02± 0.11	Ref
sdNVP/sdNVP+AZT	24	-0.19 ± 0.20	-0.11 (-0.65, 0.43)	-0.16± 0.18	-0.07 (-0.55, 0.40)	-0.13 ± 0.21	-0.11 (-0.66, 0.44)
sdNVP +AZT+ 3TC	10	0.12 ± 0.24	0.20(-0.43, 0.84)	-0.14 ± 0.21	-0.06(-0.58, 0.46)	0.05 ± 0.19	0.07(-0.44, 0.57)
cART	10	-0.10 ± 0.15	-0.02 (-0.45, 0.41)	-0.35 ± 0.17	-0.27 (-0.75, 0.21)	-0.33 ± 0.18	-0.31(-0.91, 0.29)
		Externalizing Problems Composite$_{\psi}$					
Early ART$^{\pm}$	**N**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	57	-0.07 ± 0.10	Ref	-0.11 ± 0.12	Ref	-0.15 ± 0.12	Ref
sdNVP/sdNVP+AZT	24	-0.22± 0.17	-0.15 (-0.63, 0.34)	-0.13± 0.19	-0.02 (-0.50, 0.45)	-0.21± 0.22	-0.06 (-0.63, 0.51)
sdNVP +AZT+ 3TC	10	0.12± 0.21	0.19 (-0.37, 0.75)	0.00± 0.24	0.11 (-0.45, 0.67)	0.05± 0.22	0.20 (-0.42, 0.82)
cART	10	-0.20 ± 0.17	-0.13 (-0.60, 0.35)	-0.36 ± 0.17	-0.25 (-0.75, 0.24)	-0.40 ± 0.18	-0.25 (-0.84, 0.35)
		Global Executive Composite Deficits$_{\psi}$					
Early ART$^{\pm}$	**N**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**	**LSM ± SE**	**SMD (95% CI)**
None	57	0.05 ± 0.10	Ref	0.04 ± 0.10	Ref	0.08 ± 0.11	Ref
sdNVP/sdNVP+AZT	24	-0.33 ± 0.12	-0.38(-0.76, 0.01)	-0.36 ± 0.13	**-0.40(-0.79, -0.01)**	-0.33 ± 0.25	**-0.41(-0.81, -0.00)**

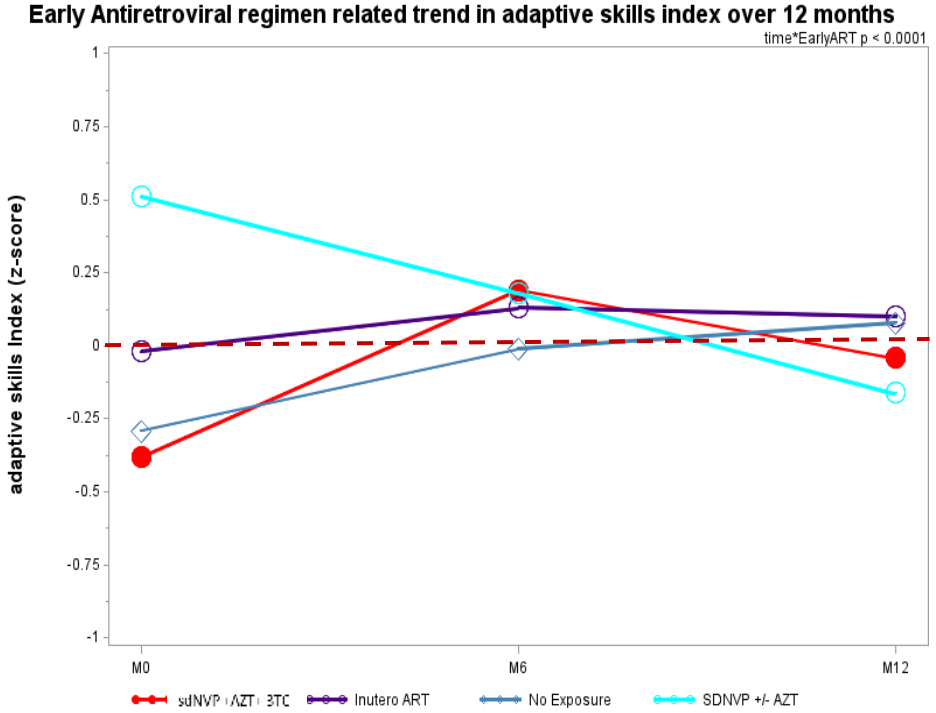
sdNVP +AZT+ 3TC	10	0.49 ± 0.24	0.44 (-0.20, 1.09)	0.38 ± 0.28	0.34 (-0.33, 1.01)	0.37 ± 0.25	0.29 (-0.35, 0.92)
cART	10	-0.54 ± 0.20	-0.59 (-1.03, -0.04)	-0.75 ± 0.20	**-0.79 (-1.32, -0.26)**	-0.74 ± 0.20	**-0.82 (-1.37, -0.28)**

Estimates shown are time averaged peripartum ART regimen vs. no peripartum ART related standardized mean difference (SMD) in respective socioemotional adjustment outcomes. Least square means and corresponding standard errors are also shown from corresponding models. SMD values reflect clinically meaningful change in age/sex standardized outcomes interpreted as: Small: SMD<|0.33|, Moderate: |0.33|<=SMD<|0.50| and Strong/Large: SMD>=|0.50|. ‡All estimates are calculated from repeated measures linear mixed models adjusted for: time, ART regimen, ART regimen*time. Multivariable model 1 is adjusted for caregiver factors (age, sex, education, parent vs. non-parent status, caregiving quality and caregiver depression). Multivariable Model 2 is adjusted for all variables in model 1 plus Current HIV-management related factors CD4 nadir. φ *:Earlier*time, p>0.10*

Figure 1: Peripartum ART Regimen Related Trend in Adaptive Skills Index over one year from 6-10 years of life among Perinatally HIV exposed and infected children from Uganda

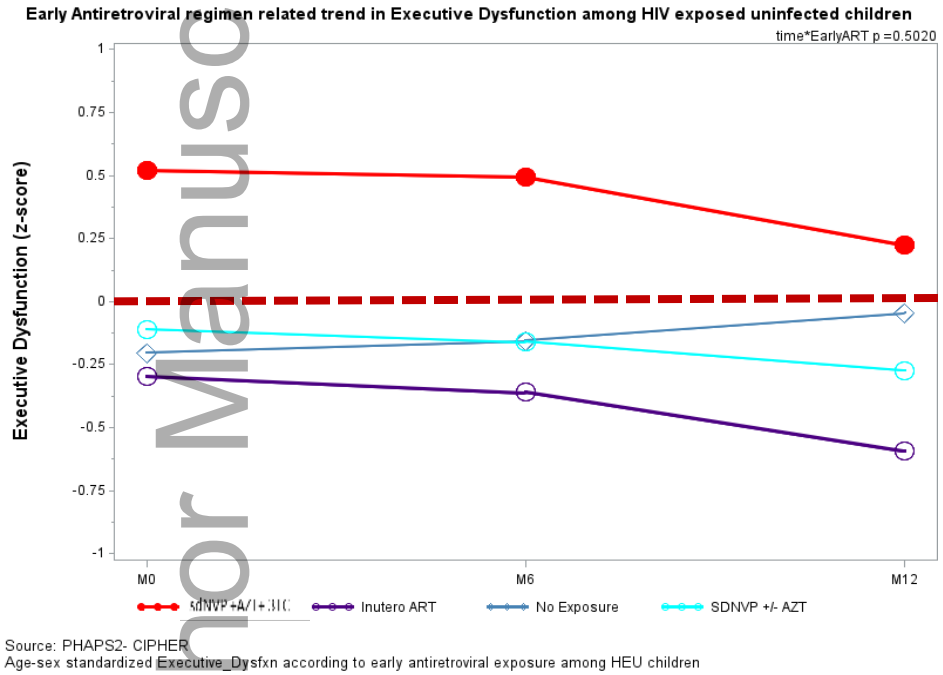


Source: PHAPS2- CIPHER
Age-sex standardized ASI according to early antiretroviral exposure among HEU children

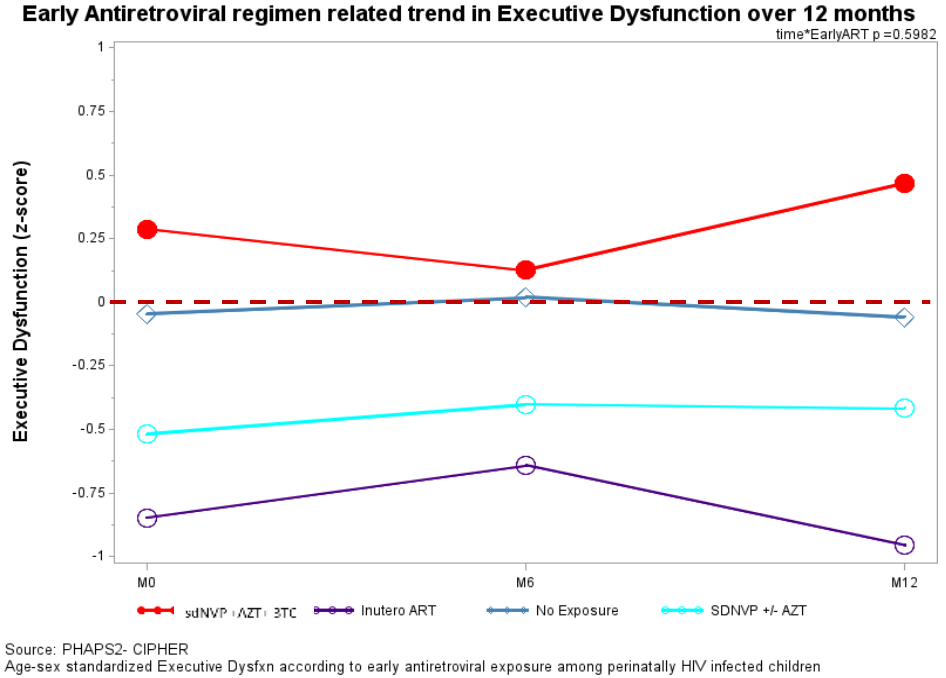


Source: PHAPS2- CIPHER
Age-sex standardized ASI according to early antiretroviral exposure among perinatally HIV infected children

Figure 2 – Peripartum ART Regimen Related Trend in Global Executive Dysfunction over one year from 6-10 years of life among perinatally HIV exposed and infected children from Uganda.



Source: PHAPS2- CIPHER
Age-sex standardized Executive_Dysfxn according to early antiretroviral exposure among HEU children



Source: PHAPS2- CIPHER
Age-sex standardized Executive Dysfxn according to early antiretroviral exposure among perinatally HIV infected children