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Nutritional and immunological correlates of memory and neurocognitive development among HIV infected children living in Kayunga, Uganda

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Abstract

Objective—To identify the nutritional and immunological correlates of memory and neurocognitive development as measured by the Mullen Scales of Early Learning (MSEL) and by the Color Object Association Test (COAT) among children in Uganda.

Design—This analysis uses baseline data collected between 2008 and 2010 from 119 HIV-infected children ages 1–6 years participating in a randomized controlled trial of an interventional parenting program in Kayunga, Uganda.

Methods—Peripheral blood draws were performed to determine immunological biomarkers. Unadjusted and adjusted linear regression models were used to relate MSEL and COAT scores to sociodemographic characteristics, weight-for-age Z-scores (WAZ), antiretroviral therapy (ART) status and immunological biomarkers.

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Results—111 children were included in the final analysis. Lower levels of CD4⁺ CD38⁺ T-cells ($p=0.04$) were associated to higher Immediate and Total Recall scores ($p=0.04$). Higher levels of CD8⁺ HLA-DR⁺ T-cells were associated with higher Total Recall score ($p=0.04$) of the COAT. Higher CD4⁺ CD38⁺ HLA-DR⁺ T-cells levels were associated with higher Gross Motor scores of the MSEL ($p=0.02$). WAZ was positively correlated to Visual Reception, Fine Motor, Expressive Language and composite score of the MSEL.

Conclusions—Overall, WAZ was a stronger predictor of neurocognitive outcomes assessed by the MSEL. CD4⁺ CD38⁺ T-cells were more specifically associated with memory-related outcomes. Future research should include immunological markers and standardized neurocognitive tests to further understand this relationship.

Keywords

HIV; COAT; MSEL; children; WAZ; immunological

Introduction

According to the Joint United Nations Program on HIV/AIDS, in 2012 there were 190,000 children under 15 years of age living with HIV in Uganda [1]. This number has been growing because more children who are born with HIV are able to live longer lives as a result of Uganda's national effort to ramp up clinical use of antiretroviral therapy (ART). Children who are perinatally infected with HIV commonly suffer from cognitive deficits, developmental delay, and behavioral, psychiatric and motor problems [2]. Developmental deficits among HIV-1 infected children can include the following areas: language, motor skills [3, 4], verbal reasoning, memory [5], visual spatial integrative ability [6, 7] and executive functioning [8]. Behavioral problems can include delinquent behavior and poor social competence [9]. It is becoming increasingly important to understand correlates of neurodevelopmental problems among HIV-infected children as life expectancy increases.

Undernutrition, commonly measured as low weight-for-age Z-scores (WAZ), has been associated with developmental delay in sub-Saharan African children with and without HIV [10–13]. A previous study showed that a low WAZ score was the most significant risk factor associated with mortality among HIV-infected Ugandan children not on ART [14]. Although the relationship between WAZ scores and certain neurodevelopmental parameters including psychomotor skills, social skills and achievement of language milestones has been studied [10–12], the association of undernutrition with working memory, receptive and expressive language, and behavioral psychiatric symptoms among HIV-infected children has not been previously examined.

Neurodevelopmental outcomes may also be affected by immunological parameters such as viral load and CD4⁺ and CD8⁺ T-cell counts, which are well-established biomarkers of the clinical stage of HIV disease [15–17]. Higher viral loads have been shown to negatively impact domains of child behavior including emotional control, task shifting and inhibition [18, 19]. In addition, markers of activated CD8⁺ T-cells, specifically CD8⁺ CD38⁺ HLA-DR⁺ T-cells subsets, have been associated with more advanced disease stages [20], and more specifically, higher levels of these markers were associated with negative behavioral

outcomes in school-aged Ugandan children [19]. Similar markers of activated CD4⁺ T-cells, namely CD4⁺ CD38⁺ HLA-DR⁺ T-cells subsets, were shown by Kapetanovic et al. to correlate negatively with IQ on the Bayley Scales of Infant Development (BSID) in children one year of age [21]. However, the relationship between these HIV biomarkers and cognition, motor skills, working memory and language development in children has not been well documented. Additionally, the impact of these immunological parameters on neurodevelopment during early childhood in particular has not been previously examined.

The most recent World Health Organization (WHO) guidelines recommend that ART should be initiated immediately in all HIV-positive children under the age of 5, regardless of CD4⁺ T-cell count values [22]. Although long-term effects of ART in young children living in rural Uganda have been associated with better neurodevelopmental outcomes [23], no studies to date have examined whether being on ART affects working memory.

Given the important neurodevelopmental deficits in memory, language, motor skills and behavior identified among HIV-infected children and the clinical relevance associated with nutrition, growth, and central nervous system (CNS) involvement [2–5], our objective was to identify nutritional and immunological correlates of cognitive development, as measured by the MSEL and COAT, among children ages 1–6 living with HIV in Kayunga, Uganda.

Methods

Study design

This is a secondary analysis of baseline data collected from HIV-infected children ages 1–6 years participating in a randomized controlled trial of a parenting intervention called Mediational Intervention for Sensitizing Caregivers (MISC). Participating children were living in Kayunga District and were referred to the study by Child-health Advocacy Uganda (CAI). After consent, 119 children were enrolled and evaluated between 2008 and 2010. All children were perinatally infected and confirmed HIV-positive with Western Blot and ELISA tests. Trial exclusion criteria included having a serious birth complication, severe malnutrition, bacterial meningitis, encephalitis, cerebral malaria or any other brain injury or disorder that might require hospitalization and which might conceal the effect of the intervention. Methods for this trial are further described in Boivin et. al [24].

Study procedures

After obtaining written consent from the parent/guardian, child neurodevelopment testing was performed in the local language of Luganda in a private and quiet setting within the project's office. Study staff administering the neurodevelopment tests had bachelor's degrees and received a 1-week training by a senior co-author following a standardized protocol. During the clinical evaluation, children's weight and age was recorded, obtaining Z-scores using CDC's child growth standard charts included in Epi Info software. Peripheral blood draws were obtained from all children in order to determine complete blood counts and immunological biomarkers, including CD4⁺ and CD8⁺ T-cell counts as well as viral load. Blood specimens drawn at Kayunga District Hospital on the same day as the neurodevelopment testing or shortly after, and were transported to the Makerere University-

Walter Reed Project (MU-WRP) for processing and storage. The Institutional Review Boards of Michigan State University, University of Michigan, the School of Medicine Research Ethics Committee at Makerere University, and the Ugandan National Council for Science and Technology approved the study.

Measures

Child demographics included age, sex, current anti-retroviral treatment use (ART: yes/no), and weight-for-age (Z-scores obtained using the Epi Info software v. 7 based on the CDC's child growth standards) [25]. Viral load was assessed using the Amplicor HIV-1 Monitor test, version 1.5 (Roche Diagnostics, Indianapolis, IN) in standard mode. HIV-associated immunodeficiency values are based on WHO classification for established HIV infection [26].

Assessment of CD4⁺ and CD8⁺ T-cell activation

Immunophenotyping of CD4⁺ and CD8⁺ T-cells was performed. Samples were processed at MU-WRP. To measure immune activation, peripheral blood mononuclear cells (PBMCs) were isolated by ficoll-hypaque gradient centrifugation. Freshly isolated PBMCs were analyzed for CD4⁺ and CD8⁺ T-cell activation using the Beckton-Dickinson FACScalibur (BD, San Carlos, California). Cells were stained with fluorochrome monoclonal antibodies: CD4⁺ or CD8⁺ T-cells PerCP-cy5.5, HLA-DR⁺ PE and CD38⁺ APC. Preset gating was applied to all samples based on a naturally occurring break in the expression pattern of the activation markers documented in healthy Ugandan children. Reference values presented for immunomarkers in Table 1 are based on a sample of 352 healthy, HIV-negative Cameroonian children [27]. HIV-associated immunodeficiency values are based on WHO immunological classification for established HIV infection [26].

Developmental Outcomes

Objective measures of child development are those in which there is a direct observation of the child performing a specific task. In these tests, motor, language and visual reception skills are frequently evaluated by a trained assessor using a standardized battery of tests that can include the use of props and toys. Two objective performance-based tests of child cognitive development were used in this study. The Mullen Scales of Early Learning (MSEL) is a comprehensive test consisting of 124 items measuring specific developmental domains of Gross Motor, Fine Motor, Visual Reception, Receptive Language and Expressive Language. Items are combined into four cognitive scales (Visual Reception, Fine Motor, Receptive Language and Expressive Language) that can be summarized to yield an overall score (the composite total MSEL score) as well as domain-specific sub-scores. The total composite MSEL score serves as a general measure of fluid intelligence reflective of general cognitive ability [28]. Age-adjusted T-scores were obtained from standardized samples from the U.S., and lower scores indicate diminished cognitive abilities. The Mullen scales have previously been adapted for use with young children in rural Uganda and demonstrate high sensitivity when used within this population [29].

The Color Object Association Test (COAT) was used to assess memory. This test uses the placement of small toys inside of small colored boxes to assess visual memory independent

of language competency [30]. The principal outcomes are the number of recalled items for assessing immediate memory and an overall total recall or learning score of correctly placed items over the course of a test, with lower scores indicating poorer performance. Although the COAT has previously been adapted and used as a valid measure of memory for Ugandan children [31]. It is an experimental measure that is currently not used for clinical interpretation.

Statistical Analysis

Distributions of demographics as well as nutritional and immunological variables were summarized. Internal consistency of the MSEL and COAT scales were evaluated using Cronbach's alpha. After checking that data did not violate linear regression assumptions (linear relationship, normal distribution of the outcome, and homoscedasticity), linear regression was used to relate the MSEL and COAT scores to demographic characteristics (sex and age), ART status, viral load and immunological biomarkers (CD4⁺ cell count %, CD4⁺ CD38⁺ %, CD4⁺ HLA-DR⁺ %, CD4⁺ CD38⁺ HLA-DR⁺ %, CD8⁺ CD38⁺ %, CD8⁺ HLA-DR⁺ % and CD8⁺ CD38⁺ HLA-DR⁺ % T-cells). Two sets of linear regression models were built. First, simple (unadjusted) models related the COAT and MSEL outcomes to viral load and immunological biomarkers, age, sex, WAZ and ART status, individually. Second, the effects of each biomarker and viral load on outcomes were adjusted for age, sex, WAZ and ART status by including the respective variables as covariates. In the unadjusted models, given the available sample size and 0.05 level of significance, correlations of 0.26 or higher in absolute value could be detected as statistically significant with power of 0.80 or greater. Since correlations less than 0.2 in absolute value are regarded as very weak, 0.2–0.39 as weak, 0.40–0.59 as moderate, 0.6–0.79 as strong and 0.8–1 as very strong [32], this analysis was well powered to detect even weak correlations. In the adjusted models with 5 explanatory variables, the detectable effect size expressed as Cohen's f-squared was 0.07. Therefore the effects of biomarkers that corresponded to at least 7% of the residual variance were detectable as statistically significant. All statistical tests were two-sided. All analyses were performed in STATA version 12 (*Stata* [computer program]. Version 12. College Station, TX 2012).

Results

A total of 111 children were included in the study. Eight children were excluded from analyses due to missing viral loads. Median age was 3.6 years (IQR=2.5, 4.7), 62 were boys (55.9%) and median weight-for-age Z-score (WAZ) was -2.2 (IQR=-3.3, -0.9). Only 35 children (31%) were currently on antiretroviral therapy (ART). Median CD4⁺ T-cells count percentage was 33.2% (IQR=22.7, 44.9) and CD8⁺ count percentage was 55.5% (42.5, 62.6). COAT median Immediate Recall and Total Memory score were 2 (IQR=2, 6) and 5 (IQR=2, 12), respectively. Median MSEL scores were 31.2 (IQR=31.2, 31.2) for Gross Motor, 32 (IQR=20, 39) for Fine Motor, 27 (IQR=20, 38) for Visual Reception, 33 (IQR=28, 50) for Expressive Language, and 70 for composite total MSEL score (IQR= 55, 82) (Tables and Figures 1 and 2). Cronbach's alpha was 0.75 and 0.84 for the COAT and MSEL, respectively.

Table 3 shows the coefficients for the explanatory variables in the two sets of models for the COAT scores described in the analysis section. In the unadjusted analysis, higher Immediate Recall scores were associated with older age ($\beta=0.99$, 95% CI=(0.52, 1.46)), and higher CD4⁺ HLA-DR⁺ T-cells levels ($\beta=0.11$, 95% CI=(0.02, 0.20)). Lower Immediate Recall scores were associated with higher CD4⁺ CD38⁺ T-cells and CD8⁺ CD38⁺ T-cells levels ($\beta=-0.08$, 95% CI= (-0.12, -0.04) and $\beta=-0.05$, 95% CI=(-0.09, -0.01), respectively). The effect of age persisted in the adjusted models over and above the biomarkers. After adjustment for age, sex, WAZ, and ART status, only CD4⁺ CD38⁺ T-cells remained significantly associated with the Immediate Recall scores.

In the unadjusted analyses, higher Total Recall scores were associated with age ($\beta=2.51$, 95% CI= (1.42, 3.60), CD4⁺ HLA-DR⁺ T-cells ($\beta=-0.24$, 95% CI=(0.03, 0.45)) and CD8⁺ HLA-DR⁺ T-cells ($\beta=0.08$, 95% CI=(0.01, 0.15)). Lower Total Recall scores were associated with CD4⁺ CD38⁺ T-cells ($\beta=-0.18$, 95% CI=(-0.27, -0.08)). Similar to the analysis of Immediate Recall Scores, the effect of age remained significant over and above biomarkers in the adjusted models. The effect of CD4⁺ CD38⁺ and CD8⁺ HLA-DR⁺ T-cells remained significant over and above age, sex, WAZ and ART status. Viral load, entered either untransformed or log-transformed, was not significantly associated with the COAT outcomes (Table 3).

Table 4 summarizes the results of models for the Mullen score. In the unadjusted analysis, age was negatively associated with Visual Reception, Fine Motor, Gross Motor, and Receptive Language. Except for Receptive Language and Gross Motor, a higher WAZ was associated with higher scores on MSEL. CD8⁺ CD38⁺ T-cells were positively associated and CD8⁺ CD38⁺ HLA-DR⁺ T-cells were negatively associated with Visual Reception in the unadjusted models. In the second set of models that included one biomarker at a time as well as age, WAZ, sex, and ART status, the significant associations between age and WAZ noted above persisted. Over and above age, WAZ, sex and ART status, the levels of CD8⁺ CD38⁺ HLA-DR⁺ T-cells were significantly related to Visual Reception scores ($a\beta=-0.19$, 95% CI (-0.35, -0.03), $p=.01$), CD4⁺ CD38⁺ HLA-DR⁺ T-cells were significantly related to Gross Motor scores ($a\beta=0.28$, 95% CI (0.02, 0.53), and the levels of CD8⁺ HLA-DR⁺ T-cells were significantly associated to Receptive Language ($a\beta=0.10$, 95% CI (0.003, 0.19), (Table 4).

Discussion

This study suggests that among Ugandan children living with HIV, immunological markers of CD4⁺ and CD8⁺ T-cell activation are poor predictors of neurocognitive outcomes as measured by the MSEL, and of memory outcomes as measured by the COAT, with the exception of CD4⁺ CD38⁺ HLA-DR⁺ T-cells for gross motor functioning, CD8⁺ CD38⁺ HLA-DR⁺ T-cells % for Visual Reception and CD8⁺ HLA-DR⁺ T-cells % on the Receptive Language score on the MSEL, and CD8⁺ HLA-DR⁺ T-cells % for Total Recall and CD4⁺ CD38⁺ T-cells % for Immediate Recall and Total Recall on the COAT. However, weight-for-age Z-scores (WAZ) were positively correlated with scores for Visual Reception, Gross Motor function, Expressive Language and composite performance on the MSEL, even after accounting for other variables. To our knowledge, this is the first study to explore the

association between performance-based measures of cognition (i.e. MSEL and COAT) and CD4⁺ and CD8⁺ T-cells expressing different activation markers among HIV-infected children in Sub-Saharan Africa.

Central nervous system (CNS) disorders, ranging from mild cognitive impairment to encephalopathy and dementia, are among the most serious long-term complications associated with HIV infection [33]. Early access to ART has significantly reduced the burden of these sequelae and prolonged life expectancy among children living with HIV [34]. Given the increasing number of children living with this chronic disease, early specific predictors of neurocognitive outcomes in children is crucial [1]. In this study, among the CD4⁺ T-cell markers that were examined, the strongest association was found between CD4⁺ T-cells co-expressing CD38⁺ and HLA-DR⁺ T-cells and gross motor function. Specifically, higher CD4⁺ CD38⁺ HLA-DR⁺ T-cells levels were correlated with higher gross motor functioning as assessed by MSEL. Also, CD4⁺ CD38⁺ T-cells appear to be a good predictors of overall memory function.

There is limited research related to the CD8⁺ T-cell biomarkers and how they relate to cognitive outcomes. Among children under the age of one, Mekmullica and colleagues reported that lower CD8⁺ HLA-DR⁺ T-cells levels were associated with more favorable psychomotor and cognitive development as measured by the Bayley Scale of Infant Development (BSID) [35]. Our results suggest that higher levels of CD8⁺ HLA-DR⁺ T-cells are associated with better memory performance, reflected in higher Total Recall scores on the COAT and with higher Receptive Language scores. Although difference in age groups and testing packages utilized in these studies may account for the associations observed, the significance of CD8⁺ HLA-DR⁺ % levels across both studies singles it out as a potential tool to screen children who might be at greater risk for poorer cognitive and behavioral outcomes. Further research is needed to determine the validity and reliability of these findings.

It has been hypothesized that immune cell activation plays a central role in the neuropathogenesis of HIV disease, with CD8⁺ T-cells freely crossing the blood-brain barrier, activating infected cells and leading to a pro-inflammatory cascade that further damages the CNS [36]. In our adjusted analysis, we found that higher levels of CD8⁺ CD38⁺ HLA-DR⁺ % were associated with lower Visual Reception and Gross Motor Scale scores on the MSEL. In previous work from our group in Uganda, we found that children with higher CD8⁺ CD38⁺ HLA-DR⁺ % levels had more significant psychiatric symptoms and poorer emotional and behavioral outcomes, as measured by the Child Behavior Checklist and Behavior Rating Inventory of Executive Function, respectively [19]. Our findings are consistent with the conclusion that higher levels of CD8⁺ CD38⁺ HLA-DR⁺ % are observed among children with neurodevelopmental delay. This leads us to hypothesize that this marker could be used to screen HIV-infected children who might benefit most from interventions that aim to improve cognitive and behavioral outcomes.

Uganda has one of the highest prevalence of infant malnutrition in the world [37]. Stunting prevalence in Uganda has been estimated at 35% (32%–38%) among children under the age of 5 [38]. In the results presented here, we found a positive association between stunting (i.e.

low weight-for-age) and the Visual Reception, Gross Motor, and Expressive Language scales and the composite total MSEL score. Previous research among HIV-infected and HIV-exposed children in Tanzania found that stunting and wasting were independently associated with poor psychomotor and neurodevelopmental outcomes [39]. Park and colleagues reported similar findings using the BSID II among children who had been internationally adopted [40]. Given the high vulnerability to poor psychomotor and neurodevelopmental outcomes among undernourished, HIV-infected children [41], stunting should be considered an accurate identifier of those children who would benefit most from early cognitive and behavioral interventions in addition to nutritional support.

High HIV RNA plasma viral load, a common marker of disease progression and severity, has been commonly associated with poorer neurocognitive performance and neurodevelopmental outcomes in adults [7, 42, 43]. We previously reported that higher viral loads were associated with more behavioral dysfunction among school-aged children from Kayunga, Uganda [19]. However, in the results presented here, viral load appears to be a poor predictor of neurocognitive functioning, suggesting that viral load could be related to subtle injury in those brain regions most strongly associated with behavior and executive function. In this sense, the myriad of neuropsychological tests currently available represent promising opportunity to gain knowledge about how viral CNS infection translates into cognitive and behavioral dysfunction in HIV-infected children. This finding needs to be considered within the extensive body of scientific research that shows an association between having a high viral load and poor neurocognitive performance in children [7, 42, 43].

Given the proven clinical relevance of and increased access to plasma viral load testing in low-income countries, future studies should continue to explore the value of this parameter as a risk factor for poor psychomotor and memory outcomes using standardized cognitive testing.

As expected when using the MSEL and the COAT, age was a strong predictor of a child cognition and memory.

Limitations

Results presented here should be viewed in light of certain limitations. First, some sociodemographic and economic factors that impact child neurodevelopment were not included in the baseline questionnaire, thus were not part of the analysis presented here. Research designs that include orphan status, parent/guardian education level, and family income, among others, can provide insight into the influence of children's social and developmental milieu.

Second, our results are based on cross-sectional data, hindering our capacity to establish causality of the reported associations. Longitudinal studies are needed to determine if these associations are consistently present over time. Standard interpretation of T-scores for the MSEL proposes that any scores at or below 7th percentile or -1.5 to -2 standard deviations below the average indicate significant delays. However, without specific norms for this Ugandan population, it is difficult to establish appropriate cut-off scores. The COAT and

MSEL scores observed in our sample are lower than those obtained by healthy community controls from Mulago Hospital, Kampala, Uganda [44] in accordance with previous reports observing poor cognitive outcomes in HIV-infected children [45].

Conclusion

Despite these limitations, our findings suggest that percentages of CD4⁺ CD38⁺, CD8⁺ HLA-DR⁺, CD4⁺ CD38⁺ HLA-DR⁺, CD4⁺ CD38⁺ HLA-DR⁺ and CD8⁺ HLA-DR⁺ T-cells can be used as predictive immunological markers of neurodevelopment in young children living with HIV. Stunting was a strong predictor of overall neurocognitive development, but not a good memory predictor. Further research is required in order to identify other immunological markers better fit for these specific tests or other tests that are commonly used among this age group. Given that children ages 1–5 years are at a critical period of cognitive development [31], it is crucial to find reliable biomarkers and anthropometric measures that can be used to identify those children who would benefit most from early neurocognitive interventions.

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References

1. Joint United Nations Program on HIV/AIDS U. Uganda: HIV and AIDS estimates; 2013. 2015.
2. Llorente AM, Brouwers P, Leighty R, Malee K, Smith R, Harris L, et al. An analysis of select emerging executive skills in perinatally HIV-1-infected children. *Appl Neuropsychol Child*. 2014; 3:10–25. [PubMed: 24236937]
3. Drotar D, Olness K, Wiznitzer M, Guay L, Marum L, Svilar G, et al. Neurodevelopmental outcomes of Ugandan infants with human immunodeficiency virus type 1 infection. *Pediatrics*. 1997; 100:E5. [PubMed: 9200379]
4. Ultmann MH, Diamond GW, Ruff HA, Belman AL, Novick BE, Rubinstein A, et al. Developmental abnormalities in children with acquired immunodeficiency syndrome (AIDS): a follow-up study. *The International journal of neuroscience*. 1987; 32:661–667. [PubMed: 2439470]
5. Levenson RL Jr, Mellins CA, Zawadzki R, Kairam R, Stein Z. Cognitive assessment of human immunodeficiency virus-exposed children. *American Journal of Diseases of Children (1960)*. 1992; 146:1479–1483. [PubMed: 1456264]
6. Boivin MJ, Green SD, Davies AG, Giordani B, Mokili JK, Cutting WA. A preliminary evaluation of the cognitive and motor effects of pediatric HIV infection in Zairian children. *Health psychology : official journal of the Division of Health Psychology, American Psychological Association*. 1995; 14:13–21.
7. Laughton B, Cornell M, Boivin M, Van Rie A. Neurodevelopment in perinatally HIV-infected children: a concern for adolescence. *J Int AIDS Soc*. 2013; 16:18603. [PubMed: 23782482]
8. Bisiacchi PS, Suppiej A, Laverda A. Neuropsychological evaluation of neurologically asymptomatic HIV-infected children. *Brain and cognition*. 2000; 43:49–52. [PubMed: 10857661]

9. Bomba M, Nacinovich R, Oggiano S, Cassani M, Baushi L, Bertulli C, et al. Poor health-related quality of life and abnormal psychosocial adjustment in Italian children with perinatal HIV infection receiving highly active antiretroviral treatment. *AIDS Care*. 2010; 22:858–865. [PubMed: 20635250]
10. Abubakar A, Holding P, Newton CR, van Baar A, van de Vijver FJ. The role of weight for age and disease stage in poor psychomotor outcome of HIV-infected children in Kilifi, Kenya. *Developmental medicine and child neurology*. 2009; 51:968–973. [PubMed: 19486107]
11. Bobat R, Coovadia H, Moodley D, Coutsoodis A, Gouws E. Growth in early childhood in a cohort of children born to HIV-1-infected women from Durban, South Africa. *Annals of Tropical Paediatrics*. 2001; 21:203–210. [PubMed: 11579858]
12. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. *PLoS medicine*. 2010; 7:e1000273. [PubMed: 20520849]
13. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*. 2008; 371:243–260. [PubMed: 18207566]
14. Munyagwa M, Baisley K, Levin J, Brian M, Grosskurth H, Maher D. Mortality of HIV-infected and uninfected children in a longitudinal cohort in rural south-west Uganda during 8 years of follow-up. *Tropical medicine & international health : TM & IH*. 2012; 17:836–843. [PubMed: 22591447]
15. Boivin MJ, Ruel TD, Boal HE, Bangirana P, Cao H, Eller LA, et al. HIV-subtype A is associated with poorer neuropsychological performance compared with subtype D in antiretroviral therapy-naïve Ugandan children. *Aids*. 2010; 24:1163–1170. [PubMed: 20425886]
16. Sanchez-Ramon S, Bellon JM, Resino S, Canto-Nogues C, Gurbindo D, Ramos JT, et al. Low blood CD8+ T-lymphocytes and high circulating monocytes are predictors of HIV-1-associated progressive encephalopathy in children. *Pediatrics*. 2003; 111:E168–E175. [PubMed: 12563091]
17. Tobin NH, Aldrovandi GM. Immunology of pediatric HIV infection. *Immunological reviews*. 2013; 254:143–169. [PubMed: 23772619]
18. Jeremy RJ, Kim S, Nozyce M, Nachman S, McIntosh K, Pelton SI, et al. Neuropsychological functioning and viral load in stable antiretroviral therapy-experienced HIV-infected children. *Pediatrics*. 2005; 115:380–387. [PubMed: 15687448]
19. Ruiseñor-Escudero H, Familiar I, Nakasujja N, Bangirana P, Opoka R, Giordani B, et al. Immunological correlates of behavioral problems in school-aged children living with HIV in Kayunga, Uganda. *Global Mental Health*. 2015; 0:1–9.
20. McCloskey TW, Kohn N, Lesser M, Bakshi S, Pahwa S. Immunophenotypic analysis of HIV-infected children: alterations within the first year of life, changes with disease progression, and longitudinal analyses of lymphocyte subsets. *Cytometry*. 2001; 46:157–165. [PubMed: 11449406]
21. Kapetanovic S, Aaron L, Montepiedra G, Burchett SK, Kovacs A. T-cell activation and neurodevelopmental outcomes in perinatally HIV-infected children. *AIDS (London, England)*. 2012; 26:959.
22. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. 2013
23. Brahmabhatt H, Boivin M, Ssempijja V, Kigozi G, Kagaayi J, Serwadda D, et al. Neurodevelopmental benefits of antiretroviral therapy in Ugandan children aged 0–6 years with HIV. *Journal of acquired immune deficiency syndromes 1999*. 2014; 67:316–322. [PubMed: 25314252]
24. Boivin MJ, Bangirana P, Nakasujja N, Page CF, Shohet C, Givon D, et al. A year-long caregiver training program improves cognition in preschool Ugandan children with human immunodeficiency virus. *The Journal of pediatrics*. 2013; 163:1409–1416. e1401–e1405. [PubMed: 23958115]
25. Dean A, Arner T, Sunki G, Friedman R, Lantinga M, Sangam S, et al. Epi Info™, a database and statistics program for public health professionals. Centers for Disease Control and Prevention. Atlanta, Georgia, USA. 2007

26. WHO. , editor. World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classificatoin of HIV-related disease in adults and chidren. Geneva, Switzerland: 2007.
27. Sagnia B, Ateba Ndongo F, Ndiang Moyo Tetang S, Ndongo Torimiro J, Cairo C, Domkam I, et al. Reference values of lymphocyte subsets in healthy, HIV-negative children in Cameroon. *Clin Vaccine Immunol*. 2011; 18:790–795. [PubMed: 21411603]
28. Mullen E. Mullen scales of early learning. Circle Pines, MN: American Guidance Service. Inc. (21). 1995
29. Busman, RA.; Page, CF.; Oka, E.; Giordani, B.; Boivin, MJ. Factors contributing to the psychosocial adjustment of Ugandan preschool children with HIV/AIDS. In: Boivin, MJ.; Giordani, B., editors. *Neuropsychology of children in Africa: Perspectives on risk and resilience*. New York: Springer; 2013.
30. Jordan CM, Johnson AL, Hughes SJ, Shapiro EG. The Color Object Association Test (COAT): the development of a new measure of declarative memory for 18-to 36-month-old toddlers. *Child Neuropsychology*. 2007; 14:21–41. [PubMed: 18097800]
31. Boivin MJ, Bangirana P, Nakasujja N, Page CF, Shohet C, Givon D, et al. A year-long caregiver training program improves cognition in preschool Ugandan children with human immunodeficiency virus. *J Pediatr*. 2013; 163:1409–1416. e1401–e1405. [PubMed: 23958115]
32. Brithish Medical Journal. Correlation and regression. 2015
33. Tardieu M, Le Chenadec J, Persoz A, Meyer L, Blanche S, Mayaux MJ. HIV-1-related encephalopathy in infants compared with children and adults. French Pediatric HIV Infection Study and the SEROCO Group. *Neurology*. 2000; 54:1089–1095. [PubMed: 10720279]
34. Ghafouri M, Amini S, Khalili K, Sawaya BE. HIV-1 associated dementia: symptoms and causes. *Retrovirology*. 2006; 3:28. [PubMed: 16712719]
35. Mekmullica J, Brouwers P, Charurat M, Paul M, Shearer W, Mendez H, et al. Early immunological predictors of neurodevelopmental outcomes in HIV-infected children. *Clinical Infectious Diseases*. 2009; 48:338–346. [PubMed: 19115969]
36. Kovacs A. Early immune activation predicts central nervous system disease in HIV-infected infants: Implications for early treatment. *Clinical Infectious Diseases*. 2009; 48:347–349. [PubMed: 19115973]
37. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*. 2008; 371:243–260. [PubMed: 18207566]
38. UNICEF: WHO; UNICEF. , editor. UNICEF. Uganda: Maternal, newborn and child survival; 2010.
39. McDonald CM, Manji KP, Kupka R, Bellinger DC, Spiegelman D, Kisenge R, et al. Stunting and wasting are associated with poorer psychomotor and mental development in HIV-exposed Tanzanian infants. *The Journal of nutrition*. 2013; 143:204–214. [PubMed: 23256148]
40. Park H, Bothe D, Holsinger E, Kirchner HL, Olness K, Mandalakas A. The impact of nutritional status and longitudinal recovery of motor and cognitive milestones in internationally adopted children. *International journal of environmental research and public health*. 2011; 8:105–116. [PubMed: 21318018]
41. Ivers LC, Cullen KA, Freedberg KA, Block S, Coates J, Webb P, et al. HIV/AIDS, undernutrition, and food insecurity. *Clinical Infectious Diseases*. 2009; 49:1096–1102. [PubMed: 19725790]
42. Ruel TD, Boivin MJ, Boal HE, Bangirana P, Charlebois E, Havlir DV, et al. Neurocognitive and motor deficits in HIV-infected Ugandan children with high CD4 cell counts. *Clin Infect Dis*. 2012; 54:1001–1009. [PubMed: 22308272]
43. Smith R, Chernoff M, Williams PL, Malee KM, Sirois PA, Kammerer B, et al. Impact of HIV severity on cognitive and adaptive functioning during childhood and adolescence. *Pediatr Infect Dis J*. 2012; 31:592–598. [PubMed: 22592486]
44. Bangirana P, Opoka RO, Boivin MJ, Idro R, Hodges JS, Romero RA, et al. Severe malarial anemia is associated with long-term neurocognitive impairment. *Clinical Infectious Diseases*. 2014 ciu293.

45. Le Doaré K, Bland R, Newell M-L. Neurodevelopment in children born to HIV-infected mothers by infection and treatment status. *Pediatrics*. 2012 peds. 2012-0405.

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Figure 1.
Boxplots of Color Object Association Test (COAT)

Figure 2.
Boxplots of Mullen Scales of Early Learning (MSEL)

Table 1

Demographic characteristics and immunological parameters of 111 children living with HIV in Kayunga, Uganda

Characteristic	Children living with HIV (n=111)	Reference* (10 th –90 th percentiles)
Age in years, median (IQR)**	3.6 (2.5–4.7)	–
Gender, n (%)		
Boys	62 (55.9)	–
Girls	49 (44.0)	–
WAZ, median (IQR)***	–2.2 (–3.3, –0.89)	–
Height (cm), median (IQR)	90.7 (83.2, 97.6)	–
Currently on ART, n (%)	35 (31)	–
CD4 count %, median (IQR)	33.2 (22.7, 44.9)	35 (26, 44)
CD8 count %, median (IQR)	55.2 (42.5, 62.6)	20 (14, 30)
CD4 ⁺ CD38 ⁺ %, median (IQR)	69 (56.2, 75.4)	79 (71, 84)
CD8 ⁺ CD38 ⁺ %, median (IQR)	52.8 (43.6, 53.3)	79 (54, 88)
CD4 ⁺ HLA-DR ⁺ %, median (IQR)	2.2 (1.4, 4.1)	5 (3, 9)
CD8 ⁺ HLA-DR ⁺ %, median (IQR)	3 (1, 4)	10 (8, 33)
CD4 ⁺ CD38 ⁺ HLA-DR ⁺ %, median (IQR)	9 (5.8, 4.2)	4 (2, 7)
CD8 ⁺ CD38 ⁺ HLA-DR ⁺ %, median (IQR)	18.5 (12.2, 30.7)	9 (6, 23)
Plasma HIV RNA (copies/mL), median (IQR)	215,201.9 (5958, 341837)	0
Log 10 plasma HIV RNA (copies/mL), median (IQR)	4.9 (2.9, 5.9)	–
HIV-associated immunodeficiency, n (%)		–
None or non-significant	69 (62)	
Mild	11 (10)	
Advanced	15 (13)	
Severe	14 (13)	

* Values based on sample of 352 healthy Cameroonian children (Sagnia et.al).

** IQR= interquartile range.

*** WAZ= weight-for-age Z-scores (WAZ).

Table 2

Summary of distributions of Mullen Scales of Early Learning (MSEL) and Color Object Association Test (COAT) scores of 111 children living with HIV in Kayunga, Uganda

Domains	Median	First quartile, third quartile (IQR)
COAT Immediate Memory	2	2, 6
COAT Total Memory	5	2,12
MSEL Gross Motor	31.2	31.2, 31.2
MSEL Fine Motor	32	20, 39
MSEL Visual Reception	27	20, 38
MSEL Receptive Language	35	26, 44
MSEL Expressive Language	33	28, 50
MSEL Composite score	70	55, 82

Table 3

Unadjusted and adjusted linear regression models for Color Object Association Test (COAT) Immediate and Total Recall scores among 111 children living with HIV in Kayunga, Uganda

Outcome	COAT Immediate Recall		COAT Total Recall	
Predictor	Simple (unadjusted) regression models	Multivariable regression models with <i>one biomarker</i> adjusted for sex, age, WAZ, ART status	Simple (unadjusted) regression models	Multivariable regression models with <i>one biomarker</i> adjusted for sex, age, WAZ, ART status
	β (95%CI)	$a\beta$ (95%CI)	β (95%CI)	$a\beta$ (95%CI)
Sex	-0.81 (-2.12, 0.50)	—	-1.17 (-4.26, 1.91)	—
Age	0.99 (0.52, 1.46)	—	2.51 (1.42, 3.60)	—
WAZ	0.12 (-0.24, 0.47)	—	0.61 (-0.20, 1.43)	—
ART	1.09 (-0.30, 2.48)	—	3.29 (0.05, 6.53)	—
CD4 ⁺ CD38 ⁺	-0.08 (-0.12, -0.04)	-0.05 (-0.09, -0.00)	-0.18 (-0.27, -0.08)	-0.11 (-0.21, -0.01)
CD8 ⁺ CD38 ⁺	-0.05 (-0.09, -0.01)	-0.03 (-0.07, 0.01)	-0.06 (-0.16, 0.03)	-0.01 (-0.10, 0.08)
CD4 ⁺ HLA-DR ⁺	0.11 (0.02, 0.20)	0.07 (-0.02, 0.16)	0.24 (0.03, 0.45)	0.14 (-0.07, 0.34)
CD8 ⁺ HLA-DR ⁺	0.03 (-0.0, 0.06)	0.02 (-0.01, 0.05)	0.08 (0.01, 0.15)	0.06 (0.00, 0.13)
CD4 ⁺ CD38 ⁺ HLA-DR ⁺	0.01 (-0.07, 0.08)	0.01 (-0.05, 0.06)	-0.01 (0.18, 0.16)	-0.00 (-0.13, 0.13)
CD8 ⁺ CD38 ⁺ HLA-DR ⁺	-0.01 (-0.05, 0.04)	0.01 (-0.04, 0.06)	-0.08 (-0.19, 0.03)	-0.02 (-0.12, 0.09)
CD4 %	-0.04 (-0.08, 0.01)	-0.03 (-0.08, 0.01)	-0.06 (-0.16, 0.05)	-0.05 (-0.15, 0.05)
CD8 %	0.02 (-0.03, 0.06)	0.03 (-0.02, 0.07)	-0.002 (-0.10, 0.10)	0.01 (-0.09, 0.11)
Plasma HIV RNA (copies/mL)	$-1.49 \times 10^{-6} (-3.94 \times 10^{-6}, 0.96 \times 10^{-6})$	$-0.58 \times 10^{-6} (-3.13 \times 10^{-6}, 1.97 \times 10^{-6})$	$-5.04 \times 10^{-6} (-10.7 \times 10^{-6}, 0.67 \times 10^{-6})$	$-1.31 \times 10^{-6} (-6.96 \times 10^{-6}, 4.34 \times 10^{-6})$
Log10 plasma HIV RNA (copies/mL)	-0.13 (-0.87, 0.62)	-0.27 (-0.52, 1.06)	-0.57 (-2.21, 1.07)	-0.58 (-1.09, 2.26)

WAZ= weight-for-age Z-scores (WAZ)

Table 4 adjusted linear regression models for Mullen Scales of Early Learning (MSEL) scores among 111 children living with HIV in Kayunga,

	Visual Reception		Fine Motor		Gross Motor	
	Unadjusted	Adjusted*	Unadjusted	Adjusted*	Unadjusted	Adjusted*
	β (95%CI)	$a\beta$ (95%CI)	β (95%CI)	$a\beta$ (95%CI)	β (95%CI)	$a\beta$ (95%CI)
	1.56 (−2.73, 5.86)	—	0.63 (−4.07, 5.33)	—	3.12 (−4.24, 10.49)	—
	−2.23 (−3.89, −0.57)	—	−2.67 (−4.40, −0.94)	—	−10.16 (−16.91, −3.40)	—
	1.75 (0.66, 2.86)	—	1.99 (0.70, 3.10)	—	1.16 (−0.84, 3.17)	—
	−2.46 (−7.45, 2.52)	—	−3.91 (−8.89, 1.07)	—	−6.52 (−16.1, 3.04)	—
	0.10 (−0.05, 0.24)	−0.02 (−0.17, 0.14)	0.12 (−0.04, 0.28)	−0.00 (−0.18, 0.16)	0.04 (−0.30, 0.37)	−0.18 (−0.48, 0.12)
	0.15 (0.01, 0.28)	0.09 (−0.05, 0.23)	−0.12 (−0.04, 0.27)	−0.05 (−0.10, 0.20)	0.16 (−0.16, 0.49)	−0.07 (−0.35, 0.22)
	−0.17 (−0.48, 0.14)	−0.03 (−0.34, 0.28)	−0.26 (−0.60, 0.08)	0.11 (−0.44, 0.22)	−0.13 (−2.35, 2.09)	0.88 (−1.18, 2.95)
	0.02 (−0.08, 0.12)	0.03 (−0.07, 0.12)	−0.01 (−0.12, 0.10)	0.01 (−0.09, 0.11)	−0.67 (−3.20, 1.86)	−0.39 (−2.68, 1.91)
DR ⁺	−0.10 (−0.30, 0.10)	0.10 (−0.16, 0.35)	−0.06 (−0.28, 0.16)	0.03 (−0.18, 0.24)	0.29 (0.00, 0.58)	0.28 (0.02, 0.53)
DR ⁺	−0.19 (−0.35, −0.03)	−0.19 (−0.35, −0.03)	−0.04 (−0.21, 0.14)	−0.02 (−0.20, 0.15)	−0.04 (−0.33, 0.25)	−0.07 (−0.35, 0.22)
	$−3.5 \times 10^{-6}$ (−12.11 $\times 10^{-6}$, 5.0 $\times 10^{-6}$)	$−4.2 \times 10^{-6}$ (−12.8 $\times 10^{-6}$, 4.5 $\times 10^{-6}$)	1.65×10^{-6} (−9.1 $\times 10^{-6}$, 9.4 $\times 10^{-6}$)	$−1.07 \times 10^{-6}$ (−10.3 $\times 10^{-6}$, 8.14 $\times 10^{-6}$)	$−2.5 \times 10^{-6}$ (−15.8 $\times 10^{-6}$, 10.8 $\times 10^{-6}$)	$−3.10 \times 10^{-6}$ (−15.4 $\times 10^{-6}$, 9.17 $\times 10^{-6}$)
	0.31 −2.21–2.83	−0.20 (−2.86–2.46)	0.98 (−1.71–3.68)	−0.24 (−3.02–2.55)	0.08 (−4.78–4.94)	0.31 (−4.22–4.83)
	Expressive Language		Receptive Language		Composite Score	
	Unadjusted	Adjusted*	Unadjusted	Adjusted*	Unadjusted	Adjusted*
	β (95%CI)	$a\beta$ (95%CI)	β (95%CI)	$a\beta$ (95%CI)	β (95%CI)	$a\beta$ (95%CI)
	3.53 (−1.92, 8.98)	—	0.33 (−3.89, 4.55)	—	5.78 (−9.73, 21.29)	—
	2.04 (−0.03, 4.11)	—	−2.52 (−4.07, −0.97)	—	−5.21 (−11.1, 0.66)	—
	2.35 (0.96, 3.75)	—	1.16 (−0.05, 2.27)	—	7.01 (3.09, 10.93)	—
	−1.55 (−7.43, 4.32)	—	−3.83 (−8.30, 0.64)	—	11.77 (−28.3, 4.74)	—
	−0.11 (−0.30, 0.08)	−0.10 (−0.30, 0.10)	0.09 (−0.06, 0.23)	0.03 (−0.18, 0.13)	0.17 (−0.37, 0.70)	−0.05 (−0.30, 0.20)
	−0.04 (−0.22, 0.14)	−0.00 (−0.18, 0.17)	−0.03 (−0.17, 0.11)	−0.10 (−0.24, 0.03)	0.17 (−0.37, 0.67)	0.01 (−0.21, 0.23)
	0.09 (−0.32, 0.49)	0.11 (−0.28, 0.50)	−0.22 (−0.53, 0.08)	−0.09 (−0.40, 0.22)	−0.56 (−1.69, 0.57)	−0.07 (−0.56, 0.42)

CD8 ⁺ HLA-DR ⁺	0.11(−0.02, 0.23)	0.09(−0.03, 0.21)	0.07(−0.02, 0.17)	0.10 (0.003, 0.19)	0.19(−0.16, 0.55)
CD4 ⁺ CD38 ⁺ HLA-DR ⁺	−0.20(−0.46, 0.06)	0.09 (−0.33, 0.16)	−0.17(−0.37, 0.03)	0.12 (−0.31, 0.07)	−0.55(−1.27, 0.18)
CD8 ⁺ CD38 ⁺ HLA-DR ⁺	−0.22(−0.43, −0.02)	−0.03 (−0.20, 0.15)	−0.05(−0.21, 0.10)	−0.08 (−0.24, 0.09)	−0.50(−1.07, 0.08)
Plasma HIV RNA (copies/mL)	10.2×10 ^{−6} (−20.9 ×10 ^{−6} , 0.4×10 ^{−6})	−6.05×10 ^{−6} (−17.0 ×10 ^{−6} , 4.85×10 ^{−6})	−1.2×10 ^{−6} (−9.4 ×10 ^{−6} , 7.1×10 ^{−6})	−3.3×10 ^{−6} (−11.8 ×10 ^{−6} , 5.2×10 ^{−6})	−15.3×10 ^{−6} (−45.9 ×10 ^{−6} , 15.2×10 ^{−6})
Log10 plasma HIV RNA (copies/mL)	−1.20 (−4.39–2.20)	−0.35 (−3.77–3.06)	0.65 (−1.80–3.10)	−0.60(−3.23–2.04)	0.71 (−8.24–9.67)

WAZ= weight-for-age Z-scores (WAZ).

* Multivariable regression model with one biomarker adjusted for: age, sex, WAZ and ART