Trajectories, predictors, and impact on neurocognition of viral control among children living with HIV in Kenya

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2023

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Program Authorized to Offer Degree

Public Health – Epidemiology

University of Washington

Abstract

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Background: Children with HIV have poorer viral control than adults. Early initiation of effective antiretroviral therapy (ART) is essential for suppressing virus and recovering immunity; however, children are less likely to attain viral suppression than adults due to inadequate treatment dosing for weight, drug resistance, and barriers to adherence. Similar to adults, during viral suppression replication-competent HIV persists in infected cells that can reactivate after discontinuation or interruption of ART. Understanding predictors and trajectories of HIV viral control and the effect of HIV viral control on neurocognitive outcomes can inform cure and differentiated care strategies for children with HIV and identify children who could benefit from interventions to improve neurocognitive outcomes.

Methods: We used samples, responses to questionnaires, and results from neurocognitive and neuropsychological assessments from the Optimizing Pediatric HAART study (5R01HD094718; MPIs: Drs. Grace John-Stewart and Dara Lehman), which enrolled a cohort of children with HIV who initiated ART by 12 months of age and have been followed for >10 years post-ART in Kenya. For Chapter 1, we used linear mixed effects models to determine predictors of HIV DNA levels. Chapter 2 involved group-based trajectory models to determine HIV viral load trajectories and Fisher's exact tests and t-tests to determine correlates of HIV viral load trajectories. For Chapter 3, we assessed the relationship between HIV viral load, HIV DNA, and cytomegalovirus (CMV) DNA and neurocognitive and neuropsychological outcomes using generalized linear models.

Results: In Chapter 1, children with 10% higher pre-ART CD4 percent had 13% and 24% lower total and intact HIV DNA levels, respectively, in adjusted models. One-log higher pre-ART HIV viral load was significantly associated with 21% higher total HIV DNA levels. Children on protease inhibitor-based first-line regimens had higher intact HIV DNA compared to children on non-nucleoside reverse transcriptase inhibitor-based regimens. One-log higher CMV DNA copies/ml was significantly associated with 16% higher intact HIV DNA levels. In Chapter 2, nearly two-thirds (63%) of children were in the sustained-low HIV, and 16% were in the sustained-very-high, 9% were in the sustained-high viral load group during the 6-24 month time period. Children in the sustained-high viral load group were more frequently on a first-line protease inhibitors and had younger caregivers compared to children in the sustained-low viral load group. From 48-96 months post-ART, 76% of children were in the sustained-low viral load group, and children in the high-to-low viral load group had younger caregivers compared to children in the sustained-low viral load group. In Chapter 3, higher pre-ART viral load, total HIV DNA levels, and intact DNA levels were associated with lower mean executive function z-score. Higher CMV DNA levels were associated with lower cognitive ability and motor z-scores. Higher total HIV DNA was associated with higher motor z-scores and higher intact HIV DNA was associated with higher attention z-scores.

Conclusions: Early initiation of effective and palatable ART is crucial for improving viral control and neurocognitive outcomes for children with HIV. Further studies investigating the link between CMV DNAemia and HIV DNA levels as well as impact of CMV prevention or suppression therapy on neurocognitive outcomes in children living with HIV are needed. Leveraging advanced epidemiologic methods to assess longitudinal viral control could inform approaches to target children with different patterns of HIV viral control. Children with higher levels of pre-ART viral load and early HIV DNA and CMV DNA levels may benefit from targeted and effective strategies to improve long-term neurocognitive outcomes.

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ACKNOWLEDGEMENTS

First, I want to thank the Optimizing Pediatric HAART (OPH) study participants, the clinical research team, and administrative team for their dedication and support. This research would not have been possible without the support of a multidisciplinary and collaborative team. I would like to express my deepest gratitude to my dissertation committee: Drs. Grace John-Stewart (Chair), Barbra Richardson, Stephen Hawes, Jennifer Slyker, Anjuli Wagner, Irene Njuguna, and Susanne May (GSR); my F31 mentors: Drs. Dara Lehman and Sarah Benki-Nugent; and Drs. Dalton Wamalwa and Christine McGrath. Their unwavering support over the last 4 years has been crucial to the development and execution of these scientific aims and my growth as a researcher. I would also like to thank my peers in Dr. John-Stewart's Peridoc group and in Drs. Wagner and Njuguna's mentorship group. Thank you to the faculty, staff, and students at the Global Center for Integrated Health of Women, Adolescents, and Children (Global WACh) and the Kenya Research and Training Center (KRTC) for their support. Lastly, I would like to thank my mentors, colleagues, family, and friends.

INTRODUCTION

An estimated 1.7 million children ages 0-14 years are living with HIV globally.[1] HIV disease in children has a more aggressive course than adult disease.[2] Among children with HIV who are not on treatment, half are estimated to die by 2 years of age.[3] Early initiation of antiretroviral therapy (ART) has resulted in improved survival, suppressed viral load, immune recovery, and reduced likelihood of opportunistic infections.[4–6]

ART clears replicating HIV, but latently infected cells – cells that have HIV DNA integrated into the genome – can reactivate upon treatment interruption, necessitating adherence to lifelong ART.[7] Existing studies have assessed predictors of total HIV DNA levels among children with HIV;[8–12] however, >90% of total HIV DNA is not replication competent due to deletions or mutations.[13] Few studies have evaluated predictors of intact HIV DNA,[14,15] which estimates replication-competent proviruses that can reactivate upon treatment interruption.[16] Determining predictors of total and intact HIV DNA levels can inform cure strategies for children with HIV.

To further investigate HIV viral control in children, we assessed patterns of HIV viral load in children after ART initiation. In programmatic settings, a third of children with HIV who are on ART remain unsuppressed.[17] Children face unique barriers to HIV viral suppression including inappropriate drug dosing for weight, unpalatable regimens, and poor adherence.[18] Leveraging advanced epidemiologic methods[19] to understand early HIV viral load trajectories and correlates associated with HIV viral load trajectories could inform differentiated care approaches to improve health outcomes for children who are not virally suppressed.

After evaluating predictors of HIV DNA levels and trajectories of HIV viral load among children with HIV, we determined the effect of early HIV DNA levels, HIV viral load, and cytomegalovirus DNA levels on neurocognitive outcomes among school-aged children with HIV. Children with HIV have poorer neurodevelopmental and neurocognitive outcomes compared to children without HIV.[20,21] Among children with HIV, early ART can improve neurodevelopmental

outcomes but does not overcome adverse effect of HIV on neurocognition. A few studies have found that early viral suppression has been associated with better neurocognitive outcomes.[22–24] Cytomegalovirus, which is a common co-infection among children with HIV,[25,26] has been associated with accelerated disease progression and poorer neurocognitive outcomes.[27,28] Understanding HIV and cytomegalovirus predictors of neurocognition could help clinicians identify of children with HIV who may benefit from interventions to improve neurocognition.

This dissertation will evaluate predictors of HIV DNA levels, characterize viral load trajectories, and determine the effect of HIV and cytomegalovirus-related biomarkers on neurocognitive outcomes among children with HIV.

CHAPTER 1: Predictors of intact HIV DNA levels among children in Kenya

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Published citation: Neary J, Fish CS, Cassidy NAJ, Wamalwa D, Langat A, Ngugi E, Benki-Nugent S, Moraa H, Richardson BA, Njuguna I, Slyker JA, Lehman DA, John-Stewart G. Predictors of intact HIV DNA levels among children in Kenya. AIDS. 2023 May 1;37(6):871-876. doi: 10.1097/QAD.0000000000003499. Epub 2023 Jan 30. PMID: 36723512; PMCID: PMC10079608.

ABSTRACT

Objective: We determined predictors of both intact (estimate of replication-competent) and total (intact and defective) HIV DNA in the reservoir among children with HIV.

Design: HIV DNA in the reservoir was quantified longitudinally in children who initiated antiretroviral therapy (ART) at <1 year of age using a novel cross-subtype intact proviral DNA assay that measures both intact and total proviruses. Quantitative PCR was used to measure pre-ART cytomegalovirus (CMV) viral load. Linear mixed effects models were used to determine predictors of intact and total HIV DNA levels (log_{10}copies/million).

Results: Among 65 children, median age at ART initiation was 5 months and median follow-up was 5.2 years; 86% of children had CMV viremia pre-ART. Lower pre-ART CD4 percent (adjusted relative risk [aRR]: 0.87, 95% confidence intervals [95%CI]: 0.79-0.97; p=0.009) and higher HIV RNA (aRR: 1.21, 95%CI: 1.06-1.39; p=0.004) predicted higher levels of total HIV DNA during ART. Pre-ART CD4 percent (aRR: 0.76, 95%CI: 0.65-0.89; p<0.001), CMV viral load (aRR: 1.16, 95%CI: 1.01-1.34; p=0,041), and first line protease inhibitor-based regimens compared to non-nucleoside reverse transcriptase-based regimens (aRR: 1.36, 95%CI: 1.04-1.77; p=0,025) predicted higher levels of intact HIV DNA.

Conclusion: Pre-ART immunosuppression, first-line ART regimen, and CMV viral load may influence establishment and sustainment of intact HIV DNA in the reservoir.

Keywords: intact DNA; HIV DNA; HIV; pediatric; HIV reservoir; cytomegalovirus

INTRODUCTION

HIV proviruses persist in long-lived immune cells in the HIV reservoir after ART has cleared replicating virus. Over 90% of HIV proviruses in the reservoir are defective and not replication-competent.[29] However, replication-competent proviruses retain the ability to reactivate, resulting in rebound viremia upon ART interruption. Most pediatric HIV reservoir studies have evaluated total HIV in the reservoir [8–12] which include both replication-competent and defective proviruses. Only a few pediatric studies have estimated cells with replication-competent HIV DNA that can be induced ex vivo using the quantitative viral outgrowth assay (QVOA),[30,31] however QVOA underestimates the size of the reservoir.[13] These studies found that larger replication-competent HIV levels at 24 weeks post-ART was associated with replication-competent reservoir size at 2 years post-ART initiation.[30,31]

Recent PCR-based intact proviral DNA assays, more accurately estimate the replication-competent reservoir by distinguishing full-length (intact) proviruses from those with large deletions (defective).[13,16,32] Few studies have investigated intact HIV DNA in children. One study in Botswana found that very early ART initiation was associated with smaller intact reservoir size.[14] Identifying predictors of intact HIV DNA, which reflect replication-competent proviurses,[13,29] can inform HIV cure strategies. We assessed predictors of intact and total (intact plus defective) HIV DNA levels in children with HIV who started ART during the first year of life.

METHODS

Children with HIV who initiated ART at <1 year of age were enrolled into the OPH study from September 2007 to August 2010 at Kenyatta National Hospital in Nairobi, Kenya. OPH was a randomized controlled trial comparing treatment interruption and continued treatment following 24 months of ART (NCT00428116).[33] HIV DNA was assessed at timepoints with HIV RNA <150 copies/ml after 12 months of ART. The study was approved by the University of Washington and

Fred Hutchinson Cancer Center Institutional Review Boards and Kenyatta National Hospital Ethics and Research Committee. Caregivers provided written informed consent for their children's participation.

HIV RNA was previously measured in longitudinal plasma samples using the Gen-Probe HIV RNA assay, lower limit of detection of 150 copies/ml.[33] Quantitative PCR was used to measure cytomegalovirus (CMV) viral loads in plasma as previously described.[9] The lower limit of detection was 50 copies/ml for CMV DNA. Undetectable HIV RNA and CMV DNA were designated a value of half the limit of detection.

Total and intact HIV proviruses were quantified using the cross-subtype intact proviral DNA assay (CS-IPDA) on DNA from cryopreserved peripheral blood mononuclear cells (PBMCs).[32] PBMC samples were selected from timepoints 12, 24, 42, 60, and 96 months post-ART initiation known to have virally suppressed HIV RNA levels (<150 copies/ml). CS-IPDA was performed in duplicate, with additional replicates performed on samples with no intact HIV proviruses detected until either intact proviruses were detected or a minimum of 10^5 cells were interrogated. Both total and intact HIV DNA levels are determined for samples with DNA shearing rates of <40% as measured by the RPP30 reference assay.[32] Data from samples with >40% DNA shearing (n=6) is limited to total but not intact HIV DNA due to shearing. In this analysis, all samples have detectable total HIV DNA. The CS-IPDA is able to detect a single copy of intact HIV DNA,[32] and thus, samples with undetectable intact HIV DNA were set to 0.5 copies over the number of cells interrogated normalized to 10^6 cells. In a prior analysis, <1% of sequences were incorrectly classified as defective when they were intact, suggesting that underestimating intact due to sequence diversity is rare.[32]

Potential predictors of HIV DNA levels included infant sex, pre-ART CD4 percent (continuous, assessed per 10% change); pre-ART HIV RNA ($\log_{10}$ copies/ml); first-line ART regimen (protease inhibitors [PI]/non-nucleoside reverse transcriptase inhibitors [NNRTI]) among

children who did and did not switch regimens; and pre-ART CMV viremia (log_{10}DNA copies/ml). Inverse probability weighting was conducted to account for differential missingness by age at enrollment into the study. All models adjusted for time on ART at HIV DNA measurement, age at ART initiation, and randomization arm. Linear mixed effects models were used to determine predictors of total and intact HIV DNA levels (log_{10}copies/million T cells). Linear mixed effects models are commonly used to analyze correlated data; we used these models to account for nonindependence of longitudinal HIV DNA measurements within individuals.[34] Adjustment variables were considered collinear if the standard error changed >10% when the variable was removed from the model. Stata version 17.0 (Stata Corporation, College Station, Texas USA) was used for all analyses.

RESULTS

Sixty-five children had PBMCs available during times of HIV RNA suppression in which total HIV DNA was measured. Of those, 59 children had HIV DNA samples with minimal shearing (0.2-22%) allowing for quantification of intact HIV DNA without risk of underestimation due to DNA shearing. Median number of HIV DNA measurements per child was 2 (IQR: 1, 3; Table 1). Half of the children were male, 45% (n=29) had a PI-based first-line regimen, and 26% (n=17) were randomized at 24 months post-ART to a short treatment interruption (median 4.3 months). Median age at ART initiation was 5.0 (IQR: 4.2-8.1) months and median follow-up time was 5.2 (IQR: 3.3-8.0) years. Most (86%) children had detectable CMV viremia pre-ART with a median CMV viral load of 3.6 (IQR: 2.9-4.0) log_{10}copies/ml. Total and intact HIV DNA decayed over time on ART, with a median total and intact HIV DNA of 3.1 (IQR: 2.8-3.3) and 2.2 (IQR: 1.1-2.3) log_{10}copies/million T cells, respectively, at 1 year post-ART initiation, which decayed to 2.8 (IQR: 2.5-3.0) and 0.9 (IQR: 0.6-1.3) log_{10}copies/million T cells, respectively, by 8 years post-ART (Supplementary Figure 1).

Children with a 10% higher pre-ART CD4 percent had 13% and 24% lower total and intact HIV DNA levels (adjusted relative risk [aRR]: 0.87, 95% confidence interval [95%CI]: 0.79-0.97, p=0.009 and aRR: 0.76, 95%CI: 0.65-0.89, p<0.001), respectively during longitudinal follow-up (Figure 1). One-log higher pre-ART HIV RNA was significantly associated with 21% higher total (aRR: 1.21, 95%CI: 1.06-1.39, p=0.004) and higher intact HIV DNA levels but this association was not statistically significant (aRR: 1.13, 95%CI: 0.90-1.42, p=0.305).

Children with PI-based first-line ART had higher intact HIV DNA compared to children with NNRTI-based regimens (aRR: 1.36, 95%CI: 1.04-1.77, p=0.025); this effect was similar in the subset of children without treatment switches (aRR: 1.32, 95%CI: 0.90-1.92, p=0.153). Infant sex was not associated with HIV DNA levels (Figure 1).

Each 1-$\log_{10}$ increase in CMV DNA copies/ml was associated with a 16% larger intact HIV DNA (aRR: 1.16, 95%CI: 1.01-1.34, p=0.041) but was not associated with total HIV DNA (aRR: 0.96, 95%CI: 0.87-1.04, p=0.317).

DISCUSSION

We assessed predictors of total and intact HIV DNA among children who started ART within the first year of life and were followed up to 8 years post-ART. Higher pre-ART HIV viral load and lower CD4 percent predicted higher total HIV DNA levels during suppressive ART, while higher pre-ART CMV viral load, first-line PI-based regimens, and lower CD4 percent predicted higher intact HIV DNA levels over serial longitudinal assessments.

We found that lower pre-ART CD4 percent predicted both higher total and intact HIV DNA levels. Our findings are consistent with a pediatric study in South Africa which found that lower pre-ART CD4 percent and higher pre-ART HIV viral load were associated with higher total HIV DNA after 1 year of ART.[35] Several studies have noted that very early initiation of ART limits HIV DNA levels.[12,36–43] However, there remain shortfalls in early infant diagnosis and treatment

initiation globally, with only 59% of children with HIV with a known HIV status in 2020.[44] Delayed diagnosis and treatment is a lost opportunity to lower HIV DNA in children with HIV.

Nearly all Kenyan infants with HIV acquire CMV by 3 months of age and, in the absence of ART, experience persistent CMV viremia.[45] In this cohort, most (86%) children had detectable CMV viremia pre-ART. We previously found that children with detectable CMV DNAemia pre-ART had larger total HIV DNA levels at 24 months post-ART than children without CMV DNA pre-ART.[9] In our current analysis, HIV DNA was assessed longitudinally for a median of 5 years post-ART, and we found that higher pre-ART CMV DNA level was associated with higher intact HIV DNA. There is evidence that the replication-competent DNA is established near the time of ART initiation.[46,47] Among children with HIV with concurrent acute CMV infection at the time of ART initiation, proliferation of activated CMV-specific CD4+ T cells may expand the population of HIV-permissive cells, leading to larger HIV intact DNA levels, consistent with our findings.[48] The effect of subclinical CMV on certain T-cell subsets – specifically effector memory T-cells – may play an important role in sustainment of intact HIV DNA.[49,50]

Children treated with first-line PI-based regimens had significantly higher intact DNA levels than children on NNRTI-based regimens. This effect persisted in the smaller subset of children who did not switch regimens, but lost statistical significance, potentially due to the limited sample size. A recent study among adults with HIV found that those on PI-based regimens had higher levels of cell-associated HIV RNA and DNA compared to adults on NNRTIs. [51] Existing literature has also found that adults on NNRTIs have lower levels of residual HIV viremia compared to those on PIs.[52] Additionally, there is evidence that adults on NNRTIs may have a longer time to viral rebound after treatment interruption,[53] which may result from the longer half-life of NNRTIs or could indicate lower levels of replication-competent reservoirs among those on NNRTIs compared to those on PIs. It will be important to further evaluate the association between regimen and total

and intact HIV DNA levels in pediatric populations and to understand how newer dolutegravir-based regimens affect HIV reservoir decay.

Limitations of this study include an inability to assess the effect of dolutegravir-based ART regimens on HIV DNA levels given the regimens in use at the time of cohort establishment. In addition, DNA measurements were limited to samples with HIV RNA below detection with HIV RNA measurements available only every 6 months. Thus, we cannot rule out viremia between timepoints. There were a limited number of longitudinal timepoints per child at which children were virally suppressed. Additionally, there was a relatively small number of samples for which pre-ART CMV viral load was quantified; however, pre-ART CMV viral load was associated with intact DNA in this analysis despite the sample size. The parent study included randomization to a short treatment interruption at 24 months post-ART initiation, however, there were no differences in total HIV DNA between children randomized to continue versus interrupt ART, by 18 months after treatment interruption,[54] and randomization arm was included in adjusted models.

In summary, we evaluated total and intact HIV DNA over a median of 5 years post-ART among children <1 year of age. Pre-treatment immune status and CMV viremia influenced HIV DNA in the long-term reservoir. CMV viral levels pre-ART specifically influenced the replication-competent intact HIV DNA.

ACKNOWLEDGEMENTS

We are grateful to the children and families who participated in this research, to the clinic and laboratory staff who provide clinical care and monitoring of this cohort, and to the Comprehensive Care Clinic and Kenyatta National Hospital where the research was conducted. We are also grateful to Julie Overbaugh at Fred Hutchinson Cancer Center for providing advice, human immunodeficiency virus (HIV) viral load testing, and technical support.

CONFLICTS OF INTEREST

Authors have no conflicts of interest to disclose.

FUNDING

This work was supported by the National Institutes of Health (NIH) (Eunice Kennedy Shriver National Institute Of Child Health & Human Development of the National Institutes of Health under Award Number F31HD106261 to JN and National Institute of Allergy and Infectious Diseases grants K01AI087369 to JAS [principal investigator (PI)] and R01 AI076105-07S1 to Julie Overbaugh [PI] and Fogarty International Center K43 TW 011422-01A1 to IN and Eunice Kennedy Shriver National Institute of Child Health and Human Development grants R01HD-23412 and K24HD054314 to G. J. S. [PI] and R01HD094718 to DAL and GJS [MPIs]), the University of Washington Center for AIDS Research (New Investigator and HIV-Associated Malignancy Awards; the Center is funded by NIH grant P30AI027757), and the University Washington Global Center for Integrated Health of Women, Adolescents and Children.

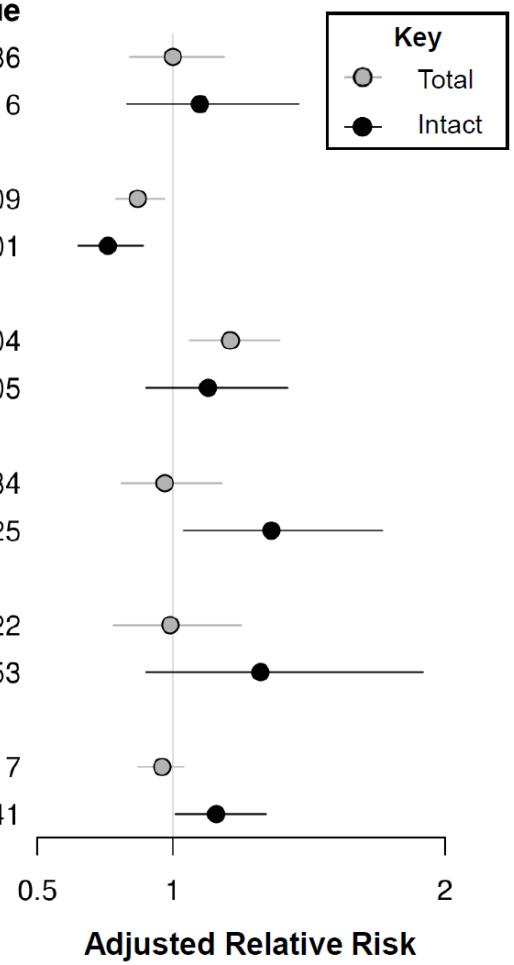
TABLES AND FIGURES

Table 1. Child characteristics

	Children with Total HIV DNA		Children with Intact HIV DNA	
	N	**Median (IQR) or n(%)**	**N**	**Median (IQR) or n(%)**
Age at ART initiation (months)	65	5.0 (4.2, 8.1)	59	4.9 (4.2, 7.9)
Male (ref: Female)	65	34 (52)	59	31 (53)
Time followed (time to last DNA measurement in years)	65	5.2 (3.3, 8.0)	59	5.2 (3.4, 8.0)
Pre-ART CD4% (every 10% change)	65	1.9 (1.4, 2.5)	59	1.8 (1.4, 2.4)
Pre-ART HIV RNA (log_{10}copies/ml)	43	6.6 (6.0, 7.0)	39	6.6 (6.0, 7.0)
First line PI-based Regimen (ref: NNRTI)				
Among all children	64	29 (45)	58	25 (43)
Among children with no switches	41	25 (61)	38	22 (58)
Treatment interruption arm in OPH study	65	17 (26)	59	16 (27)
Pre-ART Cytomegalovirus (CMV)				
CMV (>50 copies/ml) (ref: no CMV)	22	19 (86)	20	18 (90)
CMV viral load (log_{10}copies/ml)	22	3.6 (2.9, 4.0)	20	3.7 (3.4, 4.1)

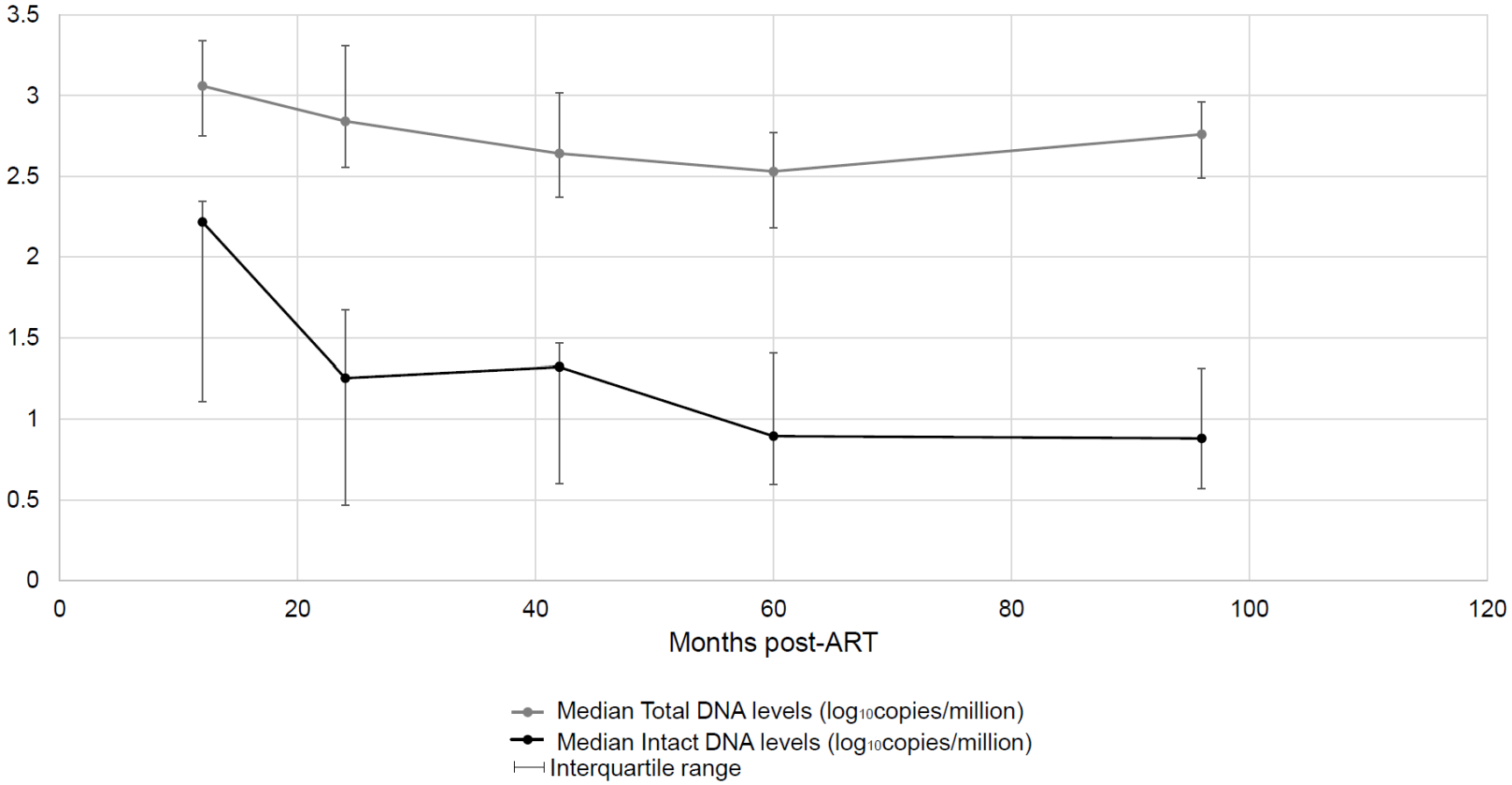
Figure 1. Predictors of total and intact HIV DNA levels (continuous log_{10} copies/million T cells) during longitudinal follow-up up to 8 years post-ART

	Adjusted Relative Risk[a] (95%CI)	p–value
Male (ref: Female)	1.00 (0.84, 1.19)	0.986
	1.10 (0.83, 1.46)	0.516
Pre-ART CD4% (per 10% change)	0.87 (0.79, 0.97)	0.009
	0.76 (0.65, 0.89)	<0.001
Pre-ART HIV RNA (log_{10}copies/ml)	1.21 (1.06, 1.39)	0.004
	1.13 (0.90, 1.42)	0.305
First line ART PI regimen (ref: NNRTI) among all children	0.97 (0.81, 1.18)	0.784
	1.36 (1.04, 1.77)[b]	0.025
First line ART PI regimen (ref: NNRTI) among children with no switches	0.99 (0.78, 1.25)	0.922
	1.32 (0.90, 1.92)[b]	0.153
Pre-ART CMV (log_{10} copies/ml)	0.96 (0.87, 1.04)	0.317
	1.16 (1.01, 1.34)	0.041



a. All models adjusted for time since ART initiation, randomization arm, and age at ART initiation; variables that were collinear were not included in the models.
b. Randomization arm was collinear with age at ART initiation. Age at ART initiation was retained.

Supplemental Figure 1. Median total and intact DNA levels (log_{10}copies/million T cells) by time post-ART



CHAPTER 2: Group-based trajectory modeling to determine long-term HIV viral load trends among children with HIV in Kenya

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ABSTRACT

Background: Identifying determinants of longitudinal viral trajectories using group-based trajectory modeling (GBTM) can inform clinical strategies and mechanisms of non-adherence among children with HIV.

Methods: Children under 12 months of age newly diagnosed with HIV were enrolled in the Optimizing Pediatric HIV Therapy (OPH; NCT00428116) from 2007-2010. Children initiated antiretroviral therapy (ART) at enrollment, and HIV viral load (VL) was assessed every 3 months for 24 months post-ART initiation and 6-monthly thereafter up to 8 years of age. VL trajectory groups were defined using GBTM. Fisher's exact and Kruskal-Wallis tests were used to determine correlates of each trajectory group compared to the sustained-low VL group.

Results: Five VL trajectory groups were identified among 89 children with 522 VL visits from 6-24 months: sustained-low VL (63% of children), sustained-very-high (16%), sustained-high (9%), low-to-high (7%), and high-with-periods-of-low (6%). Children in the sustained-high group were more frequently on a first-line protease inhibitor (PI)-based regimen (63% vs 38%; p=0.03) and had younger caregivers (median: 22 vs 28 years; p=0.02).

Among 54 children with 560 VL visits followed from 48-96 months, 5 trajectory groups were identified: sustained-low (74%), mid-range (4%), periods-of-low (7%), high-to-low (7%), and sustained-high (7%). Those in the high-to-low group had younger caregivers (21 vs 29 years; p=0.01).

Conclusions: Group-based trajectory modeling identified unique VL patterns among children with unsuppressed VL. Caregiver and regimen-related characteristics were associated with patterns of non-suppression. Younger caregivers may benefit from tailored counseling to help them support child ART adherence. Palatable regimens are necessary for viral suppression among children with HIV.

INTRODUCTION

Globally, children with HIV who are on antiretroviral therapy (ART) are less likely to achieve viral suppression than adults on ART.[55] In programmatic settings in Kenya in 2018, 67% of children 0-14 years of age who were on ART were virally suppressed, compared to >90% of adults on ART who were suppressed.[56] Children acquire HIV during a time of immune development[57] and face unique challenges to viral suppression due to dynamic growth, which affects drug dosing, unpalatable regimens, and poor adherence.[58] Thus, patterns of viral suppression can vary widely within pediatric cohorts.

Methodological approaches that assess cumulative viral suppression over a given period or assess mean HIV viral load (VL) among pre-defined subgroups can oversimplify important individual variation in VL. Alternative longitudinal modeling methods, including group-based trajectory models (GBTM), can provide a unique perspective of complex changes in VL over time. This approach clusters individuals based on longitudinal patterns and group formation is not informed by researcher assumptions.[19] GBTM has been used to characterize VL among adults with HIV. [59,60] To our knowledge, this approach has not been used to model VL in pediatric populations. Characterizing trajectories of VL among children diagnosed with HIV in the first year of life can inform strategies to identify unique groups of children who experience viral non-suppression.

METHODS

Study population

This secondary analysis was nested in a prospective cohort study of 140 children with HIV who initiated ART (2 nucleoside reverse transcriptase inhibitors [NRTIs] with either non-nucleoside reverse transcriptase inhibitor [NNRTI] or protease inhibitor [PI]) in the first year of life. Children were enrolled in the Optimizing Pediatric HIV Therapy (OPH; NCT00428116) study

between 2007 and 2010 in Nairobi, Kenya.[33] At 24 months post-ART initiation, a subset of 42 children who met specific inclusion criteria[33] were randomized to either continued or treatment interruption (21 children in each arm). Children in the interruption arm restarted ART after they met WHO ART-eligibility criteria. Most children met restart criteria 3 months after interruption, resulting in the Data Safety and Monitoring Board recommending stopping randomization and giving all children the opportunity to restart at 18-months post-interruption. Median time off ART was 4.3 months.[33] Children enrolled in the OPH study have subsequently remained in follow up on ART for over 96 months. The study was approved by the University of Washington Institutional Review Board and Kenyatta National Hospital Ethics and Research Committee. Caregivers provided written informed consent for their children's participation.

Study procedures

Plasma samples were collected every 3 months up to 24 months post-ART initiation and every 6 months thereafter for HIV VL quantified using the Gen-Probe HIV-1 viral load assay (Gen-Probe Incorporated, San Diego, California, USA) with a lower limit of detection of 150 copies/ml.[61] HIV VL trajectories were evaluated starting after 6 months on ART and, due to the subset of children with short-term interruption at 24 months, separately during two time periods: from 6-24 months and 48-96 months post-ART. There were 16 children randomized to treatment interruption who were included in the HIV VL trajectory analysis between 48-96 months post-ART, and all 16 restarted ART >6 months prior to this time period, with a median time between ART restart and first VL measure during the 48-96 month period of 21 months [IQR: 16-29]). Children included in the 6-24-month and 48-96-month analyses remained in follow up with VL data through 24 months and 96 months post-ART, respectively.

Statistical Methods

Proportions were used to determine cumulative measures of HIV viral suppression (<150 copies/ml). Initial descriptive categorization using *a priori* definitions led to 4 groups: persistent non-suppression (defined as ≥2 unsuppressed (≥150 copies/ml) VL measures), isolated viral rebound (defined as a single VL measure ≥400 copies/ml with suppressed VL immediately before and after), blips (defined as a single VL measure between 150-400 copies/ml, with suppressed VL immediately before and after), and persistent suppression (<150 copies/ml at all VL measurements in the time window assessed)

Group-based trajectory modeling (GBTM) was used to identify groups of children with similar HIV VL trajectories.[19] In contrast to the *a priori* classification, GBTM included no *a priori* assumptions about grouping, and instead solely used statistical methods to categorize groups of children. This approach allows for identification of latent clusters using maximum likelihood estimation and has been used to characterize HIV VL trajectory groups in adults.[62] The primary indicator of interest was continuous HIV VL (log_{10}copies/ml) modeled with a censored normal distribution. We hypothesized 1 to 5 HIV VL trajectory groups for each time period. Iterations of models for 1 to 5 groups were run with a different polynomial form for each group. The optimal model fit and number of groups was determined using Bayesian Information Criterion.[19]

Correlates of specific GBTM groups were assessed using Fisher's exact tests for binary correlates and Kruskal–Wallis tests for continuous variables. The sustained-low viral load group was the reference group. Correlates assessed included child factors (infant sex and infant breastfeeding [ever vs never]), child ART (age at ART initiation [continuous; months] and first-line regimen [PI vs NNRTI]), caregiver factors (caregiver age [continuous; years], caregiver education [continuous; highest year of education completed], primary caregiver [mother vs other]), and socioeconomic factors (crowding [>3 people/room vs ≤2 people/room] and household rent [Kenya shillings]). Additional variables including hospitalization, whether the child's biological parent was living, or whether the child's parent was fulfilling parental duties were collected between 42-48

months post-ART initiation and were assessed as correlates of the 48-96 month trajectory groups. Two-sided tests with $p<0.05$ considered statistically significant were used. Data were analyzed using STATA 17 SE (STATA Corp., College Station, Texas, USA).

RESULTS

Months 6-24 post-ART

Eighty-nine children (64%) remained in follow-up for 24 months post-ART initiation. Half (49%) of children were female, 40% were on a first-line PI-based regimen, and median age at ART initiation was 5 months (Table 1a). Using *a priori* defined classifications, during 6-24 months of follow-up 22 children (25%) did not suppress, 61 (69%) experienced ≥1 time of persistent non-suppression, 17 (19%) experienced ≥1 isolated virologic rebound, and 11 (12%) experienced ≥1 blip. Seven (8%) were suppressed at all 6-24 month visits (Supplementary Figure 1a).

Using GBTM, 5 HIV viral load trajectory groups were identified in the 6-24 month period: sustained-low VL, low-to-high VL, high-with-periods-of-low VL, sustained-high VL, and sustained-very-high VL (Figure 1a). Nearly two-thirds (56 [63%]) of children were in the sustained-low VL group. Fourteen (16%) were in the sustained-very-high VL group, 8 (9%) were in the sustained-high VL group, 6 (7%) were in the low-to-high VL group, and 5 (6%) were in the high-with-periods-of-low VL group.

Compared to the sustained-low reference trajectory group, a higher proportion of children in the sustained-high VL group were on a first-line PI-based regimen (63% vs 38%; p=0.03) and had younger caregivers (median age: 22 [IQR: 21-27] vs 28 [IQR: 24-32] years; p=0.02). Other correlates assessed were not significantly associated with VL trajectory groups from 6-24 months (Table 1a).

Months 48-96 post-ART

Fifty-four children (39%) were followed with VL data for 96 months. Sociodemographic characteristics were similar between children included in the 6-24 and 48-96 month analyses (Table 1A and Table 1B). Forty-three percent of children were female, 43% were on a first-line PI-based regimen, and median age at ART initiation was 5 months (Table 1b). Sixteen (30%) children had been previously randomized to ART interruption at 24 months post-ART and restarted on ART >12 months prior to the 48 month start of the analysis. During the 48-96 month window using *a priori* classifications, 6 children (11%) did not suppress, 30 (56%) experienced ≥1 time of persistent non-suppression, 17 (31%) experienced ≥1 isolated virologic rebound, 21 (39%) experienced ≥1 blip, and 3 (6%) were suppressed at all visits (Supplementary Figure 1b).

Using GBTM, 5 trajectory groups were identified in the 48-96 month period: sustained-low, mid-range, periods-of-low, high-to-low, and sustained-high VL (Figure 1b). Nearly three-quarters (40 [74%]) of children were in the sustained-low VL group. Four children (7%) were in each of the high-to-low, periods-of-low, and sustained-high VL groups. Only 2 children (4%) were in the mid-range VL group.

Compared to those in the sustained-low VL reference group, those in the high-to-low VL group had younger caregivers (median age: 21 [IQR: 19-23] vs 29 [IQR: 24-34] years; p=0.01). The 2 children in the mid-range VL group had both been previously randomized to ART interruption compared to 10 (25%) in the sustained-low group; this difference had a trend for statistical significance (p=0.08). There were no other statistically significant differences between VL trajectory group (p>0.01; Table 1b).

Comparing trajectory groups 6-24 months to 48-96 months post-ART

Fifty-two children were included in both the 6-24 month and 48-96 month GBTM analyses (Supplementary Figure 2). Eighty-three percent of children in the 6-24 month sustained-low VL group were in the 48-96 month sustained-low VL group. Sixty percent of children in 6-24 month

the sustained-high VL group and 43% of children in the 6-24 month sustained-very-high group were in the 48-96 month sustained-low group.

DISCUSSION

In this study, we found that GBTM effectively summarized complex patterns of pediatric HIV VL. In contrast to the *a priori* classifications, which included rare children with complete viral suppression at every visit and other groups with episodic blips and rebounds, GBTM clustered children with rare or episodic viral detection into a 'sustained-low VL' group. GBTM models from each of the two time periods yielded a sustained-low VL group, which included the majority of children, and distinct groups representing children with persistently high VL. Younger caregiver age was associated with being in a sustained-high and high-low-low VL group when compared to children in sustained-low groups at 6-24 and 48-96 months, respectively. Children on PI-based regimens were more frequently in the sustained-high VL group at 6-24 months.

GBTM provides a new approach for summarizing longitudinal VL when compared to approaches that use cumulative definitions of viral suppression or mean models comparing pre-set subgroups. Compared to a cumulative definition of suppressed at all timepoints, GBTM identified a much larger group of children in the low VL group at both timepoints (sustained-low trajectory group: 63% vs. suppressed at all measures: 8% at 6-24 months and sustained-low trajectory group: 74% vs. suppressed at all measures: 6% at 48-96 months). Definitions of sustained viral suppression in pediatric literature varies by VL cutoff and amount of time of sustained suppression. GBTM allows us to leverage continuous VL to define VL trajectory groups in pediatric populations. This approach created subgroups among children with high VL, which allowed us to examine differences in VL trajectories among children who would be considered unsuppressed when using a cumulative definition of non-suppression. Distinguishing unique VL trajectories among unsuppressed children could inform differentiated strategies and interventions focused on improving pediatric viral suppression. This approach could be particularly useful for

children and adolescents who experience lower rates of viral suppression compared to adults due to challenges with adherence and dosing.

Children in groups with periods of higher VL (sustained-high and high-to-low) groups in the 6-24 and 48-96 month timepoints, respectively, had younger caregivers when compared to children in the sustained suppressed group. Older adolescents and young adults (≤24 years) are less likely to be virally suppressed than older adults due to unique barriers to HIV care and lower ART adherence,[63] and child viral suppression has been linked with caregiver viral suppression.[64] Adolescence and young adulthood is a time of transition, resulting in distinct barriers to ART adherence including financial instability and changes in social support.[65,66] Interventions that focus on improving young adult adherence to ART and retention in care could improve ART adherence and viral suppression for their children. Tailoring strategies for pediatric ART adherence for younger caregivers may also improve viral suppression among children with HIV.[67]

Children in the sustained-high group from 6-24 months were more frequently on PI-based regimens compared to NNRTIs. Compared to NNRTIs, PIs generally have poorer tolerability[68,69] and palatability among pediatric populations,[70,71] which could have resulted in non-adherence to ART. Newer drug formulations that that make ART more palatable for children 6-24 months of age could improve pediatric ART adherence and, thus, viral suppression. Drug palatability should be considered as new classes of drugs – namely integrase strand transfer inhibitors (INSTIs) – become available to infants. It will also be important to understand the role of INSTIs on sustained HIV viral suppression in children when compared to previous regimens.

While this methodological approach provided a unique understanding of VL trajectories among children in this cohort, there are some important limitations. Under the ART guidelines from 2007-2010, only pregnant people who met certain criteria based on their CD4 count initiated ART. Timing of ART initiation relative to pregnancy may influence pre-ART VL in infants, which is why we assessed viral load trajectories from 6-24 months post-ART. ART guidelines have

changed and access to ART has improved over the last decade. There was substantial loss to follow up by the 48-96 month period, and children retained in the study could be different to children who were not retained, which could impact the generalizability of these findings. There was limited power, specifically at the 48-96 month timepoint, to determine correlates of trajectory groups due to the small sample size. The parent study involved a treatment interruption at 24 months post-ART initiation; therefore, we conducted a GBTM in the two separate time periods.

In conclusion, we determined longitudinal patterns of HIV VL using GBTM – a modeling approach not previously used to evaluate VL trajectories among children with HIV. This approach provides a unique perspective – particularly of unsuppressed children – that could inform effective strategies to improve pediatric viral suppression among children with distinct VL trajectories.

ACKNOLWEDGMENTS

We are grateful to the children and families who participated in this research, to the clinic and laboratory staff who provide clinical care and monitoring of this cohort, and to the Comprehensive Care Clinic and Kenyatta National Hospital where the research was conducted.

CONFLICTS OF INTEREST

No conflicts of interest declared.

SOURCES OF FUNDING

This work was supported by the National Institutes of Health (NIH) (Eunice Kennedy Shriver National Institute Of Child Health & Human Development of the National Institutes of Health under Award Number F31HD106261 to JN and National Institute of Allergy and Infectious Diseases grants K01AI087369 to JAS [principal investigator (PI)] and R01 AI076105 to JO [PI] and Fogarty International Center K43 TW 011422-01A1 to IN and Eunice Kennedy Shriver National Institute of Child Health and Human Development grants R01HD-23412 and K24HD054314 to G. J. S. [PI] and R01HD094718 to DAL and GJS [MPIs]) and K01MH121124 to ADW, the University of Washington Center for AIDS Research (New Investigator and HIV-Associated Malignancy Awards; the Center is funded by NIH grant P30AI027757), and the University Washington Global Center for Integrated Health of Women, Adolescents and Children.

TABLES AND FIGURES

Table 1. Child sociodemographic characteristics by HIV viral load trajectory groups

A. Among children with viral load data from 6-24 months post-ART (N=89)

Variable	Overall, N=89	Sustained-low VL, N=56 (REF)	Low-to-high VL, N=5		High VL with periods of low VL, N=6		Sustained-high VL, N=8		Sustained-very-high VL, N=14	
	n (%) or Median (IQR)	n (%) or Median (IQR)	n (%) or Median (IQR)	p-value	n (%) or Median (IQR)	p-value	n (%) or Median (IQR)	p-value	n (%) or Median (IQR)	p-value
Age at ART initiation (years)	5.0 (4.2-7.5)	5.0 (4.4-8.0)	4.4 (3.7-7.5)	0.51	3.9 (3.5-5.1)	0.12	5.6 (4.3-9.2)	0.73	4.8 (3.6-6.5)	0.27
Female (ref: Male)	44 (49%)	28 (50%)	3 (50%)	>0.99	2 (40%)	>0.99	5 (63%)	0.71	6 (43%)	0.77
First line PI-based Regimen (ref: NNRTI) all	36 (40%)	21 (38%)	4 (67%)	0.21	2 (40%)	>0.99	5 (63%)	0.03	4 (29%)	0.76
First line PI-based Regimen (ref: NNRTI) no switches (N=52)	30 (58%)	18 (55%)	4 (80%)	0.37	1 (50%)	>0.99	4 (80%)	0.37	3 (43%)	0.69
Ever breastfed (ref: Never breastfed)	77 (89%)	48 (87%)	5 (100%)	>0.99	4 (80%)	0.52	7 (88%)	>0.99	13 (93%)	>0.99
Child adherence reported ≥1 missed dose (ref: no missed doses)	45 (51%)	28 (50%)	3 (50%)	>0.99	4 (80%)	0.36	6 (75%)	0.26	4 (29%)	0.23
Caregiver age (years)	26.0 (23.0-31.0)	28.0 (24.0-32.0)	26.5 (24.0-29.0)	0.51	26.0 (25.0-31.0)	0.83	22.0 (20.5-27.0)	0.02	27.0 (23.0-30.0)	0.85
Caregiver years of education	9.0 (8.0-12.0)	9.0 (8.0-12.0)	8.0 (8.0-9.0)	0.41	12.5 (10.0-13.0)	0.11	8.0 (8.0-12.0)	0.77	10.0 (8.0-11.0)	0.78
Biological mother as primary caregiver (ref: other primary caregiver)	88 (99%)	56 (100%)	5 (83%)	0.10	5 (100%)	-	8 (100%)	-	14 (100%)	-
Crowding (>3 people/room; ref: no crowding)	38 (67%)	24 (67%)	3 (100%)	0.54	2 (50%)	0.60	3 (60%)	>0.99	6 (67%)	>0.99
Employment (ref: housewife or unemployed)	19 (22%)	13 (24%)	0 (0%)	0.33	2 (40%)	0.59	1 (13%)	0.67	3 (21%)	>0.99
Household rent (KSH/1000)	1.5 (1.0-3.0)	1.5 (1-2.75)	1.5 (1.5-1.6)	0.78	3.5 (1.5-6.3)	0.30	1.2 (0.8-2.5)	0.56	2.0 (1.5-3.0)	0.17
Parent receiving ARVs (ref: parent not on ARVs)	27 (31%)	15 (27%)	2 (40%)	0.61	1 (20%)	>0.99	5 (63%)	0.10	4 (29%)	>0.99

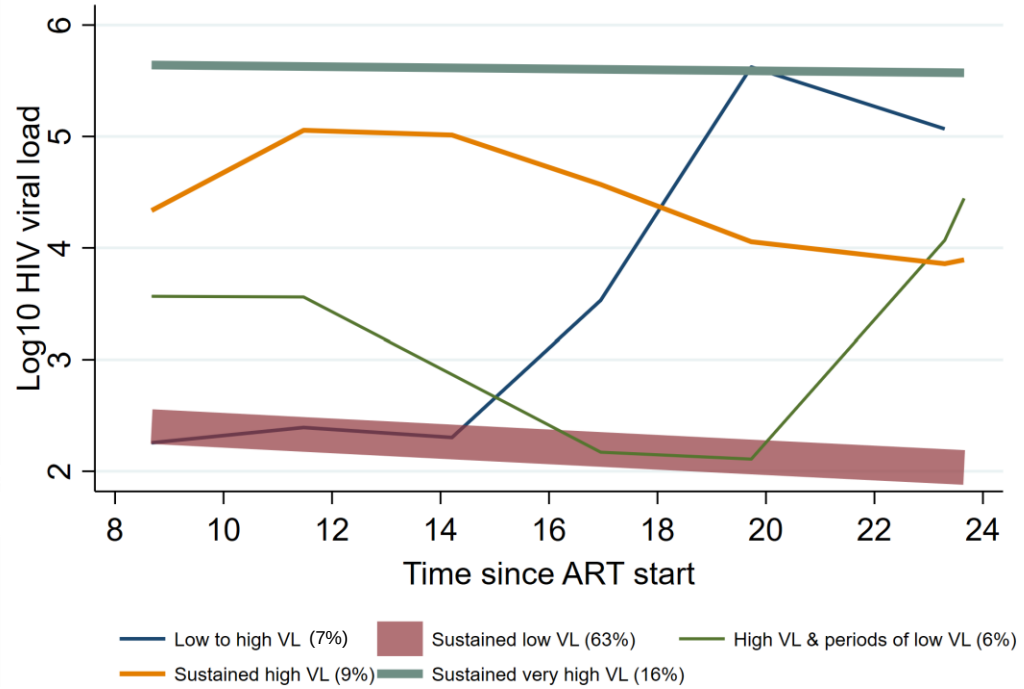
B. Among children with viral load data from 48-96 months post-ART (N=54)

Variable	Overall, N=54	Sustained-low VL, N=40 (REF)	Mid-range VL, N=2		Periods of low VL, N=4		High to low VL, N=4		Sustained-high VL, N=4	
	n (%) or Median (IQR)	n (%) or Median (IQR)	n (%) or Median (IQR)	p-value	n (%) or Median (IQR)	p-value	n (%) or Median (IQR)	p-value	n (%) or Median (IQR)	p-value
Age at ART initiation (years)	5.0 (4.4-7.6)	5.1 (4.4-8.1)	6.6 (6.2-7.1)	0.13	4.5 (4.1-5.9)	0.48	5.0 (3.8-5.0)	0.11	6.2 (3.5-9.3)	0.49
Female (ref: Male)	23 (43%)	21 (53%)	0 (0%)	0.49	0 (0%)	0.11	1 (25%)	0.61	1 (25%)	0.61
First line PI-based Regimen (ref: NNRTI) all	23 (43%)	17 (43%)	1 (50%)	>0.99	3 (75%)	0.38	1 (25%)	0.66	1 (25%)	0.66
First line PI-based Regimen (ref: NNRTI) no switches (N=52)	20 (61%)	15 (65%)	1 (100%)	>0.99	3 (100%)	0.53	0 (0%)	0.06	1 (33%)	0.54
Child OVC (assessed at 4 years post ART)	25 (48%)	18 (47%)	1 (50%)	>0.99	2 (50%)	>0.99	3 (75%)	0.61	1 (25%)	0.61
Ever breastfed (ref: Never breastfed)	48 (92%)	36 (92%)	2 (100%)	>0.99	4 (100%)	>0.99	3 (100%)	>0.99	3 (75%)	0.33
Time breastfed (months)	6.0 (4.0-18.0)	6.0 (5.0-18.0)	3.0 (3.0-3.0)	0.13	13.5 (5.8-21.0)	0.48	15.0 (10.0-28.6)	0.11	6.0 (1.5-9.0)	0.49
Child adherence reported ≥1 missed dose (ref: no missed doses)	24 (44%)	17 (43%)	2 (100%)	0.20	1 (25%)	0.63	3 (75%)	0.32	1 (25%)	0.63
Child hospitalized by 4 years post-ART (ref: not hospitalized)	39 (72%)	29 (73%)	2 (100%)	>0.99	2 (50%)	0.57	3 (75%)	>0.99	3 (75%)	>0.99
Randomized to interruption	16 (30%)	10 (25%)	2 (100%)	0.08	1 (25%)	>0.99	1 (25%)	>0.99	2 (50%)	0.30
Caregiver age (years)	26.0 (24.0-34.0)	29.0 (24.0-34.0)	24.0 (22.0-26.0)	0.87	27.5 (26.0-32.0)	0.97	20.5 (18.5-22.5)	0.01	27.0 (25.5-30.5)	0.82
Caregiver years of education	10.0 (8.0-12.0)	10.0 (8.0-12.0)	12.0 (12.0-12.0)	0.40	11.0 (10.0-14.0)	0.18	9.0 (8.0-10.0)	0.47	16.0 (12.0-20.0)	0.08
Biological mother as primary caregiver (ref: other primary caregiver)	53 (98%)	40 (100%)	2 (100%)	-	4 (100%)	-	3 (75%)	0.09	4 (100%)	-
Mother died (ref: mother living)*	2 (4%)	1 (3%)	0 (0%)	>0.99	0 (0%)	>0.99	1 (25%)	0.18	0 (0%)	>0.99
Father died (ref: father living)*	2 (4%)	2 (5%)	0 (0%)	>0.99	0 (0%)	>0.99	0 (0%)	>0.99	0 (0%)	>0.99
Mother not fulfilling parental duties (ref: mother fulfilling parental duties)	1 (2%)	1 (3%)	0 (0%)	>0.99	0 (0%)	>0.99	0 (0%)	>0.99	0 (0%)	>0.99
Father not fulfilling parental duties (ref: father fulfilling parental duties)	17 (33%)	13 (34%)	0 (0%)	>0.99	2 (50%)	0.61	1 (25%)	>0.99	1 (25%)	>0.99
Crowding (>3 people/room; ref: no crowding)	20 (61%)	14 (56%)	0 (0%)	0.46	2 (67%)	>0.99	2 (100%)	0.50	2 (100%)	0.50
Household rent (KSH/1000)	1.5 (1.0-4.0)	1.5 (1.0.0-3.5)	2.4 (1.2-3.5)	0.70	4.0 (1.8-7.3)	0.06	1.4 (1.0-1.1)	0.86	3.3 (2.0-5.0)	0.13
Parent receiving ARVs (ref: parent not on ARVs)	18 (34%)	14 (35%)	1 (50%)	>0.99	1 (25%)	>0.99	1 (33%)	>0.99	1 (25%)	>0.99

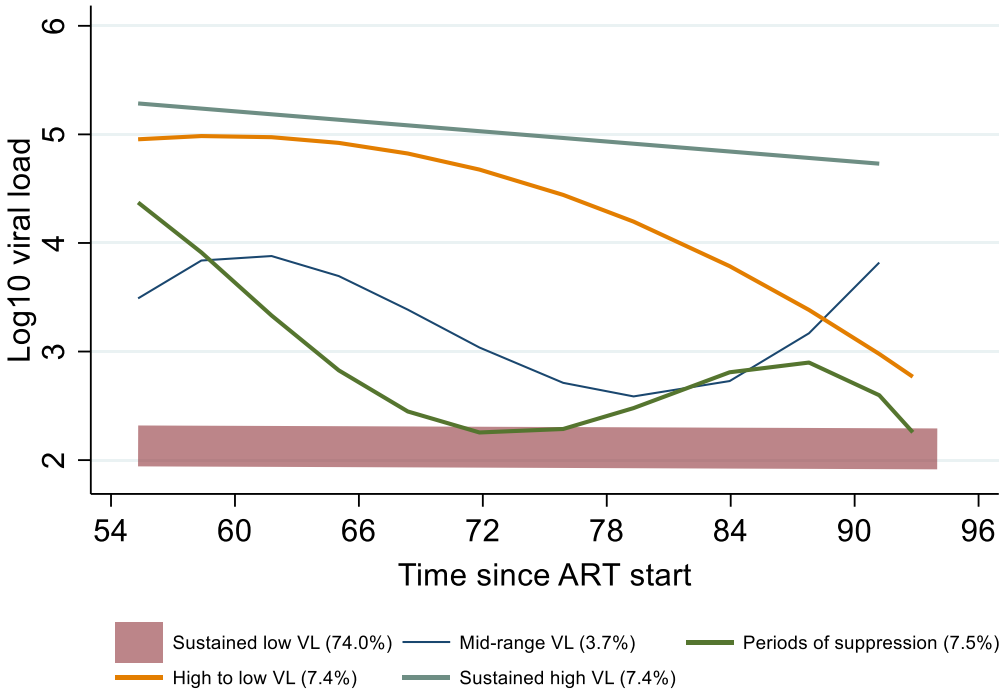
*Assessed at 4 years post-ART initiation

Figure 1. Group-based trajectory models

A. Pediatric HIV viral load groups from >6 to 24 months post-ART initiation (N=89)*



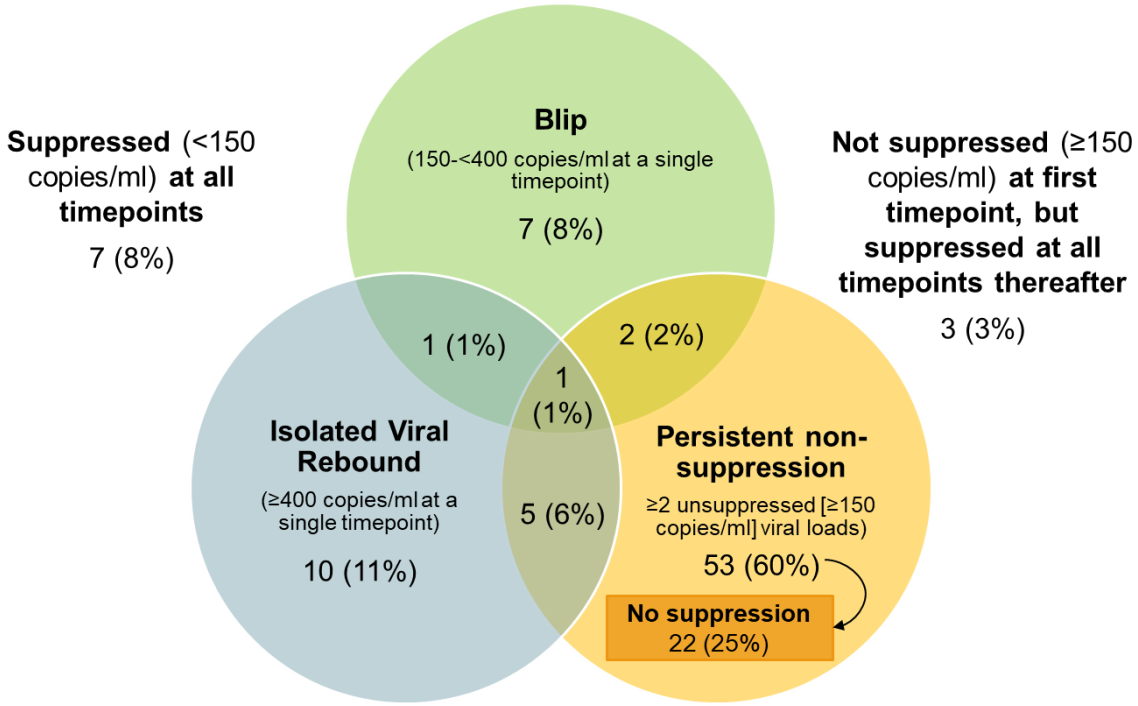
B. Pediatric HIV viral load groups from >48 to 96 months post-ART initiation (N=54)*



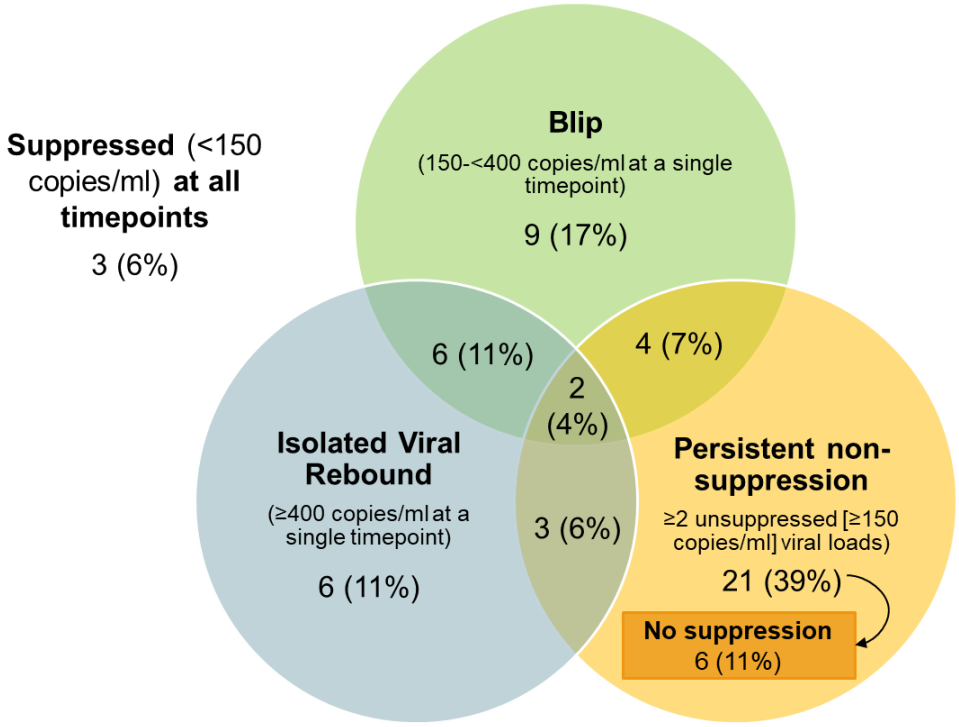
*Line thickness reflects size of group

Supplemental Figure 1. Cumulative definitions of viral suppression and non-suppression during 6-24 and 48-96 month periods

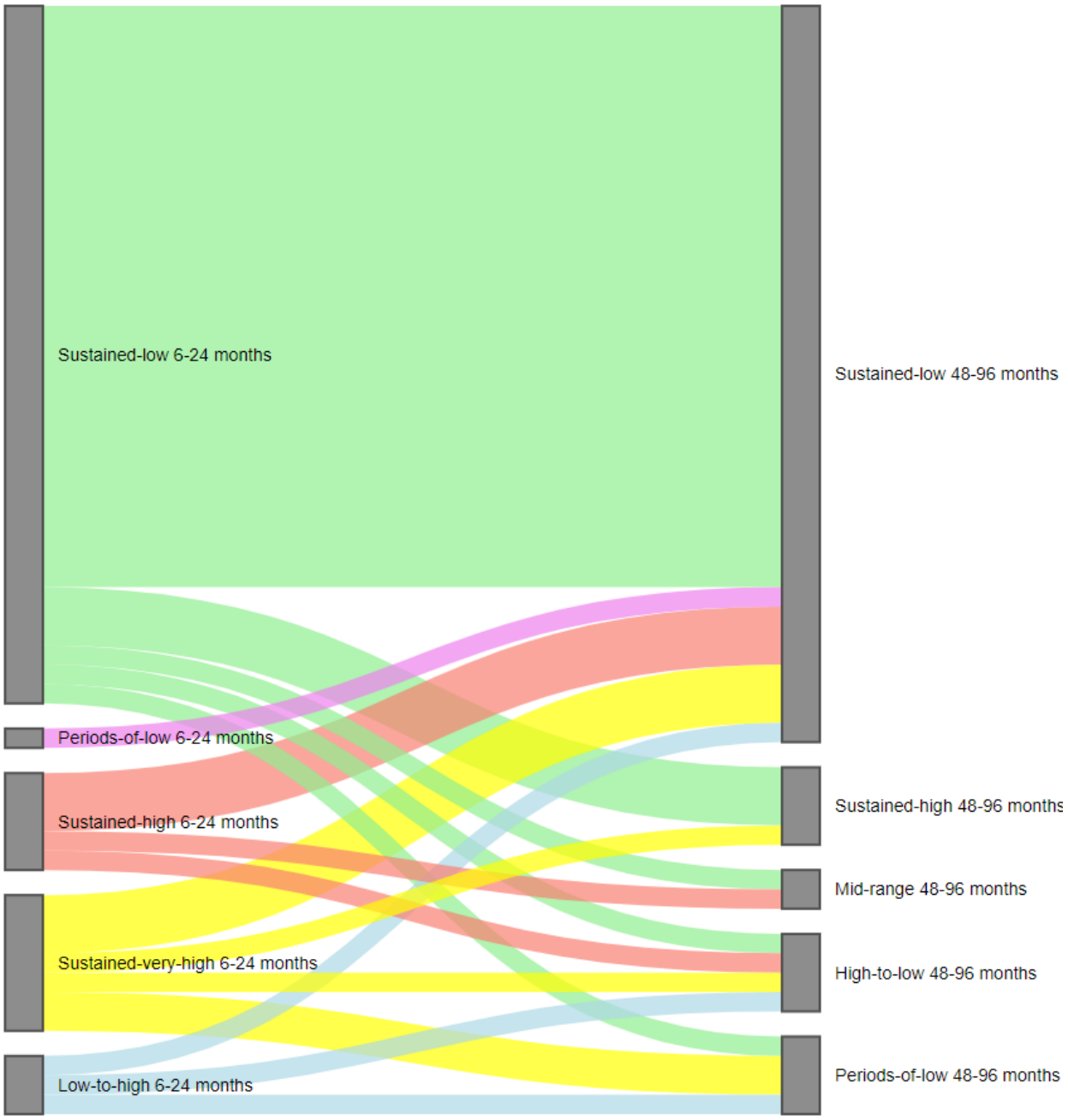
A. Characteristics of viral non-suppression 6-24 months post-ART (N=89)



B. Characteristics of viral non-suppression 48-96 months post-ART (N=54)



Supplemental Figure 2. Comparing 6-24 month trajectory groups and 48-96 month trajectory groups (N=52)



CHAPTER 3: Effect of HIV and cytomegalovirus on neurocognitive outcomes among children with HIV

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ABSTRACT

Background: Children with HIV may experience adverse neurocognitive outcomes despite antiretroviral treatment (ART). The influence of HIV viral load (VL), post-ART HIV DNA and CMV infection during infancy on neurocognition among children with HIV is poorly defined.

Methods: Children who initiated ART before 12 months of age were enrolled from 2007-2010 in Nairobi, Kenya. Blood was collected at enrollment and every 6 months thereafter. Neurocognitive assessments including the Kaufman Assessment Battery for Children 2nd Edition (KABC), Behavior Rating Inventory of Executive Functioning (BRIEF), Bruinick's-Oseretsky Test of Motor Proficiency 2nd Edition Brief Form (BOT), and Visual Test of Variables of Attention (TOVA) were conducted when children were a median of 7 years of age. Four primary outcomes included cognitive ability from KABC, executive function from BRIEF, motor from BOT and attention from TOVA. Generalized linear models were used to determine associations between HIV VL (pre-ART and cumulative), HIV DNA (by 12 months of age) and peak CMV DNA (by 24 months of age).

Results: In adjusted models, higher peak CMV DNAemia by 24 months of age was associated with lower cognitive ability (-0.30; 95% confidence interval [95%CI]: -0.56, -0.03; p=0.027) and motor z-scores (-0.24; 95%CI: -0.46, -0.01; p=0.040). Higher pre-ART HIV VL (-0.55; 95%CI: -0.99, -0.11; p=0.014), total HIV DNA (-0.65; 95%CI: -1.23, -0.07; p=0.028), and intact HIV DNA (-0.52; 95%CI: -1.00, -0.04; p=0.032) were associated with lower executive function z-scores. Higher HIV DNA levels were associated with higher motor scores (0.68; 95%CI: 0.20, 1.17; p=0.006) and higher intact HIV DNA with higher attention z-scores (0.63; 95%CI: 0.02, 1.24; p=0.042).

Among secondary outcomes, higher intact HIV DNA levels were associated with lower behavior regulation z-scores (-0.77; 95%CI: -1.37, -0.18; p=0.011) and higher pre-ART VL was associated with 0.32 lower mean nonverbal and 0.77 lower mean metacognition z-scores (-0.32; 95%CI: -0.63, -0.02; p=0.037 and -0.77; 95%CI: -1.37, -0.18; p=0.011, respectively).

Conclusion: Pre-ART VL, early post-ART total and intact HIV DNA levels, and CMV DNA in infancy predicted several neurocognitive deficits in childhood, including executive function, behavioral regulation, and metacognition. Early total/intact HIV DNA predicted improved outcomes for some measures. Our findings underscore potential long-term benefits of early viral suppression and reservoir containment on neurocognition. Impact of early suppression may differ by domain of neurocognition or be influenced by unmeasured confounders.

INTRODUCTION

Children with HIV experience neurodevelopmental and neurocognitive delays.[20,21] Early ART initiation improves neurodevelopmental outcomes,[72–74] but does not fully mitigate adverse neurocognitive outcomes.[21,75,76] Among children 5-7 years of age, there is evidence that central nervous system (CNS) damage persists despite early ART and viral suppression.[77–79]

Persistent neurocognitive deficits among children with HIV may be due to persistence of the viral reservoir, local inflammation in the CNS, or disruption of neuronal function.[80] Viral suppression during infancy and early childhood is associated with improved neurocognition among older children.[22–24] Early cytomegalovirus (CMV) infection, which is common in children with HIV, has been associated with accelerated HIV disease progression in children,[25,26] and there is evidence that CMV infection could influence neurodevelopmental and neurocognitive outcomes.[27,28] Additionally, congenital CMV can cause profound damage to the CNS and has long-term effects on neurodevelopmental outcomes.[81] Early biomarkers that predict neurocognitive or neuropsychological deficits could facilitate identification of children in need of targeted interventions. We aimed to determine the association between early viral load (VL), HIV DNA levels, and CMV DNAemia on neurocognitive and neuropsychological outcomes in school aged children with HIV.

METHODS

This secondary longitudinal analysis was nested in the OPH study (NCT00428116), which enrolled children who initiated ART by 12 months of age from September 2007 to August 2010 at Kenyatta National Hospital (KNH) in Nairobi, Kenya. OPH was a trial that randomized a subset of children who met pre-specified criteria to either treatment interruption or continued treatment after 24 months of ART.[33] Blood samples were collected every 3 months for the first 24 months of follow up and every 6 months thereafter. Neurocognitive assessments were conducted at a

median of 7 years of age. The study was approved by the University of Washington and Fred Hutchinson Cancer Center Institutional Review Boards and KNH Ethics and Research Committee. Caregivers provided written informed consent for their children's participation.

HIV VL was quantified using the Gen-Probe assay on plasma samples, which had a lower limit of detection of 150 copies/ml. HIV VL area under the curve (AUC) from birth to the neurocognitive assessment was calculated using the trapezoidal rule with cubic splines. Peak total and intact HIV DNA by 12 months of age were measured in DNA from cryopreserved peripheral blood mononuclear cells (PBMCs) samples collected after ART initiation and prior to 12 months of age using the cross-subtype intact proviral DNA assay (CS-IPDA).[32,82] The CS-IPDA was performed in duplicate, with additional replicates performed on samples with no intact HIV DNA detected until either a minimum of 10^5 cells were interrogated or intact DNA were detected. Total and intact HIV DNA levels were included from samples with DNA shearing rates of <40% as measured by the RPP30 reference assay. Total HIV DNA, but not intact DNA levels, were included from samples with ≥40% DNA shearing. All samples in this analysis have detectable total HIV DNA. The CS-IPDA can detect a single copy of intact HIV DNA;[32] therefore, samples with undetectable intact HIV DNA were set to 0.5 copies over the number of cells interrogated normalized to 10^6 cells. Peak CMV DNAemia by 24 months of age was measured by quantitative PCR on plasma with a lower limit of detection of 50 copies/ml for CMV DNA. Undetectable HIV VL and CMV DNA were designated half the value of the limit of detection.

Neurocognitive assessments included Kaufman Assessment Battery for Children 2nd Edition (KABC), Behavior Rating Inventory of Executive Functioning (BRIEF), Bruinick's-Oseretsky Test of Motor Proficiency 2nd Edition Brief Form (BOT), and Visual Test of Variables of Attention (TOVA). Four primary outcomes included cognitive ability from KABC, executive function from BRIEF, motor from BOT, and attention from TOVA. Secondary outcomes included short-term memory, visual-spatial, learning, non-verbal, and delayed memory from the KABC;

behavior regulation and metacognition from the BRIEF; and processing speed from the TOVA. All neurocognitive outcomes were normalized and presented as z-scores. Children were included in this analysis if they had one or more exposures of interest. Inverse probability weighting was used to account for differential missingness by age of enrollment. Generalized linear models were used to determine the association between pre-ART HIV VL, HIV VL AUC, total and intact HIV DNA levels, and CMV DNA levels, and neurocognitive outcomes. Adjustment variables included infant sex at birth, caregiver education, and age at neurocognitive test. Additionally, months on ART at the time of sample collection was adjusted for in analyses for total and intact HIV DNA. Benjamini-Hochberg approach was used to account for multiple comparisons for primary and secondary outcomes. Stata version 17.0 (Stata Corporation, College Station, Texas USA) was used for all analyses.

RESULTS

Of 39 children who completed neurocognitive assessments, 38 had data on pre-ART VL, 26 had HIV DNA levels measured prior to 12 months of age, and 20 had CMV DNA levels measured prior to 24 months of age. Median age at ART initiation was 4.6 (interquartile range [IQR]: 4.1-5.3) months, half of the children were female (19 [49%]), and 41% were on a first-line protease inhibitor (PI)-based regimen. The majority of caregivers (38 [97%]) were the child's biological mother. Median caregiver age was 26 (IQR: 23-34) years and median caregiver years of education was 10 (IQR: 8-12) years (Table 1).

Primary outcomes

In adjusted models, one $\log_{10}$ higher peak CMV DNAemia copies/ml was associated with 0.30 lower mean cognitive ability z-score (-0.30; 95% confidence interval [95%CI]: -0.56, -0.03; p=0.027). Peak CMV DNAemia was associated with lower mean motor z-scores (-0.24; 95%CI: -0.46, -0.01; p=0.04). Children with one $\log_{10}$ higher pre-ART HIV VL (-0.55; 95%CI: -0.99, -0.11;

p=0.014), total HIV DNA levels (-0.65; 95%CI: -1.23, -0.07; p=0.028), and intact HIV DNA levels (-0.52; 95%CI: -1.00, -0.04; p=0.032) had lower mean executive function z-scores. Total HIV DNA levels were associated with higher mean motor z-scores (0.68; 95%CI: 0.20, 1.17; p=0.006). Early intact HIV DNA levels were associated with higher mean attention z-scores (0.63; 95%CI: 0.02-1.24; p=0.042; Figure 1).

Secondary outcomes

Higher levels of intact HIV DNA were associated with lower behavior regulation z-scores (-0.77; 95%CI: -1.37, -0.18; p=0.011). Additionally, one $\log_{10}$ higher pre-ART VL copies/ml was associated with 0.32 lower mean nonverbal and 0.77 lower mean metacognition z-scores (-0.32; 95%CI: -0.63, -0.02; p=0.037 and -0.77; 95%CI: -1.37, -0.18; p=0.011, respectively). Pre-ART HIV VL, HIV VL AUC, intact and total HIV DNA, and peak CMV DNAemia were not associated with short-term memory, visual-spatial, learning, delayed memory, or processing speed z-scores (Figure 2).

DISCUSSION

Higher pre-ART VL, early total and intact HIV DNA levels, and early CMV levels were generally associated with poorer neurocognitive and neuropsychological outcomes. Children with higher levels of pre-ART VL and early total and intact HIV DNA had lower mean executive function z-scores. Cognitive ability and motor scores were lower among children with higher CMV DNAemia.

Children with higher pre-ART HIV VL had significantly lower mean executive function, non-verbal, and metacognition z-scores, which aligns with existing evidence that has found that peak or early viral suppression is associated with better cognitive[22–24] and executive function scores.[25,83] In contrast to pre-ART VL, HIV VL AUC up to age at neurocognitive assessment was not associated with any neurocognitive or neuropsychological outcomes, which aligned with our

a priori hypothesis that pre-ART VL would be a better predictor of neurocognitive outcomes than AUC. The CNS is a sanctuary site for HIV-1, with evidence of persistent HIV replication despite ART.[84–86] The CNS is protected by a highly selective semipermeable blood-brain barrier, which makes it difficult for ART to enter the CNS.[86,87] HIV VL in plasma and in cerebrospinal fluid (CSF) are not well-correlated in children and adults after long duration of ART.[84,85,88–90] Some studies have found detectable HIV VL in cerebrospinal fluid among children and adults with sustained undetectable VL.[84,91] Plasma HIV VL during acute infection, however, has been associated with viral seeding of the CNS,[92] and there is evidence that VL in plasma and CSF are correlated immediately after ART initiation.[93–95] Thus, pre-ART VL better predicts long-term outcomes of children. Our findings suggest that obtaining VL prior to initiating ART could be useful to identify children who need more intensive follow-up for neurocognitive assessments. Our findings also underscore the importance of early infant HIV diagnosis programs for improving long-term neurocognitive and neuropsychological outcomes among children with HIV. In 2021, only 52% of children with HIV were on ART;[1] innovative approaches to improve early infant diagnosis and linkage to care and treatment are necessary for improving long-term health and developmental outcomes for children with HIV.

HIV DNA is an important biomarker of HIV disease progression,[96–99] and there is evidence that HIV DNA correlates with HIV-associated neurocognitive disorders, particularly executive function, in older adults.[100] We found that children with higher levels of early total and intact HIV DNA had lower mean executive function z-scores and children with higher levels of intact HIV DNA had lower behavioral regulation z-scores in this analysis. These findings suggest that HIV DNA levels in infancy may have long-term effects on neurocognition several years later. In untreated adults, there have been strong associations between HIV DNA levels and HIV-associated neurocognitive disorders.[101] Studies have also found that HIV DNA levels have been associated with dementia[102] and neuropsychological deficits in adults; however, the latter study

found that HIV DNA did not predict future neuropsychological scores.[103] In our exploratory analysis, we found two associations that were in the opposite direction than hypothesized – total HIV DNA levels were associated with higher motor z-scores and intact HIV DNA levels were associated with higher attention z-scores. It is unlikely that these associations mean that higher levels of HIV DNA are associated with better neuropsychological outcomes. Rather, we speculate that because we measured HIV DNA at different ages and timepoints, there could be unmeasured confounders that could not be accounted for in these specific associations. It is also possible that for these neurocognitive domains, early HIV DNA markers were a surrogate marker for an unmeasured predictor of outcomes.

Few studies have evaluated CMV and neurocognitive outcomes in children, most of which have compared children with and without CMV infection, rather than assessing impact of levels of early CMV DNAemia. CMV seropositivity or infection has been associated with adverse neurodevelopmental[27] and neurocognitive outcomes, respectively.[28] The latter study, found that at 8 years of age CMV infection was associated with lower intelligence quotient butnot any other neurocognitive outcomes.[28] We found that children with higher peak CMV DNAemia by 24 months of age had lower mean cognitive ability and mean motor z-scores at 7 years of age. Our findings suggest that measures to prevent CMV infection or reduce CMV DNAemia – including early ART initiation – could improve neurocognitive outcomes among children with HIV.

There are effective interventions to support children to overcome neurocognitive and neuropsychological deficits.[104] A randomized controlled trial in Uganda found that computerized cognitive rehabilitation training can be effective for neurocognitive rehabilitation among children with HIV.[105] Additionally, caregiver training, non-computerized cognitive training, and interventions focused on improving child physical activity and nutrition have been effective in improving certain neurocognitive and neuropsychological domains.[104] Given the predictors we

identified, it may be useful to focus on providing effective evidence-based interventions to children with higher pre-ART VL, and early total and intact HIV DNA, and CMV DNAemia during infancy.

This secondary analysis leveraged data from a longitudinal cohort of plasma pre-ART VL, repeated VL, early HIV DNA, and early CMV DNA measures with neurocognitive and neuropsychological assessments conducted at a median of 7 years of age. We also had small sample sizes of children with HIV DNA and CMV DNA data which may have limited our ability to detect differences. We were not able to assess HIV VL or DNA in the CNS, which would be more biologically relevant to neurocognition. We also were unable to diagnose congenital CMV in this cohort. Peak total and intact HIV DNA post-ART by 12 months of age was used to estimate early HIV DNA levels; however, total and intact HIV DNA levels declined rapidly after ART initiation. To address this, we included time on ART by HIV DNA measurement in adjusted analyses.

CONCLUSION

Higher pre-ART VL, early HIV DNA levels, and CMV DNAemia were associated with neurocognitive and neuropsychological deficits. Efforts to improve early ART initiation could improve neurocognitive and neuropsychological outcomes for children with HIV. Findings from this study could inform predictive models to determine children with HIV who are most in need of effective interventions that could improve neurocognition.

ACKNOWLEDGMENTS

We are grateful to the children and families who participated in this research, to the clinic and laboratory staff who provide clinical care and monitoring of this cohort, and to the Comprehensive Care Clinic and Kenyatta National Hospital where the research was conducted. We are also grateful to Julie Overbaugh at Fred Hutchinson Cancer Center for providing advice, human immunodeficiency virus (HIV) viral load testing, and technical support.

CONFLICTS OF INTEREST

No conflicts of interest declared.

SOURCES OF FUNDING

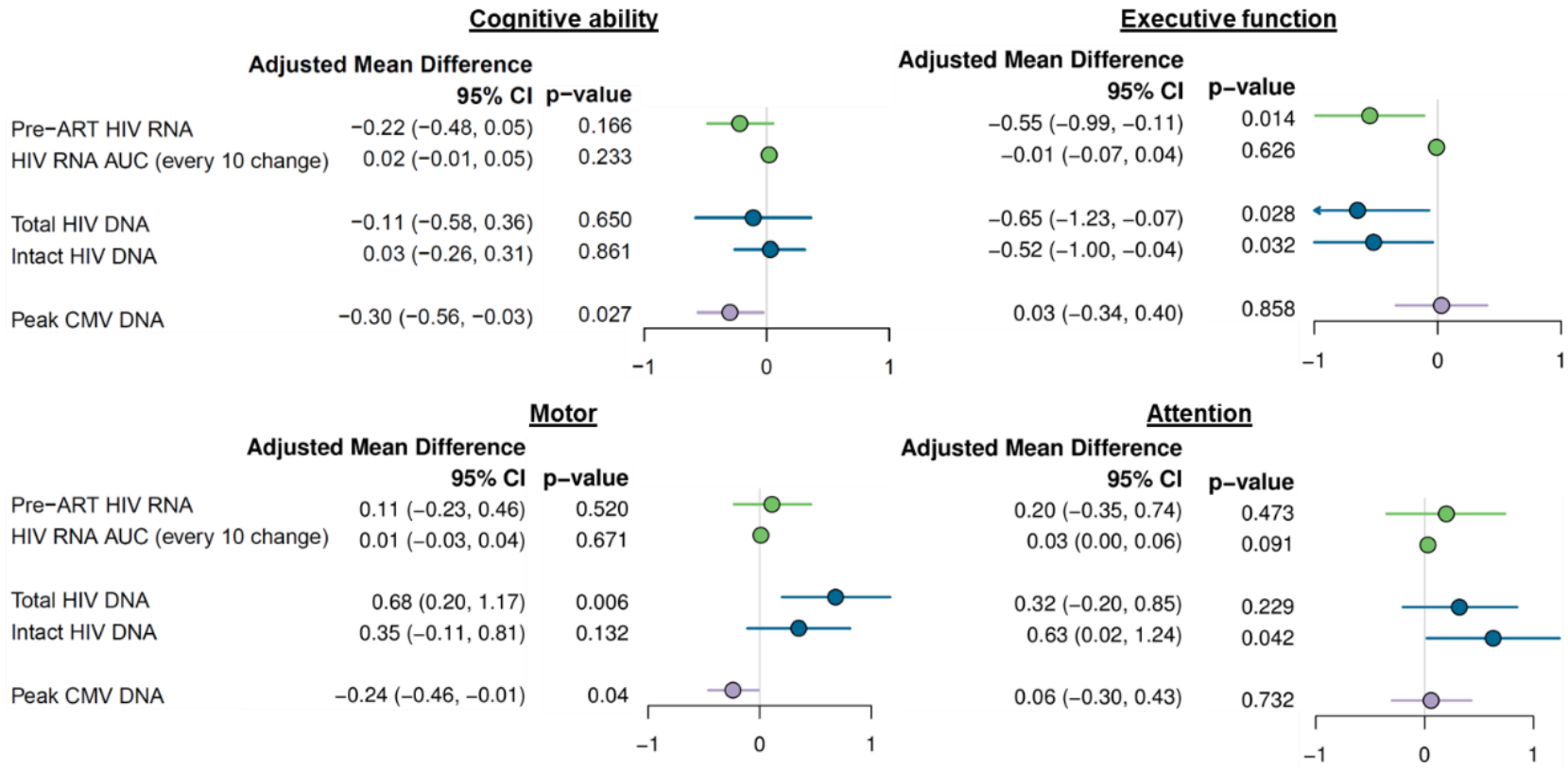
This work was supported by the National Institutes of Health (NIH) (Eunice Kennedy Shriver National Institute Of Child Health & Human Development of the National Institutes of Health under Award Number F31HD106261 to JN and National Institute of Allergy and Infectious Diseases grants K01AI087369 to JAS [principal investigator (PI)] and R01 AI076105 to Julie Overbaugh [PI] and Fogarty International Center K43 TW 011422-01A1 to IN and Eunice Kennedy Shriver National Institute of Child Health and Human Development grants R01HD-23412 and K24HD054314 to G. J. S. [PI] and R01HD094718 to DAL and GJS [MPIs]) and K01MH121124 to ADW, the University of Washington Center for AIDS Research (New Investigator and HIV-Associated Malignancy Awards; the Center is funded by NIH grant P30AI027757), and the University Washington Global Center for Integrated Health of Women, Adolescents and Children.

TABLES AND FIGURES

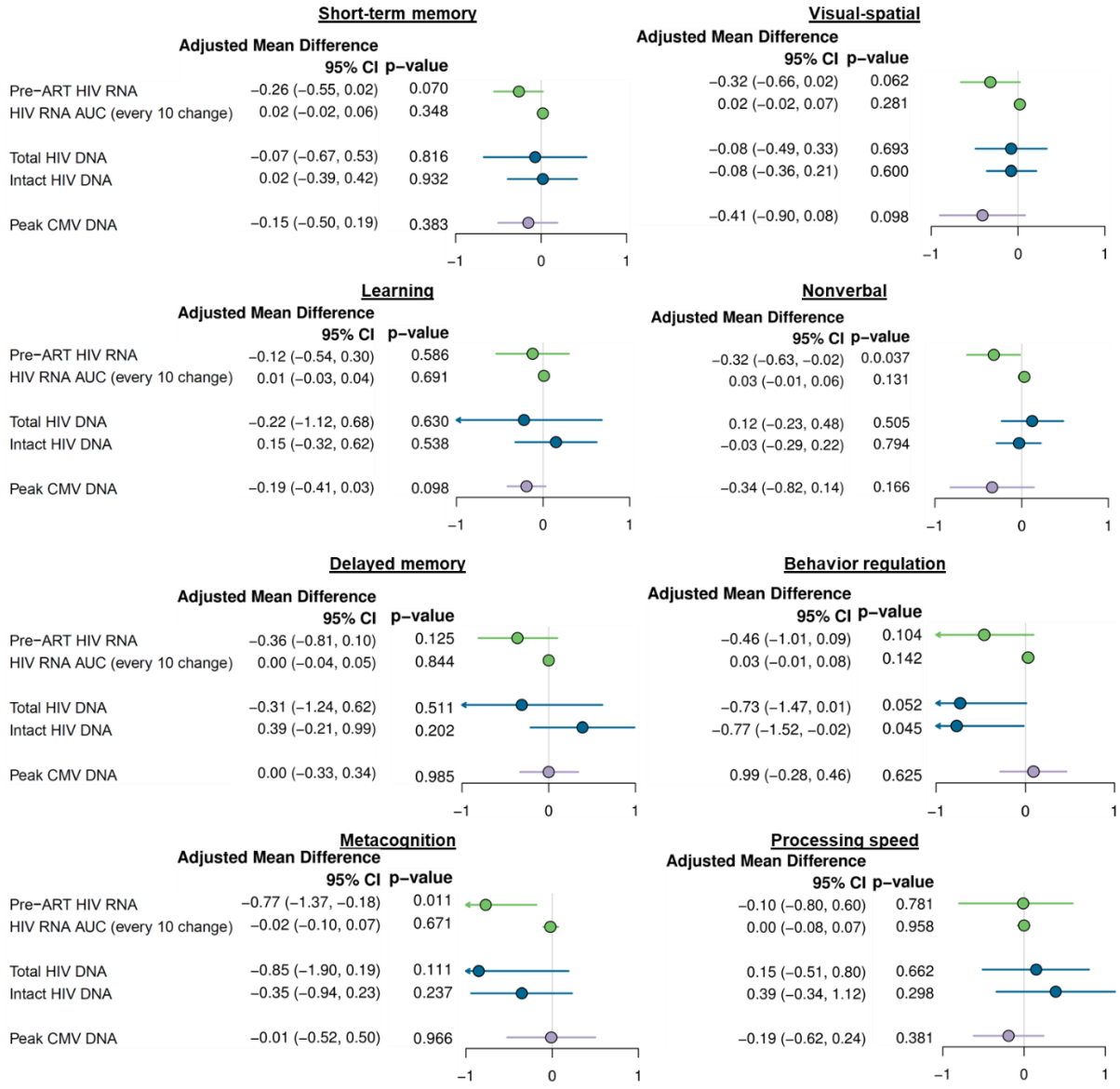
Table 1. Characteristics of children with neurocognitive assessments and at least one exposure measure (N=39)

	N	Median (IQR) N=39
Age at ART initiation (months)	39	4.6 (4.1-5.3)
Child age at neuropsychological assessment (years)	39	7.1 (6.5-7.6)
Female (REF: Male)	39	19 (49%)
PI-based regimen (all children)	39	16 (41%)
PI-based regimen (no switches)	23	15 (65%)
Ever breastfed	37	34 (92%)
Ever hospitalized	39	22 (56%)
Caregiver age (in years)	38	26.0 (23.0-34.0)
Highest level of education (binary)	39	
None/Primary		23 (59%)
Secondary/College		16 (41%)
Caregiver education number of years of education	33	10.0 (8.0-12.0)
Primary caregiver: biological mother (REF: Other)	39	38 (97%)
Crowding	26	16 (62%)
Monthly house rent (KSH)	37	1500 (1200-4000)
Early total HIV log_{10}copies/million cells (peak <12 months of age)	26	3.4 (3.2-3.6)
Early intact HIV DNA log_{10}copies/million cells (peak <12 months of age)	26	2.7 (2.4-3.1)
Baseline VL log_{10}copies/ml	38	6.6 (6.0-7.0)
VL AUC (log_{10}copies/ml-months)	38	215 (187-278)
CD4 percent	35	17.3 (14.0-24.0)
Pre-ART CMV	19	16 (84%)
Peak CMV log_{10}copies/ml	20	4.3 (3.8-5.0)

Figure 1. Predictors of neurocognitive outcomes among school-age children with HIV



Supplemental Figure 1. Predictors of secondary neurocognitive outcomes among school-age children with HIV



Supplemental Table 1. Univariate analysis

	Cognitive ability (N=39)				Executive function (N=38)				Motor (N=38)				Attention (N=32)			
	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value
Sex	19	0.28	(-0.09, 0.65)	0.138	19	-0.12	(-0.71, 0.46)	0.676	18	0.15	(-0.26, 0.57)	0.472	16	0.08	(-0.43, 0.59)	0.757
Age at ART start	46	-0.05	(-0.16, 0.07)	0.406	43	-0.11	(-0.25, 0.04)	0.150	45	0.01	(-0.10, 0.12)	0.843	38	-0.10	(-0.22, 0.03)	0.136
Age at KABC	19	-0.07	(-0.37, 0.24)	0.666	19	-0.07	(-0.47, 0.33)	0.744	18	0.28	(-0.04, 0.60)	0.089	16	-0.07	(-0.41, 0.27)	0.676
Caregiver age	19	0.07	(0.01, 0.13)	0.016	19	0.03	(-0.09, 0.15)	0.659	18	-0.01	(-0.08, 0.06)	0.877	16	-0.03	(-0.13, 0.07)	0.531
First-line regimen	19	-0.07	(-0.47, 0.34)	0.749	19	-0.20	(-0.81, 0.40)	0.510	18	0.08	(-0.38, 0.53)	0.735	16	0.32	(-0.16, 0.79)	0.189
First-line regimen (no switches)	19	-0.04	(-0.55, 0.47)	0.882	19	-0.15	(-0.87, 0.56)	0.674	18	0.31	(-0.27, 0.89)	0.292	16	0.57	(-0.12, 1.26)	0.103
Baseline CD4	21	0.07	(-0.18, 0.33)	0.574	21	0.19	(-0.20, 0.57)	0.343	20	-0.05	(-0.29, 0.19)	0.701	17	-0.36	(-0.64, -0.07)	0.014
Pre-ART HIV VL	32	0.00	(-0.24, 0.25)	0.982	31	-0.34	(-0.75, 0.07)	0.107	31	0.12	(-0.14, 0.39)	0.356	27	0.25	(-0.24, 0.73)	0.315
VL AUC	32	0.01	(-0.02, 0.04)	0.368	31	-0.01	(-0.06, 0.04)	0.769	31	0.00	(-0.03, 0.03)	0.998	27	0.00	(-0.03, 0.04)	0.811
Total HIV DNA (peak <12 months)	21	0.23	(-0.16, 0.63)	0.250	21	-0.65	(-1.37, 0.07)	0.076	20	0.60	(0.06, 1.13)	0.029	17	0.59	(0.10, 1.09)	0.019
Intact HIV DNA (peak <12 months)	21	0.02	(-0.26, 0.30)	0.881	21	-0.13	(-0.49, 0.22)	0.459	20	0.24	(0.01, 0.48)	0.044	17	0.38	(0.05, 0.72)	0.023
Peak CMV log10 DNA	19	-0.36	(-0.63, -0.09)	0.008	19	-0.07	(-0.41, 0.28)	0.711	18	-0.28	(-0.49, -0.06)	0.012	16	0.05	(-0.32, 0.41)	0.800

	Short term memory (N=39)				Visual-spatial (N=39)				Learning (N=39)				Nonverbal (N=39)			
	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value
Sex	19	0.32	(-0.18, 0.82)	0.203	19	0.32	(-0.10, 0.74)	0.137	19	0.13	(-0.39, 0.65)	0.623	19	0.24	(-0.16, 0.63)	0.240
Age at ART start	46	-0.08	(-0.27, 0.10)	0.359	46	0.04	(-0.04, 0.11)	0.346	46	-0.02	(-0.21, 0.18)	0.860	45	-0.01	(-0.09, 0.08)	0.866
Age at KABC	19	0.08	(-0.34, 0.49)	0.712	19	0.05	(-0.26, 0.35)	0.764	19	0.14	(-0.27, 0.56)	0.503	19	-0.09	(-0.39, 0.21)	0.548
Caregiver age	19	0.10	(0.00, 0.21)	0.060	19	0.02	(-0.03, 0.07)	0.445	19	0.16	(0.08, 0.24)	0.000	19	0.00	(-0.04, 0.04)	0.991
First-line regimen	19	-0.33	(-0.82, 0.16)	0.191	19	0.11	(-0.39, 0.60)	0.668	19	-0.32	(-0.81, 0.18)	0.210	19	0.20	(-0.25, 0.65)	0.391
First-line regimen (no switches)	19	-0.21	(-0.79, 0.37)	0.485	19	0.32	(-0.28, 0.91)	0.299	19	-0.39	(-1.12, 0.34)	0.293	19	0.30	(-0.21, 0.82)	0.251
Baseline CD4	21	0.12	(-0.20, 0.44)	0.470	21	0.12	(-0.07, 0.32)	0.217	21	0.04	(-0.30, 0.39)	0.806	21	0.06	(-0.11, 0.23)	0.456
Pre-ART HIV VL	32	0.03	(-0.27, 0.34)	0.832	32	-0.15	(-0.40, 0.10)	0.251	32	0.10	(-0.24, 0.44)	0.553	32	-0.11	(-0.35, 0.13)	0.358
VL AUC	32	0.02	(-0.03, 0.06)	0.506	32	0.01	(-0.02, 0.04)	0.435	32	0.02	(-0.03, 0.06)	0.409	32	0.01	(-0.02, 0.04)	0.458
Total HIV DNA (peak <12 months)	21	0.32	(-0.31, 0.94)	0.323	21	0.23	(-0.11, 0.58)	0.190	21	0.08	(-0.55, 0.72)	0.800	21	0.38	(0.07, 0.70)	0.018
Intact HIV DNA (peak <12 months)	21	0.19	(-0.08, 0.45)	0.163	21	0.04	(-0.20, 0.29)	0.741	21	-0.16	(-0.46, 0.14)	0.298	21	0.19	(0.01, 0.37)	0.043
Peak CMV log10 DNA	19	-0.30	(-0.66, 0.07)	0.108	19	-0.41	(-0.85, 0.03)	0.066	19	-0.34	(-0.62, -0.05)	0.020	19	-0.30	(-0.76, 0.17)	0.208

	Delayed memory (N=32)				Behavior Regulation (N=38)				Metacognition (N=38)				Processing Speed (N=32)			
	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value	N	Mean difference	95% Confidence Intervals	p-value
Sex	13	0.06	(-0.55, 0.68)	0.841	19	-0.46	(-1.10, 0.17)	0.149	19	-0.02	(-0.77, 0.73)	0.954	16	-0.01	(-0.70, 0.67)	0.966
Age at ART start	36	-0.04	(-0.27, 0.19)	0.752	44	-0.13	(-0.28, 0.01)	0.076	44	-0.08	(-0.26, 0.11)	0.415	38	-0.10	(-0.25, 0.06)	0.234
Age at KABC	13	0.20	(-0.13, 0.53)	0.236	19	0.00	(-0.55, 0.54)	0.994	19	-0.22	(-0.69, 0.25)	0.353	16	-0.20	(-0.74, 0.33)	0.461
Caregiver age	13	0.17	(0.03, 0.30)	0.014	19	0.01	(-0.13, 0.14)	0.938	19	-0.02	(-0.21, 0.17)	0.844	16	0.04	(-0.03, 0.12)	0.278
First-line regimen	13	-0.06	(-0.59, 0.47)	0.826	19	-0.57	(-1.21, 0.06)	0.077	19	0.24	(-0.51, 0.98)	0.535	16	-0.04	(-0.71, 0.64)	0.912
First-line regimen (no switches)	13	0.30	(-0.41, 1.01)	0.406	19	-0.73	(-1.49, 0.03)	0.059	19	0.66	(-0.51, 1.82)	0.269	16	-0.33	(-1.15, 0.49)	0.430
Baseline CD4	15	0.00	(-0.32, 0.32)	0.983	21	0.23	(-0.12, 0.58)	0.199	21	0.19	(-0.32, 0.70)	0.466	17	-0.36	(-0.84, 0.12)	0.146
Pre-ART HIV VL	26	0.01	(-0.30, 0.32)	0.968	31	-0.12	(-0.58, 0.34)	0.607	31	-0.55	(-1.00, -0.10)	0.016	27	0.11	(-0.46, 0.68)	0.700
VL AUC	26	0.03	(-0.04, 0.10)	0.418	31	0.03	(-0.01, 0.06)	0.179	31	-0.02	(-0.09, 0.04)	0.510	27	0.00	(-0.05, 0.05)	0.997
Total HIV DNA (peak <12 months)	15	0.03	(-0.54, 0.59)	0.919	21	-0.70	(-1.71, 0.32)	0.180	21	-0.70	(-1.59, 0.19)	0.123	17	0.52	(-0.18, 1.21)	0.145
Intact HIV DNA (peak <12 months)	15	0.08	(-0.10, 0.26)	0.407	21	-0.21	(-0.88, 0.46)	0.538	21	-0.16	(-0.58, 0.26)	0.446	17	0.35	(-0.05, 0.75)	0.084
Peak CMV log10 DNA	13	-0.23	(-0.69, 0.23)	0.336	19	-0.02	(-0.35, 0.31)	0.899	19	-0.08	(-0.53, 0.37)	0.725	16	-0.16	(-0.57, 0.26)	0.465

CONCLUSION

In this dissertation, we assessed predictors and trajectories of HIV viral control and the effect of early HIV and CMV on neurocognitive and neuropsychological outcomes among children with HIV. Findings from Chapters 1-3 highlighted the importance of early initiation of ART to suppress HIV viral load, recover immunity, and improve neurocognition among children with HIV. In Chapters 1 and 2, children on PIs had poorer viral control compared to children on NNRTIs. In line with adult studies, in Chapter 1, we found that children on PIs had higher levels of intact HIV DNA despite being virally suppressed. Additional research evaluating the association between regimen and HIV DNA levels in children, especially as INSTI regimens become available to children, is important for HIV cure research.

In Chapters 1 and 3, CMV was associated with higher levels of intact HIV DNA and poorer neurocognitive outcomes, respectively. While CMV is ubiquitous and often asymptomatic, CMV DNA levels could influence the establishment and sustainment of the HIV reservoir and have long-term neurocognitive effects. Further exploration of mechanisms to explain the associations between CMV and HIV reservoir and CMV and neurocognitive outcomes are warranted.

Advanced epidemiologic methods could be used to better understand HIV viral load trajectories and neurocognitive outcomes of children with HIV. In Chapter 2, GBTMs furthered our understanding of HIV viral load trajectories, especially among unsuppressed children, which could inform differentiated care strategies aimed to improve adherence and care for children with HIV. Findings from Chapter 3 could inform predictive models that assess neurocognitive outcomes in children. These models could identify children who could benefit from interventions to improve neurocognitive outcomes.

This dissertation identifies gaps and strategies to further knowledge of predictors, trajectories, and impact HIV viral control in children with HIV.

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