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HIV-associated CD4/8 depletion in infancy is associated with neurometabolic reductions in the basal ganglia at age 5 years despite early antiretroviral therapy

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Abstract

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Conflict of Interest

The content of this paper does not reflect the views or policies of NIH, NRF, or SAMRC, nor does mention of trade names, commercial projects, or organizations imply endorsement by any organization or government.

None of the funding sources had any role in the study design; the collection, analysis, and interpretation of data; the writing of the report; and in the decision to submit the paper for publication.

Author Contributions

Mark F. Cotton was part of the team that designed the CHER trial. Els Dobbels and Barbara Laughton managed the patients in the Children's Infectious Diseases Clinical Research Unit and contributed to protocol development. Barbara Laughton was principal investigator of the neurodevelopmental sub study to the CHER trial. Andre J.W. van der Kouwe, Barbara Laughton and Ernesta Meintjes were principal investigators, designed the imaging study, oversaw protocol development, data collection, data analyses, and interpretation of the findings, as well as sourced funding from the US National Institute of Child Health and Human Development, the US National Institute of Mental Health, and the RSA National Research Foundation. Aaron T. Hess developed the scanning sequences and contributed to the data management and processing operations under supervision from Andre J.W. van der Kouwe and Ernesta Meintjes. Kenneth Mbugua performed data analyses and wrote the primary report under supervision of Martha J. Holmes and Ernesta Meintjes. Francesca Little provided statistical expertise and Eva-Maria Ratai contributed to the reviewership of the report.

Objective—Investigating consequences of early or late antiretroviral therapy (ART) initiation in infancy on young brain development using magnetic resonance spectroscopy (MRS).

Design—Most pediatric HIV/ART-related neurological studies are from neuropsychological/clinical perspectives. MRS can elucidate the mechanisms underpinning neurocognitive outcomes by quantifying the brain's chemical condition through localized metabolism to provide insights into health and development.

Methods—Basal ganglia metabolite concentrations were assessed in thirty-eight 5-year-old HIV-infected children previously participating in a randomized trial comparing early limited ART to deferred continuous ART, as well as 15 uninfected controls (12 HIV exposed). Metabolite levels were compared between 26 infected children who initiated ART at/before 12 weeks and 12 who initiated afterwards, and were correlated with clinical HIV and treatment-related measures.

Results—HIV-infected children initiating ART after 12 weeks had lower creatine, choline and glutamate (p 's<0.05) than those initiating ART at/before 12 weeks. The CD4/CD8 ratio at baseline correlated with N-acetyl-aspartate ($r=0.56$, $p=0.003$) and choline ($r=0.36$, $p=0.03$) at 5 years, irrespective of treatment regimen and ART interruption. In comparison with uninfected controls, 80% of whom were HIV exposed *in utero*, children on early treatment had higher N-acetyl-aspartate ($p=0.006$) and choline ($p=0.03$).

Conclusions—Despite early ART (<12 weeks), low baseline CD4/CD8 predicts brain metabolite levels in later childhood. Also, HIV-exposure and antiretroviral exposure for preventing vertical HIV transmission may hinder metabolite health, but needs further investigation.

Keywords

Human Immunodeficiency Virus (HIV); Magnetic Resonance Spectroscopy (MRS); Basal Ganglia (BG); Antiretroviral Therapy (ART); Metabolites, N-acetyl-aspartate (NAA); choline (GPCPCh); Creatine (CrPCr); Glutamate (Glu); Cluster of Differentiation 4/8 (CD4/8)

Introduction

Human immunodeficiency virus (HIV) infection has transitioned from a fatal to a chronic disease. Sub-Saharan Africa has 10% of the world's population, but 68% of adults and 90% of children with HIV infection [1]. Combination antiretroviral therapy (ART) guidelines have evolved over time towards earlier initiation at higher CD4 counts in adults and for treatment of all children regardless of age, disease stage or CD4 count. The early data of the Children with HIV early Antiretroviral (CHER) trial, released in 2007, was pivotal in changing pediatric ART guidelines. Infants at a median age of 7.4 weeks (interquartile range, 6.6 to 8.9) and a CD4 percentage of 35.2% (interquartile range, 29.1 to 41.2) were randomized to immediate or deferred ART. Early ART was associated with reduction in mortality by 76% and HIV progression by 75% compared to deferred ART [2,3]. A neurodevelopmental sub study [4] found that infants started on early ART had significantly higher locomotor and general scores on the Griffiths Mental Development Scales at a median age of 11 months compared to infants on deferred ART. However, long-term effects of timing of ART initiation relative to acute or chronic HIV infection and long-term effects of ART on neurodevelopment and neurocognition remain unclear [4–6].

The developing brain is especially vulnerable to HIV infection due to rapid and critical brain development in the first 2 years of life [7]. Paediatric HIV and ART-associated damage has been primarily investigated from neuropsychological [4,5] and clinical perspectives [8]. Neuroimaging can potentially describe the mechanisms underpinning neurobehavioral outcomes by quantifying the structural, functional and chemical condition of the brain [9–11]. Few neuroimaging studies have assessed HIV-infected subjects stable on ART, particularly among young children [10].

Proton magnetic resonance spectroscopy (¹H-MRS) non-invasively evaluates localized brain metabolism, providing insights into brain health, development and the consequences of both long-term HIV infection [9,12] and ART. The archetypal metabolites measured include: N-Acetyl-Aspartate (NAA), a marker of neuronal integrity and viability; creatine-phosphocreatine (CrPCr), involved in the Krebs cycle; choline (GPCPCh), which comprises glycerophosphocholine (GPC) and phosphocholine (PCh), reflecting cell membrane integrity and turnover primarily in macrophages and microglia; glutamate (Glu), a primary neurotransmitter and myo-inositol (Ins) involved in cell transport and hormone-sensitive neuroreception.

The basal ganglia (BG) play important roles in cerebral development, controlled movement, hand-eye coordination, and motivation [13] but remain among the most HIV-affected regions [14,15]. ¹H-MRS studies of HIV-infected adults [16,17] and children [10,15,18,19] report altered BG neurometabolism, irrespective of symptoms. Adult studies documented reduced BG NAA levels or NAA/creatine ratios with HIV-dementia progression, as well as increased choline levels or choline/creatine ratios and Ins/creatine ratios as markers of increased neural inflammation and gliosis. However, findings in children are conflicting, possibly due to small sample sizes, wide age ranges over several developmental phases, different ethnicities, different treatment regimens and late therapy initiation, as well as study-based focus on HIV-encephalopathies and other malignancies [18,19].

We aimed to investigate the effects of different ages of ART initiation and disease severity in infancy on metabolite levels in the BG at age 5 years. We hypothesized that children who initiated ART early would show neurometabolic advantages as a proxy for brain health at preschool age compared to delayed treatment, and that poorer immune health in infancy would be associated with poorer neurometabolic outcomes at age 5 years, indicating developmental delay/damage. Strengths of this study include prospective follow-up, early access to standardized ART, uniform sociodemographics, and all children assessed within a narrow age range (5–6.4 years).

Methods

Participants

Participants were sixty-two HIV-infected Xhosa children from the randomized CHER trial in follow-up at the Children's Infectious Diseases Clinical Research Unit, Tygerberg Children's Hospital, Cape Town [2,3].

Zidovudine (ZDV, AZT, Retrovir®) was administered from 34 weeks gestation and to neonates for 7 days. Additionally, a single dose Nevirapine (NVP, Viramune®) was given to mothers in labor and to infants shortly after birth [2,3]. HIV infection was confirmed by a positive polymerase chain reaction (PCR, Roche Amplicor Version 1.5 RNA) test for HIV-1 DNA and plasma viral load (PVL) > 1000 HIV-1 RNA copies/ml. All children with CD4 percentage (CD4%) ≥ 25% were randomized to one of the following three treatment arms: a) ART-Def (ART deferred until CD4% < 25% in first year or CD4% < 20% thereafter, or if clinical disease progression criteria presented), b) ART-40W (ART initiated *early* – i.e., *before 12 weeks of age* – and interrupted after 40 weeks), and c) ART-96W (ART initiated early and interrupted after 96 weeks) [2,3].

Since our aim was to investigate potential benefits of early ART on neurometabolism at age 5 years. Also, some children in the ART-Def arm met criteria for almost immediate initiation of ART, the CHER children were grouped here into those who received ART at or before 12 weeks of age (early-ART) and those who received ART after 12 weeks (late-ART), irrespective of treatment arm.

All children underwent comprehensive clinical and immunological follow-up until 5 years of age. Additionally, a neurological examination was conducted prior to MRI scanning at ages 4.8–5.9 years. The ART regimens comprised ZDV + Lamivudine (3TC, Epivir®) + Lopinavir-Ritonavir (LPV/r, Kaletra®). Clinical follow-up occurred every 3 months. HIV-1 RNA was monitored using the Amplicor HIV-1 MONITOR PCR test (Roche Molecular Systems, Inc., Branchburg, NJ, USA) over the range of 400–750,000 copies/ml of plasma.

Seventeen HIV-uninfected Xhosa children were recruited from an interlinked vaccine trial [20] as controls. They comprised HIV exposed but uninfected (HEU) children born to HIV-infected mothers but who tested HIV-negative (PCR) at baseline and 30 days after the third dose of vaccine, and HIV unexposed and uninfected (HUU) children born to HIV-seronegative mothers (tested after 24 weeks gestation) who remained seronegative at enrollment [20].

Neuroimaging

Children received neuroimaging on the 3T Allegra MRI Scanner (Siemens, Erlangen, Germany) at the Cape Universities Brain Imaging Centre (Tygerberg Children's Hospital) between ages 5–6.4 years, without sedation, according to protocols approved by the Human Research Ethics Committees of the Universities of Cape Town and Stellenbosch. Parents/guardians provided written informed consent; children provided oral assent.

The protocol included a high-resolution T1-weighted acquisition for voxel placement and single voxel spectroscopy (SVS) in the right BG (Fig. 1A) using a real-time motion and B0 corrected point resolved spectroscopy (PRESS) sequence (1.5×1.5×1.5 cm³ voxel; TR 2000 ms, TE 30 ms, 64 averages) [21]. The voxel comprised the frontal limb of the internal capsule and part of the caudate nucleus, putamen and globus pallidus. Water unsuppressed ¹H-MRS measurements were obtained at different TE's (TE = 30/50/75/100/144/500/1000 ms, TR=4000 ms, 2 averages) to estimate tissue fraction composition [11].

Absolute metabolite levels of the corrected water-suppressed spectra were quantified using LCModel [22]. The water signals of the unsuppressed spectra were modeled (Sigma Plot, San Jose, California) to estimate the tissue fractions in the voxel [11,23]. Tissue content values and relaxation correction factors were utilized to obtain absolute quantification values [11,22] from offline frequency- and phase-corrected data.

Data were excluded if the full-width at half maximum line widths of NAA exceeded 0.075 ppm and if the SNR was below 7 [11,24]. Subjects were excluded if the mean of the SNR-dependent Cramér–Rao minimum variance bounds (CRMVB) for the chosen metabolites – NAA, CrPCr, GPCPCr, Glu, and Ins – was above 20%. A senior neuroradiologist reviewed all structural scans.

Statistical Analyses

Statistical analyses were performed in SPSS 22 (IBM, Armonk, New York). Demographic variables and clinical measures were compared between groups using analysis of variance (ANOVA). Amongst infected children, differences in metabolite levels between children receiving early and late ART were examined using a student's t-test. We also investigated possible group differences in metabolite levels between children who had normal versus abnormal neurological exams, and amongst the children receiving early ART between those with treatment interruption versus continuous treatment. As a proxy for inflammation, we compared metabolite levels and clinical variables between infected children who had any instances of virological breakthrough (VB) following ART initiation and those in whom PVL remained suppressed.

Any confounders related to a given outcome at $p < 0.10$ were entered into an analysis of covariance (ANCOVA) to determine whether group differences remained significant after controlling for these measures. Further, since ART interruption is characterized by a subsequent drop in CD4 count and increased PVL that may negate the potential benefits of early ART, we also controlled for duration of ART interruption.

Pearson's and Spearman's rank (ρ) correlations were used to assess metabolites with clinical measures recorded at enrollment and at time of scanning – CD4 count, CD4%, CD8 count, CD8 percentage (CD8%), ratio of CD4/CD8 counts – as well as cumulative period on ART, age at ART initiation, and the age at which PVL first dropped below 400 copies/ml (first PVL suppression). We did not examine associations with plasma viral load as the range was concatenated. Multiple regression analyses were used to examine the effect of confounding variables related to the outcome at $p < 0.10$ on the associations between metabolite levels and clinical measures.

We performed correction for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method [25].

Results

Sample Characteristics

Of 79 children recruited, 26 were excluded for the following reasons: 15 for scanner claustrophobia, 1 due to poor data quality because of excessive head movement and 10 due to incomplete water reference spectra (necessary for tissue fraction estimation for quantification of absolute metabolite levels). No subjects were excluded due to NAA line widths exceeding 0.075 ppm, SNR below 7 or mean CRMVBs above 20%. We thus present results for 38 HIV-infected children (age 5.0 – 6.3 years; 18 male) comprising 12 who received late ART and 26 who received early ART, as well as 15 uninfected controls (age 5.1 – 6.4 years; 7 male). Of the 26 who received early ART, 10 remained on continuous ART in line with clinical criteria governing interruption. Treatment was interrupted in 16, two of whom had not met ART restart criteria at the time of scan as they remained clinically well with CD4 percentage $\geq 20\%$. Twelve of 15 control children (80%) were HEU.

Radiological reports were normal in all except 7 children who were referred for follow up. Only in 1 child who manifested right BG malformation, (ventriculomegaly and colpocephaly) was left BG spectra obtained. Comparative analyses including and excluding this subject were performed. For the neurological examinations; 38 children were normal, 11 children had brisk limb reflexes (2 late-ART, 8 early-ART, 1 control), 3 children had lower limb spasticity (2 late-ART, 1 early-ART), and 1 control child did not undergo examination.

There were no significant differences in demographics between controls and infected children in the early or late treatment groups, and no differences in clinical measures at enrollment or at scan between children receiving early and late ART (Table 1). Due to the design of the CHER study, treatment-related measures differed between the groups. Children who initiated ART early were younger when viral load was first suppressed, but tended to be older at their nadir CD4% and peak CD8. Notably, nadir CD4 and peak CD8 measures typically occurred after interruption in the early treatment children, while nadir CD4% and peak CD8 count occurred around time of ART initiation in the late-ART group.

There were no significant differences in demographic or clinical variables amongst the early-ART children in those with and without interruption, except that cumulative period on ART was longer in children on continuous treatment (mean $\pm$ s.e. = 283 ± 6 weeks compared to 219 ± 14 weeks, $p = 0.001$) and CD8% at enrollment tended to be higher in those receiving continuous treatment compared to those in whom treatment was interrupted (36.2 ± 4.5 versus 27.1 ± 2.3 , $p = 0.07$). Interestingly, children with abnormal neurological signs had higher CD8 count at scan compared to neurologically normal children (1291 ± 153 versus 856 ± 82 , $p = 0.01$). Instances of virological breakthrough (VB) occurred in 19 children after ART initiation. Total period on ART was shorter in these children (235 ± 10 weeks compared to 263 ± 8 weeks in children without VB, $p = 0.03$), and they tended to be younger at ART initiation (13.5 ± 2.6 weeks, compared to 23.0 ± 5.0 weeks in children without VB, $p = 0.09$).

Metabolite level group comparisons

The children who initiated ART after 12 weeks of age had lower levels of CrPCr, GPCPCh, and Glu than those initiating treatment at or before 12 weeks (Table 2). Of the potential confounders considered, only birth weight had a significant association with GPCPCh ($r = 0.26$, $p = 0.05$). Differences between early and late ART groups remained significant after controlling for ART interruption and the potential confounding effects of birth weight on GPCPCh. In figure 1B we show mean and 95% confidence intervals of BG metabolite levels for early and late ART groups together with values for uninfected controls. Unexpectedly, uninfected children had lower levels of NAA (student's t-test $p = 0.006$) and GPCPCh ($p = 0.03$) than children in the early-ART group. The GPCPCh difference remained significant after controlling for birth weight. There were no significant differences in metabolite levels between uninfected children and children in the late-ART group.

Notably, GPCPCh was higher in children with abnormal neurological exams (1.12 ± 0.02 versus 1.04 ± 0.02 , $p = 0.02$), while none of the other metabolites differed significantly (all p 's > 0.2). Controlling for abnormal neurological signs did not alter the group difference in GPCPCh between children who initiated ART early and late ($p = 0.05$).

No metabolite levels differed between children on continuous early ART versus interrupted early ART (all p 's > 0.2), nor between children with or without VB (all p 's > 0.13).

Associations between metabolite levels and clinical measures in HIV-infected children

Higher CD8%, as well as lower CD4 count and lower CD4/CD8 ratio, all at enrollment, were associated with reduced NAA and GPCPCh. Higher baseline CD8 count was also associated with lower NAA (Table 3). The aforementioned associations with GPCPCh remained significant after controlling for birth weight. Figure 1C shows the relations of NAA and GPCPCh with CD4/CD8. In contrast, metabolite levels were *not* associated with any of the clinical measures at time of scan or cumulative time on ART. Similar to the higher CrPCr levels observed in children initiating ART before 12 weeks, younger age at ART initiation and at first PVL suppression were both associated with increasing CrPCr levels at age 5 years. Controlling for presence of abnormal neurological signs did not alter the associations between clinical measures and GPCPCh (partial $r = 0.35$, $p = 0.04$).

Partial correlation analysis was used to determine the degree to which the effects of immunocompromise during infancy on GPCPCh might be attributable to poorer neuronal integrity, as measured by NAA. After controlling for NAA, the relation of CD4/CD8 to GPCPCh was no longer significant (partial $r = 0.12$, $p = 0.48$), indicating that the effects of poor immune health in infancy on GPCPCh may be attributable to its effect on NAA.

After Benjamini-Hochberg FDR correction for multiple testing, only the relationships between NAA and CD8% at enrollment as well as NAA and CD4/CD8 at enrollment survived correction with $p = 0.003$ at $\alpha = 0.05$ [25].

Discussion

This is the first study to use metabolite levels as markers of neurodevelopment and brain health [5,9,11,15] *in young HIV-infected children in a narrow age range who initiated ART at different stages in infancy, of whom 95% have suppressed viral loads and 66% are neurologically normal.* Notably, children who received ART before 12 weeks of age had significantly higher mean creatine, choline and glutamate levels at age 5 years, compared to those in whom ART was initiated after 12 weeks, irrespective of ART interruption. The higher metabolite levels imply that early ART initiation affords newborns neurometabolic advantages, and by extension, neurodevelopmental benefits [4–6,11,15,17,26], which are detectable even at later ages.

In contrast, independent of when treatment was initiated, poorer immune health in infancy (baseline CD4/CD8) was associated with reduced neuronal integrity at age 5 years. This result suggests that early effects of HIV exposure and immunocompromise in utero or during the first 6–8 weeks of life may lead to either delayed neurodevelopment or brain damage that persists into early childhood [27,28]. The CD4/CD8 ratio may be a more sensitive marker than CD4 depletion from 8 weeks [29] as the ratio reflects both CD4 depletion and CD8 expansion characterized by acute HIV infection [30,31]. These results are consistent with previous studies reporting that neurological damage sustained in utero and/or during the first 6–8 weeks of life persists into early childhood, irrespective of ART, likely through toxicity, apoptosis, synaptic injury and neuronal pruning [9,14]. One study [19] postulated that HIV-associated damage is reversible through ART mitigation via observed NAA recovery. Longitudinal studies are needed to establish whether recovery occurs.

The association between GPCPCh and baseline CD4/CD8 was lost after controlling for NAA, suggesting that the effects of poor immune health in infancy on GPCPCh levels is attributable to its effect on neuronal cell density, and that HIV-1 may target neurons rather than neuronal support cells.

Although creatine levels are widely regarded as stable, there is evidence of HIV-associated decreases in the BG [15,32]. Consistent with Keller et al., [15], we found that the younger the children at time of first viral load suppression (< 400 copies/ml plasma), the higher their CrPCr levels at age 5 years. This finding was supported by higher CrPCr levels in children in the early-ART group in whom PVL would have been suppressed earlier in life. CrPCr is essential for the Krebs cycle, involving cellular transport of high energy phosphate from mitochondria [33]. Decreased CrPCr levels may reflect decreased total neurometabolism suggesting neural cell loss [15,17] and is associated with advanced neurological disease [17] or late stage dementia [16] in HIV infected adults.

GPCPCh is a precursor to the neurotransmitter acetylcholine, which influences neurogenesis, stem cell differentiation, myelin formation and synaptogenesis during early neurodevelopment [34]. Reduced levels of choline-containing compounds in children indicate lower neural cell density [15]. Elevated GPCPCh levels together with higher CD8 count at scan observed in children with abnormal neurological signs, however, provides

evidence of acute inflammation. The fact that reduced choline levels in children initiating ART after 12 weeks remain significant after controlling for abnormal neurological signs, suggests that this reduction is attributable to lower neural cell density rather than acute inflammation in the early-ART children. It is striking that reduced choline presumably sustained from delayed ART initiation persists into early childhood, despite children being stable on ART at the time of scanning with high CD4 counts and viral load suppression.

Lower glutamate levels suggest decreased neurotransmitter levels, found in excitatory glutamatergic neuronal mitochondria. Reasons for glutamate alterations are complex, but HIV-driven neuronal mitochondrial excitotoxic dysfunction is a plausible explanation for the reduced Glu levels in ART delayed children [35].

Using instances of VB as a proxy for inflammatory activity after initial ART, the similarity in metabolites between children with VB versus without VB, provides evidence that the differences seen here between children initiating ART early and late are not attributable to inflammatory activity after ART initiation.

Although metabolite levels of uninfected controls were mostly similar to children receiving early ART, NAA and GPCPCh levels were lower in controls than children in the early-ART group, similar to findings by Keller et al., [15]. This unexpected result may be attributed to 80% of the uninfected children being HEU, thus exposed to both HIV and ART, similar to observations of Cortey et al. [36]. A limitation of this study was the low number of HUU children recruited.

Contrary to our expectations, only baseline CD8% tended to be higher in children on early continuous ART versus early interrupted ART, likely reflecting more severe disease at baseline and already reaching endpoint criteria prior to planned treatment interruption. Furthermore, interruption didn't affect the similarity in metabolites between early-ART and late-ART, but the relation between CD4/CD8 and GPCPCh strengthened ($r = 0.56$, $p = 0.005$), affirming our observation that the effects of immunocompromise (decreased CD4/CD8 ratio) before 6–8 weeks persist into early childhood, irrespective of treatment strategy [27].

There were limitations in our study, such as the selectivity of MRS in measuring certain metabolites (unlike other compounds like glucose via other modalities) as well as clinical limitations such as measurement of additional lymphocyte inflammatory markers, such as CD38 and HLADR expression, all which would also have been beneficial. Some of these limitations can be explained by ethical and clinical considerations since the study was in children stable on standardized ART, 73% neurologically normal and over a narrow age range.

Our findings support an emerging philosophy of administering ART at birth to HIV exposed infants, as seen in the “Mississippi Baby” [27] and “Canadian Newborns” case series [28], as well as the CHER and neurodevelopmental sub-study findings [2–4,6].

Although various studies have shown that HIV infection causes neurometabolic alterations, these changes and their effects on different aspects of neurodevelopment remain unclear.

MRS offers a sensitive and powerful tool for measuring specific metabolite levels known to relate to neuronal and cellular integrity. Our findings support the benefits of early ART initiation into early childhood. In addition, the association of poor baseline immune health in infancy with compromised neuronal integrity at age 5 years suggests that early brain damage or developmental delay may persist into childhood, despite early ART and viral load suppression.

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Figure 1.

Figure 1A: Location of the right basal ganglia voxel on a (a) sagittal slice, (b) coronal slice and (c) axial slice. A = Anterior; P = Posterior; R = Right and L = Left.

Figure 1B: Comparison of metabolite levels (mean and 95% confidence intervals) in the right basal ganglia between HIV-infected children who initiated ART before or at 12 weeks, ART after 12 weeks and uninfected controls. mM = millimolar.

Figure 1C: Relationship between the CD4/CD8 ratio in HIV-infected infants at the time of enrollment into the CHER trial and their levels of (A) NAA and (B) GPCPCh measured at age 5 years in the right basal ganglia. mM = millimolar.

Table 1

Sample characteristics

Total N = 53	Controls (n = 15)	Early-ART (n = 26)	Late-ART (n = 12)	F / χ^2 / t (p)
<i>Demographics</i>				
Sex: M; F	7M; 8F	13M; 13F	5M; 7F	0.23 (0.89)
Handedness: R; L	13R; 2L	24R; 2L	12R; 0L	1.70 (0.42)
Age at scan (years)	5.6 (5.0 – 6.2)	5.5 (5.0 – 6.3)	5.5 (5.0 – 5.9)	0.10 (0.90)
Birth weight (kg)	3.1 (2.0 – 4.2)	3.2 (2.4 – 3.8)	3.1 (2.5 – 3.6)	0.28 (0.76)
<i>Clinical data at enrollment</i>				
CD4 count (cells/mm ³)	n/a	1799 (294 – 4050)	1963 (1085 – 3731)	0.25 (0.62)
CD4% (%)	n/a	33 (11 – 54)	38 (26 – 53)	2.06 (0.15)
CD8 count (cells/mm ³) ^a	n/a	1637 (522 – 4119)	1513 (758 – 4337)	0.16 (0.69)
CD8% (%)	n/a	31 (14 – 56)	28 (18 – 55)	0.30 (0.59)
CD4/CD8 ^a	n/a	1.3 (0.2 – 3.5)	1.5 (0.5 – 2.3)	0.78 (0.38)
Plasma Viral Loads, RNA copies/ml				
High (> 750,000)	n/a	14 (54%)	9 (75%)	
Low (400 – 750, 000)	n/a	12 (46%)	3 (25%)	1.54 (0.21)
Suppressed (< 400)	n/a	0	0	
<i>Clinical data at scan^b</i>				
CD4 count (cells/mm ³)	n/a	1182 (438 – 2993)	1378 (699 – 3220)	0.78 (0.38)
CD4% (%)	n/a	36 (19 – 55)	40 (30 – 51)	1.74 (0.20)
CD8 count (cells/mm ³)	n/a	1022 (30 – 2660)	1019 (332 – 1796)	0.01 (0.99)
CD8% (%)	n/a	31 (16 – 44)	29 (20 – 42)	0.23 (0.63)

Total N = 53		Controls (n = 15)	Early-ART (n = 26)	Late-ART (n = 12)	F / χ^2 / t (p)
CD4/CD8		n/a	1.3 (0.5 – 3.5)	1.5 (0.8 – 2.1)	0.33 (0.57)
Plasma Viral Loads, RNA copies/ml					
High (> 750,000)		n/a	0 (0%)	0 (0%)	
Low (400 – 750, 000)		n/a	1 (4%)	1 (8%)	2.63 (0.27)
Suppressed (< 400)		n/a	25 (96%)	10 (83%)	
<i>Treatment-related measures</i>					
Age at ART initiation (weeks)		n/a	8 (6 – 12)	37 (7 – 75)	54.9 (0.001)
Time on ART (weeks)		n/a	244 (96 – 313)	247 (201 – 290)	0.03 (0.86)
Continuous ART		n/a	10 (39%)	12 (100%)	
ART interrupted and restarted		n/a	14 (54%)	0 (0%)	12.8 (0.002)
ART interrupted and not restarted		n/a	2 (7%)	0 (0%)	
Age at ART interruption (weeks)		n/a	75 (47 – 108)	not interrupted	n/a
Length of Interruption (weeks)		n/a	36 (0 – 183)	not interrupted	n/a
Age at nadir CD4 count (weeks)		n/a	162 (1 – 281)	142 (21 – 295)	0.33 (0.57)
Age at nadir CD4% (weeks)		n/a	85(2 – 259)	43 (5 – 191)	4.21 (0.05)
Age at peak CD8 count (weeks)		n/a	94 (6 – 246)	46 (19 – 181)	3.98 (0.06)
Age at peak CD8% (weeks)		n/a	112 (6 – 254)	114 (19 – 269)	0.01 (0.95)
Age at first PVL suppression (weeks)		n/a	50 (30 – 213)	88 (41 – 169)	7.02 (0.01)
<i>CDC Adverse Event</i>					
Suffered adverse Event; Yes; No		n/a	12Y; 14N	6Y; 6N	0.05 (0.83)
No event		n/a	14 (54%)	6 (50%)	
Severe stage B		n/a	7 (27%)	4 (25%)	0.17 (0.92)
Stage C		n/a	5 (19%)	2 (25%)	

Total N = 53	Controls (n = 15)	Early-ART (n = 26)	Late-ART (n = 12)	F / χ^2 / t (p)
Age at event (weeks)	n/a	69 (28 – 135)	99 (26 – 268)	1.01 (0.33)
<i>Incidence of Virological Breakthrough</i> ^c ; <i>Yes, No</i>	n/a	15Y; 10N	4Y; 8N	2.82 (0.25)

All values are Median (Range) or n (% of total).

^aCD8 count at enrollment missing for one child who received Late ART.

^bClinical data at scan not available for one child who received Late ART.

^cThe PVL history profile for one child who received early ART was incomplete.

Table 2

Comparison of mean metabolite levels (mM) between children initiating ART at/before (Early-ART) and after (Late-ART) 12 weeks of age.

	Early-ART	Late-ART	<i>p</i>	<i>p^a</i>	<i>p^b</i>	<i>p^c</i>
N =	38	26	12			
NAA	5.33 (0.07)	5.16 (0.14)	0.26	0.11		
CrPcr	5.45 (0.07)	5.15 (0.13)	0.02	0.03		
GPCPCh	1.11 (0.02)	1.03 (0.04)	0.04	0.04	0.05	0.05
Ins	3.14 (0.13)	2.93 (0.11)	0.29	0.27		
Glu	7.32 (0.15)	6.75 (0.31)	0.07	0.02		

Values are Mean (s.e.)

^aControlling for duration of ART interruption

^bControlling for duration of ART interruption AND birth weight in GPCPCh

^cControlling for the presence of signs of abnormal neurology in GPCPCh

Table 3

Associations of metabolite levels in basal ganglia with clinical measures.

n = 38	NAA	CrPCr	GPCrCh	Glu	Ins
	<i>r</i>	<i>r</i>	<i>r</i>	β	<i>r</i>
<i>Clinical data at enrollment</i>					
CD4 count at enrollment	0.34*	0.20	0.33*	0.33*	-0.04 -0.15
CD4% at enrollment	0.28†	0.06	0.11	0.14	0.03 -0.22
CD8 count at enrollment ^a	-0.35*	-0.16	-0.13	-0.11	-0.19 0.09
CD8% at enrollment	-0.51***	-0.31†	-0.40*	-0.41*	-0.05 0.11
CD4/CD8 at enrollment ^a	0.56***	0.24	0.36*	0.38*	0.05 -0.26
<i>Clinical data at scan^b</i>					
CD4 count at scan	0.09	-0.02	0.20	0.18	-0.01 -0.03
CD4% at scan	-0.06	-0.07	-0.11	-0.21	0.06 0.003
CD8 count at scan	0.13	0.05	0.19	0.21	-0.01 -0.05
CD8% at scan	-0.10	-0.02	-0.08	-0.08	-0.04 -0.11
CD4/CD8 at scan	0.03	0.05	-0.01	-0.07	0.13 0.02
<i>Treatment-related Measures</i>					
Age at ART initiation ^c	-0.07	-0.33*	-0.20	-0.20	-0.11 0.05
Age at first PVL suppression ^c	-0.23	-0.43**	-0.07	0.03	-0.20 0.03
Time on ART	0.15	-0.19	-0.10	-0.02	-0.05 -0.10

† $p < 0.1$;

* $p < 0.05$;

** $p < 0.01$;

*** $p < 0.005$

r is Pearson correlation coefficient; β is the standardized regression coefficient after accounting for the potential confounding influence of birth weight on GPCrCh.

^a CD8 count at enrollment missing for one Late-ART child.

^b Clinical data at scan missing for one Late-ART child (a different child to the one for whom CD8 enrollment data were missing).

ρ_s: Spearman's rank correlation coefficient

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