Neurologic Abnormalities in HIV-1 Infected Children in the Era of Combination Antiretroviral Therapy

Lotus A. van Arnhem¹, Madeleine J. Bunders¹, Henriette J. Scherpbier¹, Charles B. L. M. Majoie², Liesbeth Reneman², Olivier Frinking¹, Bwee Tien Poll-The³, Taco W. Kuijpers¹, Dasja Pajkrt¹*

¹ Department of Pediatric Hematology, Immunology and Infectious Diseases, Emma Children’s Hospital, Academic Medical Centre (AMC), Amsterdam, The Netherlands, ² Department of Radiology, AMC, Amsterdam, The Netherlands, ³ Department of Pediatric Neurology, Emma Children’s Hospital, AMC, Amsterdam, The Netherlands

Abstract

Background: Pediatric HIV-1 infection is associated with neurologic abnormalities. In recent years, the neurological outcome of HIV-1 infected children has substantially improved with combination antiretroviral therapy (cART). However, data regarding the long-term effect of cART and neurologic outcome are limited.

Methods: In the Pediatric Amsterdam Cohort on HIV-1 study, 59 perinatally HIV-1 infected children were evaluated from 1992–2010. All children underwent neurological examination and neuro-imaging studies, including CT-scan and/or MRI imaging. Fisher exact and Kruskal-Wallis tests were used to compare clinical deviations of neuro-imaging studies with HIV-1 related parameters, including CD4⁺ T cell count, HIV-1 viral load in blood and cerebrospinal fluid (CSF), and duration of cART as well as neurologic examination.

Results: Abnormal neurologic examinations in these HIV-1 infected children included language impairment (22%), abnormal muscle tone (hyper/hypotonia) (14%) and delay in reaching developmental milestones (12%). Ventricular enlargement and sulcal widening (29%) and white matter lesions (38%) were prominent findings. White matter lesions were positively correlated with HIV-1 viral load levels. In a small follow-up sub study white matter lesions did not improve while children with ventricular enlargement and sulcal widening showed improvements whilst being treated with cART.

Conclusions: In the current era of cART HIV-1 infected children still frequently show neurologic impairments together with abnormal neuro-imaging.

Introduction

Pediatric HIV-1 infection continues to be a worldwide challenge. Although the number of HIV-1 infected children is slowly decreasing, in 2009 still 370,000 children were newly HIV-1 infected via mother-to-child-transmission (MTCT) [1].

HIV-1 associated illnesses affect multiple organs and being a neurotrophic virus, HIV-1 can severely affect the central nervous system (CNS). In the pretreatment era, classic pediatric HIV-1 encephalopathy (as characterized by impaired brain growth, motor deficits and developmental delay) was the most prevalent neuro-disability reported [2–5]. In addition, HIV-1 infected children can present with more variable neurologic symptoms, including seizures, headaches, and behavioral changes [6,7]. Neuro-imaging of (perinatally) HIV-1 infected children shows substantial abnormalities of both the white and grey matter, such as white matter lesions and calcifications [8–12].

Treatment of HIV-1 infected children with combination antiretroviral therapy (cART) has substantially improved the clinical outcome of HIV-1 infected children and resulted in a decrease of plasma and cerebrospinal fluid (CSF) HIV-1 RNA viral load (HIV VL). The incidence of HIV-1 encephalopathy has decreased significantly in the cART era [13–15]. More recently, early ART intervention proved effective in improving neurodevelopmental outcomes in infants [16]. Nevertheless, data from adult infected individuals suggest that the CNS can serve as a reservoir for HIV-1 and viral replication and immune activation can take place whilst being treated with cART, potentially associated with ongoing neurological damage [17]. Up to date there is a lack of data regarding the follow-up of neurological impairments and neuro-imaging abnormalities in HIV-1 infected children receiving long-term cART. In this study, we evaluated neurologic functioning and neuro-imaging findings in perinatally HIV-1 infected children who were treated with cART and associated HIV-1 parameters.

Methods

Ethics Statement

The ethics committee of the Academic Medical Centre, Amsterdam, approved the study. Written informed consent was

* E-mail: d.pajkrt@amc.uva.nl

† These authors contributed equally to this work.

Citation: van Arnhem LA, Bunders MJ, Scherpbier HJ, Majoie CBLM, Reneman L, et al. (2013) Neurologic Abnormalities in HIV-1 Infected Children in the Era of Combination Antiretroviral Therapy. PLoS ONE 8(5): e64398. doi:10.1371/journal.pone.0064398

Editor: Shilpa J. Buch, University of Nebraska Medical Center, United States of America

Received December 17, 2012; Accepted April 13, 2013; Published May 15, 2013

Copyright: © 2013 van Arnhem et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors have no support or funding to report.

Competing Interests: The authors have declared that no competing interests exist.
obtained from all study participants and the study was conducted according to guidelines of the declaration of Helsinki.

Subjects
Here, we describe a single-center observational cohort study. From January 1992 until May 2010 59 HIV-1 infected children treated at the Emma Children’s Hospital Academic Medical Centre in Amsterdam, the Netherlands were included in the study. These children also participated in the Pediatric Amsterdam Cohort on HIV-1 (PEACH) study, that evaluated long-term effectiveness and safety of cART in HIV-1 infected children [18]. All included children were infected via MTGT of HIV-1. None of the mothers used illicit drugs during pregnancy. Antiretroviral treatment regimes of the mothers of included patients were not documented.

Antiretroviral Treatment
Before 1996, all included children received zidovudine, monotherapy or in combination with either lamivudine or didanosine, as part of standard patient care. In 1996 cART was introduced in the Netherlands and consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) in combination with either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI). The two combinations of cART most frequently used in this study were either a combination treatment with nelfinavir, lamivudine and stavudine (mostly used from 1996 until 2002) or a combination treatment with efavirenz, abacavir, didanosine and lamivudine (mostly used from 2002 until 2005) [19].

Drug Resistance Mutations
Drug resistance mutation analysis was performed in the first available blood samples from all patients prior to start cART and were interpreted according to the 2013 IAS-USA drug resistance list [20]. HIV-1 sequence analysis was performed with the Viroseq HIV-1 genotyping kit (version 2; Abbott Laboratories, Abbott Park, IL) as reported previously [21]. Alternative switches of antiretroviral treatment regimes were based on newly acquired drug resistance mutations to cART components.

Neuro-imaging and Neurological Examination
Neuro-imaging was performed by computed tomography (CT) from 1992 until 2001 and by magnetic resonance imaging (MRI) after 2001. CT scans were performed with 5 mm slice thickness without contrast enhancement. MRI was performed on 1.5 T scanners using axial T1- and T2-weighted spin echo and FLAIR sequences. The neuro-imaging scans were evaluated by two independent radiologists and scored for presence or absence of intracerebral calcifications, ventricular enlargement, sulcal widening and number of white matter lesions using a standardized scoring list. Baseline is defined as the time of the first neuro-imaging of a child. In a sub-study, we randomly included 19 children for a follow-up neuro-imaging examination to evaluate alterations in neuro-imaging over time.

A standard neurological physical examination was performed yearly at the outpatient clinic by the treating pediatrician and pediatric neurologist and included evaluation of cranial nerves, motor function, sensory function, reflexes, and coordination. Developmental delay and language impairment was documented. Ages and dates of developmental milestones were interpreted according to the 2013 IAS-USA drug resistance list [20]. HIV-1 sequence analysis was performed with the Viroseq HIV-1 genotyping kit (version 2; Abbott Laboratories, Abbott Park, IL) as reported previously [21]. Alternative switches of antiretroviral treatment regimes were based on newly acquired drug resistance mutations to cART components.

Statistical Analyses
Data entry and management were carried out using Microsoft Office Access, 2007 (Microsoft Corp, Redmond, WA, USA). Statistical analyses were carried out using StataIC 10, 2009 (StataCorp, College Station, TX, USA). The following variables were included in the analyses: nadir CD4 T cell percentage, CD4 T cell percentage at time of neuro-imaging, peak HIV VL and HIV VL at time of neuro-imaging as well as CDC classification at time of neuro-imaging.

Results
Baseline Sub Study
At baseline which is at time of first neuro-imaging, characteristics of 59 HIV-1 infected children were evaluated as depicted in Table 1. Most children were black (68%). CDC classification B or C was diagnosed in 15 and 25 children respectively. The majority of children were moderately immune compromised at initiation of ART, with a median CD4 T cell nadir of 20% (IQR: 9% to 34%). The median age at baseline was 4.9 years (IQR: 1.5 to 8.9 years). The majority of children (n = 31) received cART at baseline, and 14 children had undetectable HIV VL in blood at baseline neuro-imaging. The median age at initiation of ART was 2.6 years (IQR: 0.8 to 5.9 years). At baseline, 12 children had been exposed to a PI-based regimen (7 patients to nevirapine, 4 patients to lopinavir/ritonavir and 1 patient to indinavir/ritonavir), 9 children to a NNRTI-based regimen (6 children to efavirenz and 3 patients to nevirapine), 10 children had been exposed to PI and NNRTI based regimens (various combinations of nevirapine or lopinavir/ritonavir and/or nevirapine or efavirenz) (see Table 1). Drug resistance mutation analyses from first available plasma samples (n = 53) could not be performed in 4 patients because of undetectable HIV VL.
There were no NNRTI or PI resistance mutations identified. In 2 patients major gene mutations associated with NRTI resistance were detected. One patient had mutations that confer high-resistance to multi-NRTIs (41L, 44D, 210W and 215Y) and one patient had a mutation that confers resistance to NRTI (184V) at baseline. The first patient was pre-treated with zidovudine prior to start of cART. Both patients were optimally HIV suppressed with their initial cART that consisted of stavudine, didanosine and nelfinavir.

In 27 patients, the following minor gene mutations were detected: 69N (NRTI), 10I, 10V, 13V, 16E, 20I, 20R, 36I, 63P, 64LV, 69K, 77I, 82I, 93L (atazanavir, ritonavir, saquinavir, indinavir, lopinavir or nelfinavir) and 150A (etravirine) in first available plasma samples prior to start cART. These mutations are not associated with drug resistance [20].

CSF analysis was performed in 29 children at time of first neuro-imaging. The median CSF HIV VL was 2.7 log copies/ml (IQR: 2.4 to 3.8 log copies/ml). There were no children with a detectable HIV VL in CSF while they simultaneously had an undetectable HIV VL in blood.

Neurological examination was abnormal in 17 of 59 children (29%) at time of first neuro-imaging (table 2). These abnormalities included hyperreflexia (10%), abnormal muscle tone, either hypertonia or hypotonia in 14%, spasticity and developmental delay in respectively 7% and 12% of the children. Language impairment was found in 13 children (22%). CT scans were performed in 20 children (prior to 2001) while MRI imaging was performed in 39 children (table 2) as first neuro-imaging test. Ventricular enlargement and/or sulcal widening was detected in 17 of 59 children (29%). White matter lesions (that can only be detected accurately by MRI imaging) occurred in 15 of 39 children (38%). Calcifications in basal ganglia were detected in one child.

HIV-1 related parameters of children with either ventricular enlargement and sulcal widening or white matter lesions were compared to those of children without abnormalities on neurological examination and neuro-imaging. Results are summarized in table 3. The incidence of ventricular enlargement and sulcal widening was higher in children diagnosed with AIDS, but did not reach statistical significance ($p = 0.07$). Other HIV-1 related parameters were not significantly associated with ventricular enlargement and sulcal widening. White matter lesions were significantly associated with higher peak HIV VL in both blood and CSF ($p = 0.04$). Additionally, there was a trend towards the

### Table 1. Characteristics of the 59 children at baseline, at time of first neuro-imaging.

| Characteristics | Male N (%) | Female N (%) |
|-----------------|------------|--------------|
| Sex             | 28 (47)    | 31 (53)      |
| Ethnicity       | 40 (68)    | 6 (10)       |
| CDC-classification | 5 (9) | 13 (22) |
| CD4 T cell % nadir | 20 | (9–34) |
| Median (IQR)    | 29         | (19–36)      |
| ART experience, N (%) | 5 (8) | 2 (3) |
| No therapy      | 2 (3)      | 12 (20)      |
| Mono or dual therapy | 9 (15) | |
| PI-based cART   | 10 (17)    | 15 (25)      |
| NNRTI-based cART| 25 (42)    | 2 (3)        |
| Unknown         | 1 (2)      | 1 (2)        |
| Duration of ART experience | 1.6 | |
| Median (IQR)    | 0.3–5.8    | 2.6          |
| Age at start ART| 0.8–5.9    | 0.8–5.9      |
| HIV VL <400 copies/ml, N (%) | 14 (24) | |
| HIV VL at start ART | 5.3 | |
| Median log copies/ml (IQR) | 4.4–6.0 | |

ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; VL, viral load; 1Other included Asian ethnicity or unknown.

doi:10.1371/journal.pone.0064398.t001

### Table 2. Neurologic examination and Neuro-imaging results, N = 59.

| Findings* N (%) |
|-----------------|
| Neurologic examination |
| Hyperreflexia | 6 (10) |
| Spasticity     | 5 (8)  |
| Hypertonia/hypotonia | 8 (14) |
| Developmental delay | 7 (12) |
| Language impairment | 13 (22) |
| Total          | 17* (29) |

**Neuro-imaging (CT, n = 20, MRI, n = 39)**

| Findings* N (%) |
|-----------------|
| Calcification   | 1 (2)  |
| Widening        | 17 (2) |
| Only ventricular enlargement | 6 (10) |
| Only sulcal widening | 6 (10) |
| Ventricular enlargement and sulcal widening | 5 (8) |
| White matter lesions | 15 (38) |
| <3 lesions      | 7 (18) |
| >3 lesions      | 2 (5)  |
| Diffuse         | 6 (15) |

*Various findings could occur simultaneously.

doi:10.1371/journal.pone.0064398.t002
presence of white matter lesions in children with a CD4+ T cell % of less than 15% \((p = 0.06)\). There was no significant association between ventricular enlargement and/or sulcal widening and CD4+ T cell %.

Duration of cART prior to neuro-imaging did not impact the presence of ventricular enlargement and/or sulcal widening or white matter lesions. Even though the median duration of cART was shorter in children with ventricular enlargement and/or sulcal widening, these differences did not reach statistical significance.

We replicated this finding in a second analysis including all children with a MRI during the whole study period, including additional 14 MRIs. There was no association with white matter lesions and duration of cART prior to MRI imaging \((p = 0.93)\) (data not shown). We investigated if presence of neurological abnormalities at physical examination was associated with presence of abnormalities as detected by neuro-imaging. Ventricular enlargement and sulcal widening were associated with developmental delay and language impairment \((p = 0.017)\) (Table 4). The analyses of white matter lesions did not show significant associations with neurological abnormalities. One child had had a toxoplasmosis infection and one was diagnosed with a congenital CMV infection, both conditions are related to abnormalities at neuro-imaging.

### Longitudinal Sub Study

Of 59 HIV-1 infected children, only 19 children were randomly included in a follow up study. Five children had a second MRI. The other 14 children had a CT scan at baseline and a follow up MRI. The median duration between CT and MRI or between 2 MRIs was 5.9 years \([IQR 1.7–9.2]\) for ventricular and/or sulcal widening and 7.5 years \([IQR 5.4–8.6]\) for white matter lesions respectively. The median duration of cART prior to second neuro-imaging was 6.3 years \([IQR 4.4–7.8]\) and 6.1 years \([IQR 5.7–10.2]\) respectively. Nine children had ventricular enlargement and/or sulcal widening at baseline and in 4 children this finding had resolved on the follow up MRI. White matter lesions detected in 4 of 5 children that underwent a second MRI remained stable over time. Ventricular enlargement and/or sulcal widening as measured with CT and MRI improved in 5 children. Statistical analyses to identify associations between ventricular enlargement

| Table 3. Neuro-imaging abnormalities and associations with HIV-1-related parameters. |
|---------------------------------|------------------|------------------|------------------|------------------|
| Variable                        | ventricular enlargement and/or sulcal widening | white matter lesions |
|                                 | (CT and MRI, N = 59) | (MRI, N = 39)    |                   |                   |
| Age                             | N = 17            | N = 15           |                   |                   |
| Median years (IQR)              | 3.3 (1.3–5.2)     | 5.3 (1.8–9.5)    | 0.136             | 0.419             |
| AIDS N (%)                      | 24 (42%)          | 9 (56%)          | 0.066             | 0.208             |
| No                              | 35 (20%)          | 30 (33%)         |                   |                   |
| CD4+ T cell % at neuro-imaging N (%)<15% | 10 (40%) | 7 (71%) | 0.308 | 0.062 |
| >15%                            | 49 (27%)          | 32 (31%)         |                   |                   |
| CD4+ T cell % nadir N (%)<15%   | 20 (40%)          | 12 (58%)         | 0.146             | 0.090             |
| >15%                            | 39 (23%)          | 27 (30%)         |                   |                   |
| HIV VL peak                     | 5.5 (4.4–6.2)     | 5.5 (4.7–6.0)    | 0.284             | 0.035             |
| HIV VL in CSF N (%) <500        | 16 (19%)          | 16 (20%)         | 0.233             | 0.035             |
| >500                            |                   |                   |                   |                   |
| Date of birth N (%)             | 19 (21%)          | 8 (50%)          | 0.279             | 0.360             |
| 1996–2003                       | 34 (29%)          | 25 (40%)         | 0.571             | 0.534             |
| >2003                           | 6 (50%)           | 6 (17%)          | 0.225             | 0.237             |
| Duration of ART                 | 0.9 (0.4–2.9)     | 3.5 (1.1–6.5)    | 0.828             | 0.702             |
| Median years (IQR)              |                   |                   |                   |                   |
| Duration PI-regimen             | 0.08 (0.02–2.8)   | 3.7 (1.3–4.9)    | 0.481             | 0.786             |
| Median years (IQR)              |                   |                   |                   |                   |
| Duration NNRTI-regimen          | 1.1 (0.4–2.9)     | 1.1 (0.1–1.3)    | 0.586             | 0.877             |
| Median years (IQR)              |                   |                   |                   |                   |

Continuous data are described as median and interquartile ranges. Statistical comparisons were made by using Fisher’s exact test for categorical data and the Kruskal-Wallis test for continuous data. AIDS, acquired immunodeficiency syndrome; HIV VL, HIV viral load; CSF, cerebrospinal fluid; cART, combination antiretroviral therapy; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; CT, computed tomography; MRI, magnetic resonance imaging.

doi:10.1371/journal.pone.0064398.t003
and/or sulcal widening ventricular widening or white matter lesions with CD4+ T cell %, HIV-1 VL and cART did not differ from baseline analyses (data not shown).

Discussion

Our study describes the neurologic abnormalities detected in a retrospective cohort of perinatally HIV-1 infected children enrolled over a period of 18 years, from 1992 to 2010. We found a significant number of children demonstrating abnormalities as detected by neurologic (29%) and neuro-imaging examinations (44%). The majority of children with neuro-imaging abnormalities presented with ventricular enlargement and/or sulcal widening (29%) or white matter lesions (39%). Neurological abnormalities such as language impairment and developmental delay were frequently detected in HIV-1 infected children and were significantly associated with ventricular enlargement and/or sulcal widening.

Ventricular enlargement and/or sulcal widening were not associated with HIV-1 related markers or duration of cART. White matter lesions were associated with HIV-1 related parameters including blood and CSF HIV VL at time of baseline neuro-imaging. Duration of cART usage was not associated with presence of white matter lesions. Furthermore, in cART treated children, white matter lesions did not improve in time. Congenital CMV and toxoplasmosis infection that are associated with HIV-1 infections were detected in 2 children. These children did not have white matter lesions. Their neurologic examinations also remained stable over time.

Previous studies reported substantial survival benefit of different cART regimes after diagnosis of HIV-1 encephalopathy and improvement of impact neuro-imaging with CNS penetrating ART[1,13,22–24]. Duration of cART usage and specific PI- or NNRTI regimes did not impact the presence of ventricular enlargement and/or sulcal widening or white matter lesions in our study. The cART regimes used in our study are reported to have a moderate to good penetration of the CNS [25]. Associations with HIV-1 related parameters and lack of associations with cART treatment imply that the initial phase of active viral replication in CNS prior to cART treatment is crucial in the development of neuro-imaging abnormalities. Nevertheless, the small sub-study showed that ventricular enlargement and/or sulcal widening decreased in approximately half of cART children. These results must be interpreted cautiously but could indicate that long term cART can indeed reduce certain neuro-imaging abnormalities, while other specific neuro imaging abnormalities such as white matter lesions may remain irreversibly present over time. Rapid initiation of treatment may be helpful to prevent progression of white matter lesions and potentially reverse widening of the sulci to improve long term neurologic outcome [16,24–26]. Lack of significant associations of neuro-imaging abnormalities with cART may be due to the small sample size in our study. Nevertheless our results show that neuro-imaging abnormalities and neurologic abnormalities at examination are still frequently detected in the era of cART in the pediatric population and suggest that rapid initiation of treatment is critical to prevent progression of neurological abnormalities. Further prospective research in larger cohorts is required to assess the long-term effect of high CNS-penetrating regimens to improve the neurological outcome in HIV-1 infected children.

Acknowledgments

We thank S Jurriaans and NKT Back for the HIV-1 gene mutation analyses.

Table 4. Associations between ventricular enlargement and/or sulcal widening and white matter lesions and neurologic examination HIV-1 infected children.

| Variable                        | N = 59 | Present (N = 17) | p-value | N = 59 | Present (N = 15) | p-value |
|--------------------------------|--------|-----------------|---------|--------|-----------------|---------|
| Hyperreflexia N (%)            |        |                 |         |        |                 |         |
| Yes                            | 6      | 4 (67%)         | 0.052   | 1      | 1 (100%)        | 0.385   |
| No                             | 53     | 13 (25%)        | 38      | 14     | (37%)           |         |
| Spasticity N (%)               |        |                 |         |        |                 |         |
| Yes                            | 5      | 3 (60%)         | 0.138   | 1      | 1 (100%)        | 0.385   |
| No                             | 54     | 14 (26%)        | 38      | 14     | (37%)           |         |
| Hypertonia/hypotonia N (%)     |        |                 |         |        |                 |         |
| Yes                            | 8      | 4 (50%)         | 0.157   | 2      | 1 (50%)         | 0.628   |
| No                             | 51     | 13 (25%)        | 37      | 14     | (38%)           |         |
| Developmental delay N (%)      |        |                 |         |        |                 |         |
| Yes                            | 7      | 5 (71%)         | 0.017   | 4      | 2 (50%)         | 0.502   |
| No                             | 52     | 12 (23%)        | 35      | 13     | (37%)           |         |
| Language impairment N (%)      |        |                 |         |        |                 |         |
| Yes                            | 12     | 7 (58%)         | 0.017   | 6      | 1 (17%)         | 0.237   |
| No                             | 47     | 10 (21%)        | 33      | 14     | (42%)           |         |

Statistical comparisons were made by using Fisher’s exact test for categorical data.

CT, computed tomography; MRI, magnetic resonance imaging.

doi:10.1371/journal.pone.0064398.t004
Neurologic Outcome in HIV Children

Author Contributions
Performed the experiments: HS BP TK DP. Analyzed the data: LA OF MB CM LR DP. Wrote the paper: LA MB HS TK CM DP.

References

1. Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO). AIDS epidemic update (2010) Available: http://www.unaids.org/globalreport/global_report.htm. Accessed 2010 Dec.

2. Epstein LG, Sharer LR, Oleske JM, Connor EM, Goudsmit J, et al. (1986) Neurologic manifestations of human immunodeficiency virus infection in children. Pediatrics 78: 678–687.

3. Cooper ER, Hanson C, Diaz C, Mendez H, Abbood R, et al. (1998) Encephalopathy and progression of human immunodeficiency virus disease in a cohort of children with perinatally acquired human immunodeficiency virus infection. J Pediatr 132: 801–812.

4. Epstein LG, Sharer LR, Joshi VV, Fojas MM, Koenigsberger MR, et al. (1985) Neurologic, neurocognitive, and brain growth outcomes in children with acquired immune deficiency syndrome. Ann Neurol 17: 488–496.

5. Schmitt B, Seeger J, Kreuz W, Enzel S, Jacobi G (1991) Central nervous system involvement of children with HIV infection. Dev Med Child Neurol 33: 535–540.

6. Angelini L, Zibordi F, Triulzi F, Cinque P, Giudici B, et al. (2000) Age-dependent neurologic manifestations of HIV infection in childhood. Neuro Sci 21: 135–142.

7. Chiriboga CA, Flishman S, Champion S, Gaye-Robinson L, Abrams EJ (2005) Incidence and prevalence of HIV encephalopathy in children with HIV infection receiving highly active anti-retroviral therapy (HAART). J Pediatr 146: 402–407.

8. Wilmshurst JM, Burgess J, Hartley P, Eley B (2006) Specific neurologic complications of human immunodeficiency virus type 1 (HIV-1) infection in children. J Child Neuro. 21: 785–794.

9. Kieck JR, Andronikou S (.2004) Usefulness of neuro-imaging for the diagnosis of HIV encephalopathy in children. S Afr Med J 94: 628–630.

10. Vinutaratama P, Oraratanachai K (2002) Clinics in diagnostic imaging (75). HIV encephalopathy and cerebral aneurysmal arteriopathy. Singapore Med J 43: 377–380.

11. Chamberlain MC, Nichols SL, Chase CH (1991) Pediatric AIDS: comparative cranial MRI and CT scans. Pediatr Neurol 7: 357–362.

12. Johann-Liang R, Lin K, Cervia J, Stavola J, Noel G (1998) Neuroimaging findings in children perinatally infected with the human immunodeficiency virus. Pediatr Infect Dis J 17: 753–754.

13. Patel K, Ming X, William PL, Robertson KR, Oleske JM, et al. (2009) Impact of HAART and CNS-penetrating antiretroviral regimens on HIV encephalopathy among perinatally infected children and adolescents. AIDS 23: 1093–10911.

14. Shanbhag MC, Rustein RM, Zouznis T, Zhao H, Chao D, et al. (2005) Neurocognitive functioning in pediatric human immunodeficiency virus infection. Arch Pediatr Adolesc Med 159: 651–656.

15. Raskino C, Pearson DA, Baker CJ, Lifschitz MH, O’Donnell K, et al. (1999) Neurologic, neurocognitive, and brain growth outcomes in human immunodeficiency virus-infected children receiving different nucleoside antiretroviral regimens. Pediatrics 104: 1–10.

16. Laughton B, Cornell M, Grove D, Kidd M, Springer PE, et al. (2012) Early antiretroviral therapy improves neurodevelopmental outcomes in infants. AIDS 26: 1615–1619.

17. Eden A, Fuchs D, Hagberg L, Nilsson S, Spudich S et al. (2010) HIV-1 viral escape in cerebrospinal fluid of subjects on suppressive antiretroviral therapy. J Infect Dis 202: 1819–1825.

18. Scherphiev HJ, Bekker V, van Leth F, Juruinans S, Lange JMA, et al. (2006) Long-term effectiveness with combination antiretroviral therapy that contains nevirapine for up to 7 years in a pediatric cohort. Pediatrics 117: 529–536.

19. Scherphiev HJ, Bekker V, Pajet D, Juruinans S, Lange JMA, et al. (2007) Once-daily highly active antiretroviral therapy for HIV-infected children: safety and efficacy of an efavirenz-containing regimen. Pediatrics 119: 707–715.

20. Johnson VA, Calvez V, Gnauthard HF, Paredes R, Pillay D, et al. (2013) Update of the drug resistance mutations in HIV-1: March 2013. Top Amir Med 21: 4–12.

21. Thao VP, Le T, Torok EM, Ven NTB, Chau TTH, et al. (2012) HIV-1 drug resistance in antiretroviral-naive individuals with HIV-1-associated tuberculous meningitis initiating antiretroviral therapy in Vietnam. Antiviral Therapy; 26: 1685–1690.

22. Sa´nchez-Ramo´n S, Resino S, Bellón Cano JM, Ramos JT, Garibdo D, et al. (2003) Neuroprotective effects of early antiretrovirals in vertical HIV infection. Pediatr Neurol 29: 218–221.

23. Wood SM, Shah SS, Steenhoof AP, Rustein RM (2009) The impact of AIDS diagnoses on long-term neurocognitive and psychiatric outcomes of surviving adolescents with perinatally acquired HIV. AIDS 23: 1859–1865.

24. Viodari A, Cooton MF, Gibb DM, Babiker AG, Stryn J et al. (2008) Early antiretroviral therapy and mortality among HIV-infected infants. N Eng J Med 359: 2233–2244.

25. Letendre SL, Ellis RJ, Ankes BM, McCutchan JA (2010) Neurologic complications of HIV disease and their treatment. Trop HIV Med 18: 45–55.

26. Smurzyński M, Wu K, Letendre S, Robertson K, Bosch RJ, et al. (2011) Effects of central nervous system antiretroviral penetration on cognitive functioning in the ALLRT cohort. AIDS 25: 357–365.