A Diffusion Tensor Imaging and Neurocognitive Study of ART-naïve and ART-treated Children in Cape Town

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University of Cape Town

The Road goes ever on and on
Down from the door where it began.
Now far ahead the Road has gone,
And I must follow, if I can,
Pursuing it with eager feet,
Until it joins some larger way
Where many paths and errands meet.
And whither then? I cannot say.

Lord of the Rings JRR Tolkien

CONTENTS

DECLARATION

I, Jacqueline Hoare, do hereby declare that this thesis is based on five journal manuscripts: four of which have been published or accepted for publication (chapter 2-5), and one under review in international journals (chapters 6). These manuscripts have been formatted uniformly for the purposes of this thesis, with regards to referencing style and use of terms. The content of each manuscript remains unchanged from that which has been either published or submitted for publication, but the introduction and conclusion of each has been edited in such a way as to underscore the coherence of the entire thesis i.e. how each chapter links to the next and the others. The manuscripts included are listed below, with a description of my contribution to each.

Chapter 2.
Systematic review of neuroimaging studies in vertically transmitted HIV positive children and adolescents. Hoare J, Ransford GL, Phillips N, Amos T, Donald K, Stein DJ. Metabolic Brain Disease. 2014. June; 29(3): 221-229

In this review, I developed a search methodology with input from my supervisors, and then conducted the database and journal search. G Ransford, T Amos and myself reviewed all papers for potential inclusion and established our level of agreement. We extracted data into a spreadsheet. Nicole Phillips checked data entry. I then analysed and summarised all the data myself, and wrote the full first draft of the manuscript. My co-authors D Stein and K Donald, reviewed the draft, made conceptual and intellectual contributions. I made all revisions prior to publication myself.

Chapter 3.
A diffusion tensor imaging and neurocognitive study of HIV-positive children who are HAART-naïve "slow progressors". Hoare J, Fouche JP, Spottiswoode B, Donald K, Philipps N, Bezuidenhout H, Mulligan C, Webster V, Oduro C, Schrieff L, Paul R, Zar H, Thomas K, Stein D. J Neurovirol. 2012 Jun; 18(3): 205-12.

This manuscript was the first based on the empiric data obtained from this research project. I was the sole principle investigator- I initiated the work, obtained all funding, recruited study staff, examined a proportion of participants myself, oversaw the development of the database, did imaging analysis with the help of JP Fouche and Bruce Spottiswoode, and then wrote the full first draft of the manuscript. A project of this nature was contributed to by others, including my supervisors Prof Dan Stein and other experts Prof Heather Zar and Prof Robert Paul. Dr Kevin Thomas and Leigh Schrieff advised on the selection of tests for the neurocognitive battery and checked quality of neurocognitive battery administration. Nicole Phillips over saw patient recruitment and other administrative duties for the project. Dr Kirsten Donald, Dr H Bezuidenhout and Dr Christine Mulligan were involved in examining and assessing patients physically and neurologically. Charity Oduro and Victoria Webster were the study neuropsychologists who helped by testing participants and overseeing neuropsychological test scoring and data entry. All authors read and approved the manuscript. I managed all revisions.

Chapter 4.

Clinical associations of white matter damage in cART treated HIV positive children in South Africa. Hoare J, Fouche JP, Phillips N, Joska JA, Donald K, Thomas Kevin, Stein Dan J. J Neurovirol. 2015 Apr; 21(2): 120-8. doi: 10.1007/s13365-014-0311-1.

This manuscript followed the first (chapter 3), described above. The idea for examining clinical associations of white matter damage in cART treated children was based on my initial hypothesis that there would be a number of factors which contributed to neurocognitive impairment/white matter damage in the cental nervous system in children vertically infected with HIV. The literature for HIV and neuroimaging studies in adults treated with ART has become more substantial, but for vertically infected children with HIV it is still small, particularly studies using novel imaging techiques such as DTI. JP Fouche again helped with imaging statistical analysis. Dr Kirsten Donald, a pediatric neurologist, was involved in examining and assessing patients physically and neurologically. As this data was based on the main project, the oversight of the work again was all mine. I also cleaned and analysed the data with help from N Phillips, and wrote the full first draft of the manuscript.

My co-authors D Stein, K Thomas and J Joska, reviewed the draft, made conceptual and intellectual contributions. I made all revisions prior to publication myself.

Chapter 5.

White matter microstructural changes in ART naïve and ART treated children and adolescents infected with HIV in South Africa. Hoare J, Fouche JP, Phillips N, Joska JA, Paul R, Donald K, Thomas Kevin, Stein Dan J. Article has been accepted for publication in *AIDS (*AIDS-D-15-00165R1).

This manuscript was based on the large parent project, on which I was the principle investigator. This manuscript was the final imaging analysis from the large parent project, and is the largest neuroimaging study , using novel neurimaging techniques, of HIV infected children published to date. I have received support for the neurocognitive aspects from Kevin Thomas and Nicole Phillips, who scored and entered the neurocogntive data. Kirsten Donlad conducted neurological examinations on all of the paticipants. I collated all this data, together with the clinical data, and did the imaging statistical analysis with the help of JP Fouche. I wrote a full draft of this manuscript, and then sent on to my co-authors John Joska, Kirsten Donald and Dan Stein for comments and input. My supervisor, Dan Stein, continued to advise and support . Rob Paul was involved as an international neuroAIDS expert. All authors read and approved the manuscript. I managed all revisions of this manuscript.

Chapter 6.

Applying the HIV Associated Neurocognitive Disorder diagnostic criteria to HIV infected youth

Hoare J, Phillips N, Joska JA, Paul R, Donald K, Stein Dan J, Thomas Kevin. Under review in *Neurology.*

This manuscript was based on the large parent project, on which I was the principle investigator. Participants were tested under the supervision of Kevin Thomas and Nicole Phillips- the latter again scored and entered the neuropsychological data. Kirsten Donlad conducted neurological examinations on all of the paticipants. I collated all this data,

together with the clinical data, and did the statistical analysis with the help of Kevin Thomas. I wrote a full draft of this manuscript, and then sent on to my co-authors for comments and input. My supervisor, Dan Stein, continued to advise. Rob Paul has remained involved as an international neuroAIDS expert. All authors read and approved the manuscript. I have managed all revisions of this manuscript to date

I confirm that no part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in this or any other university. I hereby grant the University of Cape Town free license to reproduce this thesis in whole or part for the purposes of research or teaching.

This thesis is presented for examination in fulfilment of the requirements for the degree of Doctor of Philosophy in Psychiatry.

Signed,

Signed by candidate

Signature removed

Jacqueline Hoare August 2015

ABSTRACT

Title: A Diffusion Tensor Imaging and Neurocognitive Study of ART-naïve and ART-treated Children in Cape Town

Background

Human Immunodeficiency Virus (HIV) infection in children is becoming a chronic disease due to the widespread introduction of antiretroviral treatment (ART). Due to the longer duration that children are living with HIV, neurocognitive impairment has become more prevalent and can significantly impact on quality of life, but remains understudied in older HIV infected children residing in sub-Saharan Africa (SSA). This dissertation addressed 5 key questions in the field: 1) to investigate the effect of HIV on white matter integrity and neurocognitive function in children and adolescents vertically infected with HIV, compared to a HIV negative healthy control group 2) the neurocognitve performance and CNS white matter integrity in ART naïve HIV infected children and adolescents with slow progression 3) the socio-demographic, clinical and laboratory correlates of CNS white matter damage in HIV infected children 4) compare neurocognition and white matter integrity in ARV naïve slow progressors, children stable on ART and children with a clinical diagnosis of HIV encephalopathy 5) and use a comprehensive neurocognitive battery and an assessment of functional competence to determine whether a spectrum of neurocognitive disorders can be detected in HIV-infected children and adolescents.

Methods

The published literature was reviewed to summarise what is already known in the field of neuroimaging in vertically acquired HIV, and to highlight possible future directions in using neuroimaging and neurocognitive testing to understand the spectrum of neurocognitive disorders in HIV infected children. A cross-sectional clinical cohort study was initiated in Cape Town, in which 120 participants, including a HIV negative healthy control group for comparison, completed clinical and neurocognitive assessments. HIV infected children were either stable on antiretroviral treatment (ART) for a minimum of 6 months or ART naïve. Neuroimaging was completed sucessfully on 105 children in the cohort study. We compared 75 children vertically infected with HIV aged 6 to 16 years, including both children on antiretroviral therapy (ART) and ART-naïve, with 30 matched controls using diffusion tensor imaging (DTI) measures. We then used the detailed neurocognitive battery, an assessment of

adaptive functioning and the AAN system for diagnosing ND to establish whether this system could detect a spectrum of ND in HIV infected older children and adolescents.

Results

The reviewed literature suggests the relationship between cognitive impairment and central nervous system damage in HIV infected children, as seen by neuroimaging remains incompletely understood. Larger cohort studies describing in detail the cognitive, behavioural and neuroimaging findings in older children with vertically infected HIV are needed, which include information on socio-environmental factors. DTI , as a measure of white matter integrity, can detect subtle central nervous system (CNS) changes secondary to HIV, and is therefore ideally suited to address these questions. In this study, ART naïve "slow progressors" performed poorly compared to controls on Performance and Verbal tasks of the Wechsler Intelligence Scale for Children and on tests of visuospatial processing, visual memory and executive function. "Slow progressors" had white matter damage in the corpus callosum and the superior longitudinal fasciculus, compared to controls. Significant associations were found between failing first line ART regimen, socio-demographic factors, nutritional-hematological status, HIV-relevant clinical variables, cognitive functioning and white matter integrity in children stable on ART. When comparing HIV uninfected (30) children to HIV infected children (75) this DTI study found damaged neuronal microstructure in the HIV infected children. Neuronal damage, of the right external capsule, was associated with poor fronto-striatal cognition scores. Children with a clinical diagnosis of encephalopathy (HIVE) had greater white matter damage when compared ART treated children without encephalopathy. DTI also found significant myelin loss in ART naïve children when compared with ART treated children. Using the AAN criteria for HAND we found that 45.35% of the HIV infected children had a ND. HIV infected children were also more likely to have impaired competence in various domains of functioning.

Conclusion

Despite the use of ART and improved virological control with immune reconstitution, there are still a significant percentage of children in this study who were found to have ND.
Our findings also suggest that children on ART remain at risk for developing CNS disease, and that this risk extends to physically well ARV naïve slow progressors. ART naïve slow progressors, who receive limited attention from heath care services, as they are thought to be

'well', were found to have neurocognitive impairment and white matter microstructural damage. The current findings also underline the possible association of first line treatment failure with white matter brain dysfunction in children on ART.

The AAN HAND criteria designed for adults was able to identify children and adolescents with important functional cognitive impairments who don't fit criteria for HIVE and would therefore not have been identified otherwise. This has major clinical implications in terms of the importance of managing HIV infected children. Further work to identify cases and delineate mechanisms of disease and treatment response is needed. This might take the form of larger prospective studies, incorporating control groups. Such studies could better elucidate disease mechanisms with a view to developing screening tools and targets for therapeutic interventions.

Acknowledgements

The work reported in this thesis was funded by the South African National Research Foundation Thuthuka Programme, the Carnegie Corporation (Institute Infectious Diseases and Molecular Medicine (IIDMM)), the Biological Psychiatry Special Interest Group of the South African Society of Psychiatrists, and the Medical Research Council self initiated research grant.

I would like to acknowledge and thank the following people: the patients and their families for agreeing to participate in this study; Dr Kirsten Donald, Dr H Bezuidenhout and Dr Christine Mulligan for their invaluable assistance with patient assessment; Mss Victoria Webster and Charuty Oduro for conducting the neuropsychological assessments, together with Bulelwa Mtukushe. I thank Bulelwa Mtukushe for your dedicated fieldwork in recruitment; JP Fouche for your assisstance with neuroimaging analysis, and Nicole Philipps for being a superb project manager.

A number of people generously gave of their time and expertise as consultants: Professor Rob Paul, Dr Kevin Thomas, Dr Kirsten Donald and Dr Bruce Spottiswoode.

I thank my supervisor Professors Dan Stein, who must be one of the finest role models around.

I thank the staff of the sites where this work was conducted, and the facility managers of Nolungile site C in Khayalitsha, Woodstock Community Health Centres, Red Cross Children's and Groote Schuur Hospitals

I would specially like to thank my colleagues at Groote Schuur Hospital for their support and especially Dr John Joska. To my husband, Douglas and daughter, Arwen thank you for all your encouagement and faith in me.

Jacqueline Hoare

Cape Town
August 2015

Chapter 1.

Introduction;

Conceptualising a spectrum of HIV-associated Neurocognitive Disorders in vertically infected HIV positive children in South Africa

CONTEXT

Human Immunodeficiency Virus (HIV) infection has had a huge impact on global child health. There are an estimated 3.2 million HIV infected children of whom over 90% are in sub-Saharan Africa (SSA) (UNAIDS 2014). The majority of children are infected by mother-to-child transmission of the virus (Wachsler-Felder and Golden 2002), and infection at this young age can have a profound effect on the child's physical and mental development. Without treatment over 50% of children die before their second birthday (Newell et al. 2004). The introduction of antiretroviral therapy (ART) has altered the course of HIV infections reducing mortality and morbidity (Sutcliffe et al. 2008), resulting in increasing numbers of HIV infected children surviving into adolescence, and shifting the research/clinical focus to long-term health outcomes. Yet little is known about the effects of HIV and antiretroviral treatment (ART) on the developing brain, and the neurodevelopmental and behavioral outcomes of adolescents vertically infected with HIV. The WHO guidelines changed in 2013 to advise that all children less than 5 years old receive ART (UNAIDS 2014). However, new guidelines have yet to be fully scaled up, especially in SSA countries, and therefore many children who are eligible for ART are not receiving treatment. In addition, there are some asymptomatic children (slow progressors) aged 5 years and older that have not yet started ART and who have been clinically and immunologically stable (2006), and therefore receive little attention form health care services.

NEUROBEHAVIOURAL COMPLICATIONS OF HIV

Studies in children infected with HIV have shown a pattern of cognitive deficits in attention, executive function, working memory and processing speed domains (Boivin et al. 1995; Nozyce et al. 2006), similar to adults (Castellon et al. 1998). A spectrum of neurocognitive disorders has been described in the adult HIV literature, with well-defined criteria for diagnosis (Heaton et al. 2010), however no such criteria have been proposed in the paediatric neuroAIDS literature. Behavioural and psychiatric problems in HIV infected children have been described including lethargy, social withdrawal, anxiety, depression, hyperactivity and impulsivity (Govender et al. 2011; Nozyce et al. 2006). The severity of neurological outcomes is mediated by a complex interplay of factors (Valcour et al. 2011), including disease severity and viral load (Lobato et al. 1995), socioenvironmental factors, and ART use. ART seems to improve or even reverse some of the neurological pathology seen in HIV infected children, but as most of these studies have been done in developed countries, no current data is available for the children living in Sub Saharan Africa (Van Rie et al. 2008).

Some studies have remarked that even if a child is virologically suppressed on ART, neurological improvement is not guaranteed as most anti-retroviral agents do not cross the blood-brain barrier readily and are postulated to allow a consequent viral reservoir in the brain (Letendre et al. 2004; Staprans et al. 1999). Deficits in cognition and behaviour have been documented early in infants (Chase et al. 2000), and even in clinically 'asymptomatic' HIV infected children (Boivin et al. 1995; Nozyce et al. 2006). The increasing number of HIV-infected children surviving into adolescence has resulted in a rising concern about these chronic neurobehavioral deficits (Laughton et al. 2013) and their impact on school and daily functioning (De Baets et al. 2007). Traditionally, adolescence is viewed as the age when abstract thought develops, together with improvements in planning, problem solving and cognitive flexibility (Kuhn 2006). These tasks would be key to the adolescent developing independence and decision-making at this crucial time of development.

There are very few published studies describing the clinical profile of older children vertically infected with HIV and even fewer studies which have ART naïve children. Children who are vertically infected with HIV, but who remain clinically and immunologically stable for a long period – are called "slow progressors". Few studies in the field of HIV neuropsychology have focused on ART-naïve slow progressors specifically. An article reviewing all studies in SSA evaluating development/cognition/behaviour in HIV infected children as their primary outcome highlighted the lack of data in this field as well as the fact that all except one had focused on children less than 6 years of age (Abubakar et al. 2008). Figures were generally small and not all studies took biomedical markers into account in their analysis.

Describing a spectrum of neurocognitve disorders in pediatric neuroAIDS would be an important first step to increasing awareness of some of the difficulties that our children are experiencing in the class-room and at home. Understanding correlates of central nervous system (CNS) white matter damage could inform management guidelines, development of screeening tools and appropriate educational interventions.

DIFFUSION TENSOR IMAGING

Diffusion Tensor Imaging (DTI) is a non-invasive magnetic resonance imaging (MRI) technique that provides quantitative information on the tissue microstructure. It is a powerful technique for investigating subtle changes not seen on conventional imaging (Filippi et al. 2001), and white matter abnormalities seen in adult HIV (Pomara et al. 2001; Ragin et al. 2004). DTI measures the diffusion of water molecules in the brain (Jones et al. 2013). Water naturally flows along the path of least resistance, which is parallel to axon long axes in healthy brain white matter due to myelin barriers, known as anisotropic diffusion. Diffusion therefore depends on cortical architecture as well as fibre density, diameter, orientation, myelination and disease (Beaulieu 2002). DTI-derived metrics include fractional anisotropy (FA), mean diffusivity (MD) , radial diffusivity (RD) and axial diffusivity (AD). FA represents the degree to which water diffusion displays a main direction (Jones et al. 2013) and is measured on a scale of 0-1 where zero represents free diffusion in all directions, and one directed diffusion. Due to myelin sheaths FA values are high in white matter and used as an accepted measure of the microstructure integrity (Neil 2008). MD measures the diffusion magnitude, RD measures water diffusion perpendicular to axons and is therefore an indicator of myelin loss, and AD measures movement parallel to axons, and thus serves as a marker of axonal damage (Song et al. 2002). Healthy white matter with organised linear tract fibres demonstrates higher FA and low MD compared to grey matter and diseased tissue that disrupts the local architecture.

DTI has been used in HIV infected adults with some success showing reduced FA in frontostriatal pathways specifically in the corpus callosum, superior longitudinal fasciculus, cingulum and sagittal stratum, internal capsule (Hoare et al. 2011; Pomara et al. 2001) some associated with cognitive impairment, motor deficits and HIV-dementia stage in adults with HIV (Hoare et al. 2011). However, it is a relatively novel approach in HIV-infected children, despite being particularly suitable as it is non-invasive, quick, with no known harmful effects, and sensitive to early neurological damage in HIV adults. The technique uses standardised methods of interpretation (Filippi et al. 2001).

Availability of nutritional measures may be particularly important for our analyses, given a prominent role of nutrition in brain development (BentonILSI Europe a.i.s.b.l. 2008). Micronutrient deficiency has been sown to affect HIV disease progression and growth in children (Friis 2006). Nutritional covariates may be essential to understanding the

neuroimaging data. Other factors may include socio-demographic status, HIV-relevant clinical variables and ART treatment regimen.

RESEARCH AIMS AND OBJECTIVES

Given that the prevalence of HIV is extremely high in South Africa children; that these children are at risk of developing neurocognitive disorders; and that there are questions about a broader spectrum of neurocognitive disorders other than HIV encephalopathy alone - it is important to begin to understand these issues in our context.

The overall aim of this thesis is to characterise a spectrum of neurocognitive disorders in pediatric neuroAIDS using a detailed neurocognitive battery and Diffusion Tensor Imaging. This aim will be addressed through the following **objectives**:

1) To describe the effect of HIV on white matter integrity and neurocognitive function in children and adolescents vertically infected with HIV, compared to a HIV negative healthy control group

2) To describe the neurocognitve performance and CNS white matter integrity in ART naïve HIV infected children and adolescents with slow progression.

3) To study the socio-demographic, clinical and laboratory correlates of CNS white matter damage in a group of HIV infected children, using DTI.

4) To determine whether performance on neurocognitive testing correlates with damage to CNS white matter.

5) To compare neurocognition and white matter integrity in ARV naïve slow progressors, children stable on ART and children with a clinical diagnosis of HIV encephalopathy.

6) To determine whether a comprehensive neurocognitive battery, an assessment of functional competence, and the American Academy of Neurology (AAN) system for diagnosing neurocognitive disorder in adults could detect a spectrum of neurocognitive disorder in HIV-infected older children and adolescents.

Prior to this study, there have been no published studies of cognitve impairment in HIV infected children utilising a detailed neuropsychological test battery and DTI. There is a paucity of work in Africa in general, with differences in methodologies making comparison across regions difficult. Regional differences include the predominance of clade C HIV in South Africa, which has been suggested to account for differences in neurocognition. In

addition, there is a small literature addressing the issue of the effects of ART on neurocognition and white matter damage.

OVERALL DESCRIPTION OF THE PROJECT

This project arose out of the Division of Liaison Psychiatry, where we started a clinic in ward G4 at Groote Schuur Hopital to attend to the mental health needs of chronically ill adolescants. A need to understand neurocognitive problems in HIV infected children more fully, arose out of our work with our Pediatric colleagues in G4. What resulted was the research protocol that forms the basis of this thesis. I then obtained funding from three local sources- the National Research Foundation Thuthuka programme, the Biological Psyciatry Special Interest Group of the South African Society of Psychiatrists, and the Carnegie Corporation. We began working in two centres, Red Cross Hospital and Groote Schuur Hospitals infectious disease clinics, and asked participants to come to Groote Schuur Hospital for testing. Neuroimaging was conducted at the Cape Universities Brain Imaging Centre (CUBIC). In 2007 the CUBIC was set up as a joint initiative between Siemens, Stellenbosch University, University of Cape Town, and the Medical Research Council. The centre contains a Siemens 3T Magnetom Allegra MRI scanner (Siemens, Erlangen, Germany), the first dedicated MRI of its kind in Africa (figure 1), and capable of performing diffusion tensor imaging. The environment at CUBIC was adjusted to become more amenable for testing children. These adjustments included creating a 'mock scanner' (Figure 2) for the children to become accustomed to lying still in smaller spaces, creating demonstration videos in Xhosa and painting the scanning room with animal pictures. DTI imaging analysis was conducted at CUBIC (figure 3).

A graphic representation of the project and how aspects of the work related to distinct manuscripts is presented in Figure 1. These manuscripts are what comprise this thesis.

Other work and projects have arisen subsequent to this parent project commencing. Pilot data from the overall project was also instrumental in our group successfully competing for an RO1 grant of the National Institutes of Health, with Prof Heather Zar as Principle Investigator.

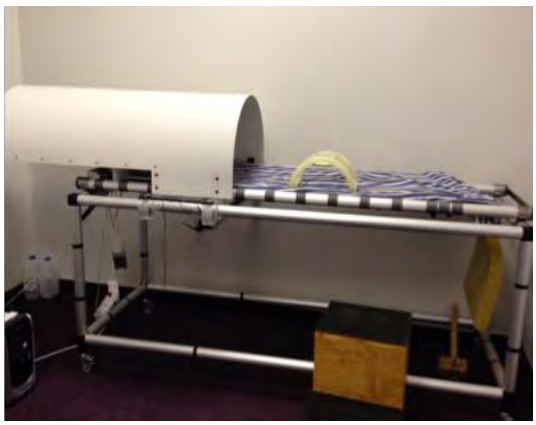


Figure1: Mock MRI scanner

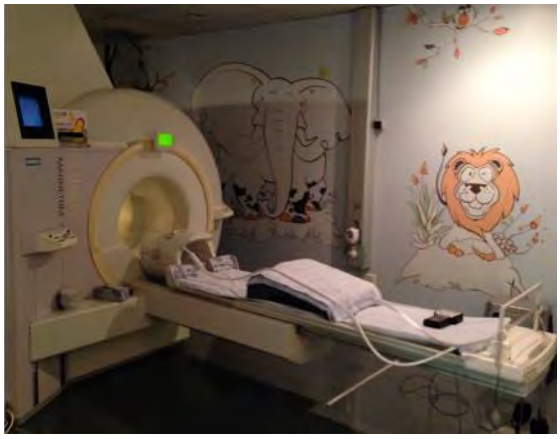
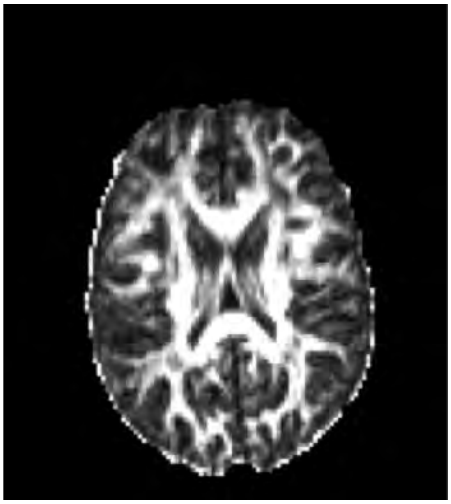
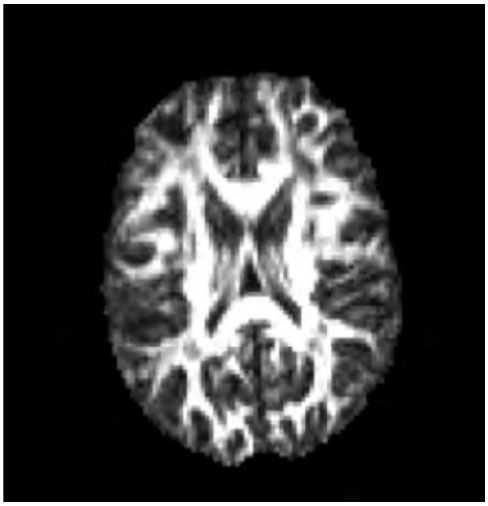


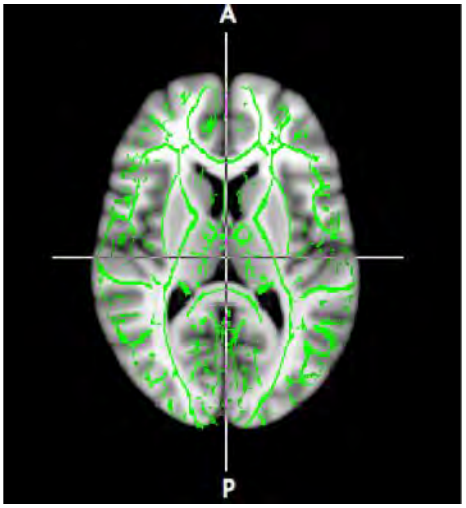
Figure 2: CUBIC 3T MRI scanner for DTI



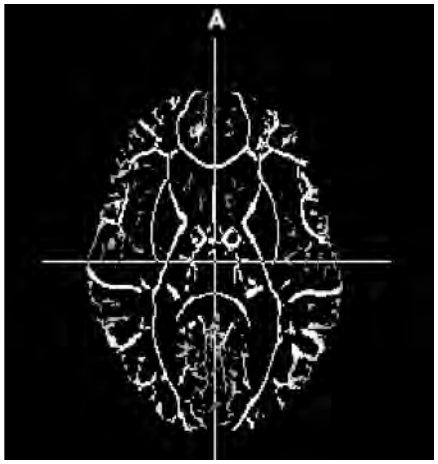
1. Pre-processing for eddy Current and motion Correction; FA, MD, AD, RD Image maps created



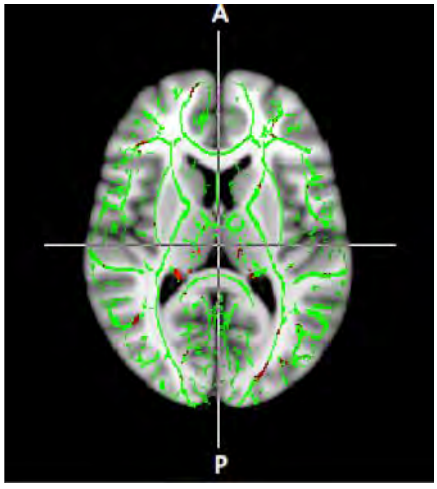
2. FA images aligned to Common target FA template Using nonlinear registration



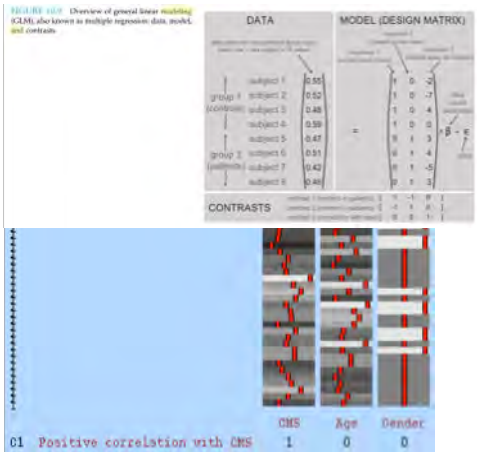
3. Mean FA skeleton created and aligned to MNI152 image template



4. FA data projected onto the mean FA skeleton, and a distance map was created to calculate the subject FA voxels to skeleton distance



5. An ROI analysis was Performed where values for FA, MD, AD and RD were Extracted for different Brain regions



6. A voxelwise analysis of multi-subject diffusion data was performed on FA, MD, AD and RD images using a general linear model

Figure 3: TBSS steps in DTI image process

OUTLINE OF THE THESIS

The thesis is based on five journal manuscripts: three of which have been published, one has been accepted for publication and one under review in an international journal. The publications collectively cover the full breadth of my PhD topic; each publication answers one or more key objectives of the thesis, which together address the aims and research question of the thesis. Each publication captures all the material related to the objective(s) it answers, including relevant literature reviewed, data analyzed and reported, and the overall discussion of these findings. Organizing the material of the thesis in this way facilitates my task of integrating the findings in the final chapter that synthesizes the results and discusses the implications of them. As such the inclusion of these publications together with an introduction chapter and a concluding discussion chapter would form a coherent body of research work, which addresses in full the scope of my research proposal. The coherence of this thesis revolves around three main points. Firstly, the thesis centres around my own role as the single principle investigator. In this respect, I initiated and led the project from conception to completion. Secondly, the thesis involves a single project. Each chapter describes an analysis conducted on the same cohort of patients enrolled into this study. Thirdly, there is a distinctly unifying theme to this thesis, namely, the description of neurocognitive disorders and white matter damage as seen by DTI in a cohort of vertically infected HIV postitve children, and the impact of a number of variables, including the use of ART, on these disorders. This thesis has therefore resulted in a series of manuscripts, of which four have been published to date. They represent an evolution in the work.

Chapter 2 provides a comprehensive background and literature review relevant to the topic and is presented in the format of a journal article The purpose of this review is to summarise what is already known in the field of neuroimaging in vertically acquired HIV, and to highlight possible future directions in using neuroimaging and neurocognitive testing to understand the spectrum of neurocognitive disorders in HIV positve children.

Chapter 3 addresses objectives one, two and four of the thesis and is presented as a journal article. We compared asymptomatic HIV infected children with ‘slow progression’ with matched controls on a neurocognitive battery as well as DTI. There are few neuropsychological or neuroimaging studies of children with “slow progression”. Previous

studies findings have been inconsistent, with some reporting no cognitive deficits, whilst others did report significantly poorer performance in the children with slow progression.

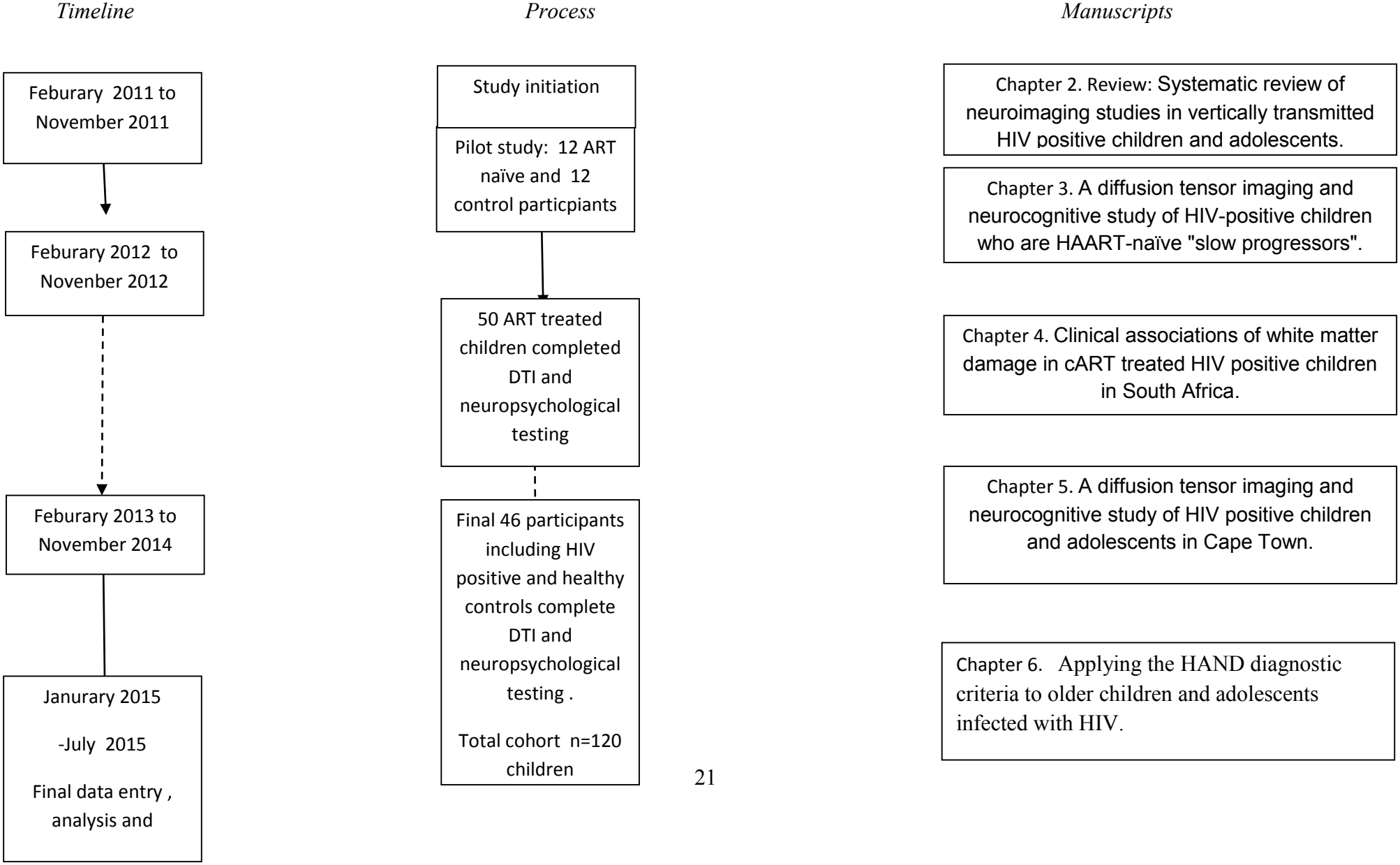
A range of factors may contribute to white matter damage in vertically infected HIV infected children. In chapter 4, which addresses objective three and is presented as a journal article, we explored associations between a number of these factors and DTI measures in ART treated children. It is important to examine the effects of HIV disease in the context of treatable clinical variables such as anemia and nutritional status.

In chapter 5, which addresses objectives one, two, four and five and is presented as a journal article, we compared vertically infected HIV infected children, including both children on ART and ART-naïve, with matched controls on a fronto-striatal cognition battery and DTI measures. DTI is ideal for studying the impact of HIV infection on older children and adolescents due to the sensitivity of DTI to white matter integrity and the impact of HIV on white matter. Despite evidence of enhanced sensitivity to HIV infection, DTI methodology has not been applied in a large cohort of pediatric HIV.

The last chapter (6) addresses objective six and is presented as a journal article. The article seeks to investigate whether the American Academy of Neurology (AAN) criteria for assessment of HIV associated neurocognitive disorders (HAND) in HIV infected adults could be used to detect a spectrum of neurocognitive disorders in HIV infected older children and adolescents. Neurocognitive impairment can have deleterious effects on children's ability to function, however there are still no diagnostic criteria for a spectrum of neurocognitive disorders (ND) secondary to HIV infection for children.

The concluding chapter (7) provides an integrative narrative of the thesis that synthesizes the findings from all chapters to answer the aims and objectives of the thesis. Conclusions of the thesis as a whole are drawn, the implications of the research for pediatric NeuroAids field in general are discussed and priorities for future research are identified.

Figure 1. Outline of study and project process and manuscript preparation and publication



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Chapter 2.

Systematic review of neuroimaging studies in vertically transmitted HIV positive children and adolescents

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ABSTRACT

One of the most serious consequences of vertical HIV-infection is its impact on the central nervous system (CNS). Although much work has been done to elucidate the complex mechanism of HIV associated neurotoxicity, several questions remain unanswered. The purpose of this review is to summarise what is already known in the field of neuroimaging in vertically acquired HIV, addressing three aims and to highlight possible future directions in using neuroimaging and neurocognitive testing to understand the spectrum of neurocognitive disorders in HIV positve children. Here we aim to address several clinically relevant questions in pediatric neuroHIV, using the current evidence base by conducting a systematic review. We aim to investigate what is known about the relationship between cognitive impairment and central nervous system damage in HIV as seen in neuroimaging studies, and to search for any evidence in the neuroimaging literature which suggests a spectrum of neuocognitive disorders in vertically infected HIV. Secondly, we aim to enquire whether children with a clinical diagnosis of encephalopathy are clearly distinguishable from HIV positive children without encephalopathy on neuroimaging and neurocognitive testing. Finally aim to investigate what is known about the effect on the CNS of antiretroviral therapy in paediatric HIV. Three separate databases were searched and two investigators systematically evaluated the titles, abstracts, and keywords associated with each individual article to determine those that may have met the inclusion and exclusion criteria. Following this process 11 studies were included in the review. Thus there was limited available data to address the three questions posed.

INTRODUCTION

One of the most serious consequences of human immunodeficiency virus (HIV)-infection is its impact on the central nervous system (CNS). Children vertically infected (from mother to child during pregnancy, delivery, or breastfeeding) with HIV display CNS disease more frequently than adults, and may present with a CNS problem in the first two years of life as the initial diagnostic indicator of HIV infection (Tardieu et al., 2000).

Evidence of CNS damage in vertically infected children are confirmed by work done at autopsy, with reported neuropathological findings corresponding to clinical disease in paediatric patients include impaired brain growth, reactive gliosis, myelin pallor, calcifications of the basal ganglia, cortical and cerebral atrophy with neuronal loss and ventricular enlargement as well as abnormalities of cerebral vasculature (George et al., 2009).

From a clinical perspective the patterns of CNS pathology in HIV infected children may include (1) HIV related encephalopathy, both progressive and static forms, (2) other neurological complications of HIV including periheral neuropathies, myopathies, seizures (3) non-HIV related CNS impairment, which is related to these children's complex medical histories (metabolic, endocrine, systemic illnesses, toxic side effects of drugs, secondary CNS infections) and social situations (4) lastly there is evidence in the literature which is suggestive of HIV related CNS compromise, characterized by global cognitive functioning within normal limits, but with significant impairments in selective developmental or cognitive domains, (Brouwers et al., 1995). Developmental delays, loss of previously acquired milestones, cognitive impairment, microcephaly, weakness, spastic paresis, and hyperreflexia are the most common manifestations of HIV encephalopathy in children (Belman et al., 1992). On the other end of the spectrum 'slow progressors' are typically defined in the research literature as children or adolescents who were receiving no or minimal therapy (defined as single or dual nucleoside therapy) before the age of 10 years and who had maintained CD4 counts above 25% for the first decade of life.

While clinicians have clinical criteria for the diagnosis of HIV related encephalopathy and 'slow progression' in vertically infected HIV positive children, little is known about the cognitive functioning and neuroimaging findings of the even larger cohort of children who are stable on combination anti-retroviral therapy (cART) and who do not meet diagnostic criteria for encephalopathy or slow progression. The adult literature on HIV related CNS damage supports a spectrum of HIV associated neurocognitive disoders or HAND. HAND includes

HIV associated dementia , mild neuorcognitive disorder and asymptomatic neurocognitive disorder (Antinori et al., 2007). The criteria have clinical utility, as they allow for the conceptualisation of a range of functional impariment, and are helpful in the formulation of individual management plans for adult pateints with HIV related cognitive problems. Similar spectrum criteria are needed in pediatric HIV in order to better understand the impact, and social and educational management needed to support the large number of children in Sub-Saharan Africa with vertically infected HIV.

Neuroimaging is likely to have a role in defining the spectrum of neurocognitive disorders in HIV positive children and adolescents. Conventional neuroimaging currently has two roles in the evaluation of HIV positive children. Firstly, in the detection of cerebral atrophy and other early signs of encephalopathy and secondly, for excluding or investigating secondary CNS complications associated with HIV such as opportunistic infections and tumours (Gavin & Yogev, 1999). In resource limited settings computed tomography (CT) is more accessible than Magnetic Resonance Imaging (MRI). However MRI studies are more sensitive and specific in detecting primary HIV related brain changes (Avison et al., 2002).

Data on the other neuroimaging modalities in pediatric HIV are at an early stage. The purpose of this review is to summarise what is already known in the field of neuroimaging in vertically acquired HIV, addressing three aims and to highlight possible future directions in using neuroimaging and neurocognitive testing to understand the spectrum of neurocognitive disorders in HIV positve children. Here we aim to address several clinically relevant questions in pediatric neuroHIV, using the current evidence base by conducting a systematic review. We aim to investigate what is known about the relationship between cognitive impairment and central nervous system damage in HIV as seen in neuroimaging studies, and to search for any evidence in the current literature which suggests a spectrum of neuocognitive disorders in vertically infected HIV. Secondly, we aim to enquire whether children with a clinical diagnosis of encephalopathy are clearly distinguishable from HIV positive children without encephalopathy on neuroimaging and neurocognitive testing. Finally aim to investigate what is known about the effect on the CNS of antiretroviral therapy in paediatric HIV.

METHODS

The databases searched were PubMed, Medline, and Scopus. The keywords used were all possible combinations of the following words and phrases as both text words and MeSH terms: “adolescents”, “children”, “pediatric”, “human immunodeficiency virus”, “HIV”, “acquired immunodeficiency syndrome”, “AIDS” “neuroimaging”, “computed tomography”, “CT”, “magnetic resonance imaging”, “MRI”, “magnetic resonance spectroscopy”, “MRS”, “positron emission tomography”, “PET”, “diffusion tensor imaging”, “DTI”, “functional magnetic resonance imaging”, “fMRI”, “single-photon emission computed tomography”, and “SPECT”. A total of 904 unique articles were found.

Two investigators systematically evaluated the titles, abstracts, and keywords associated with each individual article to determine those that may have met the inclusion and exclusion criteria. If there was confusion or ambiguity regarding an article, it was reviewed independently by two investigators and rejected or retained based on the consensus of the two investigators. Of the 904 articles found, 258 were not considered relevant to the current review, meaning that the articles did not refer to either HIV or neuroimaging, 427 included only HIV or neuroimaging separately, and 219 included both HIV and neuroimaging together. Note that EEG and angiography studies were not considered neuroimaging for the purpose of this study and were not included in the HIV and neuroimaging group. Studies were not considered to qualify in the HIV and neuroimaging group if HIV was mentioned in an indirect fashion, such as a study looking at the clinical implications of MRI and listing HIV as one of the implications.

The inclusion criteria were as follows: the article had to be written in English, the study had been an original scientific investigation, and the study had to be investigating neuroimaging. Additionally, a minimum of five subjects had to be included, the subjects had to be children within the age range 0-years to 18-years-old, the subjects had to be diagnosed with HIV-infection, and the subjects had to be naïve to or on ART. In order to obtain a clearer depiction of the range of impairment, the subjects must not have been selected on the basis of impairment. The study had to include a group of vertically infected children.

The exclusion criteria were as follows: the subjects had to be without opportunistic infections and medical conditions that could confound neurological/neuroimaging abnormalities, such as seizures, hemophilia, and genetic conditions eg. Downs Syndrome, cancers, etc.

Subjects had to be without a diagnosed psychological disorder, and could not have abused substances. The subjects could have been HIV encephalopathic or non-encephalopathic. It should be noted that multiple exclusion criteria may have been applied to any particular article however; the article was eliminated by the first exclusion criteria that it met in this process.

The studies included in the HIV and neuroimaging group were then filtered based on the more specific inclusion and exclusion criteria listed above.

One article could not be obtained in full text and was unable to be assessed beyond its title and was therefore rejected. The studies were first eliminated if they were not written in English (16 articles). Then, those studies that were not original scientific investigations were eliminated (21 articles). One study was rejected because the subjects were animal models and not human beings. Next, studies that exclusively investigated or included adult subjects without reporting on the pediatric group separately (subjects over 18-years-old) were eliminated (76 articles). Studies that investigated less than five subjects were then eliminated (39 articles). These studies were usually case reports that included subjects with opportunistic infections or other medical conditions. Thereafter, studies including subjects with opportunistic infections, medical conditions that could confound neurological/neuroimaging abnormalities, psychological disorders, or those born to mothers who abused substances during pregnancy were rejected (21 articles). Additionally, three studies were rejected because the subjects were not considered to be naïve or stable on ART and two were eliminated for not reporting the results of the neuroimaging findings.

18 studies were considered to meet the inclusion and exclusion criteria based on the information provided in the abstracts and methods of the articles. Two investigators then separately extracted data from these remaining studies. If it was revealed during the data extraction that studies violated the inclusion and exclusion criteria, those studies were rejected. In this process, six studies were further eliminated as they included children with opportunistic infections, other medical conditions that could confound neurological/neuroimaging abnormalities, psychological disorders, or those born to mothers who abused substances during pregnancy and one study was eliminated for including subjects over 18-years-old. After systematic review of the 904 articles 11 studies met the required inclusion and exclusion criterion.

Figure 1 outlines the process by which the final number of included studies was determined and shows the number of articles remaining after each elimination step (noted in parenthesis).

Figure 1

Diagram outlying the process of including or rejecting articles based on the inclusion and exclusion criteria

RESULTS

Of the 11 studies included in the review, six studies used CT, four used MRI, two used magnetic resonance spectroscopy (MRS), one used positron emission tomography (PET) and one used diffusion tensor imaging (DTI). Independent variables examined most commonly were the presence or absence of HIV encephalopathy; children who were symptomatic versus those who were not, and those with more CNS lesions were compared to those with less CNS damage. Dependent variables were the various neuroimaging markers as well as cognitive function and viral load (See table 1).

Table 1: Summary description of included studies.

Study ID	Study type	Modality	Independent Variable	Dependent Variable
Depas 1995	Cross sectional	PET	Groups: asymptomatic versus symptomatic / vertically	PET + cerebral function
Pavlakis 1995	Cross sectional	MRI and MRS	Groups: Encephalopathic children versus non-encephalopathic children (children with acquired immunodeficiency/AIDS)	MRI and MRS / Brain abnormalities
Wolters 1995	Cohort	CT	Groups: encephalopathic versus non-encephalopathic + Children with Symptomatic HIV Disease	CT scan abnormalities + Differential Receptive and Expressive Language Functioning
Scarmato 1996	Cross sectional	MRI	Groups: progressive encephalopathic, non-encephalopathic, control, children with cerebral atrophy from causes other than HIV infection	MRI + central brain atrophy
Wolters 1997	Prospective	CT	Groups; Encephalopathic vs. non-encephalopathic / children with symptomatic HIV disease, immune status	CT + brain scan abnormalities+ Receptive and expressive language functioning, and language dysfunction
Brouwers 2000	Cohort	CT	Groups; children with intracerebral calcifications versus those without and children with symptomatic HIV disease	CT + viral load in plasma and cerebrospinal fluid (CSF)
Nozyce 2006	Cohort	CT and MRI	Groups; previous treatment with ARV	CT and MRI + Behavior problems and cognitive functioning
Martin 2006	Cross sectional	CT	Groups: patients with CT abnormalities within normal limits (WNL) and patients	CT + Cognitive functioning

			with minimal to moderate abnormalities (MMA) + Vertically Acquired HIV	
Tahan 2006	Prospective and Cross sectional	CT	Groups: HIV-infected versus sero-reverters	CT + Neurological Profile and Neurodevelopment
Prado 2011	Prospective	MRI and MRS	Groups: HIV-infected versus controls / HIV encephalopathy	Image evaluation, quantitative MR techniques
Hoare 2012	Cross sectional	DTI	Groups: HIV –infected slow progressors versus controls	DTI, fractional anisotropy, mean diffusion, axial diffusion, radial diffusion, and cognitive function

NOTE: Study type; cross sectional (Cross-sectional studies involve data collected at a defined time); cohort (a form of longitudinal study, the population under investigation consists of individuals who are at risk of developing a specific disease or health outcome); prospective (A prospective study is a cohort study that follows over time a group of similar individuals)

Most of the studies had sample sizes of less than 50. The largest study had 236 CT scans. However no quantitative neuroimaging techniques were used in this study. CT scans were evaluated by neuroradiologists who examined basal ganglia/subcortical calcification, cortical atrophy, white matter abnormalities, and focal mass lesions. Only two studies (Martin et al., 2006 and Hoare et al., 2012) examined older HIV+ve children, with both studies mean age was greater than 10years. Four studies did not report on whether the children were stable on their ARV regimens. Hoare et al., 2012 is the only study to examine ART naïve children exclusively. Two other studies included ART naïve children; Wolters et al., 1997 compared 15 ART naïve children to 21 children with encephalopathy. Brouwers et al., 2000 only included three ART naïve children. Half of the studies did not report on the number of children with encephalopathy in the different groups. Five studies compared HIV+ve children with encephalopathy to non-encephalopathic children or controls. A number of the studies did not report on disease stage, CD4 count and viral loads.

The main findings of the 11 studies, including the strengths and limitations of each of the studies have been summarized in Table 2.

The most frequent brain abnormalities reported in vertically infected HIV across the included studies were ventricular enlargment, cortical and subcortical atrophy, involvement of the basal ganglia, frontal white and frontal grey matter abnormalities, calcifications and damage to the corpus callosum.

Table 2: Descriptive statistics for included studies.

Study ID	HIV + Sample size	Mean age (Yrs.)	Number Stable[a]	Number ART Naïve	Number Encephalopathic	Number of Controls	CDC Stage or WHO stage	MEAN CD4 Count	Mean Plasma Viral Load
Depas 1995	8	4	8	0	***	0	3 patients were P-2 B/D-1; five were P-2/A	***	***
Pavlakis 1995	25	E = 4.3; NE = 7.6	***	***	8	9	***	E = 539; NE 494	***
Wolters 1995	36	5.5	21	15	21	20	Class P2	***	***
Scarmato 1996	34	5.3	***	***	9	42	***	***	***
Wolters 1997	Baseline = 44; 24-months = 17	Baseline = 5.4; 24-months = 8.5	***	***	Baseline = 24/44 (55%); 24-months = 4/17	0	***	***	***
Brouwers 2000	39	3.6	36	3	***	0	***	***	5.0112
Nozyce 2006	274	***	27	0	***	0	Stage 1 or 2	1529	***
Martin 2006	41	11.2	41	0	***	0	***	608.3	100,588.0

Study ID	HIV + Sample size	Mean age (Yrs.)	Number Stable[a]	Number ART Naïve	Number Encephalopathic	Number of Controls	CDC Stage or WHO stage	MEAN CD4 Count	Mean Plasma Viral Load
Tahan 2006	88	***	***	***	***	84	***	***	***
Prado 2011	9	6	9	0	9	9	***	745 +/- 321	42,791 +/- 15,792
Hoare 2012	24	10.4	12	12	0	12	Stage 1 or 2	585	All undetectable except one (vL=24,032)

*NOTE: [a]On HAART or ARV regiments for 4 weeks or more. *** = Not Available*.

Table 3: Summary of findings for the included studies

Study ID	Summary of findings	Study limitations	Study strengths
Depas 1995	Hypometabolism in the temporal and occipital lobes was greater in the symptomatic group, but was evident, predominantly on the right side of the brain, in the asymptomatic group. In symptomatic children, hypermetabolism was evident in the caudate and lenticular nuclei.	The sample size was small. Study did not use controls. The procedure for analyzing the scans was not the same for all patients. The results were not statistically analyzed or correlated, so the results may not be significant.	Qualitative analysis was performed by two independent individuals.
Pavlakis 1995	MRS of the encephalopathic children had lower NAA/Cr ratios in the regions of the basal ganglia and white matter compared to non-encephalopathic and control children. However, only the ratio in the basal ganglia region was significantly different from non-encephalopathic and control children.	Study did not describe necessary demographics of the patients. The number of children included in the groups is small. Only relative metabolite data is reported	Automated quantitative imaging methods. Control group used.
Wolters 1995	HIV-infected children scored significantly lower in expressive and receptive language tests compared to sibling controls. The encephalopathic children scored significantly lower in expressive and receptive language tests, as well as overall functioning, than non-encephalopathic children. Greater severity of CT scan ratings correlated with deficits in expressive and receptive language scores for both encephalopathic and non-encephalopathic children.	Not all scans were performed with the same machine. Study does not describe ARV regimens. The study used a broad age range. Breaking down the data into subgroups for comparisons resulted in somewhat small subgroup sizes. The use of two different language tests may also be a possible limiting factor.	Two neurologists, independently rated the scan using a semi quantitative rating system A comparison group of uninfected siblings of the HIV-infected patients used as controls Patients who have previously taken ARV treatments were compared to those naive to treatment.

Study ID	Summary of findings	Study limitations	Study strengths
Scarmato 1996	Encephalopathic children only evidenced significantly greater ventricular- and bicaudate-brain ratios, while bifrontal-brain ratios were not significantly different, compared to non-encephalopathic and control children. HIV-disease in encephalopathic children is associated with basal ganglia and subcortical atrophy on MRI Planimetry.	Study does not identify if all MRI results were reported by the same observer(s). Study does not include demographics of the patients, or any information of patient use of ARV treatment.	All MRI measurements were performed by observers blinded to the clinical status of the patients. Study used controls.
Wolters 1997	Expressive language scores remained significantly lower than receptive language scores for the overall sample. The severity of CT scan ratings was significantly greater in the encephalopathic than the non-encephalopathic children. The severity of CT scan ratings did not significantly change from baseline to the 6- or 24-month evaluations. For the encephalopathy group, greater severity of CT scan ratings significantly correlated with expressive, but not receptive, language scores at both baseline and 6-month evaluations.	Only 39% (17 out of 44) of children who initially received a baseline evaluation were administered a third language assessment after 24 months of antiretroviral therapy. A group of controls was not included in this longitudinal study. Environmental variables were not controlled for in this study. Detailed data regarding CT scan findings is not provided. Sample size is not the same for each evaluation.	A pediatric neurologist, blinded to the clinical status of the patient, rated the scan for presence and severity of various brain abnormalities. The study compared the following subgroups from baseline to 6 months: encephalopathic versus non-encephalopathic, vertically versus transfusion-acquired infection, previously treated versus untreated.
Brouwers 2000	The most common CT scan abnormality was cortical atrophy, and basal ganglia and/or frontal white matter calcifications. Only vertically infected children had intracerebral (basal ganglia) calcifications. Higher CD4 was significantly correlated with less cortical atrophy.	Study did not use controls. The study did not indicate if the same neurologist rated all of the CT scans. The population size was small and not all patients were vertically	Study separated vertically infected and transfusion infected patients. Neurologist was blind to the patients' clinical status. A previously described highly reliable semi

Study ID	Summary of findings	Study limitations	Study strengths
	Plasma viral load did not correlate with any CT abnormalities. CSF viral load was significantly correlated with a higher degree of cortical atrophy.	infected. The population had a wide age range.	quantitative technique was used to rate the severity of brain abnormalities.
Nozyce 2006	HIV-infected children scored significantly lower on cognitive and behavioral testing than norms, cognitive and behavioral findings did not significantly correlate with CT or MRI imaging findings.	The study did not include a control group. CT and MRI were not evaluated by the same individual. No automated quantitative imaging techniques.	The study population was large.
Martin 2006	Ventricular enlargement and subarachnoid dilatation were the most common abnormalities identified, followed by intracerebral calcifications and white matter abnormalities. The MMA groups scored significantly lower compared to both the WNL group and theoretical population means on cognitive tests.	The sample size is small. The numbers of patients in the WNL and MMA groups are not reported. The patients are not compared to a control group.	A pediatric neurologist blinded rated the scans according to the severity on a 100mmvisual analog scale as previously described byDeCarli et al. (1993).
Tahan 2006	The most common CT abnormality was calcification of the basal ganglia, ventricle asymmetry, and cerebral atrophy.	Methods describing in depth neuroimaging protocols are not given. Not all children received CT scans. CT data was not analyzed in comparison to neurodevelopmental test scores. Immunologic and virological data is not provided. The	The same neuropediatric team made all neurological evaluations.

Study ID	Summary of findings	Study limitations	Study strengths
		study does not disclose if the patients are on ARV therapy or are naive to HAART.	
Prado 2011	In regards to 1H-MR spectroscopy, N-acetyl aspartate/creatine ratios were not statistically different between the HIV-infected and control groups in the regions of interest or the overall NAA/Cr ratio. However, significantly increased choline/creatine ratios were observed bilaterally in both the frontal gray and white matter, in the left parietal white matter, and in the overall CHO/Cr ratio. In regards to relaxometry, lengthening of the relaxation time was found bilaterally in both the frontal gray and white matter of the lateral ventricles, the corpus callosum, and the centrum semiovale.	Patient data analysis is somewhat limited by the age ranges included and the difference of age between control and HIV-positive patients. Study investigated a small sample size.	Prospective. Quantitative MR techniques. Study used healthy controls. All children included in this study underwent a psychological and an extensive neurological evaluation by a specialist within a fortnight from the MRI exam. Both the patient and control groups were matched by social economics status and had the same geographical distribution and origin.

DISCUSSION

The most frequent brain abnormalities reported in vertically infected HIV across the included studies were ventricular enlargment, cortical and subcortical atrophy, involvement of the basal ganglia, frontal white and frontal grey matter abnormalities, calcifications and damage to the corpus callosum. These findings were derived from 11 empirical research studies. We will now discuss the current literature in vertically acquired pediatric HIV, based on the neuroimaging and neurocognitive findings in the 11 empirical research studies.

First, the relationship between cognitive impairment and central nervous system damage in HIV as seen by neuroimaging remains incompletely understood. Five out of the 11 studies examined the relationship between cognitive function and neuroimaging markers of CNS damage. While HIV-infected children frequently scored significantly lower on cognitive and behavioural testing in a number of the studies, cognitive and behavioural findings did not always correlate with CT or MRI imaging findings in all the studies (Nozyce et al., 2006). Others found that children with more white matter lesions on CT, scored significantly lower on cognitive tests compared to HIV positive children with few white matter lesions (Martin et al., 2006). For HIV positive children with encephalopathy, greater severity of CT scan ratings significantly correlated with expressive language scores (Wolters et al., 1997). A correlation was found between poor performance on a test of executive function and a test of attention with DTI in the corpus callosum and the superior longitudinal fasiculus in a study of slow progressors (Hoare et al., 2012). Studying the factors associated with cognitive and behavioural problems and HIV related CNS damage is complicated because of the influence of a number of environmental factors and nutritional factors. The aetiology of neuroimaging detected brain abnormalities in HIV-infected children is likely to be multifactorial, including factors such as prenatal drug exposure, difficult family environment, level of maternal education, changes in caregivers, CNS infections, nutrition, and poverty. Information on these factors was not available in a number of the studies and therefore could not be explored in this review. The inclusion of a control group matched for age, gender, socioeconomic status, living conditions and quality/level of education and excluding children exposed to prenatal drugs would help in determining the aetiology of CNS damage.

Secondly, while 6 studies mentioned the inclusion of children with HIV encephalopathy only 4 studies corrlelated the presence or absence of encephalopathy with neuroimaging or cognitive findings. Wolters et al 1997 found the severity of CT scan ratings was significantly

greater in the encephalopathic than the non-encephalopathic children, however the discrepancies between expressive and receptive language scores were not significantly different between the encephalopathy and non-encephalopathy groups (Wolters et al., 1997). Using MRS, Pavlakis and colleagues found that only the ratio in the basal ganglia region was significantly different from non-encephalopathic and control children (Pavlakis et al., 1995). Encephalopathic children only evidenced significantly greater ventricular- and bicaudate-brain ratios on MRI, while bifrontal-brain ratios were not significantly different, compared to non-encephalopathic children. The lack of clear differences may be due to variations in the clinical diagnosis of encephalopathy or perhaps an indication that the CNS damage in clinically well HIV positive children may be worse than previously suggested.

Thirdly, patients included in the studies were receiving varied ARV regimens. The ARV regimens were often not considered when interpreting the results of the reported studies. Differences in findings may be related to (1) the type of antiretroviral therapy the patients were receiving (i.e. differences in the CNS permeability of the therapy and differential effectiveness of the treatment in the systemic and CNS compartments); (2) the stage of the disease (i.e. in the later stages the blood brain barrier may be less intact, thereby creating a greater comparability between the CNS and the rest of the body systems; (Ellis et a1., 1997) and (3) the variation of viral load in the sample (Ellis et a1., 1997). It is not yet clear whether CNS damage such as cortical atrophy or white matter damage (Brouwers et al., 2000), is an appropriate surrogate marker for CNS drug efficacy in antiretroviral therapy.

Limitations of the current study include small number of studies included, being unable to do a metaanalysis due to diversity of imaging modalities and methods used. Few studies employed statistical methods to analyze the data rather than simply presenting descriptive findings. Patient data analyses are somewhat limited by the age ranges included, given the difficulty of studying the brains of children and comparing findings across age ranges as their brains are developing and changing rapidly, especially in early childhood (Marsh et al., 2008). A lack of descriptive variables and small number of controls make interpretation of the aetiology of the CNS damage difficult.

These results reveal that abnormalities can be detected by PET and DTI in both symptomatic and asymptomatic HIV-infected children. The effect of antiretroviral therapy on MR imaging in children with HIV encephalopathy is not yet clear. The African context is uniquely situated to investigate this question as antiretroviral therapy was historically only initiated once

disease was established. The Children with HIV Early Antiretroviral Therapy (CHER) trial found that early HIV diagnosis and early antiretroviral treatment reduced early infant mortality by 76% and HIV progression by 75 % (Violari et al., 2008). Following these findings, the WHO has recommended the introduction of antiretroviral therapy in all children under the age of 5 years diagnosed as being HIV positive. It would be of clinical use if imaging could be used to monitor the effect of long term antiretroviral therapy in HIV positive vertically infected adolescents with or without encephalopathy, particularly to determine if white matter signal changes and cerebral atrophy are reversible (George et al., 2009).

Large cohort studies describing in detail the cognitive, behavioural and neuroimaging findings in older children with vertically infected HIV are needed which include information on factors such as level of education, nutrition, and poverty. These sudies will allow us to better describe the spectrum of neurocognitive disorders in HIV infected children and adolescants. The varieties of available neuroimaging techniques have the potential to identify the underlying neurological disease process involved in vertically transmitted HIV. DTI, as a measure of white matter integrity, can detect subtle changes and is therefore ideally suited to investigating CNS damage secondary to HIV, in order to address these questions. The different imaging techniques could act as neuroimaging biomarkers to track the progression of neurological disease in children born with HIV, and thereby aid in answering a number of questions in vertically acquired HIV, including the timing of CNS injury and understanding of the neurobiological mechanisms for CNS injury in HIV.

In the next chapter I will go on to addresss the neurocognitve performance and CNS white matter integrity in ART naïve HIV infected children and adolescents with slow progression.

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Chapter 3

A Diffusion Tensor Imaging and Neurocognitive Study of HIV positive children who are ART-naïve 'slow progressors'.

Hoare J, Fouche JP, Spottiswoode B, Donald K, Philipps N, Bezuidenhout H, Mulligan C, Webster V, Oduro C, Schrieff L, Paul R, Zar H, Thomas K, Stein D. J Neurovirol. 2012 Jun; 18(3): 205-12.

ABSTRACT

There are few neuropsychological or neuroimaging studies of HIV-positive children with "slow progression". "Slow progressors" are typically defined as children or adolescents who were vertically infected with HIV, but who received no or minimal antiretroviral therapy. We compared 12 asymptomatic HIV-positive children (8 to 12 years) with matched controls on a neuropsychological battery as well as diffusion tensor imaging (DTI) in a masked region of interest analysis (ROI) focusing on the corpus callosum, internal capsule and superior longitudinal fasciculus. The "slow progressor" group performed poorly compared to controls on Performance and Verbal tasks of the Wechsler Intelligence Scale for Children and on tests of visuospatial processing, visual memory and executive function ($p = <0\ .05$). "Slow progressors" had lower fractional anisotropy (FA), higher mean diffusivity (MD) and radial diffusivity (RD) in the corpus callosum ($p = <0.05$), and increased MD in the superior longitudinal fasciculus, compared to controls. A correlation was found between poor performance on a test of executive function and a test of attention with corpus callosum FA, and a test of executive function with lowered FA in the superior longitudinal fasiculus. These data suggest that demyelination as reflected by the increase in RD may be a prominent disease process in paediatric HIV infection

INTRODUCTION

For our pilot study, in order to establish the feasability of using diffusion tensor imaging (DTI) in children, we recruited a small cohort of 'slow progressors' . If DTI could be sensitive enough to detect white matter damage in 'slow progressors' compared to neighbourhood matched controls, we could be more confident in its utility in HIV infected children. In the HIV literature, "slow progressors" are typically defined as children or adolescents who were (a) vertically infected with HIV, but who (b) received no or minimal therapy (defined as single or dual nucleoside therapy) before the age of 10 years, and who (c) remained clinically and immunologically stable for the first decade of life (e.g., had maintained CD4 counts above 25% over that period) (Bagenda et al. 2006). As a result these children receive limited attention from health services. In South Africa, as many as 35% of all infected children are believed to meet these criteria for slow progression (Archary et al. 2010), yet little is known about the neurocognitive characteristics of these children.

Regarding neuropsychological aspects, few studies have, in fact, described the cognitive profile of older (> 6years) vertically-infected HIV-positive children. Previous studies have focused mainly on younger children and described specific cognitive impairments associated with HIV infection. They highlighted deficits in the domains of visual perceptual and visual motor skills, attention, executive functions, memory, and language (Nozyce et al 2006; Fundaro et al. 1998; Boivin et al. 1995). A recent article reviewing studies in sub-Saharan Africa (SSA) that used development, cognition, and behaviour in HIV-positive children as their primary outcomes highlighted the paucity of data in this field as well as the fact that all except one study had focused on the pre-school age group (Abubakar et al. 2008).

Even fewer studies in the field of HIV neuropsychology have focused specifically on antiretroviral therapy (ART) naïve slow progressors. The findings have been inconsistent, with some reporting no cognitive deficits, whilst others did report significantly poorer performance in the children with slow progression. Bagenda et al. (2006) investigated Ugandan children, aged 6-12 years, who were asymptomatic, vertically HIV-infected and who were ART-naive. They compared those children to a control group of HIV-negative children. Although scoring slightly lower on academic achievement measures and showing more signs of acute illness and malnutrition, the patient group did not differ from the control group on tests of sequential processing, simultaneous processing, and memory. Another study

of asymptomatic HIV-infected children reported relatively normal performances on tests of general intellectual functioning and language, in the presence of executive function impairments (Bisuacchi et al. 2000; Brown and Lourie 2000).
These data suggest that even though a child might be described as asymptomatic, HIV infection may still have an impact on particular aspects of CNS function, leading to discrete and subtle deficits in specific cognitive domains (De Baets et al. 2007). The deficits could have far reaching impacts on the child's academic performance and hence schooling outcome. These findings underscore the importance of investigating specific cognitive domains in addition to general intellectual functioning.

Structural brain imaging studies of HIV in children have shown a range of abnormalities including cortical atrophy with ventriculomegaly and basal ganglia calcifications on CT, and MRIs show white matter lesions (DeCarli et al 1993; George et al 2009). Atrophy is more frequently seen in younger children, and the amount of atrophy correlates with plasma HIV RNA levels (Angelini et al. 2000). Atrophy has also been noted in asymptomatic children, although to a lesser degree (Gavin and Yogev 1999). Should neuropsychological impairments exist in slow progressors, these should be underpinned by loss of white matter integrity.

Whereas traditional MRI only describes the location and extent of white matter damage, DTI is capable of examining the microstructural integrity and directionality of the white matter. We regarded it as particularly suitable for use with this sample of children because it is a non-invasive technique that allows for rapid data collection, and because it has with demonstrated utility in studies of HIV-positive adults (Filippi et al. 1999; Pomara et al. 2001). Traditional scalar metrics derived from DTI data include fractional anisotropy (FA), which represents axon integrity and/or packing density, and mean diffusivity (MD), which represents the mean water mobility within the white matter. High FA and low MD values are typically associated with healthier neural microstructure and better cognitive performance, whereas low FA and high MD values are indicative of white matter damage. Axial diffusivity (AD) and radial diffusivity (RD) are additional DTI-derived metrics corresponding to diffusion parallel and perpendicular to the direction of the white matter tract, respectively. Myelin loss (measured in DTI by an index of RD) and axonal damage (measured in DTI by an index of AD) are both observed in white matter injuries, such as might occur in HIV. Because AD and RD have potential for use in differentiating between axonal injury and myelin loss (Song et al. 2002),

separate analyses of the changes in these two indices may provide insight into the underlying mechanisms of white matter damage associated with HIV infection in children. Hence, in the current study one of our specific aims was to determine whether, in this group of slow progressors, clade C HIV affects the DTI indices of FA, MD, RD, and AD in specific regions of interest, selected based on a previous DTI study of HIV-positive adults from the same community (Hoare et al. 2011) (viz., the corpus callosum, internal capsule, and superior longitudinal fasciculus).

This study aimed to examine neuropsychological and DTI in slow progressors compared to matched controls. The neuropsychological battery assessed general intellectual functioning as well as specific domains of cognition. DTI was used to access myelin loss and axonal damage.

METHODS

Subjects and sampling

We recruited 12 HIV-positive slow progressor children from the Infectious Diseases clinic at Red Cross War Memorial Children's Hospital in the Western Cape Province of South Africa. A study coordinator identified potentially eligible candidates, and then invited those children and their parents/guardians to participate. Eligible children were those who had attended the Infectious Disease clinic at least once, who were ART-naïve, and who met the inclusion criteria listed below.

We also recruited a control group of 12 HIV-negative children, matched to the patient group on age, gender, and race. These children were resident in the same community as the HIV-positive children in order to control for socio-economic status and quality of education.

After enrolment, parents/ guardians provided full informed consent for the child to participate in the study, and the child provided assent. Parents/guardians were compensated for transport costs and loss of time. Ethical approval was obtained from the University of Cape Town's Faculty of Health Sciences research ethics committee.

HIV-infected children had to satisfy the following criteria in order to be included in the study: age between 8 and 12 years; positive diagnosis of HIV infection (including initial and

confirmatory tests); ART-naïve and asymptomatic (i.e., no AIDS-defining stage 4 illness and a CD4 count of >25%). Individuals with any of the following were excluded from participation: an uncontrolled medical condition, such as poorly controlled diabetes mellitus, epilepsy, or active tuberculosis requiring admission; an identified CNS condition (other than HIV), such as TB meningitis and bacterial meningitis, documented cerebrovascular accident, and lymphoma; a positive history of drug or alcohol exposure in pregnancy; a history of head injury with of loss of consciousness greater than 5 minutes, or any radiological evidence of skull fracture; a history of perinatal complications such as hypoxic ischemic encephalopathy or neonatal jaundice requiring exchange transfusion, or neurodevelopment disorder not attributed to HIV; contra-indications to MRI (such as metal in the body and claustrophobia.

Instruments and Measures

Neuropsychological Assessment

Each participant was assessed using a standardized battery of neuropsychological tests commonly used in paediatric neuropsychology clinical assessment and research internationally and in South Africa. Test instructions were translated and back-translated into Xhosa, and we took steps to ensure test administration maintained compliance with International Test Commission guidelines (Bartram 2001).

The test battery comprised the following instruments: the *Wechsler Abbreviated Scale of Intelligence* (*WASI*; Axelrod 2002) measured general intellectual functioning; the *Grooved Pegboard Test* (*GPT*; Rourke et al. 1973) measured eye-hand coordination and motor speed; the Symbol Search, Digit Symbol - Coding, and Digit Span subtests from the *Wechsler Intelligence Scale for Children - Fourth Edition* (*WISC-IV*; Wechsler, 2003) measured information processing speed, attention and concentration, and working memory; the *Color Trails Test* (*CTT*; Williams et al. 1995) measured visual attention and cognitive flexibility; the *Rey Complex Figure Test* (*RCFT*; Kirkwood et al. 2001) measured visuospatial processing and visual memory; a category fluency test and the NEPSY-II Inhibition subtest (Brooksab et al. 2009) measured elements of executive functioning (generativity and inhibition of automatic responses, respectively).

Brain Imaging Protocol

Diffusion-weighted images were acquired at the Cape Universities Brain Imaging Centre (CUBIC) on a 3T MRI scanner (Siemens Magnetom Allegra, Erlangen, Germany). The diffusion-weighted images were acquired in an axial orientation with the following parameters: 1.8x1.8x2.0 mm^3 spatial resolution, 220 mm FOV, TR = 8800ms, TE = 88 ms, 65 slices, 0% distances factor and twofold GRAPPA acceleration. Gradients were applied in 30 directions with b = 1000 mm/s^2 and three volumes were acquired without any diffusion-weighting. This sequence was repeated three times.

For analyses of these data, images were imported into the FSL (FMRIB Software Library) 4.1.8 toolbox (Smith 2004) and corrected for eddy current distortions. The images were then imported into MATLAB (Mathworks, Natick, MA) for further pre-processing. The three acquisitions were linearly co-registered with the b = 0 mm/s^2 image of the first average used as a reference. For each of the three co registered acquisitions, outliers were determined relative to the tensor estimate by calculating the Z-value at the 25th and 75th percentiles, and ignoring values three standard deviations away from the mean. The three acquisitions were then averaged and exported to the tract-based spatial statistics toolbox (TBSS; Smith 2004) of FSL for voxel wise analysis. Fractional anisotropy (FA), mean diffusivity (MD), radial (RD) and axial diffusivity (AD) images were created by fitting a tensor model to the averaged data. Brain extraction was performed with FSL BET (Smith 2004). A study-specific paediatric FA template was created for the purpose of registering data to MNI space.

After images were pre-processed with the TBSS pipeline, a region-of-interest mask (ROI) was created. For mask creation the JHU-White matter atlas (Mori et al. 2005) included with FSL was used.

Statistical Analyses

All analyses were completed using SPSS version 19. All data upheld assumptions underlying parametric statistical tests, and did not need to be transformed in any way. Alpha was set at .05.

The first analytic step involved a series of independent-samples *t*-tests comparing neuropsychological test performance in the HAART-naïve and control groups. Although the research design focused on hypothesis testing, the public health context of this research, together with the small sample sizes, results in an increased concern about missing real effects rather than concern for the strict control of alpha values (i.e., Type II vs. Type I errors; Jacobson & Jacobson, 2005).

Three ROI were defined for statistical analysis: The corpus callosum, superior longitudinal fasciculus, and the internal capsule. Statistical analysis was performed at 5000 permutations with threshold free cluster enhancement (TFCE) in FSL's randomise. An unpaired two-sample t test was performed to compare DTI matrices between the two groups ($p < 0.05$ corrected for multiple comparisons).

The third analytic step involved a series of correlations describing the relationship between white matter integrity FA, as measured by diffusion tensor imaging, and neuropsychological test performance. The correlation coefficient used here was Pearson's r.

RESULTS

Sample Characteristics

Overall, these data suggest that the HIV-positive ART-naïve and HIV-negative healthy control groups were homogeneous and well-matched. Regarding age at testing, all children were between 8 and 12 years, and there were no significant between-group differences (patients: $M = 10.40$, $SD = 1.45$; controls: $M = 9.83$, $SD = 1.16$), $t(1, 11) = 0.85$, $p = .411$, Cohen's $d = 0.40$. Regarding level of education at the time of testing, all children had successfully completed between 1 and 5 years of formal schooling, and there were no significant between-group differences (patients: $M = 3.00$, $SD = 1.00$; controls: $M = 2.43$, $SD = 1.27$), t (1, 11) = 1.01, $p = .311$, Cohen's $d = 0.48$.

All participants were right-handed and of low socioeconomic status. All participants except one (a female in the ART-naïve group, who had a home language of English) had a home language of isiXhosa. Regarding race, all participants except one (the same female in the HAART-naïve group, who was coloured) were Xhosa. The difference in sex distribution

across groups was not statistically significant, χ^2 (1) = 1.57, p (two-tailed) = .210. The mean CD4 count in the HIV positive group was 585.

Neuropsychological Test Performance

Patients performed significantly more poorly than controls on tests of general intellectual functioning, visuospatial processing, visual memory, and semantic fluency. There were also trends toward significantly poorer performance by patients than controls on tests of motor functioning, processing speed, and cognitive flexibility. See Table 1

Table 1

Neuropsychological Test Performance: Between-group differences

Domain / Test / Subtest	ART-naïve (n = 12)	Healthy control (n = 12)	t	p
General intellectual functioning				
WASI				
Verbal IQ	87.78 (15.24)	101.20 (14.26)	1.98	.032*
Performance IQ	73.67 (8.85)	85.70 (9.89)	2.78	.007**
Motor functioning				
Grooved Pegboard Test				
Dominant hand z-score	3.32 (4.65)	-0.31 (2.19)	-1.76	.051†
Non-dominant hand z-score	2.86 (3.68)	0.17 (2.58)	-1.52	.077†
Processing speed				
WISC-IV Processing Speed Index	75.00 (14.04)	83.50 (7.62)	1.66	.057†
Attention and concentration				
WISC-IV Digit Span Forward SS	6.78 (2.17)	8.10 (3.07)	1.07	.149
Color Trails Test Trail 1 raw score	118.88 (99.90)	79.50 (24.19)	-1.21	.122
Working memory				
WISC-IV Digit Span Backward SS	6.00 (2.50)	5.20 (1.23)	-0.90	.191
Visuospatial processing				
WASI Block Design T-score	36.00 (5.55)	57.10 (18.25)	3.32	.002**
Rey Complex Figure Test copy z-score	-4.51 (1.64)	-2.37 (2.28)	2.05	.032*
Memory – visual				
Rey Complex Figure Test				
Immediate recall T-score	36.75 (6.74)	38.30 (5.27)	0.55	.296
Delayed recall T-score	29.88 (8.49)	36.40 (6.85)	1.81	.045*
Executive function				
Semantic fluency: Animals total	6.38 (3.25)	9.00 (2.26)	2.02	.030*
Color Trails Test Trail 2 raw score	203.38 (46.61)	168.10 (40.04)	-1.73	.051†
NEPSY-II Inhibition: Inhibition SS	5.75 (1.98)	7.10 (3.35)	1.01	.165
WASI Matrix Reasoning T-score	6.12 (2.04)	35.30 (17.53)	0.11	.456

Note. Means are presented with standard deviations in parentheses. The p-values presented are for one-tailed hypothesis tests. WASI = Wechsler Abbreviated Scale of Intelligence; WISC-IV = Wechsler Intelligence Scale for Children, Fourth Edition. SS = age-adjusted scaled score.

†$p < .10$.*$p < .05$. **$p < .01$.

Diffusion Tensor Imaging

Table 2 presents' summary data for significant changes in FA, MD, and RD (there were no significant changes in AD) for the three ROI (corpus callosum, internal capsule and superior longitudinal fasciculus). In the patient group, we observed decreased FA (figure1) and increased RD (figure 2) and MD (figure 3) in the corpus callosum. We also observed increased MD in the superior longitudinal fasciculus. There were, however, no significant changes in all four DTI parameters in the internal capsule.

Figure 1. Significant decreases in the corpus callosum FA of HIV+ ART-naïve children when compared to HIV- controls.

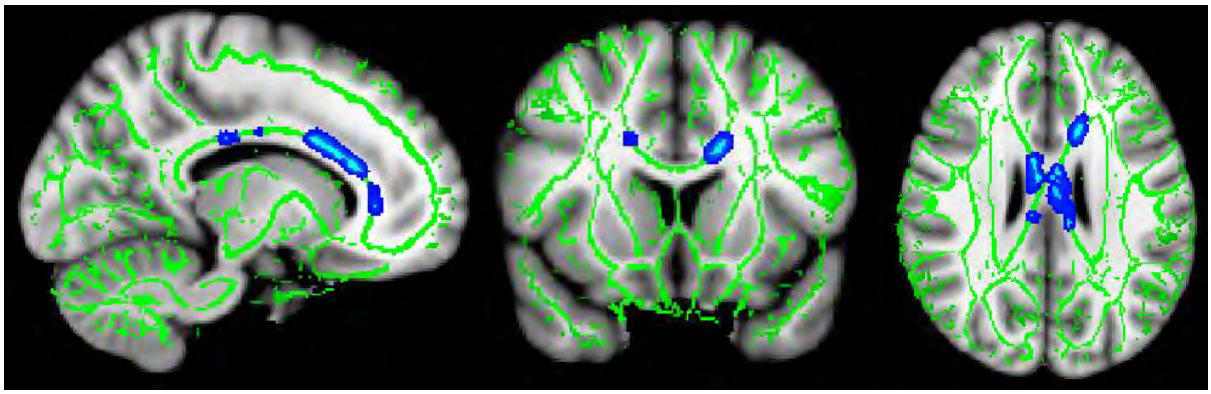


Figure 2. Significant RD increases in the corpus callosum genu and body of HIV+ ART-naïve children when compared to HIV- controls

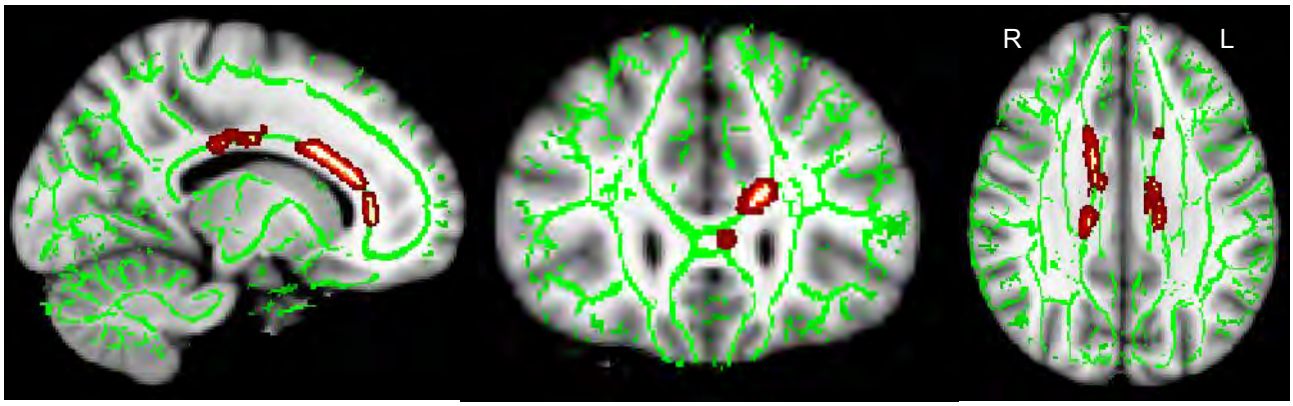


Table 2

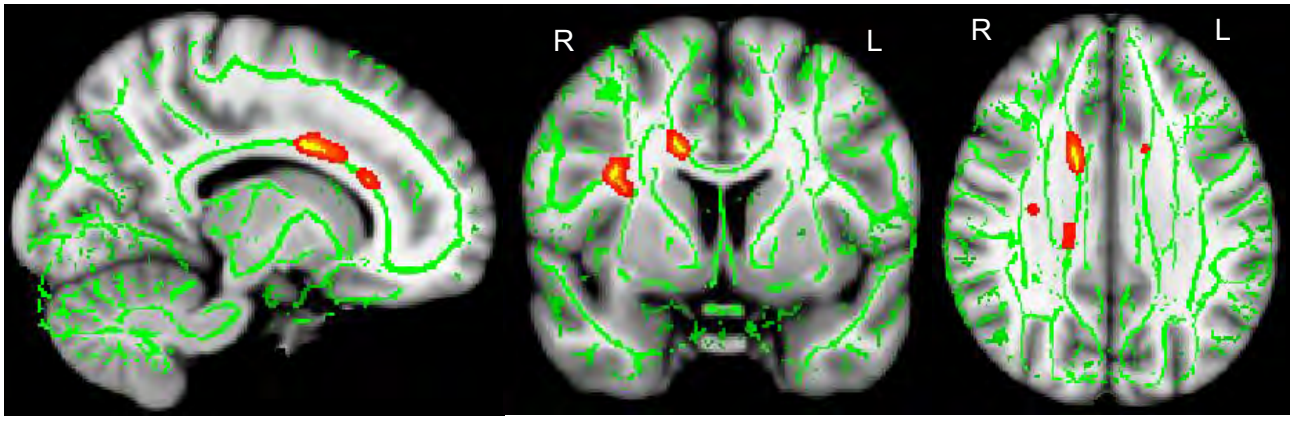
Summary of significant findings in the ROI analysis

Anatomy	MNI coordinates	Cluster size (mm^3)	Mean FA Controls (SD)	Mean FA HIV+ ART-naive (SD)	P-value
Decreased FA in HAART-naive children compared to controls					
Corpus callosum body	-13; 15; 26	230	0.598709 (0.054)	0.539064 (0.062)	0.0362
	8; 2; 26	73	0.724581 (0.038)	0.653235 (0.068)	0.0302

Anatomy	MNI coordinates	Cluster size	Mean RD Controls (SD)	Mean RD HIV+ HAART-naive (SD)	P-value
Increased RD in HAART-naive children compared to controls					
Corpus callosum genu	-14; 28; 19	268	4.93086×10^{-4} (7.60×10^{-5})	5.65688×10^{-4} (6.85×10^{-5})	0.0216
Corpus callosum body	-14; -11; 33	175	4.73741×10^{-4} (6.42×10^{-5})	5.334×10^{-4} (4.22×10^{-5})	0.025
	17; -8; 36	110	5.18632×10^{-4} (7.57×10^{-5})	5.80854×10^{-4} (5.05×10^{-5})	0.0332
	8; 2; 26	89	4.49184×10^{-4} (9.40×10^{-5})	5.2622×10^{-4} (1.09×10^{-4})	0.0334
	14; 3; 32	76	4.59883×10^{-4} (3.60×10^{-5})	5.16298×10^{-4} (3.83×10^{-5})	0.0314

Anatomy	MNI coordinates	Cluster size	Mean MD Controls (SD)	Mean MD HIV+ HAART-naive (SD)	P-value
Increased MD in HAART-naive children compared to controls					
Right superior longitudinal fasciculus	33; 1; 20	124	7.16474×10^{-4} (5.32×10^{-5})	7.65064×10^{-4} (5.45×10^{-5})	0.0266
Corpus callosum body	14; 5; 32	83	7.67364×10^{-4} (3.67×10^{-5})	8.18203×10^{-4} (2.12×10^{-5})	0.0366

Figure 3. Significant MD increases in the corpus callosum and right superior longitudinal fasciculus of HIV+ ART-naïve children when compared to HIV- controls.



Associations between Neuropsychological and Neuroimaging Data

Table 3 presents the statistically significant correlations between DTI measures of white matter integrity and neuropsychological test performance. Correlations between lower FA values in the corpus callosum and superior longitudinal fasciculus, and poorer neuropsychological test performances were found for Colour trails 1 and semantic fluency.

Table 3

Significant Correlations between White Matter Integrity and Neuropsychological Test Performance

	Brain region			
	Corpus callosum		Superior longitudinal fasciculus	
Neuropsychological test	Genu	Splenium	Left hemisphere	Right hemisphere
Color Trails Test Trail 1	----	.479 (.044)	----	----
Semantic Fluency	.471 (.048)	.474 (.047)	.579 (.010)	----

Note. Pearson's *r* values are presented, with *p* values in parentheses

DISCUSSION

The purpose of this study was to examine, relative to a group of demographically matched HIV-negative controls, the neuropsychological and DTI characteristics of 12 HIV-positive, slow-progressing, ART-naïve children. Our data showed that, compared to HIV-negative matched controls, both cognitive function and white matter integrity were altered in asymptomatic children infected with HIV.

Our results suggest that the asymptomatic patients perform more poorly on Performance and Verbal tasks of the *WASI*. In addition, asymptomatic patients performed significantly worse than the control groups on Visuospatial processing, semantic fluency and visual memory ($p < .05$). However in a study of neurologically asymptomatic HIV positive children conducted in Europe a different pattern of cognitive impairment was noted. Executive function impairments were present in all infected children, whereas memory and visuo-spacial deficits were evident only in those with full-blown AIDS. Language abilities and overall intelligence were spared (Bisuacchi et al. 2000). While a study of Ugandan children who were long surviving and ART naïve, found that they did not differ significantly in neurologic and cognitive assessments when compared with age- and gender-matched HIV-negative children (Bagenda et al. 2006). The findings of this study suggest that even in neurologically asymptomatic children, neuropsychological evaluation can identify impairment of specific cognitive functions. However a larger study examining cognitive function in slow progressors will need to be conducted to resolve the differences noted in these studies and whether some of the differences may be clade specific.

This is the first study of which we are aware that has utilised DTI to examine the effects of HIV on white matter in HIV positive children. A number of DTI studies of HIV positive adults have reported white matter damage to the corpus callosum (Wu et al. 2006; Chang et al. 2008; Thurnher et al. 2005). In a study of Clade C HIV positive adults from the same community, decreased FA was found in the corpus callosum, superior longitudinal fasciculus, and cingulum and sagittal stratum (Hoare et al. 2011). The region of interest (ROI) analyses revealed that RD was affected to a much greater extent than AD by HIV infection, which may suggest that demyelination is the prominent disease process in white matter in paediatric HIV. Both MD and RD were increased in the corpus callosum while FA was increased. Taken together these DTI parameters indicate poor directional diffusion in the corpus callosum. The reported white matter abnormalities identified with RD in this study agrees

with previous literature. Autopsy studies have demonstrated diffuse white matter damage in HIV positive adults which appeared as pallor in sections stained for myelin damage (Price et al. 1988). Myelin pallor and subsequent gliosis has even been observed at the asymptomatic stage of infection (Gray et al. 1996).

A correlation between poor performance on a test of executive function and a test of attention with corpus callosum FA, and a test of executive function with lowered FA in the superior longitudinal fasiculus was found. This is consistent with a previous DTI study in adults showing found a correlation with executive function and DTI parameters in frontal white matter and in the superior longitudinal fasciculus (Sasson et al. 2011). Decline in the white matter integrity of the corpus callosum was associated with poor performance in tests of memory and executive function (Voineskos et al. 2010). Taken together, the findings here are consistent with a view that decline in the microstructural integrity of white matter fibres accounts for cognitive decline in slow progressors.

Limitations of our study are the sample size is small and our data are cross sectional and as such are not able to address the question of whether changes in DTI parameters represent an early marker of subsequent cognitive decline.
Despite these limitations, the findings here suggest demyelination in the corpus callosum giving an indication that this medial brain region is differentially affected by in HIV disease in children. Future work could usefully focus on DTI as a quantitative imaging biomarker larger cohort studies. Studies examining response to ART treatment in children infected with HIV will be important to determine whether DTI abnormalities observed reflect reversible or more advanced irreversible injury. A range of factors may contribute to white matter damage in vertically infected HIV positive children. Correlates of white matter damage and neurocognitive decline need to be sought, including measures of viral load, CD4 count, age, ART treatment and nutrional status. These factors may well be critical in determining the overall impact of pediatric HIV on brain function, and in particular on white matter integrity.

In the next chapter, I will go on to address the socio-demographic, clinical and laboratory correlates of CNS white matter damage in a group of HIV infected children on ART, using DTI.

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Chapter 4

Clinical associations of white matter damage in cART treated HIV positive children in South Africa.

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ABSTRACT

A range of factors contributes to white matter damage in vertically infected HIV positive children. These may include combination anti-retroviral treatment (cART) regimen, socio-demographic factors, nutritional-hematological status, HIV-relevant clinical variables, and cognitive functioning. We explored associations between a number of these factors and diffusion tensor imaging (DTI) measures in 50 cART treated children aged 6 to 15 years. Fractional anisotropy (FA), mean diffusion (MD), radial (RD) and axial diffusion (AD) were derived from 48 cerebral white matter regions. Significant associations between a number of the clinical variables and white matter integrity were found. Decreased FA, a measure of neuronal damage, was associated with being on second line cART, low hemoglobin and younger age. Children with increased MD, a measure of neuronal damage, were younger, had reduced albumin and hemoglobin, and increased viral load. Decreased AD, a measure of axonal damage, was associated with increased viral load and total protein, decreased albumin and hemoglobin, younger age, poorer frontostriatal cognition and being on second line cART. Increased RD, a measure of myelin loss, was associated with younger age, low current CD4 count, low albumin and hemoglobin, and higher viral load and total protein. The current findings underline the possible association of first line treatment failure with white matter brain dysfunction in pediatric neuroHIV, and the importance of examining the effects of HIV disease in the context of treatable clinical variables such as anemia and nutritional status.

INTRODUCTION

HIV-associated neurological complications are common in South African children, and include a wide spectrum of disorders, (Govender et al. 2011). The specific consequences of HIV infection in the central nervous system (CNS) of vertically infected children could be associated with multiple factors. These factors may include socio-demographic status, nutritional-hematological status, HIV-relevant clinical variables and cognitive functioning. Additionally, combination anti-retroviral treatment (cART) has had a substantial and positive impact on long-term morbidity, and mortality in pediatric HIV (Willen 2006). The age, at which treatment is initiated, adherence to cART, and past treatment failures is also likely to be critical in moderating the impact of HIV on the CNS.

The long duration of therapy needed for vertically infected HIV positive children aims for maximal efficacy, minimal toxicity, and the prevention of development of drug resistance. Adherence and access to treatment may be more difficult in resource poor settings, such as countries in sub-Saharan Africa. Poor adherence to cART has been associated with treatment failure (Sebunya et al. 2013). First-line treatment failure may be a key factor associated with CNS damage in vertically infected HIV positive children.

HIV disease variables such as CD4 count; viral load and a clinical diagnosis of HIV encephalopathy (HIVE) may be associated with white matter damage of the CNS in vertically infected HIV. A higher CD4 count during the first years of life appears to be predictive of improved future school adaptation and cognitive abilities (Tardieu et al. 1995), with higher CD4 counts also significantly correlated with less cortical atrophy (Brouwers et al. 2000). However studies of HIV-infected Ugandan school-age children with CD4 cell counts above WHO thresholds for cART have also demonstrated significant cognitive and motor deficits, with higher plasma viral loads associated with even poorer functioning (Ruel et al. 2012). Brain atrophy has been more frequently described in younger children and the amount of atrophy correlated to plasma viral load (Angelini et al. 2000). HIVE has been associated with more basal ganglia and subcortical atrophy on MRI than HIV positive children without encephalopathy (Brouwers et al. 1995).

Variables such as socio-demographic factors, nutritional-hematological status and cognitive variables may also be associated with white matter damage of the CNS in vertically infected HIV. Children with the lowest neuropsychological functioning and cortical atrophy have been found to be at higher risk for disease progression (Pearson et al. 2000). Factors which

have been associated with mortality among HIV-infected children in rural Zambia include younger age, anemia and lower weight for age (Sutcliffe et al. 2011). Iron deficiency is a widespread problem in low socioeconomic countries. There is evidence that iron deficiency in early life adversely affects brain development (BentonILSI Europe a.i.s.b.l. 2008). Micronutrient deficiency may also affect HIV disease progression and growth in children (Friis 2006). Serum albumin has independently predicted mortality in HIV infected children in a resource-poor setting (Mofenson et al. 2003). Gender has also been documented to be a factor predicting lower scores on neuropsychological tests in HIV positive children (Smith 2006). The studies described above report global grey and white matter changes associated with a number of HIV specific disease factors and factors specific to the child. Our study builds on this work by examining the clinical associations of vertically transmitted HIV with subtler microstructural white matter using Diffusion tensor imaging (DTI).

DTI can be regarded as particularly suitable for use with this sample of children because it is a non-invasive technique that allows for rapid data collection (Hoare et al. 2012). DTI is able to examine the microstructural integrity and directionality of the white matter, and therefore capable of providing information on the location of white matter microstructural changes correlated with clinical variables in vertically infected HIV positive children. DTI has been used successfully in pediatric populations to document injury to white matter tracts after prematurity (Feldman et al. 2010), in multiple sclerosis (Vishwas et al. 2013), and as a consequence of moderate-to-severe traumatic brain injury (Wu et al. 2009). Reported neuroimaging findings in pediatric HIV include calcifying microangiopathy, atrophy and ventricular dilatation on CT scan, and white matter lesions and atrophy on MRI (Mitchell 2001). There are a number of reports describing the neurodevelopmental outcomes of vertically infected younger HIV positive children, however the neurologic features in older children are not as well described (Angelini et al. 2000).

Traditional scalar metrics derived from DTI data include fractional anisotropy (FA), which represents axon integrity and/or packing density, and mean diffusivity (MD), which represents the mean water mobility within the white matter. High FA and low MD values are typically associated with healthier neural microstructure, whereas low FA and high MD values are indicative of white matter damage. Axial diffusivity (AD) and radial diffusivity (RD) are additional DTI-derived metrics corresponding to diffusion parallel and

perpendicular to the direction of the white matter tract, respectively. Myelin loss (measured in DTI by an index of RD) and axonal damage (measured in DTI by an index of AD) are both observed in white matter injuries, such as have been previously described in adults with HIV (Hoare et al. 2011). Because AD and RD have potential for use in differentiating between axonal injury and myelin loss (Song et al. 2002), separate analyses of the changes in these two indices for each clinical predictor or disease variable may provide insight into the underlying mechanisms of white matter damage associated with HIV infection in children.

This study aimed to examine the clinical associations of white matter microstructural damage in vertically infected HIV positive children on cART utilizing DTI. We hypothesized that HIV disease and treatment variables, including higher viral load, lower CD4 count, HIVE and second line cART regimen would be associated with white matter damage as measured by DTI. Furthermore, we hypothesized that white matter damage would be associated with socio-demographic factors including, younger age, poorer nutritional status and more impairment on frontostriatal mediated cognitive tasks. For this particular study we did not investigate associations with controls, as the focus was on identifying predictors in a HIV-infected cohort.

METHODS

Subjects and sampling

We recruited 50 HIV-positive children and adolescents between the ages of 6 and 15 years, from the Infectious Diseases clinics at Red Cross War Memorial Children's Hospital, Groote Schuur Hospital and the community HIV antiretroviral clinics in the Western Cape Province of South Africa. A study coordinator identified potentially eligible candidates, and then invited those children and their parents/guardians to participate. Eligible children were those who had attended the Infectious Disease clinic at least once, had initial and confirmatory HIV tests, who were using cART for a minimum of 6 months, and who met the inclusion criteria listed below.

After enrolment, parents/ guardians provided full informed consent for the child to participate in the study, and the child provided assent. Parents/guardians were compensated for transport

costs and loss of time. Ethical approval was obtained from the University of Cape Town's Faculty of Health Sciences research ethics committee.

Individuals with any of the following were excluded from participation: an uncontrolled medical condition, such as poorly controlled diabetes mellitus, epilepsy, or active tuberculosis requiring admission; an identified CNS condition (other than HIV), such as TB meningitis and bacterial meningitis, documented cerebrovascular accident, and lymphoma; a positive history of drug or alcohol exposure in pregnancy; a history of head injury with of loss of consciousness greater than 5 minutes, or any radiological evidence of skull fracture; a history of perinatal complications such as hypoxic ischemic encephalopathy or neonatal jaundice requiring exchange transfusion, or neurodevelopment disorder not attributed to HIV; contra-indications to MRI (such as metal in the body and claustrophobia.)

Instruments and Measures

Biological variables

Biological variables examined as potential correlates of white matter damage of the central nervous system (CNS) included age, sex, HIV encephalopathy, first or second line cART treatment, HIV disease measures and nutritional status. Ethics approval was obtained for the taking of blood from HIV positive participants. Blood was taken by an individual licensed to draw blood such as a phlebotomist or medical officer. Blood taking was scheduled to coincide with the child's clinical blood taking schedule as part of their normal standard of care. cART schedule was be obtained from the participant's medical folders or managing clinician. First line regimen for all children in this study was the South African Department of Health first line regimen for children older than 3 years and weighing more than 10kg is, Abacavir (ABC) + lamivudine (3TC) + efavirenz (EFV). When children had virological failure on the first line regimen, resistance testing was done and the children were changed to a sensitive combination (though may require up to 4 agents depending on resistance patterns). The general principle of changing at least 2 of the drugs in the regimen for the second line was applied. A pediatric neurologist conducted the physical examination and assessment of encephalopathy. A diagnosis of HIVE was based on the presence of at least one of the following findings in a pediatric patient with no concurrent illness, other than HIV infection, that could explain the findings: (1) Failure to attain, or loss of, developmental milestones, (2)

acquired microcephaly as demonstrated by head circumference measurement and (3) acquired symmetrical motor deficits manifested by 2 or more of the following: paresis, pathological reflexes, ataxia or gait disturbance.

Neuropsychological Assessment

Each participant was assessed using a standardized battery of neuropsychological tests commonly used in paediatric neuropsychology clinical assessment and research internationally and in South Africa. Test instructions were translated and back-translated into Xhosa, and we took steps to ensure test administration maintained compliance with International Test Commission guidelines (Bartram 2001). The neuropsychological measures were adjusted for age.

The test battery comprised the following instruments: the Symbol Search, Digit Symbol - Coding, and Digit Span subtests from the *Wechsler Intelligence Scale for Children - Fourth Edition* (*WISC-IV*; Wechsler, 2003) measured information processing speed, attention and concentration, and working memory; the *Color Trails Test* (*CTT*; Williams et al. 1995) measured visual attention and cognitive flexibility; a category fluency test and the NEPSY-II Inhibition subtest (Korkman et al. 2007) measured elements of executive functioning (generativity and inhibition of automatic responses, respectively).

To ensure areas of cognition relevant to neuroHIV were accounted for, a fronto-striatal cognitive score was generated for each participant. The fronto-striatal cognitive performance score used here was a measure of HIV-related neurocognitive disorder. Cognitive compromise related to this area of the brain has been associated HIV disease progression (Castelo, Courtney, Melrose, & Stern, 2007). This score encompassed tests from the following cognitive domains: processing speed, attention, and executive functioning. The individual neuropsychological tests used to test each of the domains listed above are presented in Table 1. Converting the neuropsychological test raw scores into Z-scores, and then calculating the average across the tests did this. These Z-scores were calculated in Excel using the mean and standard deviation generated by a HIV negative control group matched for age and years of education.

Table 1:

Neuropsychological tests administered for each cognitive domain.

Cognitive Domain	Neuropsychological test scores
Processing Speed	WISC-IV - Processing Speed Index
Attention	Colour Trails 1
	Digit Span - Forward and Backward
Executive Function	Category Fluency
	Phonetic Fluency
	Colour Trails 2
	Nepsy – Inhibition and Switching

NOTE: WISC-IV = Weschler Intelligence Scale for Children – Fourth UK Edition.

MRI Data acquisition

MRI was acquired at the Cape Universities Brain Imaging Centre (CUBIC), Cape Town on a 3T MRI scanner (Siemens Magnetom Allegra, Erlangen, Germany) within 7 days of the physical examination and neuropsychological testing. Diffusion-weighted images were acquired with the following parameters: 1.8 x 1.8 x 2.0 mm^3 spatial resolution, 220m FOV, TR=8800 ms, TE=88 ms, 65 slices, no distance factor and twofold GRAPPA acceleration. The gradients were applied in 30 directions with b=1000 mm/s^2 and 3 b=0 mm/s^2 images were acquired as well. This sequence was repeated three times.

Data processing

Images were eddy corrected by utilizing the FMRIB Software Library (FSL) 4.1.8 (Smith et al. 2002). After eddy correction images were imported into Matlab R2008b (Mathworks, MA). The three acquisitions were co-registered by using the first b=0 mm/s^2 as the reference image. Outliers were determined by calculating the Z-value of the tensor estimates at the 25^{th} and 75^{th} percentiles. Data points more than 3 standard deviations were discarded. The

acquisitions were then averaged and exported to the FSL for further processing. Fitting a tensor model to the diffusion-weighted images created fractional anisotropy and mean, axial and radial diffusivity maps. Brain extraction was performed with FSL BET. A study-specific template was created by affine registration of each individual's FA image to the FMRIB58 template, after which images were concatenated and averaged. The template was then affine registered to MNI space and every subject's FA image was non-linearly registered to the template. These transforms were then applied to the original FA, MD, AD and RD images. Masks were created to delineate white matter regions by utilizing the JHU white matter atlas (Mori et al. 2005), with a FA threshold of 0.2. From these regions DTI measurements for MD, AD, RD and FA were extracted and imported into Statistica 10.0 for statistical analysis

Statistical analysis

For each of the 48 cerebral white matter regions, a multiple regression model was used to examine the effects of white matter with age, gender, encephalopathy status, first or second line cART, viral load, current CD4 count, hemoglobin, total protein, albumin and fronto-striatal function. The continuous variables in this model were age, CD4 count, viral load, hemoglobin, total protein, albumin and fronto-striatal cognition score. The presence/absence of HIVE (absence = 0; presence =1), cART regimen (first line = 0; or second line = 1) were treated as categorical variables. In this model the diffusion measurement in each ROI was the dependent variable and the other covariates were independent predictor variables. The model used was the following: $Y = \beta_0 + \beta_1$ (age) + β_2 (CD4 count) + β_3 (viral load) + β_4 (hemoglobin) + β_5 (total protein) + β_6 (albumin) + β_7 (fronto-striatal score) + β_8 (HIVE) + β_9 (CART) + ε. In this model Y represents the diffusion measurement of the ROI's and β the regression coefficient for each predictor. False discovery rate (FDR) was utilized to control for multiple comparisons at a level of 0.1 (Benjamini et al. 1995) with a p-value of .05.

RESULTS

Sample demographics

The participants were all vertically infected children on cART with a mean age of 9.58 (2.08) years. The majority of the children were black African, with isiXhosa as their predominant home language. The mean number of years of education completed was 2.76 years (SD 1.80), with 44% of the group having done a grade repeat. Only one child had completed 9 years of education. After the one child with 9 years completed education the next highest was 6 years completed education.

Table 2: Demographic description of cohort

Sample demographics (N = 50)

Variable	
Gender (Male: Female)	23: 27
Age in years	
M (SD)	9.58 (2.08)
Range	6 - 15
Education in years	
M (SD)	2.76 (1.80)
Range	1 - 9
Repeated grades (Yes: No)	22: 28
Race (Black African: Coloured: White: Other)	47: 3: 0: 0
Language (isiXhosa: English: Other)	44: 5: 0

NOTE: M = Means, SD = Standard Deviation, Education = number of year completed.

Clinical correlates of white matter damage

We examined the effects of HIV disease markers, first or second line cART treatment, nutritional markers, hemoglobin, fronto-striatal cognition and age on DTI measures of FA, MD, AD and RD (Table 3 and figure 1).

Fractional anisotropy

Decreased FA, a measure of neuronal damage, was associated with being on second line cART, low hemoglobin and younger age. Second line cART when compared to first line CART was significantly associated with decreased FA in the left superior corona radiata (p=. 007) and right posterior corona radiata (p=. 005). Lower hemoglobin was associated with decreased FA in the fornix (p=. 016), left corticospinal tract (p=. 005), left inferior cerebellar peduncle (p=. 005), right cingulum (p=. 008), bilateral superior fronto-occipital fasciculus (left p=. 006; right p=. 004) and left uncinate fasciculus (p=. 01). Younger age was associated with decreased FA in the fornix (p=. 02, right corticospinal tract (p=. 005), left inferior cerebellar peduncle (p=. 005 and left superior fronto-occipital fasciculus (p=0.002).

Mean diffusivity

Children with increased MD, a measure of neuronal damage, were younger, had reduced albumin and hemoglobin and increased total protein and viral load. High viral loads were associated with increased MD in the corpus callosum body (p=. 031) and splenium (p=. 006) and the left superior longitudinal fasciculus (p=. 029). Lower albumin was associated with increased MD in the corpus callosum body (p=. 001) and splenium (p=0.0007), left superior longitudinal fasciculus (p=. 027) and right superior fronto-occipital fasciculus (p=. 004). Higher total protein was associated with increased MD in the corpus callosum body (p=. 01) and splenium (p=. 001) and left superior longitudinal fasciculus (p=. 021). Lower hemoglobin was associated with increased MD in the bilateral inferior cerebellar peduncles (left p=. 001; right p=. 008), right fornix/stria terminalis (p=. 014) and bilateral superior fronto-occipital fasciculus (left p=. 01; right p=. 002). Younger age was associated with increased MD in the corpus callosum body (p=. 01) and splenium (p=. 002), right fornix/stria terminalis (p=. 014) and bilateral superior fronto-occipital fasciculus (left p=. 01; right p=. 014).

Axial diffusivity

Decreased AD, a measure of axonal damage, was associated with increased viral load and total protein, decreased albumin and hemoglobin, younger age, poorer frontostriatal cognition and being on second line cART. Higher viral load was associated with a decreased AD in the corpus callosum splenium (p=. 012) and left superior longitudinal fasciculus (p=. 005). Second line CART was associated with decreased AD in the left inferior cerebellar peduncle (p=. 026). Higher Total protein was associated with decreased AD in the corpus callosum body (p=. 019) and corpus callosum splenium (p=. 001) and left superior longitudinal fasciculus (p=. 003). Lower albumin was associated with decreased AD in the corpus callosum body (p=. 009) and splenium (p=. 002), left superior longitudinal fasciculus (p=. 012) and the left inferior cerebellar peduncle (p=. 005). Lower hemoglobin was associated with decreased AD in the right sagittal striatum (p=. 003). Poorer Fronto-striatal cognition was associated with decreased AD in the left inferior cerebellar peduncle (p= .037). Younger age was associated with decreased AD in the corpus callosum splenium (p=. 008) and left superior longitudinal fasciculus (p=. 012) and increased AD in the left inferior cerebellar peduncle (p=. 01).

Radial diffusivity

Increased RD, a measure of myelin loss, was associated with younger age, low current CD4 count, low albumin and hemoglobin and higher viral load and total protein. Low current CD4 count was associated with increased RD in the right superior fronto-occipital fasciculus (p=. 043). Higher viral load was associated with increased RD in the corpus callosum body (p=. 025) and splenium (p=. 007) and right superior fronto-occipital fasciculus (p=. 047). Younger age was associated with increased RD in the middle cerebellar peduncle (p=. 01), corpus callosum body (p=. 007) and splenium (p=. 002) and bilateral superior fronto-occipital fasciculus (left p=. 001; right p=. 008). Total protein was associated with increased RD in the corpus callosum body (p=. 03) and splenium (p=. 004). Lower albumin was associated with increased RD in the corpus callosum body (p=. 001) and splenium (p=. 001) and right superior fronto-occipital fasciculus (p=. 002). Lower hemoglobin was associated with increased RD in the bilateral inferior cerebellar peduncles (left p=. 007; right p=. 005) and bilateral superior fronto-occipital fasciculus (left p=. 002; right p=. 001).

Table 3 illustrates the regression coefficients and p-values for clinical variables with significant effects on DTI measurements.

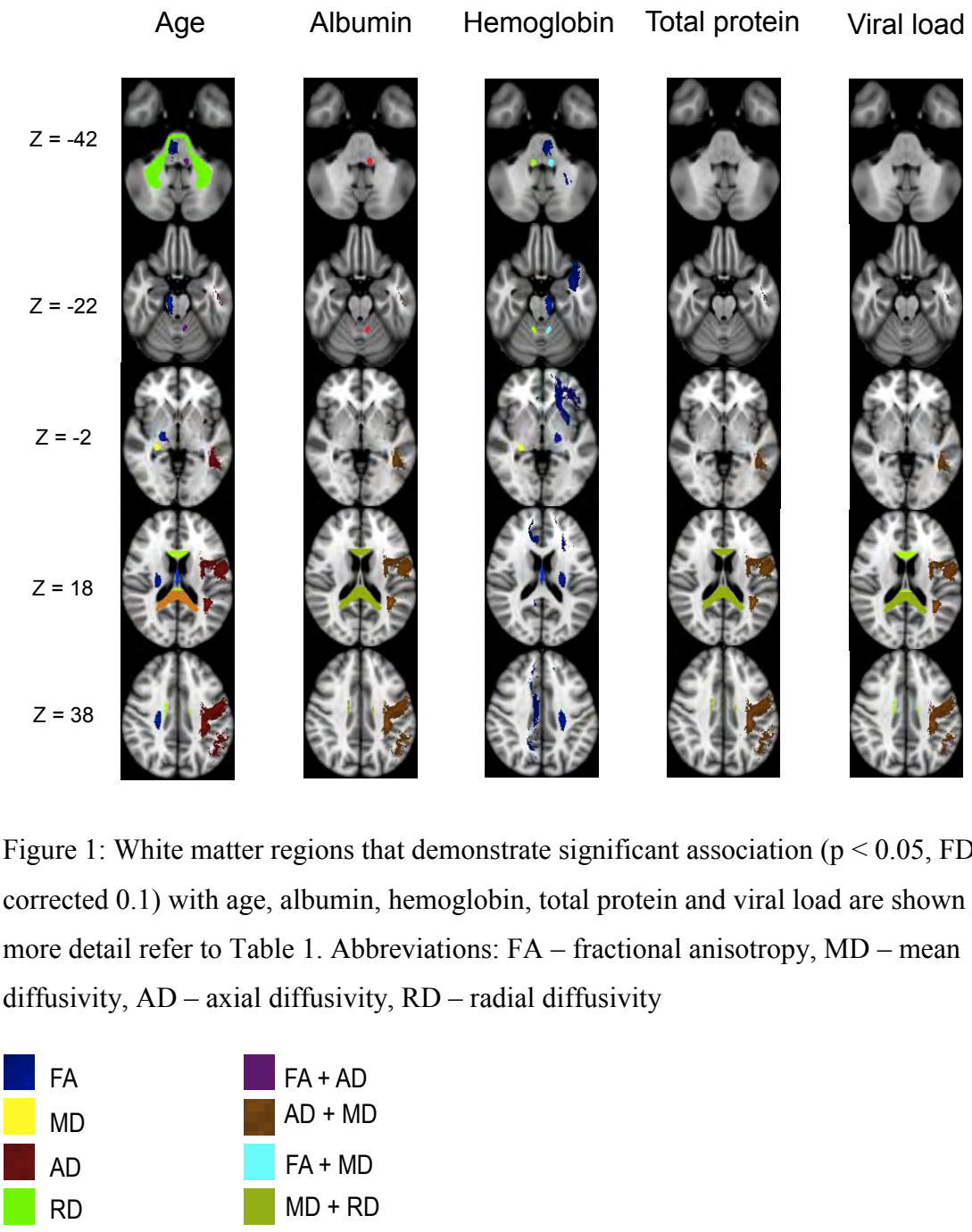

Figure 1: White matter regions that demonstrate significant association ($p < 0.05$, FDR-corrected 0.1) with age, albumin, hemoglobin, total protein and viral load are shown here. For more detail refer to Table 1. Abbreviations: FA – fractional anisotropy, MD – mean diffusivity, AD – axial diffusivity, RD – radial diffusivity

Table 3. Clinical variables that displayed a significant association with DTI metrics ($p < 0.05$, FDR corrected 0.1).

DTI measure	Clinical variable	White matter region	B Coefficient	R^2-value	p-value
FA	**Age**	Fornix	0.0058	0.647	0.0132
		RH CST	0.0059	0.650	0.0054
		LH ICP	0.0054	0.640	0.0053
		LH SFOF	0.0082	0.656	0.0018
	First line cART	LH SCR	0.0184	0.609	0.0071
		RH PCR	0.0189	0.695	0.0051
	Hemoglobin	Fornix	0.0086	0.647	0.0156
		LH CST	0.0096	0.592	0.0054
		LH ICP	0.0084	0.640	0.0048
		RH CG	0.0109	0.736	0.0075
		RH SFOF	0.0140	0.660	0.0039
		LH SFOF	0.0107	0.656	0.0057
		LH UF	0.0120	0.633	0.0097
MD	**Age**	CC Body	-0.00002	0.420	0.0106
		CC Splenium	-0.00003	0.544	0.0022
		RH Fornix/ST	-0.000006	0.470	0.0141
		RH SFOF	-0.00001	0.586	0.0142
		LH SFOF	-0.00001	0.692	0.0100
	Viral load	CC Body	0.00007	0.420	0.0313
		CC Splenium	0.0001	0.544	0.0055
		LH SLF	0.0002	0.529	0.0292

	Albumin	CC Body	-0.00002	0.420	0.0005
		CC Splenium	-0.00002	0.544	0.0007
		LH SLF	-0.00003	0.529	0.0274
		RH SFOF	-0.00001	0.586	0.0044
	Total protein	CC Body	0.000008	0.420	0.0103
		CC Splenium	0.00001	0.544	0.0014
		LH SLF	0.00002	0.529	0.0208
	Hemoglobin	RH ICP	-0.00002	0.532	0.0077
		LH ICP	-0.000008	0.512	0.0097
		RH Fornix/ST	-0.00001	0.470	0.0144
		RH SFOF	-0.00002	0.586	0.0022
		LH SFOF	-0.00002	0.692	0.0097
AD	**Viral load**	CC Splenium	-0.0001	0.492	0.0115
		LH SLF	-0.0002	0.447	0.0048
	Age	CC Splenium	-0.00002	0.492	0.0075
		LH ICP	0.000006	0.537	0.0097
		LH SLF	-0.00004	0.447	0.0121
	First line cART	LH ICP	0.00002	0.537	0.0256
	Total protein	CC Body	-0.00001	0.346	0.0188
		CC Splenium	-0.00001	0.492	0.0007
		LH SLF	-0.00002	0.447	0.0034
	Albumin	CC Body	0.00002	0.346	0.0089
		CC Splenium	0.00002	0.492	0.0016
		LH ICP	0.000005	0.537	0.0049

		LH SLF	0.00003	0.447	0.0118
	Hemoglobin	RH SS	0.00002	0.549	0.0026
	Fronto-striatal cognition	LH ICP	0.00002	0.537	0.0367
RD	**Current CD4**	RH SFOF	-4.645E-08	0.676	0.0429
	Viral load	CC Body	0.00007	0.471	0.0253
		CC Splenium	0.0001	0.551	0.0070
		RH SFOF	0.00005	0.676	0.0470
	Age	MCP	-0.000007	0.491	0.0098
		CC Body	-0.00002	0.471	0.0070
		CC Splenium	-0.00003	0.551	0.0022
		RH SFOF	-0.00001	0.676	0.0076
		LH SFOF	-0.00001	0.704	0.0013
	Total protein	CC Body	0.000006	0.471	0.0308
		CC Splenium	0.00001	0.551	0.0041
	Albumin	CC Body	-0.00002	0.471	0.0008
		CC Splenium	-0.00002	0.551	0.0011
		RH SFOF	-0.00001	0.676	0.0022
	Hemoglobin	RH ICP	-0.00002	0.570	0.0052
		LH ICP	-0.00001	0.573	0.0067
		RH SFOF	-0.00003	0.676	0.0006
		LH SFOF	-0.00002	0.704	0.0018

Abbreviations: FA: Fractional anisotropy; MD: Mean diffusivity; RD: Radial diffusivity; AD: Axial diffusivity; LH: Left hemisphere; RH: Right hemisphere; CST: Cortico-spinal tract; ICP: Inferior cerebellar peduncle; SFOF: Superior fronto-occipital fasciculus; SCR: Superior corona radiata;

PCR: Posterior corona radiata; CG: Cingulum; UF: Uncinate fasciculus; ST: Stria Terminalis; SLF: Superior longitudinal fasciculus; SS: Sagittal stratum; MCP: Middle cerebellar peduncle

DISCUSSION

In this study, we found significant associations between HIV treatment and disease variables, as well as demographic and nutritional factors, on brain white matter integrity in children. Neuronal damage, as reflected by decreased FA, was associated with being on second line cART, low hemoglobin and younger age in the fornix, cerebellar peduncles, fronto-occipital fasciculus and the cingulum. Children with increased MD, reflecting neuronal damage, in the corpus callosum, fornix, fronto-occipital fasciculus and superior longitudinal fasciculus were younger, had reduced albumin and hemoglobin, increased total protein and increased viral load. Axonal damage, as reflected by decreased AD, was associated with increased viral load; younger age, poorer frontostriatal cognition and being on second line cART. Myelin loss, as reflected by increased RD, was associated with younger age, low current CD4 count, low albumin and hemoglobin, higher viral load and increased total protein in the superior fronto-occipital fasciculus, corpus callosum and the cerebellar peduncle. The presence of FA, MD, RD and AD changes suggests a combination of axonal injury and myelin loss in vertically transmitted HIV positive older children and adolescents on cART.

A previous DTI study of HIV positive children, who were slow progressors, found increases in RD that were more significant than the decreases in AD (Hoare et al. 2012). The results suggested that the alteration in these two diffusivities is different, that demyelination as reflected by the increase in RD may be the prominent disease process associated with HIV infection in HIV, and axonal injury may occur at a weaker level in slow progressors (Hoare et al. 2012). However in this study of cART treated children we found both decreased AD and increased RD in a number of the white matter regions reported, indicating that both axonal injury and myelin loss (Song et al. 2002) may be involved in this group of children.

HIV relevant clinical variables such as higher serum viral load emerged as a more frequent predictor than CD4 count, and was associated with high MD values indicative of white matter damage, and low AD and high RD indicating axonal injury and myelin loss. These results may indicate that HIV infection in children may have a direct neuropathogenic effect that operates independently of the severe immunocompromise (Ruel et al. 2012). Low CD4 count was associated with high RD in the fronto-occipital fasciculus only. HIV encephalopathy did

not predict damage to white matter in this study. This finding may indicate that white matter damage is significant in both the encephalopathic and non-encephalopathic HIV positive children. Being on second line cART was associated with low FA, indicative of white matter damage, and low AD indicating axonal injury. HIV resistance mutations have been reported after failure of first-line antiretroviral treatment among children in resource-poor regions (Sigaloff et al. 2011). Findings in this study indicate a poorer outcome for the CNS in children who have failed first line cART.

Socio demographic factors such as younger age emerged as a strong predictor and was associated with low FA and high MD, values indicative of white matter damage, and low AD and high RD indicating axonal injury and myelin loss. A DTI study correlating of white matter diffusivity and anisotropy with age during childhood and adolescence found statistically significant decreases in MD and increases in AD with age in a group of healthy children and adolescents (Schmithorst et al. 2001). The changes observed in this study with may reflect an increase in white matter density and organization with increasing age, or the 'survivor effect' with children surviving into adolescence having less severe disease. Sex was not associated with damage to white matter in this study.

Nutritional –hematological factors, hemoglobin, total protein and albumin, were commonly associated with low FA and high MD, values indicative of white matter damage, and low AD and high RD indicating axonal injury and myelin loss in a number of white matter regions. Serum albumin has been found to be a strong independent predictor of mortality in HIV infected women after adjustment for known disease markers (Feldman et al. 2003). Serum albumin has also been inversely associated with IL-6 levels, a pro inflammatory cytokine, which has been shown to up regulate HIV replication (Ward et al. 1999). Adult ART naive patients with low baseline serum albumin had an eight times increased risk of death than those with higher serum albumin levels and patients with CD4 count of less than 100 cells were two times at higher risk of death compared to higher CD4 counts (Bhowmik et al. 2012). Mild anemia was found to be common in ARV-naïve Thai and Cambodian children without advanced HIV. However, iron deficiency prevalence was low; with the majority of cases caused by anemia of chronic disease (Kosalaraksa et al. 2012).

A previous DTI study of HIV positive children, found in a region of interest analysis, that ART naïve children had lower FA, higher MD and RD in the corpus callosum, and increased MD in the superior longitudinal fasciculus, compared to HIV negative controls (Hoare et al.

2012). White matter dysfunction in these regions can have cognitive and behavioral implications. A correlation has been found between in a DTI study between poor performance on a test of executive function and a test of attention with reduced corpus callosum FA (Hoare et al. 2012). Nearly half of the HIV positive children in this study had already done a grade repeat. Given how difficult it is to get to repeat a grade in the South African education system, this represents severe functional/adaptive deficits in the classroom. DTI performed on normal children ages 5–18 found significant positive correlations with IQ for FA in specific white matter areas, indicating that cognitive function correlated with greater fiber organization in the pediatric population (Schmithorst et al. 2005). Poorer fronto-striatal cognition was associated with axonal damage of the cerebellar peduncles in this study. Damage to the cerebellum can present with the cerebellar motor syndrome, yet cerebellar lesions can also result in the cerebellar cognitive affective syndrome, including executive, visual spatial, and language impairments, and affective dysregulation (Stoodley and Schmahmann 2010). The fornix has correlated with associative learning and memory in DTI studies of traumatic brain injury (Kinnunen et al. 2011). Reduced FA and increased MD has been observed in superior fronto-occipital fasciculus to be associated with impaired visual information processing (Bagga et al. 2013). Most commonly reported brain regions involved in this study were the corpus callosum, the superior fronto-occipital fasciculus, the cerebellar peduncles and the fornix. Other regions involved were the corticospinal tract, the corona radiate, the cingulum and the sagittal stratum.

Limitations of this study are that albumin, hemoglobin and total protein are indirect measures of nutrition and inflammation. While serum albumin is widely used to indicate nutritional status it has not consistently predicted malnutrition outcomes in HIV positive women with higher CD4 counts (Dusingize et al. 2012). More direct serum markers of inflammation and malnutrition may be more accurate. However in resource limited settings albumin, hemoglobin and total protein measures are more affordable and accessible. 50 participants may be an adequate sample for a neuroimaging study, but a small sample for a regression analysis.

The findings emphasize the need for early identification of adherence problems or resistance to first line cART in HIV infected in children living in sub-Saharan Africa, improved access

to support with issues relating to poor adherence, and the integration of antiretroviral treatment programs with other health-care services, such as nutritional support, and the importance of examining the effects of HIV disease in the context of treatable clinical variables such as anemia. A larger cohort study comparing HIV infected children stable on ART with those who are ARV naive slow progressors, and including a larger number of children with a clinical diagnosis of HIVE is needed to clarify the DTI findings observed in this study.

In the next chapter, I will go on to compare neurocognition and white matter integrity in ARV naïve slow progressors, children stable on ART and children with a clinical diagnosis of HIV encephalopathy.

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Chapter 5

White matter microstructural changes in ART naïve and ART treated children and adolescents infected with HIV in South Africa.

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ABSTRACT

The objective of the study was to describe the effect of HIV on white matter integrity and neurocognitive function in children vertically infected with HIV, compared to a HIV negative healthy control group. We compared 75 HIV infected children aged 6 to 16 years, including children on antiretroviral therapy (ART) and those who are ART-naïve, with 30 controls on diffusion tensor imaging (DTI) and a neuropsychological battery sensitive to fronto-striatal pathology. In a secondary analysis we compared 'slow progressor' ART-naïve children, children on ART without a clinical diagnosis of encephalopathy and children on ART with HIV encephalopathy (HIVE). Compared to controls (n=30), HIV infected children (n=75) displayed decreased fractional anisotropy (FA) and axial diffusion (AD), and increased mean diffusivity (MD) and radial diffusion (RD), indicating damaged neuronal microstructure. Within the HIV infected group, children with HIVE (n=14) had poor white matter integrity when compared to ART-treated children without encephalopathy (n=41), and there was significant myelin loss in ARV naïve children (n=20), compared with ART treated children. ART treated children had significant axonal damage in the corpus callosum (p=0.009). Children infected with HIV, irrespective of treatment status, displayed significantly poorer white matter integrity and impaired cognition compared to HIV negative controls. Our findings suggest that despite immune recovery in children on ART they remain at risk for developing central nervous system (CNS) disease, and that initiation of ART as early as possible may reduce the risk of developing white matter damage in ARV naïve slow progressors.

INTRODUCTION

The introduction of antiretroviral therapy (ART) has altered the course of Human Immunodeficiency Virus (HIV) infections in vertically infected children, reducing mortality and morbidity (Sutcliffe et al. 2008), and resulting in increasing numbers of children surviving into adolescence. Nevertheless, due to the longer duration that children are living with HIV, neurocognitive impairment has become more prevalent and can significantly impact quality of life. Neurocognitive impairment remains understudied in older HIV infected children. Infection by mother-to-child/vertical transmission of the virus (Wachsler-Felder and Golden 2002) occurs at a critical stage in brain development and can have a profound effect on the child's rapidly developing central nervous system (CNS). Clinically, in the pre-ART era, the most common primary HIV-associated paediatric CNS disease was HIV encephalopathy (HIVE) (Lobato et al. 1995) (Fleishman et al. 2005). However CNS compromise may present with milder cognitive or behavioural deficits in the absence of full criteria for a diagnosis of HIVE. There are guidelines for assessment of HIV-associated neurocognitive disorders (HAND) in HIV-infected adults, however there are no current guidelines for the assessment of a spectrum of neurocognitive disorders in children and adolescents. Neurocognitive impairment occurs in over 50% of untreated children with HIV (Govender et al. 2011; Van Rie et al. 2008; Wachsler-Felder and Golden 2002) and these deficits may remain prevalent despite ART (Valcour et al. 2011). The severity of the CNS outcomes in any particular child may be mediated by a complex interplay of factors (Valcour et al. 2011) including disease severity, viral load (Lobato et al. 1995; Nozyce et al. 2006) and ART use.

There has been extensive debate about the correct time to initiate ART in children, with updated guidelines established in 2013 to advise that all children younger than 5 years old receive ART irrespective of their clinical stage or immunological status**.** Unfortunately, not all children who need these life-saving therapies receive them (UNAIDS 2014). In addition, there are some asymptomatic children aged 5 years and older who are not eligible for ART under current guidelines, as they have been clinically and immunologically stable (2006)**.** These individuals have been described in the literature as "slow progressors", characterized as children or adolescents who were vertically infected with HIV, with CD4 count > 25% despite having received no or minimal therapy (defined as single or dual nucleoside therapy) before the age of 10 (2006). Given that there are a number of ART-naïve slow progressors in Sub-Saharan Africa (SSA), it is important to understand the long-term CNS outcomes in

these children (Archary et al. 2010). There are varied reports on neurocognitive deficits in 'slow progressor' ART-naïve children (Hoare et al. 2012; Nozyce et al. 2006).

Structural changes on neuroimaging have been frequently reported in children with neurological disease secondary to HIV (Hoare et al. 2013). Magnetic resonance imaging (MRI) studies have demonstrated that even children on early ART may present with superficial and deep white matter signal abnormalities suggesting that damage occurs soon after infection (Ackermann et al. 2014). Diffusion tensor imaging (DTI) provides a distinct method to assess the microstructural integrity of white matter (Wycoco et al. 2013). DTI is a non-invasive technique with rapid acquisition times, making it particularly amenable for use in children. The diffusion of water molecules depends on cortical architecture as well as fibre density, diameter, orientation, myelination and disease (Beaulieu 2002). Common DTI metrics include fractional anisotropy (FA) and mean diffusivity (MD). Higher MD and lower FA are traditionally representative of poorer directional diffusion indicating damaged neuronal microstructure (Wycoco et al. 2013). In the context of adult HIV, poorer cognitive performance is typically associated with decreased FA and increased MD (Hoare et al. 2011; Pomara et al. 2001; Wu et al. 2006). A number of studies using DTI in adult HIV have demonstrated reduced FA and increased MD in frontal white matter and the corpus callosum among adults infected with HIV (Gongvatana and Schweinsburg 2009; Hoare et al. 2011). Other DTI metrics that can be measured are radial diffusion (RD) which measures water diffusion perpendicular to axons and is therefore an indicator of myelin loss, and axial diffusion (AD) which measures movement parallel to axons, and thus serves as a marker of axonal damage (Song et al. 2002). Despite evidence of enhanced sensitivity to HIV infection, DTI methodology has rarely been applied in pediatric HIV. A previous small DTI study in vertically infected children included slow progressors only; these findings can therefore not be generalized to children on ART or those with HIVE (Hoare et al. 2012).

This study aims to determine the white matter microstructural integrity and neurocognitive functioning among HIV infected children compared to a HIV negative healthy control group, as measured by DTI and a neuropsychological battery sensitive to fronto-striatal pathology. The secondary aim was to conduct a within group comparison of the HIV infected children, comparing three groups: 'slow progressor' ART-naïve children, children stable on ART without encephalopathy and children with a clinical diagnosis of HIVE.

METHODS

Subjects and sampling

We recruited 75 HIV infected children and adolescents between the ages of 6 and 16 years, from the Infectious Diseases clinics at Red Cross War Memorial Children's Hospital, Groote Schuur Hospital and the community HIV antiretroviral clinics in the Western Cape Province of South Africa. A study co-ordinator identified potentially eligible candidates, and then invited those children and their parents/guardians to participate. Eligible children were those who were vertically infected, had attended the Infectious Disease clinic at least once, had initial and confirmatory HIV tests, and who met the criteria listed below. Children on antiretroviral treatment were eligible if they had been using ART for a minimum of 6 months. We also included HIV infected children who were ART-naïve and asymptomatic (i.e., no AIDS-defining stage 4 illness and a CD4 count of >25%). Children who attended the HIV clinic for routine monitoring and follow up and who were not on ART were included in this group if they were within the age bracket of the study and fulfilled the other inclusion and exclusion criteria of the study as a whole.

A control group of 30 HIV-negative children, matched to the patient group on age, gender, and ancestry were recruited from the same community as the HIV infected children in order to control for socio-economic status and quality of education.

Individuals in any of the groups with any of the following were excluded from participation: an uncontrolled medical condition, such as poorly controlled diabetes mellitus, epilepsy, or active tuberculosis requiring admission; an identified CNS condition (other than HIV), such as TB meningitis and bacterial meningitis, congenital cytomegalovirus, documented cerebrovascular accident, and lymphoma; a positive history of drug or alcohol exposure in pregnancy; a history of head injury with loss of consciousness greater than 5 minutes, or any radiological evidence of skull fracture; a history of significant perinatal complications such as hypoxic ischemic encephalopathy or neonatal jaundice requiring exchange transfusion, or neurodevelopment disorder not attributed to HIV; contra-indications to MRI (such as metal in the body and claustrophobia.)

After screening, parents or guardians provided full informed consent for the child to participate in the study, and the child provided assent at an age-appropriate level. Parents or

guardians were compensated for transport costs and loss of time. Ethical approval was obtained from the University of Cape Town's Faculty of Health Sciences research ethics committee.

HIV infected children were examined clinically including a full neurological examination and were assessed for the diagnosis of HIVE. A pediatric neurologist (KD) conducted the physical examination and assessment of encephalopathy. A diagnosis of HIVE was based on the presence of at least one of the 1994 CDC diagnostic criteria, (1) acquired microcephaly as demonstrated by head circumference measurement and (2) acquired symmetrical motor deficits manifested by 2 or more of the following: paresis, pathological reflexes, ataxia or gait disturbance. A clinical neuroradiologist examined all the MRI scans to look for evidence of other secondary causes of CNS effects (including congenital infections such as CMV). Blood samples were obtained in accordance with the clinic schedule for viral load and CD4 count.

Neuropsychological Assessment

Each participant was assessed using a standardized battery of neuropsychological tests commonly used in paediatric neuropsychology clinical assessment and research in South Africa. The neuropsychological battery was selected to be sensitive to fronto-striatal pathology (Table 1). Test instructions were translated and back-translated into Xhosa, and we took steps to ensure test administration maintained compliance with International Test Commission guidelines (Bartram 2001). The neuropsychological measures were adjusted for age by using age-adjusted scaled scores in the scoring of the tests. The test battery comprised the following instruments: the Symbol Search, Digit Symbol - Coding, and Digit Span subtests from the *Wechsler Intelligence Scale for Children - Fourth Edition* (*WISC-IV*; Wechsler, 2003) (Wechsler 2003). These tests measured information processing speed, attention and concentration, and working memory. The *Color Trails Test* (*CTT*) measured visual attention and cognitive flexibility; a category fluency test and the NEPSY-II Inhibition subtest (Brooks et al. 2009) measured elements of executive functioning (generativity and inhibition of automatic responses, respectively). The Fronto-striatal cognitive performance scores were derived by converting all neuropsychological test raw scores into *Z*-scores, and then calculating the average across the tests included. These Z-scores were calculated in SPSS using the mean and standard deviation from the control group of the larger sample.

Diffusion Tensor Imaging

Data acquisition

Diffusion-weighted imaging was performed at the Cape Universities Brain Imaging Centre (CUBIC) at Tygerberg on a 3T Siemens Allegra MRI scanner within 7 days of the neurocognitive assessment. Imaging was performed with a single-channel transmit-receive head coil in an axial orientation. The parameters were as follow: TR=8800 ms, TE=88ms, FOV of 220 mm, 1.8x1.8x2.0 mm^3 spatial resolution, 65 slices, 0% distance factor and 2x GRAPPA acceleration. There were 30 gradient directions with b=1000 mm/s^2 and 3 directions with b=0 mm/s^2. The sequence was repeated 3 times per subject to allow for redundancy in data acquisition, therefore acquisitions with bad image quality were excluded.

Data preprocessing

Images were corrected for eddy current distortions with FSL 4.8. Images were then imported into MATLAB R2012b for further preprocessing. Images were affine registered to the averaged b=0 mm/s^2 image of the first acquisition. For each of the co-registered acquisitions, outlier data points were calculated by determining the Z-values at 25^{th} and 75^{th} percentiles of the datasets and excluding any data that were at least 3 standard deviations from the mean. The corrected acquisitions for each subject were then averaged and exported to FSL 4.8 for further processing. Fractional anisotropy (FA), mean diffusivity (MD), axial (AD) and radial diffusivity (RD) images were created with a linear fit of the tensor model to the diffusion-weighted data.

After brain extraction with FSL BET, FA images were analyzed according to the standard tract-based spatial statistics (TBSS) pipeline (Smith et al. 2006), except that an appropriate individual FA image from the cohort was identified as a study-specific target for registration. The adult template included with TBSS is not appropriate for pediatric samples. To accomplish this, each subject's FA image was registered to every other subject. A score that represents the mean square spline coefficients of displacement across all dimensions characterized the warp parameters. The target image with the minimum mean displacement from every other subject was chosen as the representative image of the group.

Statistical analysis

Each subject's FA image was registered to the target image space and then up sampled to MNI space, taking into account the previous estimated registration parameters. From this an average FA image was created and thinned to generate a mean FA skeleton. This skeleton represents the center of white matter tracts common to this cohort. The skeleton was thresholded at 0.2 and FA, MD, AD and RD data were projected onto the skeleton before statistical analysis with FSL randomize. The four groups (HIV negative healthy controls, ART-naïve HIV infected children, HIV infected children on cART treatment and HIV positive children on ART treatment with HIVE) were compared with an ANCOVA model, including age, gender and level of education as covariates of interest for each of the 48 cerebral white matter regions. FSL randomize was performed on the data with threshold-free cluster enhancement (TFCE) at 5000 permutations, $p < 0.05$, corrected for multiple comparisons (Benjamini and Hochberg 1995). Regions that demonstrated significant differences in white matter microstructure between healthy controls and HIV infected children were exported to SPSS 22.0 for a partial correlation analysis with fronto-striatal scores ($p < 0.05$). Age, gender and level of education were controlled for in the analysis.

RESULTS

The HIV infected group and HIV-uninfected healthy control group were well-matched on demographic variables. (see Table 1). For all of the between-group comparisons described below, Levene's test was not significant. The HIV infected children performed significantly poorer on the global cognition score compared to the HIV uninfected controls ($p = .001$).

Of the 75 HIV infected children recruited for study, 20 were ART naïve slow progressors and 14 were assessed as having a clinical diagnosis of HIVE. The remaining 41 HIV infected children were stable on cART for a minimun of 6 months (see Table 2). In a between-group comparison amongst the HIV infected children, significant differences were found for years of completed education ($p=0.003$) and CD4 count, with the ART naïve slow progressors having the lowest CD4 count ($p=0.005$). However there were no significant differences in viral load or fronto-strial cognition between the HIV infected groups

Table 1: Demographic information and fronto-striatal cognition scores for HIV-positive children compared to HIV-negative controls.

	Group		
Variable	HIV-infected (N = 75)	HIV-negative (N = 30)	*P*
Gender (M / F)	44 / 31	15 / 15	.343
Ethnicity (Black African / mixed ancestry)	69 / 4	28/ 2	.786
Home language (isiXhosa / English / Afrikaans / Other)	64 / 5 / 3 / 1	28 / 1 / 1 / 0	.741
Age			.607
Mean (Std Dev)	10.03 (2.41)	9.76 (2.31)	
Range	6 - 16	6 - 16	
Education[a]			.870
Mean (Std Dev)	3.14 (1.86)	3.07 (1.94)	
Range	0 - 9	0 - 8	
Fronto-striatal Cognition			
Mean (Std Dev)	-.42 (.67)	.08 (.53)	.001**
Range	-3.51 – 1.18	-1.08 – 1.06	

NOTES: a: education indicated as of years of completed schooling. **indicates significant differences between the two groups at the 0.01 level with regards to the relevant variable.

Table 2: Clinical information for HIV infected subgroups.

	HIV-infected group			Oneway ANOVA
Variable	ART -naive[a] (N = 20)	ART-treated (N = 41)	HIVE (N = 14)	*p*
Age				.011
Mean (Std Dev) Range	9.35 (1.72) 6 - 11	10.76 (2.55) 6 – 16	8.86 (2.18) 6 – 13	
Education[b]				.003*
Mean (Std Dev) Range	2.72 (0.96) 1 – 5	3.73 (2.01) 0 – 9	1.93 (1.59) 0 – 5	
CD4 count				.005*
Mean (Std Dev) Range	615.65 (210.14) 230 – 1139	1013.53 (552.84) 257 – 2793	1061.00 (464.72) 394 – 1869	
Viral load[c]				.714
Mean (Std Dev) Range	24425.33 (45104.33) 0 – 150 000	29952.53 (150538.73) 0 - 904105	263.57 (953.59) 0 – 3575	
Frontostriatal cognition Mean (Std Dev) Range	-0.44 (0.93) -3.51 – 0.43	-0.37 (0.54) -1.77 – 1.18	-0.53 (0.64) -2.16 – 0.43	.741

NOTES: a: ART-naïve participants; b: education indicated as years of completed schooling; c: a viral load of zero (0) = lower than detectable. *indicates significant differences between the three groups at the 0.05 level with regards to the relevant variable

Abnormalities in fractional anisotropy and mean diffusivity

Results of the ANCOVA examining DTI indices between healthy control (30) and HIV infected group (75) revealed decreased FA, indicating damaged neuronal microstructure, in the left cerebral peduncle and fornix of the HIV infected group (p=0.001)(Table 4). Post-hoc comparisons between HIV infected groups revealed that HIV infected ART-naïve children (20) had lower FA in the fornix (p=0.008) and corpus callosum splenium (p=0.002) compared to controls. HIV infected children on ART treatment (40) also demonstrated lower FA in the corpus callosum body and fornix (p=0.002), and HIVE children (14) had lower FA in the cerebral peduncle (p=0.001) when compared to healthy controls. Between HIV infected groups, HIVE children displayed lower FA in the bilateral posterior limb of the internal capsule (p=0.025) and corpus callosum genu (p=0.049) compared to ART-naïve children, and lower FA in the left anterior corona radiata and left anterior limb of the internal capsule (p=0.046) when compared to HIV infected ART-treated children.

Further, results of the ANCOVA contrasting the healthy control (30) and HIV infected groups (75) found increased MD, indicating damaged neuronal microstructure, in the left superior corona radiata (p=0.001)(Table 4). Between-group analyses showed that HIV infected ART-naïve (20) patients had higher MD in the bilateral external capsule (p=0.001), left fornix/stria terminalis (p<0.001), corpus callosum body (p=0.001) and left anterior corona radiate (p=0.038) when compared to healthy controls. In addition higher MD in the left external capsule (p=0.009) and corpus callosum body (p=0.012) was found when HIV infected ART-naïve children were compared to those on treatment. For patients with HIVE (14), higher MD was demonstrated in the left external capsule (p<0.001) when compared to healthy controls as well as HIV infected ART-treated children (41). There was also higher MD in the right sagittal stratum (p=0.048) and superior corona radiata (p=0.049) of patients in children with HIVE when compared to healthy controls.

Table 3: Significant fractional anisotropy and mean diffusivity changes between controls and HIV infected groups at p <0.05

White matter anatomy	Local maxima MNI coordinates	Cluster (voxels) size	P value
Abnormalities in fractional anisotropy (FA) p < 0.05			
FA changes between HIV infected (75) and healthy control groups (30)			
LH CP and Fornix	-12; -18; -16	53878	0.001
HC (30) > HIV infected ART-naïve (20)			
Fornix	-21; -16; 44	42850	0.008
CC Splenium	-13; -52; 19	89	0.002
HC (30) > *HIV infected ART-treated (40)*			
CC Body and Fornix	23; 12; -18	63926	0.002
HC (30) > HIVE (14)			
LH CP and Fornix	-11; -18; -18	80085	0.001
HIV infected ART-naïve (20) > HIVE (14)			
LH PLIC	-19; -15; 2	1225	0.043
RH PLIC	23; -18; -1	766	0.045
CC Genu	-10; 29; 0	87	0.049
HIV infected ART-treated (41) > HIVE (14)			
LH ACR and LH ALIC	-18; 27; -6	1171	0.046
Abnormalities in mean diffusivity (MD) p < 0.05			
MD changes between HIV infected and HC			
LH SCR	-20; -6; 40	15823	0.001
HIV infected ART-naïve > HC			
RH EC	32; -20; 2	8144	0.001
LH Fornix/ST and LH EC	-32; -20; -9	7734	< 0.001
CC Body	9; 13; 22	4006	0.001
LH ACR	-16; 23; -9	262	0.038

HIV ART-naïve > ART treated			
LH EC	-30; -6; 18	1724	0.009
CC Body	17; 13; 29	912	0.012
	-20; -3; 42	829	0.005
HIVE > HC			
LH EC	-33; -10; 8	4163	< 0.001
RH SS	40; -41; -7	73	0.048
RH SCR	24; 5; 35	23	0.049
HIVE > HIV infected ART-treated			
LH EC	-33; -4; 10	564	0.004

Results are shown at $p < 0.05$ corrected for multiple comparisons. Abbreviations are as follow: LH: Left hemisphere; RH: Right hemisphere; CP: Cerebral Peduncle; CC: Corpus Callosum; PLIC: Posterior Limb of Internal Capsule; ACR: Anterior Corona Radiata; ALIC: Anterior Limb of Internal Capsule; SCR: Superior Corona Radiata; EC: External Capsule; ST: Stria Terminalis; SS: Sagittal Stratum; HIVE: HIV Encephalopathy; HC: Healthy Controls

Abnormalities in axial and radial diffusivity

Results of the ANCOVA revealed decreased AD, a measure of axonal damage, in the left superior corona radiata and fornix (p=0.002)(Table 5). HIV infected ART-naïve children showed lower AD in the right superior longitudinal fasciculus (p=0.046) when compared to healthy controls. HIVE children had lower AD in the corpus callosum genu (p=0.017) when compared to healthy controls. Among HIV infected ART-treated children, AD in the corpus callosum body (p=0.009) was significantly lower than HIV infected ART-naïve children.

Results of the ANCOVA contrasting the healthy control (30) and HIV infected groups (75) found increases in RD, a measure of myelin loss, in the left external capsule ($p<0.001$) and superior corona radiata (p=0.001) and right posterior limb of internal capsule (p=0.001) and cerebral peduncle (p=0.027)(Table 5). Abnormalities were also evident in the corpus callosum splenium (p=0.024). Higher RD was found in the right sagittal stratum and fornix ($p<0.001$) of HIV infected ART-naïve patients when compared to healthy controls, as well as higher RD in the posterior limb of the internal capsule, external capsule (p=0.007) and superior corona radiate (p=0.003) when compared to HIV infected patients on treatment. For HIV infected ART-treated patients, higher RD was demonstrated in the left anterior corona radiata (p=0.038) when compared to healthy controls. For HIVE children, higher RD was found in the left sagittal stratum ($p<0.001$) and corpus callosum body (p=0.019) when compared to healthy controls, as well as higher RD in the bilateral anterior corona radiata (p=0.004) and left sagittal stratum (p=0.006) when compared to HIV infected patients on treatment but without HIVE.

Association of mean diffusivity with measures of cognition

There were no significant associations of cognitive scores with the above-mentioned white matter regions for FA, AD and RD. However for MD of the right external capsule, there was a modest negative association with cognition ($r = -0.262$; $p = 0.036$).

Table 4: Significant axial and radial diffusivity changes between controls and HIV infected groups at $p < 0.05$

White matter anatomy	Local maxima MNI coordinates	Cluster size (voxels)	P value
Abnormalities in axial diffusivity (AD) $p < 0.05$			
AD changes between HIV infected (75)		*and HC(30)*	
LH SCR and Fornix	-19; -4; 39	30924	0.002
HC > HIV ART naive			
RH SLF	35; -34; 27	174	0.046
HC > HIVE			
CC Genu	-4; 27; 4	2193	0.017
HIV ART-naïve > HIV ART-treated			
CC Body	17; 13; 29	674	0.022
	-20; -3; 42	634	0.009
Abnormalities in radial diffusivity (RD) $p < 0.05$			
RD changes between HIV infected (75)		*and HC (30)*	
LH EC	-34; -10; 6	9026	< 0.001
RH PLIC	27; -17; 17	7519	0.001
LH SCR	-20; -6; 40	2381	0.001
RH CP	10; -16; -17	1251	0.027
CC splenium	-12; -41; 14	714	0.024
HIV ART-naïve > HC			
RH SS and Fornix	40; -24; -13	26419	< 0.001
HIV ART-naïve > HIV ART-treated			
LH PLIC and EC	-25; -17; 14	2489	0.007
LH SCR	-20; -3; 41	1970	0.003
	-27; 7; 27	56	0.048
HIVART-treated >HC			
LH ACR	-23; 34; 1	970	0.038

HIVE > HC			
LH SS	-40; -23; -11	17518	< 0.001
CC genu	17; 23; 24	1499	0.019
HIVE > HIV infected ART-treated			
LH ACR	-24; 11; 17	1738	0.004
RH ACR	31; 8; 11	423	0.017
LH SS	-39; -25; -9	1037	0.006

Results are shown at $p < 0.05$ corrected for multiple comparisons. Abbreviations are as follow: LH: Left Hemisphere; RH: Right Hemisphere; SCR: Superior Corona Radiata; SLF: Superior Longitudinal Fasciculus; CC: Corpus Callosum; PLIC: Posterior Limb of Internal Capsule; CP: Cerebral Peduncle; EC: External Capsule; PTR: Posterior Thalamic Radiation; RIC: Retro lenticular part of Internal Capsule; SS: Sagittal Stratum; ACR: Anterior Corona Radiata; HIVE: HIV Encephalopathy; HC: Healthy Controls

DISCUSSION

There were three main findings here; first, children with HIV display a range of damage to neuronal microstructure and fronto-striatal cognitive impairment when compared to negative controls, irrespective of age or treatment status. Second, children on the severe end of the neurocognitive spectrum with HIVE have more damage to neuronal microstructure. Thirdly, while the use of ART did not protect children from axonal damage, we found that children off treatment, irrespective of clinical status, had more damage to neuronal microstructure than those on ART. Specifically, ART appears to reduce the myelin loss associated with HIV but not axonal damage. This may indicate that ART is able to reduce the inflammatory response in the CNS, but is unable to reverse existing axonal damage.

In this study we found damage to neuronal microstructure when comparing HIV infected older children (n=75) and adolescents to an HIV negative control group (n=30). A recent study by Sarma et al. (2013) identified multiple areas of white matter atrophy among a small cohort of perinatally-infected adolescents, including the corpus callosum, external capsule, ventral temporal white matter, cerebral peduncles, and basal pons when compared to controls (Sarma et al. 2014). A number of these white matter regions are consistent with findings in our study. Using a composite cognitive score we found significantly poorer performance in the HIV infected children compared to controls. In linking neurocognitive deficits to white matter microstructural integrity, however we found a modest correlation with MD in the external capsule only. A DTI study of traumatic brain injury has previously found an association of poor white matter integrity with poor performance on tests of memory, attention and executive function (Kraus et al. 2007). Studies in children with HIV have shown a typical pattern of cognitive deficits in attention, executive function, working memory and processing speed domains (Boivin et al. 1995) A previous small study examining vertically infected children receiving ART, found that HIV patients with neuroimaging abnormalities were more likely to present with compromised social skills and have lower mean general intelligence scores (Thomaidis et al. 2010). The composite cognitive score in this study also did not find significant differences

between HIV infected groups. While aggregate global cognitive scores are commonly utilized in HIV research among adults (Heaton et al. 2010), it is possible that cognitive difficulties are masked by the utilization of the composite scores in paediatric HIV.

Comparisons between subgroups in the HIV infected children found the children with a clinical diagnosis of HIVE (n=14) to have greater damage to neuronal microstructure when compared to both ART naïve and ART treated HIV infected children. In today's era of ART we would expect few children with HIV infection to experience CNS manifestations indicative of encephalopathy, however 18.7 % (n=14) of the HIV positive children stable on ART in this study had a clinical diagnosis of HIVE. Previous studies using MRI to compare encephalopathic and non-encephalopathic children have found children with HIVE to have significantly greater ventricular- and bicaudate-brain ratios, with basal ganglia and subcortical atrophy on MRI (Hoare et al. 2013). Children with HIVE have higher CD4+ cell counts than the ART-naïve group and equal CD4+ counts to the ART-treated group.

Children stable on ART (n=41) without encephalopathy had lower AD in the corpus callosum when compared to ART naïve children. These findings indicate that children with previous impaired immune function, prior to treatment with ART, may be at greatest risk for axonal damage secondary to HIV, or that ART is unable to reverse existing axonal damage. A recent publication using MRI found that white matter structural abnormalities occur very early after birth, and that even initiation of ART by eight weeks of life they be too late to prevent HIV from damaging white matter in the CNS (Ackermann et al. 2014). Thus, the window of opportunity for ART to prevent white matter damage may be very small, and despite immune recovery in children on ART they remain at risk for CNS disease.

Damage to neuronal microstructure, as reflected by higher MD and RD was found when HIV infected ART-naïve children were compared to those on ART. Consistent with a previous region of interest DTI study of ART naïve slow progressors conducted by our team, the ART naïve participants in this study (n=20) had damage to neuronal microstructure in the corpus callosum and the superior longitudinal fasciculus (Hoare et al. 2012). In the context of immunological stability, slow progressors have previously been identified as healthy and expected to have favorable

outcomes relative to age-matched HIV infected peers, who have experienced a significant decline in CD4 count and subsequently required treatment with ART. However, this study suggests that while slow progressors exhibit intact peripheral health, there is evidence of significant brain dysfunction in this cohort when compared with HIV infected children stable on ART. Given the age of the children in the ART treated cohort, they would have qualified for ART either due to low nadir CD4 count or developing an AIDS related illness requiring treatment with ARV's. Low nadir CD4 count have previously been associated with neuropsychological impairment (Ellis et al. 2011), yet treatment with ARVs in this cohort is associated with lower MD and RD when compared to ART naïve slow progressors. MD increases measure higher diffusion out of the axon and together with increases in RD in the ARV naïve group, may suggest that demyelination or inflammation is the prominent disease process in white matter in ARV naïve children. These findings differ from the changes in AD described above and suggest that myelin loss is more prominent in the ARV naïve cohort when compared to children treated with ART for a minimum of 6 months. The ARV naïve group had the lowest mean CD4 count of 615. Immune recovery is typically achieved after initiation of ART from the nadir (Ellis et al. 2011), and this is reflected by the current higher mean CD4 count of the ART treated group of 1013,53. Despite the fact that children on treatment in this study were well on clinical examination, the persistence of detectable viral loads in some of the children on ART was likely to have impact on their ongoing disease state and certainly on the developing central nervous system. Having children on treatment does not guarantee that the virus will be controlled. Factors, which may impact viral load, include adherence and resistance. Adherence is especially problematic in the adolescent age group. This study highlights once again the need for attention to be paid to adherence and viral suppression in order to optimally protect the brains of these vulnerable children. Although cross-sectional design hampers drawing conclusions about causality, our findings suggest that initiation of ART as early as possible, even in slow progressors who exhibit intact peripheral health, might reduce the risk of developing damaged neuronal microstructure of the CNS.

In addition to the cross-sectional design a number of limitations deserve mention. The subgroup analyses varied in size, and the ART and HIVE groups in particular were unequal. In addition, the children in the sample were on differing ART regimens, of

varying ages, gender and level of education, which may have impacted the outcomes, though education was controlled in regression analyses. The composite cognitive score may have been too narrow a measure of cognition, however, this was an effort to reduce the multiple comparisons that were performed in the 48 white matter brain regions. In addition, the lack of data on duration of ART, other than a minimum of 6 months treatment and nadir CD4 meant the relationship between ART and DTI measures should be interpreted with caution. There are limitations associated with DTI as a quantitative imaging technique. Changes in anisotropy could be due to cellular structures such as axons and myelin, or due to varying macroscopic organization of the fibers, and significant subject movements during a scan it can influence the results obtained by DTI. Although other congenital infections and incidental CNS abnormalities were excluded as far as possible on history, clinical examination and on clinical review of the MRI scans, it remains a possibility that there may be some overlapping effects of undiagnosed conditions such as congenital CMV.

This is the first study to examine HIVE, ART treated and ARV naïve slow progressors in HIV-infected children, and investigate the association with white matter microstructure using DTI. The study took place in South Africa, one of the countries most affected by HIV/AIDS with the highest rate of new HIV infections in the world (UNAIDS 2014). Our findings suggest that children on ART remain at risk for developing CNS disease, and that initiation of ART as early as possible might reduce the risk of developing damaged neuronal microstructure in the CNS of slow progressors who exhibit intact peripheral health. These findings provide additional support that all children infected with HIV should be considered for antiretroviral treatment regardless of age or CD4 count to potentially prevent neurocognitive impairment. Future work could usefully focus on DTI measures as a quantitative imaging biomarkers in larger longitudinal studies. Correlates of white matter damage need to be sought, including whether measures of central viral load, treatment duration, details of ART prescription and treatment adherence impact on the long term development of neurocognitive disorders in pediatric HIV. This work will ensure that more robust data on the spectrum of neurocognitive and neuroimaging changes in HIV infected children are available to inform

The next chapter will investigate whether a comprehensive neurocognitive battery, an assessment of functional competence, and the American Academy of Neurology (AAN) system for diagnosing neurocognitive disorder in adults can detect a spectrum of neurocognitive disorder in HIV-infected youth.

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Chapter 6

Applying the HIV Associated Neurocognitive Disorder diagnostic criteria to HIV infected youth

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ABSTRACT

The American Academy of Neurology (AAN) proposes guidelines for assessment of HIV-associated neurocognitive disorders (HAND) in HIV-infected adults. However, there are still no diagnostic criteria for a spectrum of neurocognitive disorders (ND) secondary to HIV infection for children. It is unclear whether the HAND criteria can be effectively utilized in children and adolescents. We used a comprehensive neurocognitive battery, an assessment of functional competence, and the AAN system for diagnosing ND in a cohort of HIV-infected children and adolescents (n=86) and HIV-negative controls (n=34) to establish whether this system could detect a spectrum of ND in HIV-infected older children and adolescents. Compared to a well-matched control group of HIV-negative children, HIV-infected children performed significantly more poorly on tests of verbal IQ, full scale IQ, processing speed, finger tapping, verbal memory, expressive language, cognitive flexability and inhibition. HIV-infected children were also more likely to have impaired total competence on the Child Behaviour Checklist (CBCL). Using the AAN criteria for HAND, we found that 45.35% of the 86 HIV-infected children could be diagnosed with a ND. Furthermore, children with HIV encephalopathy (HIVE) were 9.4 times more likely to have a diagnosis of a major ND compared to HIV-infected children without HIVE. The AAN HAND criteria designed for adults was able to identify children and adolescents with important functional cognitive impairments who don't fit criteria for HIVE and would therefore not have been identified otherwise. This has major clinical implications in terms of the importance of managing HIV infected children.

INTRODUCTION

As neurocognitive impairment (NCI) can have deleterious effects on children's ability to function socially and scholastically, it is important for clinicians to be able identify and manage these impairments. In South Africa, differences in treatment guidelines and differential access to antiretroviral treatment (ART) over the past two decades have resulted in great variability in disease severity and ART exposure among HIV-infected children and adolescents (Laughton et al. 2013). Recently circulated national guidelines for the initiation of ART in South Africa now recommends treatment for the <5 years age group (with older children needing to meet clinical or immunological criteria). However, until this year, official national policy only recommended immediate treatment for infants under 12 months of age. As a result a number of adolescents in South Africa would have initiated ART only after immune compromise or after the diagnosis of HIV encephalopathy (HIVE), resulting in neurocognitive deficits that remained permanent despite ART (Laughton et al. 2013; Patel et al. 2009). While other HIV-infected children have never initiated ART as they are slow progressors who have not yet met the current clinical criteria to be eligible for ART(2006).

In the adult literature, there is growing understanding of the spectrum of cognitive disorders secondary to HIV infection, and their impact on functional competence (Rackstraw 2011). The American Academy of Neurology (AAN) system proposes four categories of HIV associated neurocognitive disorders in adults (HAND). However, for children there are still no diagnostic criteria for a spectrum of neurocognitive disorders secondary to HIV infection. The aim of this study was to apply the AAN system for diagnosing NCI in a cohort of HIV-infected youth and to thus establish whether this system was able to detect a spectrum of neurocognitive disorders (ND). A secondary outcome was to establish whether the AAN system could detect higher rates of major neurocognitive disorders (ND) in HIV infected youth with HIVE.

METHODS

Subjects and sampling

120 children and adolescents were recruited for the study. We recruited 86 HIV-infected children and adolescents (aged 6-16 years) from the Infectious Diseases clinics at Red Cross War Memorial Children's Hospital, Groote Schuur Hospital and the community HIV antiretroviral clinics in the Western Cape Province of South Africa. Potentially eligible candidates, and their parents/guardians were invited to participate. Eligible children were those who were vertically infected, had attended the Infectious Disease clinic at least once, had initial and confirmatory HIV tests, and who additionally met the criteria listed below. Children on antiretroviral treatment were eligible if they had been using ART for a minimum of 6 months. We also included HIV-infected children who were ART-naïve and asymptomatic slow progressors (i.e., no AIDS-defining stage 4 illness and a CD4 count of >25%). A control group of 34 HIV-negative children, matched to the patient group on age, gender, and ancestry were recruited from the same community as the HIV-infected children in order to control for socio-economic status and quality of education. Individuals with any of the following were excluded from participation: a history of significant perinatal complications such as hypoxic ischemic encephalopathy or neonatal jaundice requiring exchange transfusion, a positive history of drug or alcohol exposure in pregnancy; an uncontrolled medical condition, such as poorly controlled diabetes mellitus, epilepsy, or active tuberculosis requiring admission; an identified CNS condition (other than HIV), such as TB meningitis and bacterial meningitis, documented cerebrovascular accident, and lymphoma; a history of head injury with loss of consciousness greater than 5 minutes, or any radiological evidence of skull fracture; or neurodevelopment disorder not attributed to HIV.

Procedure

After screening, parents/guardians provided informed consent for the child to participate, and the child provided assent at an age-appropriate level. Parents/guardians were compensated for transport costs and loss of time. Ethical

approval was obtained from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee.

HIV-infected children were examined clinically (including a full neurological examination) and were assessed for the diagnosis of HIVE by a pediatric neurologist (KD). A diagnosis of HIVE was based on the presence of at least one of the two physical criteria in the 1994 CDC diagnostic criteria: (1) acquired microcephaly as demonstrated by head circumference measurement, and (2) acquired symmetrical motor deficits manifested by 2 or more of the following: paresis, pathological reflexes, ataxia or gait disturbance (Donald et al. 2015). Neurodevelopmental criteria were not used as they may have confounded the neurocognitive assessment. Our aim was to establish whether the AAN system could detect higher levels of major ND in children with a clinical disgnosis of HIVE. Blood samples were obtained in accordance with the clinic schedule for viral load and CD4 count. Median income by area of residence was used as a measure of socioeconomic status (SES). The median was taken from the City of Cape Town household income census data (2011) for small areas inside the city.

Neurocognitive Assessment

Each participant was assessed using a battery of standardized neurocognitive tests commonly used in paediatric neuropsychological assessment and research in South Africa. Tests were administered in the children's home language. Test instructions were translated and back-translated into Xhosa, and we took steps to ensure test administration maintained compliance with International Test Commission guidelines (Bartram 2001). Where possible the neurocognitive measures were adjusted for age by using age-adjusted scaled scores in the scoring of the tests. General intellectual functioning was measured using the Wechsler Abbreviated Scale of Intelligence (WASI)(Axelrod 2002). The Fingertip Tapping subtest from the NEPSY-II (Davis and Matthews 2009) and the Grooved Pegboard Test (Bryden and Roy 2005) measured psychomotor speed and coordination. Subtests from the Wechsler Intelligence Scale for Children (WISC-IV)(Wechsler 2003) measured information processing speed. The Rey Complex Figure Test (RCFT)(Watanabe et al. 2005) measured visuo-constructional ability. Expressive Language was measured using the the Boston Naming Test – Short Form-South Africa (BNT-SF-SA), category and phonemic fluency (Roth 2011). Learning and memory (visual and verbal) were

measured using the immediate and delayed recall trials of the RCFT and the Hopkins Verbal Learning Test-Revised (HVLT-R)(Benedict et al. 1998). Measures used to assess aspects of executive functioning included: the WISC-IV Digit Span backwards (DS backwards) subtest, the Color Trails Test 2 (CTT2) and the NEPSY-II Inhibition subtests.

Assessment of Functional competence

The Child Behaviour Checklist (CBCL)(Achenbach et al. 2007) Total Competence Index required a parent or caregiver to rate the child's competency at completing age-appropriate everyday activities successfully and independently. This instrument is suitable for use in children aged 6-18 years, and has good cross cultural validity(Weisz et al. 1993). Higher scores on this scale indicate better functioning. A score of 36-40 (borderline range) indicates that the child requires assistance with what should be age-appropriate everyday activities. A score of 35 or less (clinical range) indicates significant impairment social, school or other everyday activities.

Statistical Analyses

Regarding sociodemographic characteristics, HIV-infected participants and HIV-negative participants were compared using independent-samples *t*-tests (for continuous variables) and Pearson's chi-square test (for categorical variables). In the creation of neurocognitive domains, we set .60 as the lower bound of Cronbach's alpha. Composite neurocognitive scores were then created for each domain, using a method described by Ferrett et al. (2010). The composite scores for each domain were required to use the AAN system for diagnosing HAND. Regarding neurocognitive and adaptive functioning outcome variables, the two groups were compared using independent-samples *t*-tests.

Determination of neurocognitive disorder status

We used scores on the above mentioned neurocognitive domains, together with an evaluation of functional competence, to classify participants into one of four HAND categories, based on the updated AAN criteria (Antinori et al. 2007): No impairment; ANI (diagnosed if the participant scored more than 1 SD below the mean on at least 2 cognitive domains); minor ND (diagnosed if the participant scored more than 1 SD below the mean on at least 2 cognitive domains, and had a CBLC Total Competence

Index score of ≤ 40); and major ND (diagnosed if the participant score more than 2 SD below the mean on at least 2 cognitive domains, and had a CBCL Total Competence Index score of ≤ 35).

RESULTS

The two groups were well matched on sociodemographic characteristics (see Table 1). Compared to a well-matched control group of HIV-negative children, HIV-infected children performed significantly more poorly on tests of verbal IQ and full scale IQ. Regarding parental report on the CBCL Total Competence Index, the analysis detected a significant between-group difference, with HIV-infected participants showing lower levels of competence in completing age-appropriate everyday activities (see Table 2).

Table 1

HIV-infected versus HIV-negative Groups: Sociodemographic characteristics (N = 120)

Variable	Group		t / χ^2	p	ESE
	HIV-infected (n = 86)	HIV-negative (n = 34)			
Age (years)	9.80 (2.39)	9.71 (2.15)	-0.21	.84	0.04
Education (years)[a]	2.92 (1.88)	2.97 (1.85)	0.14	.89	-0.03
Sex (M:F)	47:39	18:16	0.03	.87	0.02
Neighborhood median income[b]			5.52	.36	0.22
0	8	1			
1-4800	0	0			
4801-9600	0	0			
9601-19200	0	0			
19201-38400	57	29			
38401-76800	1	2			
76801-153600	9	2			
153601-307200	1	0			
307201-6114400	1	0			
Race (Black African: Coloured)	79:5	32:2	< 0.001	.99	0.001
Language (X: E: A:O)	72:8:3:1	31:1:2:0	2.17	.54	0.14

Note. For the variables *Age* and *Education*, means are presented with standard deviations in parentheses; for *Language*, X = isiXhosa, E = Englishman = Afrikaans, O = other. All t values and their associated p values are based on the assumption of equal variances, because in each case Levene's test for equality of variances was non-significant. All p values are two-tailed. ESE = effect size estimate (in this case, Cohen's d [for continuous variables] or Cramer's V [for categorical variables];

[a]n = Highest level of education completed.

[b]n = the variable reflects data for income derived from participation in formal economic activities (City of Cape Town Census information, 2011); it does not include income derived from participation in the informal economy (e.g., from working as a street vendor, taxi driver, or home-based care worker). Data are given in South African rands (ZAR). At the conclusion of recruitment, the US$: ZAR exchange rate was 1:11.56.

[c]n = the language self-reported by the participant as his/her home language.

Table 2

HIV-infected versus HIV-negative cognitive and behavioral functioning (N = 120)

Variable	Serostatus: Positive (n = 86)	Serostatus: Negative (n = 34)	t	p	ESE
General Intellectual Functioning					
WASI[b]					
Verbal IQ	76.99 (12.11)	87.41 (15.07)	3.93	**< .001*** **	.80
Performance IQ	83.05 (11.04)	84.53 (12.11)	0.64	.26	.13
Full Scale IQ	83.01 (7.87)	87.85 (8.69)	2.93	.002**	.59
CBCL					
Total Competence[s]	35.73 (6.72)	39.77 (9.41)	2.51	.005**	.53

Note. Means are presented with standard deviations in parentheses. All t and p values are based on the assumption of equal variances. All p values are one-tailed. CBCL = Child Behavior Checklist; ESE = effect size estimate; in this case, Cohen's d. *p < .05. **p < .01. ***p < .001. Bolded p values indicate p < .001 (i.e., a statistically significant difference at the Bonferroni-corrected value, .05/43 = .001).

Eight neurocognitive domains were created, however three of those domains (Visuoconstructional Ability, Cognitive Flexibility and Working Memory) were each estimated by a single test (ROCF Copy, Colour trails test part 2 and Digit Span-Backward, respectively) (see Table 3)

Regarding performance on the neurocognitive test battery, the analyses detected significant between-group differences (in favor of the HIV-negative controls) in the domains of psychomotor speed and co-ordination, verbal memory, expressive language, cognitive flexability and inhibition (see Table 3).

Table 3
Neurocognitive Test Performance within Composite Cognitive Domains (n=120)

Variable	Cronbach's α	Serostatus Positive (*n* = 86)	Negative (*n* = 34)	*t*	*p*	ESE
Psychomotor Speed and Coordination[a]	.66	-0.30 (0.83)	< .001 (0.90)			
WISC-IV Processing Speed Index[b]		70.31 (13.05)	76.56 (10.95)	2.46	.01*	.50
NEPSY-II Fingertip Tapping						
Dominant hand		9.13 (2.51)	9.44 (2.38)	0.61	.27	.12
Non-dominant hand		8.70 (2.32)	9.91 (2.48)	2.52	.005**	.51
Grooved Pegboard Test[c]						
Dominant hand		80.20 (29.37)	77.74 (24.72)	-.43	.34	.09
Non-dominant hand		95.19 (33.60)	97.18 (36.12)	.28	.39	.06
Visuoconstructional/Visuospatial Ability						
RFCT Copy[d]		18.45 (7.84)	20.03 (7.36)	1.00	.16	.20
Expressive Language[e]	.82	-0.33 (0.96)	0.01 (0.84)			
BNT-SA-SF[f]		6.48 (2.04)	7.09 (1.76)	1.51	.07	.31
Category Fluency[g]		14.76 (6.48)	17.09 (5.65)	1.81	.04*	.37
Phonemic Fluency[h]		12.05 (6.67)	13.91 (6.07)	1.39	.08	.28
Verbal Memory[i]	.60	-0.31 (1.04)	< .001 (0.64)			
HVLT-R						
Trials 1-3 Total		18.12 (6.52)	18.38 (5.97)	0.20	.42	.04
Delayed Recall		5.77 (2.76)	7.09 (3.60)	2.14	.035*	.43
Recognition		10.14 (2.62)	10.85 (1.37)	1.92	.02*	.30
Visual Memory[d]	.93	-0.18 (1.01)	< .001 (0.98)			
RCFT						
Immediate Recall		10.51 (5.67)	11.38 (5.60)	0.74	.23	.15
Delayed Recall		9.96 (5.44)	11.02 (5.01)	0.96	.17	.19
Working Memory						

Variable	Cronbach's α	Serostatus Positive (n = 86)	Negative (n = 34)	t	p	ESE
WISC Digit Span Backward[b]		6.55 (2.14)	6.97 (2.17)	0.95	.17	.19
Cognitive Flexibility						
Color Trails Test Part 2[c]		223.22 (128.84)	167.97 (63.63)	-2.4	.01*	.48
Inhibition	.62	-0.33 (0.71)	-0.02 (0.78)			
NEPSY-II						
Inhibition[b]		5.74 (2.96)	6.94 (3.17)	1.94	.03*	.39
Switching[j]		5.13 (2.95)	6.31 (2.84)	1.94	.03*	.40
Naming[k]		6.00 (3.51)	6.44 (3.79)	0.60	.27	.12

Note. For each domain, data presented in the third and fourth columns are *z*-scores (means, with standard deviations in parentheses). For each subtest, data presented are age-adjusted scaled scores, *T*-scores, or raw scores (see details in foot scripted notes below). WISC-IV = Wechsler Intelligence Scale for Children; RCFT = Rey Complex Figure Test; BNT-SA-SF = Boston Naming Test, South African Short Form; HVLT-R = Hopkins Verbal Learning Test – Revised.

Of the 86 HIV infected youth, 18 youth stable on ART had a clinical diagnosis of HIVE, 45 were stable on ART with no diagnosis of HIVE and 23 were ART naïve slow progressors (see Table 4). Using the AAN criteria for HAND, we found that 45.35% of the 86 HIV-infected youth could be diagnosed with a ND (either ANI, minor ND, or major ND), 7.14% (n=6) had a major ND, 32.59% (n=28) had a minor ND, and 5.81% (n=5) had ANI. Findings for ARV-naïve slow progressors (26%) and controls were similar when using the AAN criteria (29%) (Table 4).
Analysis of the data presented in Table 4 investigated the association, within the sample of HIV-infected youth (n=86), between being diagnosed with HIVE and presenting with a major ND. Pearson's chi-squared test of contingency detected a significant association, χ^2 (1) = 8.15, p = .004, Cramer's V = .31. The odds ratio for this analysis was 9.42 (95% CI = 1.57, 56.62), suggesting that those HIV-infected youth with a diagnosis of HIVE were more than 9 times more likely to be observed as experiencing a major ND as those with no clinical diagnosis of HIVE.

Table 4

Cross-Tabulation: HAND Diagnosis and Diagnosis via Clinical subgroup (n = 120)

	Serostatus / Clinical subgroup			
Diagnosis by Testing	HIV-negative (n = 34)	ART-naïve SP (n = 23)	HIV+ART+ (n = 45)	HIVE (n = 18)
Major ND	0 (0)	1 (4.35)	1 (2.22)	4 (22.22)
Minor ND	6 (17.64)	3 (13.04)	19 (42.22)	6 (33.33)
ANI	4 (11.76)	2 (8.70)	3 (6.67)	0 (0)
No impairment	24 (70.59)	17 (73.91)	22 (48.89)	8 (44.44)

Note. Data presented are raw numbers, with percentages in parentheses. ART-naïve SP = HIV-positive ART-naïve slow progressors; HIV+ART+ = HIV-positive ART-treated; HIVE = HIV encephalopathy; ND = neurocognitive disorder; ANI = asymptomatic neurocognitive impairment.

DISCUSSION

We report on the first detailed evaluation of a spectrum of HIV-associated neurocognitive disorders in HIV-infected youth in South Africa. The youth included in this study were infected by perinatal transmission of the virus (Wachsler-Felder and Golden 2002), and infection at this young age can have a profound effect on a child's neurocognitive function. Using the American Academy of Neurology criteria, we found a prevalence of ND of 45.35% in our group of HIV-infected youth. Specifically we noted rates of major ND of 7.14% and of mild ND of 32.59%. Youth with a clinical diagnosis of HIVE were 9.4 times more likely to have a diagnosis of a major ND compared to HIV-infected youth without a clinical diagnosis of HIVE. Furthermore, our detailed neurocognitive battery found impaired functioning on tests of verbal IQ, full scale IQ, processing speed, finger tapping, verbal memory, expressive language, cognitive flexability and inhibition in HIV infected youth when compared to a well matched control group. HIV-infected youth were also more likely to have their parents report them demonstrating impaired competence in completing age-appropriate everyday activities across various domains of functioning.

The most commonly reported measure of neurocognitive outcome in HIV-infected youth is general cognition. HIV-infected children and adolescents perform more poorly in general cognition assessments than negative controls (Hoare et al. 2012; Ruel et al. 2012), although some studies have found no significant differences between groups (Koekkoek et al. 2008; 2006). Our neurocognitive battery also included a number of tests assessing specific cognitive domains, as global cognitive scores may overlook subtle deficits in one or more domains specific to HIV infected children. In keeping with previous studies HIV infected children were found to perform significantly poorer in executive function tasks (Koekkoek et al. 2008; Ruel et al. 2012), particularly in terms of cognitive flexibility and inhibition. Poor executive function could lead to significant difficulties with daily activities (Poletti 2009). HIV-infected youth in this study also had difficulties with processing speed, verbal memory, motor coordination and expressive language, again in keeping with findings from previous studies (Ruel et al. 2012; Smith et al. 2012; 2006). Processing

speed has been associated with improved capacity for working memory, reasoning and accuracy in solving arithmetic word problems, and consistently predicts performance on cognitive tasks (Kail and Ferrer 2007). While lower scores on visual–spatial processing and visual memory have been described in HIV infected children (Smith et al. 2002), this study did not find significant impairment compared to HIV negative controls. As children transition to adolescence, language and reading skills are essential building blocks for literacy and future academic success (Laughton et al. 2013). There is evidence that language is negatively affected in HIV infected children (Hoare et al. 2012; Smith et al. 2012), with our study finding significant impairment in expressive language. In addition to multiple environmental challenges, many HIV-infected children perform significantly worse than matched controls on tasks of processing speed, memory, attention and visual–spatial processing (Abubakar et al. 2008; Hoare et al. 2012; Smith et al. 2012; 2006). Poor executive functioning in adolescents having to cope with multiple environmental challenges could translate into significant functional impairment (Poletti 2009).

We measured functional competence, as part of the AAN criteria of assessing everyday functional impairment, and found HIV infected youth to have significantly lower scores on the CBCL Total Competence Index. These kinds of instruments could provide a meaningful way of assessing how youth function in their own environments (Laughton et al. 2013). The CBCL measures the ability to function effectively in school, home and social settings (Achenbach et al. 2007). Cognitive assessments alone may not be the appropriate measurement tools to assess the ability of children and adolescents to function in real-life situations (Laughton et al. 2013). Thus the inclusion of an assessment of everyday functioning is an important part of diagnosing a ND in HIV infected youth.

Using the AAN criteria, the CBCL and a comprehensive pediatric neurocognitive battery, we were able to detect a full spectrum of ND in HIV-infected youth. Previous studies have reported on both subtle NCI in HIV infected school-age children (Koekkoek et al. 2008) as well as the presence of HIVE (Van Rie et al. 2008), suggesting a spectrum of ND. Despite the use of ART and improved virological control with immune reconstitution, there are still a significant percentage of youth in this study who were found to have a ND. The prevalence of HIVE has

reportedly decreased from 40% to 18% in the post ART-period(Shanbhag et al. 2005), suggesting that ART is associated with improved neurocognitive outcomes in children with vertically acquired HIV infection(Shanbhag et al. 2005). In this study the AAN system for diagnosing HAND was able to detect higher rates of ND in children with a diagnosis of HIVE. Understanding the spectrum of cognitive and adaptive functioning problems in HIV-infected youth is an important step in conceptualizing effective management strategies for education, social support, adherence and sexual health. Many of the burdens, such as stigma, living with chronic illness, bereavement and caretaker changes are well-documented challenges that youth infected with HIV need to negotiate (Sherr et al. 2014). Guidelines for assessment, diagnosis and treatment of HIV-associated neurocognitive disorders (HAND) in HIV-infected adults suggests it is appropriate to assess neurocognitive functioning in all patients with HIV, not just the symptomatic, particularly if there is evidence of deterioration in functioning (Group 2012). The AAN system has previously been applied to adult samples of HIV-infected individuals in South Africa, with results of the application suggesting that HAND is highly prevalent in primary care settings(Joska et al. 2011).

Although higher rates of NCI were detected in the HIV-infected group, worryingly the AAN criteria also found NCI in the negative controls. Of the ART-naïve slow progressors, who receive little attention from health services, 26.09% were found to have a ND. Significant motor and cognitive deficits have previously been found in HIV infected ART-naive Ugandan children with CD4 cell counts of >350 cells/μL (Ruel et al. 2012). The ART naïve/slow progressors group differed little from the control group. A possible reason for these findings is that controls were recruited from low-SES families. A large number of youth living in SSA are not fulfilling their developmental potential. Many of these youth are exposed to multiple risks which may include poverty, malnutrition, poor health, and unstimulating home environments, which detrimentally affects their cognitive, motor, emotional and social development (Grantham-McGregor et al. 2007).

Limitations of this study include the small size of the HIV-negative control group, and the fact that normative neurocognitive data for isiXhosa speaking children do not exist. However, we recruited HIV-negative controls from the same community, schools and clinics as the HIV infected youth. Further limitations are the lack of data

on duration of ART, other than a minimum of 6 months treatment, highest viral load and nadir CD4. While applying an adult-based classification to HIV infected youth may not be ideal, we used a paediatric neurocognitive battery to determine functioning in the various domains. The AAN HAND criteria could not detect higher rates of ND in the ART naïve slow progressors.

Conclusion

In summary, we found that a high prevalence of ND exists in a sample of HIV-infected older children and adolescents in SSA. Many studies have reported that prevalence of NCI in HIV infected children has decreased dramatically since the introduction of ART. However, despite the rollout of ART in South Africa the recognition of ND in nearly 46% of the HIV infected group is an indication that these problems remain important to identify and probably largely under recognized. While applying an adult-based classification to HIV infected youth may not be ideal, this study has started the process of identifying a spectrum of ND in HIV infected youth. This is especially important in adolescents where their ability to function in school will directly affect their economic/social trajectory as adults. Further research into the impact of ART on neurocognitive function utilising prospective cohort studies in SSA is needed. A study of whether initiation of ART in slow progressors could prevent or reverse NCI is also needed (Ruel et al. 2012).

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Chapter 7.

Summary and Conclusions

In this cross-sectional clinical cohort study conducted in Cape Town in which HIV infected children and a HIV negative healthy control group for comparison, completed clinical, neuroimaging and neurocognitve assessments, we found evidence of a spectrum of cognitive disorders and significant white matter microstructural damage secondary to HIV. As summarized in earlier chapters, the need for further investigation into a possible spectrum of HIV-associated neurocognitive impairment (NCI) in HIV infected older children and adolescents in South Africa is important as the highest number of infected children in the world live in South Africa, and that HIV exerts a neurotoxic effect on the developing brain. A systematic review of cognitive studies in HIV infected children in SSA found the lack of data from SSA especially urgent for children above the age of two, and recommend that future studies use an extensive battery of neurocognitive tests, as the effects of HIV on cognition appear to be wide spread (Abubakar et al. 2008). In the adult literature there is a growing understanding of HIV associated neurocognitive disorders (HAND), and its impact on functioning with guidelines for assessment, diagnosis and treatment (Rackstraw 2011).

There are still no diagnostic criteria for a spectrum of neurocognitive disorders secondary to HIV infection for children. The American Academy of Neurology (AAN) system for HIV infected adults proposes four categories: “normal”, “asymptomatic neuropsychological impairment (ANI)”, “mild neurocognitive disorder (MND) and “HIV-dementia (HAD)”. Using these same critetia we were able to establish the presence of ANI, minor ND and major ND in this cohort of vertically infected children. This study found the adverse neurotoxicity of HIV infection in children to manifest frequently as NCI across a number of cognitive domains, that have a negative effect on a range of behavioural and functional outcomes, such as school performance and impaired activities of daily living such as self care. Using the AAN criteria for HAND we found that 45.35% of the HIV infected children had a ND. Adherence to antiretroviral treatment (ART) is the key to the successful treatment of children infected with HIV, as well as containment of drug resistance. This study was able to demonstrate significant white matter microstructural damage using diffusion tensor imaging (DTI), in children who failed first line ART. Despite the impact that ART has had on reducing severe forms of HIV encephalopathy (HIVE), milder forms of NCI were found to be prevalent in this study. These milder

forms of NCI were also found to exert a deleterious effect on adaptive functioning. DTI used in this study found an association with poor nutrition and white matter microstructural damage.

With ART, many HIV infected children now face a future once thought impossible. HIV has become as a major chronic illness of childhood. During adolescence they face unique psychosocial stressors that differ from other chronic childhood illnesses. Studies have described an increased prevalence of mental health disorders among HIV infected children (Domek 2009). Overall, we found that HIV infected children and adolescents to have poorer neurocognitive and adaptive functioning outcomes than uninfected peers, particularly those with HIVE. Children with HIV also displayed a range of damage to neuronal microstructure and fronto-striatal cognitive impairment when compared to negative controls, irrespective of age or treatment status. A number of adolescents in South Africa would have initiated ART only after immune compromise or after the diagnosis of HIVE, resulting in neurocognitive deficits that remained permanent despite ART (Laughton et al. 2013; Patel et al. 2009). Children on the severe end of the neurocognitive spectrum with HIVE had more damage to neuronal microstructure. There is also evidence form this study that HIV infected adolescents are at risk of executive dysfunction. Executive dysfunction may increase the risk of substance use, risk-taking behaviors, including sexual risk, and poorer ART adherence (Laughton et al. 2013). Lower CD4 counts, higher viral loads and the history of failing first line ART in this study was associated with poorer white matter microstructural integrity, further demonstrating the need for supporting adherence.

HIV infected children who are slow progressors and ART-naïve performed poorly on neurocognitive testing, in addition some met diagnostic criteria for HAND using the AAN criteria. ART naïve slow progressors also had more damage to neuronal microstructure than those on ART. Specifically, ART naïve children had greater myelin loss than children stable on ART. This may indicate that ART is able to reduce the inflammatory response in the CNS. These children receive little attention form health care services; our study provides compelling evidence that this should change, and that initiation of ART as early as possible may reduce the risk of developing white matter damage in ARV naïve slow progressors. It is possible that ART initiation in school-aged children and adolescents may be too late to reverse existing

impairment, however ART may play a role in preventing any further CNS microstructural damage or cognitive decline. ART has been associated with a decline in CSF HIV RNA and an improvement in neurologic status in HIV infected children. However the development of genotypic mutations was different in CSF and plasma, suggesting different viral evolution and the need for ART in children to include agents with activity in the CNS (McCoig et al. 2002). A recent publication from the PREDICT Study reported that in HIV-infected children surviving beyond one year of age without ART, neurodevelopmental outcomes were similar with ART initiation at CD4 15–24% vs. < 15%; but both groups performed worse than HIV-uninfected children. Worryingly these findings suggest a positive effect of ART initiation on neurodevelopment may remain in infancy(Puthanakit et al. 2013).

LIMITATIONS

This study has a number of limitations. First, the study is cross-sectional and thus unable to describe the long-term outcomes of HIV on neurocognitive functioning and the effects of long-term ART on the developing brain. We were unable to establish whether clinical associations of white matter damage observed in this study are predictive of future decline in white matter integrity. Larger longitudinal cohorts of imaged HIV infected children are needed to better understand the association between neurocognitive performance and brain structure. Another limitation was the lack of objective measures of adherence. Adherence is an important behavioral outcome, which could impact on neurocognitive outcomes as well as white matter integrity.

RECOMMENDATIONS

A number of important clinical issues should be considered as a result of this research. Evidence of neurotoxicity in ART naïve slow progressors suggests that the scale-up of ART accessibility in South Africa should include these children. Children with HIV infection will need comprehensive, multidisciplinary and coordinated care that includes attention to the NCI and adaptive functioning problems found in this study. Children presenting with risk factors such as low CD4, high viral load, failing first line ART and scholastic difficulties should be offered neurocognitive screening as part of routine HIV care and referral to supportive services or formal assessments where appropriate (Laughton et al. 2013). HIV infected adolescents are likely to face future physical and psychological health consequences related to the cognitve and

adaptive functioning challenges they face if mental health care is not made a priority in the fight against HIV (Domek 2009). This care may need to take into account that parents/caregivers share the infection. The impact of HIV on the family surpasses that of virtually all other chronic conditions. This is compounded by the stigma surrounding HIV(A and M 1991). The integration of HIV and maternal, neonatal, child health and nutrition services, including family planning is recognized as a key strategy to reduce child morbidity and mortality and control the HIV epidemic (Lindegren et al. 2012). Practical solutions can go hand-in-hand with the scale-up of ART accessibility in South Africa. These include mental health services, community education, school-based programs, colaboration with the Department of Education, and strengthening families to provide a safe and secure home environment for HIV infected children (Domek 2009). HIV infected adolescents should be provided with adherence support, reproductive health counseling, mental health and educational/vocational planning. Evaluations of the effectiveness of these interventions in improving adherence and reducing risk-taking behaviors should be undertaken.

FUTURE RESEARCH

There is little data on the spectrum of NCI specific to the adolescent age group, with few studies from low- and middle-income countries, which have the highest prevalence of HIV infected adolescents. Much of the current evidence is from younger children, yet evidence from these studies provides valuable information, as neurocognitive problems occurring at younger ages are likely to persist into adolescence. There is a lack of longitudinal cohort studies designed to assess the long term outcomes of HIV on neurocognitive functioning in HIV infected older children and adolescents. Little is known about the complex nature of recovery of the brain after initiation of ART, and there is inadequate evidence of the effects of long-term ART on the developing brain. A study of whether initiation of ART in slow progressors could prevent or reverse neurocognitive deficits is needed (Ruel et al. 2012)

Psychosocial development and behaviour during adolescence are profoundly influenced by HIV, with concerns related to treatment adherence, stigmatization, risk taking, and the position of young people within family and social support systems. There are potentially complex - but still poorly understood - interactions between each

of these factors; research is required to understand the health and development of HIV-infected adolescents in this context. Thus, there is an urgent need for longitudinal research assessing the long-term effect of ART and timing of ART initiation on neurocognitive outcomes of vertically HIV-infected older children and adolescents, particularly in SSA.(Laughton et al. 2013). The development of an appropriate screening tool for HAND can now be done as this study has provided a detailed neurocognitive characterisation of impairments seen in HIV infected children.

Currently I am the investigator in charge of the neuroHIV sub study of the Cape Town Adolescent Antiretroviral Cohort (CTAAC), whose goal to investigate markers of chronic disease processes and progression in perinatally HIV-infected SA adolescents focusing in four key areas (general adolescent development; neurocognitive function; pulmonary disease; and cardiovascular function). CTAAC is NIH R01 funded, with Prof Heather Zar as the principal investigator. The aim of the neuroHIV sub study is to describe the neuropsychiatric status of children over time through neuroimaging and neurocognitive testing and associations with features of HIV disease and its management. CTAAC is a prospective, descriptive cohort study of 520 HIV-infected adolescents on ART ages 9 to 14 years being followed 6-monthly for 36 months. The cohort will be followed-up at the Clinical Research Unit (CRU) at Red Cross War Memorial Children's Hospital (RCWMCH). The nested neuroHIV substudy is enrolling 200 adolescents, ages 9 to11 years for neuropsychiatric evaluation and neuroimaging at baseline and 36 months. CTAAC recruitment started in August 2013. The methods used in the neuroHIV sub study are the same as the PhD study decribed here, however a number of measures have been added based on what we have learnt from this PhD study. The international HIV dementia scale (IHDS), a commonly used screening tool for HAND in HIV infected adults will be assessed for its feasibility to screen for NCI in HIV infected children. The IHDS includes three sub-tests: a non-dominant finger-tapping test, a non-dominant Luria hand sequence, and a four-word recall test. The IHDS was validated in South African adults with a with a sensitivity of 85% and specificity of 54% (Joska et al. 2011a). Behavioural outcomes will be examined in greater detail, including measuring adherence, substance use and risk taking behaviour. Risk taking behaviour will be related to tests of executive

function, gambling tasks, and impulse control studied using functional magnetic resonance imaging (fMRI).

CONCLUSION

This area of work is highly relevant to South Africa, and other parts of the low-middle income world, where prevalence of HIV/AIDS is high, and where there has been vertical transmission. This study was able to describe a spectrum of ND in older children and adolescents infected with HIV. The observed compromise to the CNS was supported by neuroimaging findings as measured by DTI. The AAN HAND criteria designed for adults was able to identify children and adolescents with important functional cognitive impairments who don't fit criteria for HIVE and would therefore not have been identified otherwise. This has major clinical implications in terms of the importance of managing HIV infected children. From this work we hope to develop a rapid screening tool and shorter neurocognitive battery to facilitate the detection and diagnosis of ND in pediatric HIV.

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