

# **Neurocognitive Disorders in Young Adults Commencing Highly Active Anti-retroviral Treatment in the Western Cape**

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DOCTOR OF PHILOSOPHY  
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## **Supervisors**

Professor Alan J. Flisher  
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“And having seen the multitudes, he was moved with compassion for them, that they were faint and cast aside, as sheep not having a shepherd”

Matthew 9:36 (Young's Literal Translation)

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## DECLARATION

I, John Anton Joska, do hereby declare that this thesis is based on six journal manuscripts: four of which have been published or accepted for publication (chapter 2-5), and two are under review in international journals (chapters 6 and 7). 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.**

Joska, J.A., Hoare, J., Stein, D.J. and Flisher, A.J. (in press). "The neurobiology of HIV dementia: implications for practice in South Africa". *African Journal of Psychiatry*

I wrote this paper as part of my background reading and preparation for this project. I reviewed all the relevant literature myself, summarised and edited the material, and drafted the manuscript in its entirety. The other authors made conceptual and intellectual contributions to the theme of the piece, as well as to final drafts.

### **Chapter 3.**

Joska, J.A. Gouse, H., Paul, R.H., Stein, D.J. and Flisher, A.J. "Does Highly Active Anti-Retroviral Therapy Improve Neurocognitive Function? A systematic review". *Journal of Neurovirology*. 2010 Apr;16(2):101-14.

In this second review, I developed a search methodology with input from my supervisors, and then conducted the database and journal search. Both Hetta Gouse and myself reviewed all papers for potential inclusion and established our level of agreement. We extracted data into a spreadsheet. I then analysed and summarised all the data myself, and wrote the full first draft of the manuscript. My co-authors reviewed the draft, made conceptual and intellectual contributions, and edited the final draft.

### **Chapter 4.**

Joska, J.A., Westgarth-Taylor, J., Myer, L., Hoare, J., Thomas, K.G.F., Combrinck, M., Paul, R.H., Stein, D.J. and Flisher, A.J. "Characterisation of HIV Dementia among individuals starting antiretroviral therapy in South Africa". *AIDS and Behavior*. 2010 Jul 8. [Epub ahead of print]

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 statistical analysis with the help of Landon Myer, and then wrote the full first draft of the manuscript. A project of this nature was contributed to by others, including my supervisors (AJF and DJS) and other experts (RHP and MC). Jackie Hoare was involved also in examining and assessing patients, and together with Marc Combrinck and myself, in the consensus panel scoring. Jenny Westgarth-Taylor was the study neuropsychologist who helped by testing some participants and overseeing neuropsychological test scoring and data entry. All authors read and approved the manuscript. I managed all revisions.

## **Chapter 5.**

Joska JA, Combrinck M, Valcour VG, Hoare J, Leisegang F, Mahne AC, Myer L, Stein DJ. "Association between apolipoprotein E4 genotype and human immunodeficiency virus-associated dementia in younger adults starting antiretroviral therapy in South Africa". *Journal of Neurovirology*. 2010 Sep 8. [Epub ahead of print].

This manuscript followed the first, described above. The idea for genetic analysis was based on my initial hypothesis that there would be a number of factors which contributed to neurocognitive impairment in PLWHA. The literature for Alzheimer's disease and apolipoprotein E is substantial, but for HIV it is still small. Victor Valcour is a colleague who has published in this area, and he was consulted on our findings. Marc Combrinck, and expert in old age medicine, assisted with the conceptualisation. The genotyping was performed by Felicity Leisegang. Celia Mahne, together with Jackie Hoare were at this stage both assisting with participant assessment, while Landon Myer again helped with statistical analysis. 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 Marc Combrink and Landon Myer, and wrote the full first draft of the manuscript. I made all revisions prior to publication myself- this involved some re-analysis and data cleaning and re-organisation.

## **Chapter 6.**

Joska, J.A., Westgarth-Taylor, J., Hoare, J., Thomas, K.G.F., Paul, R.H., Myer, L. and Stein, D.J. "Screening for HIV Dementia using the International HIV Dementia Scale in South Africa". Under review in *Aids Patient Care and STDs*.

This manuscript was similarly based on the large parent project, on which I was the principle investigator. I have received support for the neuropsychological aspects from Kevin Thomas and Jenny Westgarth-Taylor- the latter again scored and entered the neuropsychological data. I collated all this data, together with the clinical data, and did the statistical analysis with the help of Landon Myer. I wrote a full draft of this manuscript, and then sent on to my co-authors for comments and input. My supervisor, Dan Stein, continues to advise and support (after Alan's passing). Rob Paul has remained involved as an international neuroAIDS expert, and Jackie Hoare, my clinical fellow, continued to examine and assess participants with me. All authors read and approved the manuscript. I managed all revisions of this manuscript to date.

#### **Chapter 7.**

Joska, J., Westgarth-Taylor, J., Hoare, J., Thomas, K.G.F., Combrinck, M., Paul, R.H., Myer, L. and Stein, D.J. Neuropsychological outcomes in young adults commencing anti-retroviral treatment in South Africa. Under review in *Neurology*.

This last manuscript required significant administrative input to ensure that I retained as many participants as possible. It involved reviewing all the participants seen at baseline, and re-assessing them. I had to ensure ongoing involvement from all team members, including fieldworkers who were looking for participants in the community. Jackie Hoare, and myself, with assistance from Dot Feast reviewed all participants. They were re-tested under the supervision of Kevin Thomas and Jenny Westgarth-Taylor- the latter again scored and entered the neuropsychological data. I collated all this data, together with the clinical data, and did the statistical analysis with the help of Landon Myer. I wrote a full draft of this manuscript, and then sent on to my co-authors for comments and input. My supervisor, Dan Stein, continues to advise and support (after Alan's passing). Rob Paul has remained involved as an international neuroAIDS expert. All authors read and approved the manuscript. I 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

John Anton Joska

01 November 2010

## **ABSTRACT**

### **Title: Neurocognitive Disorders in Young Adults Commencing Highly Active Anti-retroviral Treatment in the Western Cape**

#### **Background**

HIV-associated neurocognitive disorders (HAND) remain prevalent in the era of highly active anti-retroviral therapy (HAART). It is not known whether HAND are as prevalent in South Africa as in other regions, and whether individuals with HAND in South Africa will respond to HAART.

#### **Methods**

The published literature was reviewed to elucidate potential mechanisms of the development of HIV-associated dementia (HAD)- the most severe form of HAND- and to establish the effect that HAART has exerted on HAND across diverse studies. A prospective clinical cohort study was initiated in Cape Town, in which 170 participants completed baseline clinical and neuropsychological assessments. Laboratory investigations included apolipoprotein E (APOE) genotyping. The performance of the International HIV Dementia Scale (IHDS) as a brief screening tool was analysed using a receiver operating characteristic (ROC). At one-year, 105 participants were re-assessed for neuropsychological change.

#### **Results**

The reviewed literature suggests that HAD is likely mediated by a range of HIV-related factors (including possible difference in HIV sub-type) and host-related factors. In addition, while neurocognitive improvements are reported in most prospective studies, these are dogged by differences in methodology and approach. In this study, 25.4% of participants met criteria for HAD at baseline and this was associated with lower levels of education and male gender. The APOE4 allelic variant was not associated with those who had developed HAD, despite this variant being common in Cape Town. The IHDS performed reasonably well on ROC analysis, detecting 86% of dementia cases using a cut-off score of  $\leq 11$ . At one-year follow-up, participants had improved significantly on neuropsychological assessment, including a small sub-sample who had not initiated HAART. Significant associated factors were male gender, the use of HAART and worse baseline neuropsychological test performance.

#### **Conclusion**

HAND- including HAD- are common conditions in South Africa, where HIV clade C is predominant. 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 targets for therapeutic interventions.

## **Glossary of Terms**

American Academy of Neurology (AAN)

Anti-retroviral treatment (ART)

Apolipoprotein E (APOE)

Asymptomatic neurocognitive impairment (ANI)

Alcohol Use Disorders Identification Test (AUDIT)

Centers for Epidemiological Study- Depression scale (CES-D)

Composite z-score based on combinations of neuropsychological tests (NPZ)

Combination anti-retroviral treatment (CART)- same as “Highly active anti-retroviral therapy” (HAART)

CNS Penetration Effectiveness (CPE)

Immune Reconstitution Inflammatory Syndrome (IRIS)

Human immuno-deficiency virus (HIV)

HIV Dementia Scale (HDS)

HIV-associated neurocognitive disorders (HAND)

Highly Active Anti-retroviral Therapy (HAART)

HIV-associated dementia (HAD)- same as HIV- dementia (HIV-D)

HIV- dementia (HIV-D)

Mild neurocognitive disorder (MND)

Minor cognitive and motor disorder (MCMD)

International HIV Dementia Scale (IHDS)

Non-nucleotide reverse transcriptase inhibitors (NNRTI's)

Memorial Sloan Kettering staging (MSK)

Mini International Neuropsychiatric Interview (MINI)

Patient's Assessment of Own Functioning ( PAOFI)

People living with HIV/AIDS (PLWHA)

Quality of Life and Satisfaction Scale (QLESQ)

## **Acknowledgements**

The work reported in this thesis was funded by the South African National Research Foundation Thuthuka Programme, the Medical Research Council of South Africa, the Biological Psychiatry Special Interest Group of the South African Society of Psychiatrists, and the University of Cape Town Health Sciences Faculty.

I would like to acknowledge and thank the following people: the patients and their families for agreeing to participate in this study; Drs Celia Mahne, Hetta Gouse, Jackie Hoare, Dot Feast and Rory Leisegang for their invaluable assistance with patient assessment; Mss Jean Luyt and Jennifer Westgarth-Taylor for conducting the neuropsychological assessments, together with Ms Judy Xala, Mr Teboho Linda and Ms Andiswa Gidani. I thank Sisters Dorothy Magwaxaza and Doris McEwan for their dedicated fieldwork in recruitment; and sister Lorraine Adendorff for being the smiling face greeting participants at the Unit.

A number of people generously gave of their time and expertise as consultants: Professors Rob Paul, Victor Valcour, Marc Combrinck, and Landon Myer.

I thank my supervisors, Professors Dan Stein and Alan Flisher, who must be two of the finest investigators around. Alan sadly passed away before this thesis was complete; I owe much of what I can do to his guidance. I hope you would have liked it.

I thank the staff of the sites where this work was conducted, and the facility managers of Nolungile site C in Khayalitsha, Mitchell's Plain and Woodstock Community Health Centres.

I would specially like to thank my colleagues at Groote Schuur Hospital for their support and especially Dr Don Wilson. To my wife, Louise, who has done this before with aplomb, and listened patiently to all the stories; to Danny, Rach and Bennie, thanks for all the laughs and the rough-and-tumble.

John Joska  
Cape Town  
November 2010

## **Chapter 1.**

### **Introduction**

Contextualising the problem of HIV-associated Neurocognitive Disorders in South Africa

## Context

Human immuno-deficiency virus (HIV) infection constitutes a global pandemic with approximately 50 million HIV-positive people worldwide [1]. The majority of people infected with HIV live in sub-Saharan Africa and South Africa bears the burden of having the largest number of HIV-infected individuals in the world. According to data gathered during the National HIV and Syphilis Antenatal Sero-prevalence Survey in 2008, the national rate of HIV infection in South Africa was 29.3% [2]. The Western Cape reported the lowest estimate of 16.1%. In 2009, 500 000 people were newly infected with HIV in South Africa [2].

HIV infects the brain early in the course of infection [3]. A range of neurological and psychiatric sequelae follow neuro-invasion. Psychological distress and psychiatric disorders are the result of a combination of biological and psycho-social factors. Psychiatric disorders in people living with HIV/AIDS (PLWHA) are not only more common, but they exert a significant effect on many health-related outcomes. PLWHA are more likely to suffer from a mental disorder than the general population [4-7]. Mental disorders, such as depression have been consistently linked with lowered likelihood of receiving HAART [8,9], poorer medication adherence [10-12], and if untreated, greater mortality [13,14]. In addition, mental health in HIV-positive populations have been associated with decreased quality of life [15] and may increase high-risk behaviours for the further transmission of HIV [16,17]

Neurological involvement in HIV remains prevalent, with as many as 60% of PLWHA going on to develop some form of HIV associated neurocognitive disorder (HAND) in their lifetime [18] Prior to the widespread use of highly active anti-retroviral therapy (HAART), infection with HIV resulted in HIV-associated dementia (HAD) in about 15% of individuals [19] The advent of HAART has substantially altered the nature of these disorders, although they frequently persist [18,20]. Specifically, HAART has reduced the incidence of HAD, but the prevalence appears to be increasing. More recently, efforts to predict response to HAART have intensified. In South Africa, PLWHA are only able to access HAART at CD4 cell counts <200 cells/ml, unless they are pregnant, co-infected with tuberculosis, or have a stage 4-defining illness. This means that substantial organ damage may occur before immune-reconstitution. In the case of the brain, it is unclear how much of this is reversible.

There are other unique aspects of the HIV epidemic, and its relation to brain involvement. Firstly, the epidemic in sub-Saharan Africa is predominantly spread by sexual- and mainly heterosexual- transmission; secondly, a substantial proportion of

those infected are women; and thirdly, there are limited resources for care, and for mental health care in particular[1]. From the point of view of the brain and neurocognitive disorders, there are questions regarding the HIV subtypes predominant in South Africa, and their propensity to cause neuropsychological impairment. The subtypes of HIV-1 are distributed differently across the world, and indeed in Africa. These subtypes, or clades, differ in a number of respects, including in the sequencing of potentially neurotoxic viral proteins, such as the tat (transactivating transcriptor protein). Some studies have suggested that clade C tat is less neurotoxic than other clades (such as B) [21]. Clade C is highly predominant in South Africa.

Clinic services in South Africa have faced an exponential growth in demand for care. In particular, as HAART has become more readily available, the numbers of individuals entering care has risen dramatically. Despite the increase in availability of HAART, many do not receive it, and AIDS-related mortality remains unacceptably high- nearly 400 000 PLWHA died from AIDS in South Africa in 2009 [2]. In addition to increasing access to all PLWHA who require HAART, other questions remain: how can we prevent irreversible neurological brain damage from HIV infection? To what extent does HAART reverse this damage? Should we be starting HAART sooner? How can we screen PLWHA for stage 4 disease who would not otherwise qualify for HAART? Who are those most at risk for severe forms of HAND? This thesis is an attempt to address some of these questions.

### **Research Aims**

Given that the sero-prevalence of HIV is extremely high in South Africa; that PLWHA are at risk of developing neurocognitive disorders; and that there are questions about the differences in the HIV epidemic in sub-Saharan Africa and the neurological effects of HIV in clade C HIV- it is important to begin to understand these issues in our context.

The overall aim of this thesis is to characterise PLWHA neurocognitive disorders in individuals before and one year after commencement of HAART. In particular, this thesis aims to study the socio-demographic, clinical and laboratory correlates of HAND in a group of patients about to commence HAART, with a view to describing features potentially associated with a diagnosis of HIV dementia. Once these patients have commenced HAART, the study further aims to follow them up at one year, in order to describe the impact of HAART on neuropsychological functions, and variables which may be associated with either improvement or deterioration. To date, there have been no published studies of HAND in Southern Africa utilising a detailed neuropsychological test battery. 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 HAART on neurocognition, with few prospective treatment effect cohort studies.

### **Overall description of the project**

This project arose out of the Division of Neuropsychiatry. A need to understand neurocognitive problems in PLWHA more fully arose out of our work with colleagues in Infectious Diseases. 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 Psychiatry Special Interest Group of the South African Society of Psychiatrists, and the Medical Research Council. We began working in three primary health care centres, and asked participants to come to Groote Schuur Hospital for testing. 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. A small number of participants who received neurocognitive and neuromedical testing, were imaged at the Cross University Brain Imaging Centre (CUBIC). From this work, two master's students were able to obtain data for the registration and completion of dissertations- Jackie Hoare described imaging correlates of neurocognitive disorders in 44 participants; and Celia Mahne (under the supervision of Marc Combrinck) described the correlation between peripheral blood markers of neuro-inflammation and magnetic resonance spectroscopy. 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 Robert Paul as Principle Investigator.

### **Neuropsychological testing**

Particular challenges presented themselves regarding neuropsychological assessment of this patient group. Specifically, it is not possible to simply administer neuropsychological tests that were developed in North America (where most have been developed) into other regions of the world. Rather, significant effort is required to ensure the neuropsychological measures are culturally relevant and that the local population understands the task demands so that the cognitive measures measure the same brain systems on which they were developed to measure. I went about the process of selecting an appropriate battery in several ways. First, I met with three local expert neuropsychologists to establish their views on how tests needed to be adapted, which brain functions they considered needed to be

measured in people living with HIV in our population, and lastly, which measures they considered would be appropriate. Second, I met with the study neuropsychologist who had been working on a large study of HIV encephalopathy in the Western Cape, and established her views on the above matters. Lastly, I reviewed the published literature on the issue of international neuropsychological assessment. The battery used in this work was ultimately based on that used at the HIV Neurobehavioural Research Center (located at the University of California, San Diego). The battery consists of measures now well-established to sample the domains of function affected by HIV. It also utilises multiple measure for each of several domains of function. Once we had identified the battery, and having considered the opinions of local experts, I set about first forwards and back-translating all test instructions. We also reviewed word-lists for appropriateness and made changes as needed. For example, we switched the “gemstones” list in the Hopkin’s verbal learning test for “vegetables”. Few data on the psychometric properties of these tests exist for South Africa. Norms are available for Uganda. The work of Anne Shuttleworth Edwards has demonstrated that both the quality and level of education of participants may affect test performance. This makes adaption to local settings critical. In this regard, early in the course of the study we employed isi-Xhosa speaking technicians who were carefully trained and supervised to test study participants. The imperative to generate local normative data were paramount, and I set about generating these during the course of the study. Completed normative data were not available during analysis of these data, but I was able to make use of first 50, then later approximately 100 demographically matched controls.

### **Coherence of the thesis**

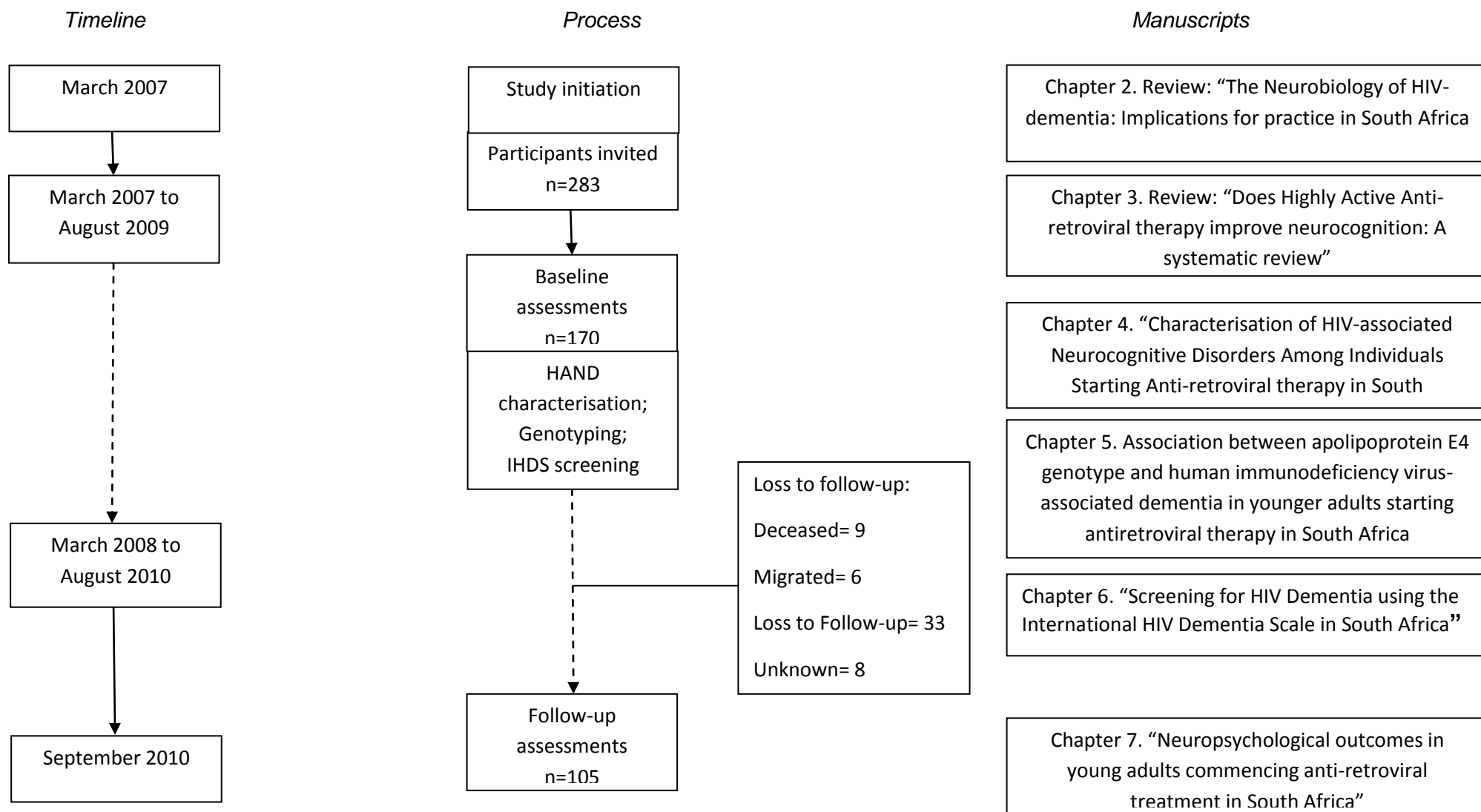
The coherence of this thesis revolves around three main points. Firstly, the thesis centres around my own role as the single principle investigator. In this respect, I initiated and led the project from conception to completion. Secondly, the thesis involves a single project. Each chapter describes an analysis conducted on the same cohort of patients enrolled into this study. Thirdly, there is a distinctly unifying theme to this thesis, namely, how neurocognitive disorders may vary between individuals and over time in PLWHA, and the impact of a number of variables, including HAART, 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.

I have included two review papers (chapters 2 and 3). The first, currently in press, provides a theoretical overview of the neurobiology of HIV-dementia, and how this might be relevant to PLWHA in South Africa. The issue of clade diversity has been brought to the fore in this

piece. The second review is systematic, and has been published. This piece examines in more detail how HAART might impact on neurocognitive disorders over time. This review also highlights how disparate research methodologies might affect our understanding of these conditions across different research settings.

In chapter 4, I seek to describe the prevalence and associated features of HAND in primary health care settings in Cape Town. It is not known whether clade C HIV produces similar rates of HAND as in North America and Europe. Also, the effect of socio-demographic, nutritional and related laboratory measures is not known. The relationship between the development of HIV-associated dementia (HAD) and the apolipoprotein E (APOE) allelic variant has been reported in studies in North America with differing results. I sought to describe the prevalence of the APOE genotype and its association with HAD in this population (chapter 6). The importance of screening for HIV-dementia has been emphasised above. In this regard, I investigated in chapter 6 whether the International HIV Dementia Scale (IHDS) performs adequately in our setting, in a receiver operating curve analysis. The last chapter (7) seeks to investigate whether the use of HAART was associated with improvement in neuropsychological function. Studies to date have generally reported significant improvements, but there is a paucity of prospective studies, and none have been conducted in Southern Africa.

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



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## **Chapter 2.**

### **The neurobiology of HIV dementia: implications for practice in South Africa**

Joska, J.A., Hoare, J., Stein, D.J. and Flisher, A.J. (in press).

In Press in: *African Journal of Psychiatry*

## **Abstract**

In this review, the neuropathogenesis of HIV dementia (HIV-D) is discussed in the context of the local epidemic in SA. HIV-D continues to be prevalent in the era of highly active anti-retroviral therapy. HIV neuro-invasion into the central nervous system may result in the development of separate HIV genotypes in an individual through compartmentalisation. The blood brain barrier continues to limit penetration of anti-retroviral drugs into the cerebrospinal fluid. Individuals with active neuro-inflammation appear to respond well to HAART. In some cases low grade neuro-degeneration persists with consequent clinical deterioration. In South Africa, the emergence of a sub-epidemic of HIV-D is being driven by various factors, including the incomplete coverage of HAART to all who need it, the late stage presentation of people living with HIV/AIDS (PLWHA) and a co-occurring methamphetamine epidemic. Differences in viral subtype do not appear to confer protection against HIV-D. Implications for PLWHA who are at risk for HIV-D in South Africa are explored, with a view to providing suggestions for improving practice and research into this area.

## Introduction

Infection with human immuno-deficiency virus type 1 (HIV) is an important health concern globally but especially in South Africa, which has the highest number of people living with HIV/AIDS (PLWHA) [1]. HIV is known to cause adverse neurological sequelae (also known as “neuroAIDS”) in a substantial proportion of individuals. Despite improved survival and quality of life following the use of highly active anti-retroviral therapy (HAART), neuroAIDS remains prevalent. In this review, we describe the neurobiology of dementia associated with HIV. In particular, we describe the process of neuroinvasion, subsequent neurodegeneration and treatments. We further aim to address how the sub-epidemic of neuroAIDS may impact individuals and services in South Africa and suggest strategies for approaching this problem.

NeuroAIDS remains prevalent, with as many as 60% of PLWHA going on to develop some form of HIV associated neurocognitive disorder (HAND) in their lifetime [2]. Furthermore nearly 90% of individuals have autopsy evidence of neuropathology. Neuro-invasion is likely to occur early in the course of HIV infection following dissemination of HIV into lymphoid tissues and cells [3]. The blood brain barrier not only limits ongoing passage of HIV into the central nervous system (CNS) following this early peak in viral load, but also acts as a barrier to drug penetration [4]. The active infection of CNS microglial cells results in the development of a neurotoxic inflammatory cascade, which involves primarily the sub-cortical white matter and striatum [5]. Neurocognitive dysfunction follows the disruption of these circuits, while neuronal apoptosis produces cortical atrophy [6]. Early response to HAART is probably associated with a rapid suppression of acute neuro-inflammation (an “encephalopathy” syndrome), while delayed improvement may occur following immune reconstitution and restoration of white matter [7].

Numerous clinical variables are now known to be associated with the development of HIV-D, including low CD4 cell count and late stage disease [7]. These are especially relevant in South Africa where the epidemic is greatest and HAART coverage is yet incomplete. The abuse of methamphetamine not only increases HIV risk behaviour, but is independently associated with neurotoxicity [8,9]. While the use of HAART has resulted in a dramatic reduction in the incidence of HIV-D, PLWHA with milder HAND do not qualify for treatment. Mild neurocognitive disorder may be a predictor of HIV-D, or at least is associated with neuropathological changes of HIV encephalitis [10]. Non-HAART treatments including the use of lithium and memantine have been

studied but results are not robust enough to warrant widespread use [8,11]. In a resource-limited setting such as South Africa, broad screening for HIV-D should be routine, and the use of HAART in PLWHA who have demonstrable neurocognitive disorder should be strongly considered. The implications at an individual, community and societal level of untreated or persistent neurocognitive disorders in large numbers of individuals are substantial.

### **Prevalence of HIV dementia: Epidemiology and challenges**

HAND remains prevalent despite HAART and clinical characteristics may be different. Prior to the widespread use of HAART, infection with HIV resulted in HIV-D in about 15% of individuals [12]. Less severe forms of HAND were found in about 30-60% of PLWHA [12,13]. The advent of HAART has substantially altered the nature of these disorders, although they do persist [2,14]. Specifically, HAART has reduced the incidence of HIV-D, but with longer life span, the prevalence appears to be increasing [7,15]. Not only does this persistence suggest either ongoing neurotoxicity, but it has implications for PLWHA who may be employed and/or need to adhere to HAART. In addition, the clinical presentation of HIV-D has changed [6], and it has been suggested that the subcortical features previously thought to be characteristic, may be less prominent [16]. As experience in the neuropsychological features of HAND has grown, it has become more apparent that cortical deficits occur frequently.

More recently, efforts to predict the neurocognitive response to HAART have intensified. Currently it is thought that people who initiate HAART prior to severe immunosuppression and achieve plasma viral suppression [17], CSF viral suppression [4] and who use CSF penetrating regimens accrue the most benefit [4,18]. In South Africa, home to the largest number of PLWHA, and despite one of the biggest anti-retroviral rollout programmes internationally, PLWHA still enter treatment late, if at all. Reasons include limited access to HAART, confusing government and media messaging regarding treatment, and socio-cultural issues (such as belief systems regarding the causes of HIV). Accordingly, the profile of this group of individuals resembles a pre-HAART cohort, with a high incidence of HIV-D. The only local study of community prevalence of HIV-D, conducted in the Western Cape reported a rate of 23.5% of HIV-D according to the HIV Dementia Scale [19]. This compares to the rate found in a study in Uganda, where the prevalence was 31% [20].

In addition to the lateness of presentation of PLWHA, clade-specific differences may contribute to the development of HAND. HIV subtype (or clade) B is predominant in North America and Europe, while clade C is found in about 90% of South Africans [21,22]. The question of whether viral clade is responsible for differences in HAND has not been well-studied in clinical populations. For instance, the neurovirulence of HIV clade C has been associated with less severe forms of neurocognitive impairment in some studies, but with equally deleterious effects in others [23-25]. Variability has been attributed to differences in the dicysteine motif within the neurotoxic region of B-Tat, producing a greater degree of Tat-induced apoptosis [26,27]. However, other viral proteins, such as gp120, may be as neurotoxic. The clade sequence, levels of proviral DNA and tat protein, together with their impact on neuropsychological functions and neuro-imaging findings, is the subject of a study currently being conducted by our group.

### **Neuro-invasion and compartmentalisation**

Neuro-invasion probably occurs early in HIV infection, and is associated with dissemination of HIV into lymphoid tissues, which include the CD4 helper cells and circulating monocytes [3]. All five main types of cell in the CNS are susceptible to the effects of infection, but it is thought that only perivascular macrophages and microglia are actively or productively infected [28]. These cells are derived from bone marrow. Both are immune-competent in the CNS, with microglia arising from the mesoderm- as such they bear receptors related to the mononuclear phagocytic system [29]. In the resting state they are branched, while in the activated state, they are rounded or “amoeboid” [28]. Active or productive infection implies that HIV is constantly being produced from the cell surface, while non-productive infection implies that HIV DNA is incorporated into host cell, but not actively extruded. This latter type of infection, together with the restricted nature of the blood-brain barrier has led to the idea that the CNS is a “sanctuary” site or reservoir of HIV infection. The blood brain barrier is a selectively permeable membrane formed by a continuous cellular barrier with tight junctions. The passage of circulating immuno-competent cells into the CSF is tightly regulated, as is that of drugs.

A number of theories of neuro-invasion have been proposed, but it seems likely that the majority of invasion arises from the passage of HIV inside penetrating circulating macrophages (“Trojan Horse theory”) and from transcytosis- a process of HIV being actively transported through endothelial cells [30]. Perivascular macrophages are probably the cell line most infected and are also readily replenished from the

circulating peripheral population [31]. These cells, together with some microglia, fuse to make up the multi-nucleated giant cells (MNGC) which are the hallmark of HIV-encephalitis [28]. HIV encephalitis is the underlying neuropathological correlate of HIV-D. MNGC express CD14 and CD15 receptors, and these are noted on neuropathological examinations [32].

Following early CNS invasion, HIV probably re-enters the CNS throughout the course of infection during periods of either high viral load or systemic illness [28]. Despite this, phylogenetic reconstruction has suggested that HIV derived from various sites in the CNS in an individual more closely resembles its own sequences, than HIV derived from peripheral tissues [33]. In addition, unproductive infection of cell lines other than microglia and macrophages suggests that genetic drift of HIV may be small. These ideas again have led credence to the notion of the CNS as a sanctuary site.

While most PLWHA have evidence of neuroAIDS, few will develop frank clinical features. This might be explained by certain host factors (such as genetic predisposition) and the differing neuropathogenicity of HIV. One possible mechanism is differing “fusogenic” potential. In the CNS, cells bearing the chemokine receptor CCR5, as well as CD4, are prone to being fused into MNGC. Highly neuropathogenic forms of HIV might require lower levels of expression of these proteins, possibly leading to more rapid and/or greater MNGC formation [34]. Furthermore, certain HIV strains might evolve into separately neuropathogenic strains through the development of envelope glycoproteins which lower the need for host CCR5 and CD4 receptors.

There has been conflicting data on whether the viral load of HIV in the CSF (as opposed to the periphery) is associated with greater rates of HAND. Some studies have established that improvement in neuropsychological function is correlated with both high CNS penetration of HAART, as well as lower CSF viral loads [4]. A more recent study reported that while penetrating HAART regimens were correlated with lower CSF viral loads, these regimens resulted in poorer neuropsychological performance [35]. The relevance of the penetration of anti-retrovirals is explained below. It must be noted that in the public sector in South Africa, there is access to only a limited number of these agents.

### **Mechanisms of neuro-degeneration**

Neurodegeneration follows a breakdown in the usual interplay between neuroprotection and neurotoxicity. These involve interactions between various protective and toxic host compounds (such as nerve growth factors and glutamate) and the effect of the inflammatory process invoked by HIV. An understanding of the role of CNS chemokines and neurotransmitters has begun to shed light on possible mechanisms of HIV neurotoxicity. Chemokines are cellular cytokines secreted in the CNS and which play various roles including cell migration, differentiation, activation and proliferation. These processes are constantly occurring. Two main families of chemokines are especially relevant to HIV:  $\alpha$ - and  $\beta$ -chemokines [28].  $\alpha$ -chemokines (also known as CXC family chemokines) are all expressed in brain, mainly by neurons. The activated CXC receptor increases intra-cellular calcium, and may be key to excitotoxic damage [36]. In contrast,  $\beta$ - chemokines (CCR family chemokines) are only weakly expressed in brain. Despite being weakly expressed, certain  $\beta$ -chemokines play a crucial role in the development of HAND- these include monocyte chemoattractant protein-1 (MCP-1). Higher levels are thought to predict HIV-D over time [7]. Other members of the  $\beta$ - chemokine group may be neuroprotective, such as CCL4, which protects neurons from gp120 induced apoptosis [36]. It can then be seen that when HIV infects and activates CD14/16 bearing cells, pro-inflammatory cytokines are increased, leading to the development of both MNGC and related glial and neuronal cell damage.

Two main theories of HIV-associated neurodegeneration have been proposed. The direct injury hypothesis posits that neuronal injury occurs directly through the effect of toxic HIV proteins - gp120 and tat protein in particular - or through virus-host interactions, whereby gp120 activates glutamate receptors or TNF expression [37]. In the “bystander” effect hypothesis, damage occurs due to immune activation out of keeping with levels of HIV in the CNS. This amplification arises out of chemokine activation, the inflammatory activation of uninfected cells and the migration of infected T-cells into the CNS following chemo-attraction [38].

The result of either direct or bystander effects is the neuropathological entity known as HIV encephalitis. This entity is now established to underlie well defined clinical HIV-D [10,39]. In this process, the activation of inflammatory chemokines and cytokines, including TNF and nitric oxide synthase, leads to the production of free radicals, which in turn leads to astrocyte apoptosis [40]. Astrocytes play a key role in the removal of excitatory amino acids from synapses, leading to a loss of the neuroprotection/neurodegeneration balance. High levels of inflammation and

neurotransmitter dysregulation may lead to clinical features of “encephalopathy”, while neuronal apoptosis and degradation of white matter may lead to less reversible deficits characterised by clinical slowing and subcortical effects.

The role of transcriptional transactivator (tat), a toxic viral protein bears mention. Tat has been associated with neuronal nuclear toxicity, alteration of blood brain barrier tight junctions and the upregulation of pro-inflammatory cytokines [41]. It has been found that tat expression differs between HIV clades, and further proposed that a defect in the dicysteine motif in tat in clade C leads to lower levels of neurotoxicity [42]. The implications are that in regions where clade C is predominant, such as South Africa, lower rates of HAND might be expected. Recent work in India, where clade C is also predominant has not supported this idea. Rates of HAND comparable to regions where clade B occurs, was reported in a clinical sample [43]. A large study into potential clade differences is underway by our group.

### **Clinical mediators of HIV dementia**

A number of clinical factors are associated with the development of HIV-D, and are reviewed in this section, with a particular focus on their relevance to SA. These include the presence of neurological impairment, the abuse of methamphetamine, depression, female gender, low CD4 count and advancing age [7,44-46].

Substance abuse and dependence are linked to both the acquisition of HIV, as well as to compounded neurocognitive effects. The use of intravenous opiates, especially heroin has been associated with the epidemic in North America, and to the co-infection with hepatitis C. In South Africa, heroin use is fairly restricted, although it has the potential to grow [47]. In addition, hepatitis C is thought to be very uncommon in South Africa, as reported by one published study in Kwazulu Natal [48]. The effect of methamphetamine (MA) on both HIV risk behaviour and neuropsychological outcomes is increasingly being studied, and is of especial relevance locally. In a sample of more than 4500 adolescents at Cape Town schools, 12-13% had used MA at least once, and MA was associated with high risk sexual behaviour [9]. There is now good evidence for the deleterious effect of MA on neuropsychological function [49]. It has been proposed that when MA abuse and HIV co-exist, additive neurotoxic effects, such as increased ischaemic events and microglial activity may occur [8]. The implications for South Africa where MA abuse is problematic are enormous.

Also of relevance to South Africa, is that the majority of PLWHA attending clinics are women, and again, that many present with late stage HIV/AIDS. The preponderance of women is a feature that differentiates the Southern African epidemic from the global one. There are some well known gender effects in neuropsychiatry, with depression being more common in women [50]. As depression is commonly co-morbid with HAND, a greater burden of disease in South Africa might be expected. There is also substantial evidence that advancing age is associated with a greater vulnerability to developing HAND, and that this may be aggravated by the use of protease inhibitor containing regimens [8]. As with persons with Alzheimer's dementia, the amyloid protein has been implicated. Specifically, high levels of  $\beta$ -amyloid have been observed in HIV neuropathology, as well as increases in amyloid precursor protein and gamma secretase. Tat may inhibit an amyloid degrading enzyme [8]. With the largest HIV epidemic in the world, and the accordingly large numbers of PLWHA entering treatment, we will face an ageing population with these co-factors.

### **Pharmacotherapy of HIV-D**

Conceptually, the approach to treatment of HIV-D could be considered from either a preventive or curative view. Drug treatments can then be considered to be either directly anti-retroviral, or adjuvant. Current WHO guidelines recommend the use of HAART when either CD4 cell counts fall below 200, or a stage 4 disease-defining illness is present. Among these conditions is HIV encephalopathy. Once HIV-D is established, it may be difficult to reverse neuronal loss. This raises two central issues regarding the treatment of HIV-D: first, whether the brain is a long term reservoir of HIV, and therefore if ongoing low grade neuro-inflammation is occurring; and second, whether less severe forms of HAND either predict or progress to HIV-D. We will now address each of these issues.

First, regarding whether the brain is a reservoir for HIV, there has been much interest in the issue of the penetration of antiretrovirals through the blood brain barrier. The ability of these agents to pass into the brain, depending on their protein-binding, molecular size and lipophilicity has been categorised into a "CNS Penetration Effectiveness" (CPE) rank system [51]. In this system, anti-retroviral drugs are categorised into groups according to the above criteria, with scores of 0.5, 1 and 2 being assigned to the three groups. The nucleotide/nucleoside reverse transcriptase inhibitors lamivudine and stavudine, for example, have a rank score of 0.5. Of the non-nucleotide/nucleoside reverse transcriptase inhibitors, nevirapine has a score of

1 and efavirenz 0.5. These drugs are the first line treatments in South Africa. While several studies have shown that regimens with a relatively high CPE rank (>2) resulted in better neurocognitive outcomes [4], it is not well known whether these regimens may produce neurotoxicity by virtue of their penetration, whether the benefits will persist, or if the HAART-related improvements to date have been observed in individuals with poor baseline neuropsychological performance or worse levels of immunosuppression. Long term studies which examine both the neurocognitive profile, CPE rank, as well as potential measures of antiretroviral neurotoxicity will be needed to resolve these issues. In a well known study, the addition of abacavir to an existing HAART regimen was not associated with improved neurocognitive outcomes [7]. First line treatments in South Africa achieve acceptable CPE ranks scores of between 1.5 and 2. Using a nevirapine-based regimen, a score of 2 is reached by adding the rank scores of lamivudine (0.5), stavudine (0.5) and nevirapine (1).

Second, whether or not less severe forms of HAND result in, or progress to HIV-D, are less clear. Different patterns of HIV-D have now been proposed [15]. It is suggested that some more acute and fulminant forms may be more sensitive to treatments. Nonetheless, only a small proportion of individuals will recover complete neuropsychological function. If, however, HAART could be initiated at higher CD4 counts, where less severe HAND is present, it is possible that progression to less reversible deficits may be prevented. Once HIV-D is present, effective HAART regimens should be used in the first instance. Prospective studies of these issues are sorely needed.

The issue of antiretroviral toxicity has been addressed to a limited extent in the literature, and is based on theories of systemic toxicity, scanty magnetic resonance imaging (MRS) studies, and in vitro evidence [52-54]. This type of antiretroviral neurotoxicity is independent of the phenomenon of neuroIRIS (immune reconstitution syndrome), which is thought to be rare but may result in worsening neurocognitive function despite HAART use [55]. NeuroIRIS may occur from between two and six weeks of HAART, and reflects an inflammatory response following cellular immune reactivation. IRIS is most consistently associated with opportunistic infections (OI's), such as Cryptococcus, tuberculosis or JC-virus. In a small number of individuals, clinical neurological deterioration occurs in the absence of OI's, and is thought to reflect a direct HIV-related immune response.

Adjuvant treatments for HIV-D are less well established. Treatment trials of known drugs such as memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, and lithium, a complex mood stabiliser with glycogen synthase kinase-3 (GSK-3) function, have been conducted with some promising results [11,56]. While lithium may not be an ideal agent in South Africa, and particularly in PLWHA, due its narrow therapeutic index, its beneficial effects may be considered as therapeutic possibilities for other drugs. Putative treatments, based on theories of HIV neurotoxicity include chemokine receptor blockers, anti-oxidants, caspase inhibitors and entry inhibitors, but any novel agent would need to penetrate the blood brain barrier.

### **Outcomes in HIV-D**

The effective use of HAART has clearly been associated with a significant reduction in the disease burden of HIV-D, as noted above. The incidence of HIV-D has decreased substantially [7]. In untreated HIV-D the mean survival is about six months [57]. With HAART, this has increased to two years, even in PLWHA with low CD4 counts at baseline [58]. Given that more than 20% of individuals in primary care in the Western Cape have HIV-D, there are significant implications for care [19].

Nath and colleagues have proposed a number of subtypes of HIV-D that have emerged in the HAART era, including a subacute progressive type, a chronic inactive type, a chronic active type and a reversible type [15]. Other groups have reported “reversibility” or “treatment response” in about 30% of PLWHA with HIV-D [59]. The converse is true that more than half have persistent deficits. This means that on the one hand, a substantial number of PLWHA with HIV-D will improve, and that the use of HAART is imperative, but also that a larger number will require additional treatment support. The implications for PLWHA who are economically or socially active are enormous. To date, there are no published cohort studies in South Africa, and this is needed.

### **Approaches to detection, treatment and research in resource-limited settings**

Given that South Africa has the largest HIV epidemic in the world, and that despite putative clade differences in neurotoxicity, it is likely that a substantial proportion of individuals entering late stage HIV/AIDS will develop HIV-D. Our approach should be three-fold:

- (1) Wide-spread screening programmes:

This will involve the use of screening tools for HIV-D being integrated into primary health care. This is in order to detect covert cases of HIV-D in busy clinics, where awareness of milder HIV-D may be low. Several instruments exist, including the HIV Dementia Scale and the Brief Cognitive Neuroscreen [60,61]. A brief tool that has been validated for use in Uganda, and that can be taught to non-neurologist staff, is the International HIV Dementia Scale (IHDS) [62]. Using a cut-off score of 10, the IHDS has a sensitivity and specificity of 80% and 55% respectively. The integration of the IHDS will involve training in its use, and information regarding what to do with positive screens. At clinics, where more extensive neuropsychological testing is not available, patients who screen positive may be evaluated by more experienced clinicians on a referral basis, and then initiated on HAART. Additional treatment support and follow-up should be considered. Where additional neuropsychological resources are available, referral may be an option. We recommend the use of a test battery containing tests of the following domains: attention and concentration, verbal memory, psychomotor function and executive function. Tests within these domains may include the digit symbol coding test for attention, the Hopkins auditory verbal learning test for verbal memory, the grooved pegboard test for psychomotor speed, and the color trails tests 1 and 2 for executive function. These tests are widely used and are readily taught to technical staff with a minimum of necessary equipment.

### (2) Consideration of offering HAART to individuals with higher CD4 counts

There is debate as to whether HAART should be started in PLWHA with higher CD4 counts. What is known, is firstly, that mild neurocognitive disorders (MND) are common, occurring in about 20-30% of PLWHA, depending on disease stage [13]. Secondly, the presence of MND correlates with neuropathological changes similar to HIV-D [39]. Thirdly, the presence of MND is likely to produce significant functional impairment [63]. Whether or not MND predicts progression to HIV-D is less clear. We propose that PLWHA with documented MND and functional impairment are at risk of this deterioration, and should be initiated on HAART. Prospective cohort studies are needed to define the outcomes more clearly.

### (3) Prospective cohort treatment effects studies

To date, there have been no published cohort treatment effect studies in South Africa, and relatively few in Africa. Studies of PLWHA entering ARV care should be conducted with a view to describing the course and progression of neuropsychological impairment, the impact on function, and the effect of anti-retrovirals on cognition. Careful clinical characterisation may further allow for the

development of predictive variables and potential biomarkers of HIV-D. In this way, a greater understanding of potential risk factors may be developed. The effect of anti-retrovirals on cognition is receiving greater attention and these studies should carefully document these [15].

## **Conclusion**

The involvement of the CNS in HIV- known as “neuroAIDS”- has profound implications in South Africa where the largest number of infected people lives. Several clinical implications emerge from this review. Screening for HAND should be routinely performed in all individuals entering care, and in those with higher CD4 counts in whom a neurocognitive disorder is suspected. They should be followed up in order to describe the course and impact of neuroAIDS. In this way, we can begin to address the massive burden that dementia-associated with HIV infection poses.

Locally relevant research directions should include investigations into either “mechanisms” or “treatments”. Prospective cohorts in South Africa should be studied, with a view to gathering not only data on neurocognitive problems and their course, but also the underlying host-viral interactions, such as clade-specific differences. Treatment programme research needs to consider ways of addressing the burden of disease of neurocognitive disorders, and may involve studies of epidemiology, but also of providing supportive and therapeutic approaches to individuals with neurocognitive problems. The problem of HIV dementia will not abate with widespread HAART, and novel solutions to address this burden are urgently needed.

There is an a priori assumption that HAART improves neurocognitive function, following immune-reconstitution. Prospective empiric studies need to be reviewed to document this outcome. In particular, questions remain as to the extent that HAART improves neurocognition; whether some domains of function improve more than others; and whether or not response to HAART can be predicted based on demographic, clinical or other disease variables. This will be discussed in the next chapter.

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### **Chapter 3.**

#### **Does Highly Active Anti-Retroviral Therapy Improve Neurocognitive Function?**

##### **A systematic review**

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## **Abstract**

Highly active anti-retroviral therapy (HAART) reduces the incidence of HIV-dementia (HAD), while the overall prevalence appears to have increased. Recent changes to diagnostic nosology have emphasised the presence of neurocognitive deficits. Uniform methods of ascertaining neuropsychological impairment and excluding confounding causes are critical to between-study comparison. We conducted a systematic review on all studies which use single cohort prospective treatment effect design that reported on the neurocognitive or neuropsychological profile of individuals commencing HAART. We included 15 relevant studies. A large number of studies using observational or cross-sectional designs were excluded, as these do not allow for a within-subject description of pre- and post-HAART predictive factors. Eleven studies reported a significant improvement in neurocognitive status or neuropsychological profile over an average study period of six months. Variable or non-reporting of HAART regimens in these studies did not allow for an analysis of individual agent or regimen effectiveness. While HAART does improve cognition, it does not appear to fully eradicate impairments. The methods used in this research differ widely and therefore comparison across studies is difficult. Studies examining the long term effects of HAART on HIV-associated neurocognitive disorders (HAND) using uniform methods of data collection are needed, together with clear reporting of HAART regimens.

## Introduction

Prior to the widespread use of highly active anti-retroviral therapy (HAART), infection with HIV resulted in HIV-associated dementia (HAD) in about 15% of individuals [1]. Less severe forms of HIV-associated neurocognitive disorders (HAND) are found in about 30-60% of people living with HIV/AIDS [1,2]. The advent of HAART has substantially altered the nature of these disorders, although they frequently persist [3,4]. Specifically, HAART has reduced the incidence of HAD, but the prevalence appears to be increasing [5,6]. In addition, the clinical presentation of HAD has changed [7]. It has been suggested that the sub-cortical features previously thought to be characteristic, may be less prominent [8]. More recently, efforts to predict response to HAART have intensified. Currently it is thought that people who initiate HAART and achieve plasma viral suppression [9], CSF viral suppression [10] and who use CSF penetrating regimens accrue the most benefit [10,11].

Central to the characterisation and description of HAND is the use of a universal diagnostic classification. The original criteria of the American Academy of Neurology, proposed in 1991, recognised two main forms: that of HAD, and a less severe minor cognitive and motor disorder (MCMD) [12]. This system emphasised the presence of behavioural and personality changes. The limitations of this approach, particularly in the face of both a growing understanding of HAND and the use of HAART, include a lack of emphasis on the cognitive deficits in HIV, as well as the presence of these deficits in the absence of overt functional decline in some individuals [13]. These limitations were addressed in a set of newer research criteria, proposed by the HIV Neurobehavioural Research Center (HNRC) and published in 2007 [14]. They now include a category of asymptomatic neurocognitive impairment (ANI), and also address the more widespread neurocognitive deficits which are thought to occur in HIV. The ANI category together with mild neurocognitive disorder (MND), require that neuropsychological deficits corresponding to at least one standard deviation below age-appropriate norms in at least two cognitive domains exist. A diagnosis of HAD is made when two or more domains reveal deficits of at least two standard deviations below the norm [15]. In addition, other causes of cognitive disorder need to be excluded, and some measure of function must be provided. Widespread use of this approach would go a long way to standardise studies of HAND, but may not always be possible or practical, particularly in resource-limited settings.

These clinical case definitions are now known to represent the underlying neuropathology, namely HIV encephalitis, and demonstrate a sensitivity and

specificity of 67% and 92%, respectively, for the HNRC categories [16,16,17]. As indicated above, these neuro-pathological changes are now thought to involve various cognitive domains. In fact, if systemic disease factors are controlled for, HAD is characterised by severe deficits in learning, motor coordination, verbal fluency, and memory, while moderate deficits are observed in attention and processing speed [18]. These represent a range of deficits across subcortical and cortical domains. In order to ascertain whether neuropsychological deficits are indeed related to HIV-related neuropathology, it is therefore necessary to assemble a range of neuropsychological tests that measure the brain regions thought to be typically affected by HIV [4]. Clinical and research batteries differ widely in their selection of tests, duration and spread across cognitive domains.

Other factors which impact on HAART-related outcomes include study design, longitudinal construct validity of neuropsychological testing, and numerous treatment and disease variables. In a recent substantive review, Cysique and Brew clearly delineate differences between cross-sectional cohort designed studies, prospective observational cohort studies and prospective treatment effect studies [19]. The cross-sectional studies are largely limited by uncontrolled cohort effects (see [3,20], while the prospective observational studies tend to include cohorts already on HAART who have either switched regimens or followed neuropsychological changes whilst on HAART (see [21,22]. Prospective treatment effect cohort studies offer the advantage of describing a range of pre-treatment variables, which may either predict or be associated with positive or adverse outcomes. These are then carried into the study in a case-controlled manner. The issue of longitudinal construct validity refers to whether tests or sub-tests can be considered appropriate for measuring neuropsychological functions over time. It is possible that some functions may improve de facto, but that change over time may also be affected by the specific function reaching a plateau due to persistence of deficits, practice effects, the severity of the deficit at baseline or disease-specific factors [23-25].

Disease-specific factors which may impact on outcomes in HAND include viral resistance, HAART-related neurotoxicity, central nervous system (CNS) penetration of HAART, the effects of ageing and co-morbidities, viral clade, and molecular biology. In particular, it is well established that HAART has reduced the incidence of severe forms of HAND, such as HAD, while there is clear evidence that at least milder forms persist (see [3,26]. Viral resistance may follow individual non-adherence and systemic resistance, the infection of individuals with resistant strains of virus, or the development of intra-individual (CNS in particular) resistance [27,28]. CNS

compartment resistance, whereby the CNS acts like a reservoir of HIV, may be affected by limited or even differential penetration of individual anti-retroviral drugs (see below) [29]. The issue of anti-retroviral toxicity has been addressed to a limited extent in the literature, and is based on theories of systemic toxicity, scanty MRS studies, and in vitro evidence [30-32]. This type of anti-retroviral neurotoxicity is independent of the phenomenon of neuroIRIS (immune reconstitution inflammatory syndrome), which is thought to be rare but may result in worsening neurocognitive function despite HAART use [33].

A related factor is the penetration of anti-retrovirals through the blood-brain barrier. The ability of these agents to pass into the brain, depending on their protein-binding, molecular size and lipophilicity has led to the development of a “CNS Penetration Effectiveness” (CPE) rank system [34]. While several studies have shown that regimens with a relatively high CPE rank (>2) resulted in better neurocognitive outcomes (see Letendre et al., 2004; [10], it is not well known whether these regimens may produce neurotoxicity, whether the benefits will persist, or if the HAART-related improvements to date have been observed in individuals with poor baseline neuropsychological performance or worse levels of immunosuppression. A recent prospective treatment effect study reported that regimens containing a higher CPE rank score were effective in suppressing CSF viral loads but were associated with worse neurocognitive performance [35]. Long term studies which examine both the neurocognitive profile and the CPE rank, as well as potential measures of anti-retroviral neurotoxicity, will be needed to resolve these issues.

The question of whether viral subtype or clade is responsible for differences in HAND has not been studied well enough in clinical populations. For instance, the neurovirulence of HIV clade C has been associated with less severe forms of neurocognitive impairment in some studies, but with equally deleterious effects in others [36-38]. Variability has been attributed to differences in the dicysteine motif within the neurotoxic region of B-Tat, producing a greater (or lesser) degree of Tat-induced apoptosis [39,40]. However, other viral proteins such as gp120 may be as neurotoxic. The clade sequence, levels of proviral DNA and tat protein, together with their impact on neuropsychological functions and neuro-imaging findings, is the subject of a study currently being conducted by our group. These clade and viral neurotoxicity studies are needed to better understand mechanisms of HAND. However, where these studies are conducted across different regions with differing culture and language effects on neuropsychological test performance, the need for standard approaches to clinical characterisation of HAND becomes more pressing. A

possible clade-specific difference has already emerged in our preliminary work, wherein we found that HIV positive participants performed as well as HIV negative controls on the grooved pegboard test, a measure consistently used to ascertain whether HIV associated subcortical neuropathology exists [41,42]

Given that prospective treatment effect studies afford many advantages to better understand the impact of viral, treatment and other individual factors on HAART, this systematic review will undertake to examine all such published studies. In particular, the methods of classifying of HAND will be discussed with a view to describing an approach which allows for comparison across studies.

## **METHODS**

### **Search strategy**

The search for studies was conducted using four approaches:

- 1) Using a key word search of the following databases conducted on 12 March 2009:
  - i) a. PUBMED: HIV [mh] OR Acquired Immunodeficiency Syndrome [mh] OR AIDS [tw] OR HIV [tw]) AND (Anti-retroviral Therapy, Highly Active [mh] OR HAART [tw]) AND (Cogniti\* Disorders [mh] OR memory [mh] OR memor\* [tw] OR cognition [mh] OR cogniti\* [tw] OR AIDS Dementia Complex [mh] OR Neuropsychological Tests [mh]). All clinical types of studies on adults were included.
  - ii) PsycINFO: AIDS and HAART and NEURO.\*
- 2) Reviewing the reference sections of articles found in this way and searching for relevant publications.
- 3) Using a hand search to review the tables of contents of key journals, searching for relevant publications. These key journals included: AIDS, AIDS and Behaviour, AIDS Care and STDs, Archives of Neurology and Neurology.
- 4) Personal communication with key researchers in the field. This was defined as first authors of studies included.

The search strategy and retrieved articles are shown in figure 1.

### **Inclusion and exclusion criteria**

We included peer-reviewed published studies in which a clinical sample received a neuropsychological and neuromedical assessment before or during early treatment (defined as within one month of commencement) of HAART, and again within 24 months. A minimum treatment period of two months was required. A clear categorisation of EITHER a neurocognitive disorder OR of global/overall neuropsychological status in patients needed to be reported at both time points. The included studies were defined as prospective treatment effect cohort studies.

We excluded cross-sectional and prospective observational studies where comparisons were made between treatment-naïve and HAART-treated groups or where neuropsychological changes over time were assessed in participants already using HAART/ not HAART naïve. Our primary aim was to describe the pre-treatment factors which may predict or be associated with HAND outcomes. In addition, the dynamics of neuropsychological profile and neuropsychological change are known to be different in individuals who are not HAART naïve (for example, see Robertson, Su et al. (2007), wherein it is reported that treatment interruption resulted in improved neuropsychological function). We also excluded studies of children and studies where the neurocognitive status OR neuropsychological profile was not reported at the two time points.

### **Study sorting**

All articles retrieved on electronic search were loaded into a single Reference Manager™ database (See figure 1). Duplicates were removed. This left 108 studies. Using the criteria set out above, the database was reviewed by two of the authors, independently and respectively (JJ and HG), to ascertain reliability of inclusion and exclusion. The kappa was 0.76. Where there was disagreement, the non-included study was discussed and a decision made as to its suitability. After this stage, 15 studies were identified. The papers were reviewed to establish suitability in terms of two criteria: they needed to report neuropsychological or neurocognitive profile in the same sample at the two time points. Duplicate publications from the same dataset were omitted. Once the electronically retrieved articles had been sorted, these were

reviewed and data extracted using a spreadsheet with key fields. The reference sections of papers reviewed in this way were then screened for other potential studies. Additional studies were discussed between the two reviewers and data extracted. Finally, we wrote to all first authors requesting their willingness to review the reference list and to suggest any papers or studies that they felt needed to be included. A final list of 15 studies was reached.

We also reviewed the quality of studies using a simple Likert-type scale of three areas: (1) assessment - did the study utilise a neuropsychological test battery including at least 3 domains of function, and was this repeated both before initiating and after a period of time on HAART; (2) reporting - did the study report on the full neuropsychological assessment both before and after HAART, and did it indicate whether the first assessment occurred prior to initiating HAART; and, (3) confounders - did the study report on the assessment of potential confounding factors such as neurological conditions, psychiatric disorders or substance misuse. Each domain was rated on a scale of 0 for no, 1 for partly, and 2 for yes. In this way, high quality studies could be viewed as scoring between 4 and 6, intermediate quality studies between 2 and 3, and lower quality studies less than 3.

## **RESULTS**

### **Nature of studies**

The majority of studies identified were conducted in the USA, where clade B is predominant (n=11); the remainder consists of one each completed in Brazil and Thailand, and two in Uganda (Table 1) [43-46]. Where reported, almost all studies were done in infectious diseases clinics or in research projects that were associated with such clinics. Sample sizes of HIV positive individuals included in these studies range from 14 to 126, with one large study including 303 individuals. The mean sample size was 69, with a median of 49. Most studies report good follow-up rates, with only two studies managing to review less than 80% of recruited subjects (67.7% and 71.4% respectively). The mean age of participants was 37.05 years, and ranges from 29.7 to 45.2 years. Studies report a wide range of gender distribution, while the mean percentage of men included was 66%. Seven studies reported on ethnicity/racial distribution of participants. For the majority that did, either “non-white” or “African American” groups were listed. The percentages of these “non-white” participants ranged from 18-78%. In most cases, the degree of immuno-suppression

at study entry was significant, with a mean CD4 cell count in all included studies of 179.2 (53-392.2). This mean improved to 285.8 after HAART use (148.5-337. Note that the post-HAART CD4 count for the study reporting a higher CD4 count at study entry was not provided). Similarly, the pre-HAART mean viral load in log<sub>10</sub> copies was 4.64 and improved to 3.29 post-HAART.

### **Measures**

The clinical assessment of neurocognitive disorders requires the exclusion of confounding causes (see table 2). Most studies (n=10) utilise either a psychiatric history or make use of rating scales to exclude participants who suffer from psychiatric disorders. Of those that use rating scales, two use the Centres for Epidemiology rating scale for Depression (CES-D), and one each use the Hamilton Depression Rating Scale and Thai Depression Inventory. Patients with current psychiatric disorders are generally not included in studies of HAND. Similarly, four of the 13 studies do not formally report on the screening of substance use disorders. Those that do, use a combination of self-report and clinician-interview, with only two using formal drug testing procedures. Only two studies formally report on the exclusion of concomitant neurological problems. Most utilise some type of standardised clinical or neurologic examination. Only one study, which aimed to correlate the use of HAART with magnetic resonance spectroscopy findings, utilised formal neuro-imaging to exclude intra-cranial pathology [47]. Regarding the reporting of functional assessment, only four studies note this, with three reporting impairment of function using the Karnofsky score. In these, the scores range from 66 to 84.

The prevalence of neurocognitive disorder is noted in nine studies, with three utilising the Memorial Sloan Kettering (MSK) score. Many of the studies recruited participants from specialised clinics, and in most cases, sought to include people with established HAND. The prevalence of people who had normal MSK ratings ranges from 4% to 69%, while 21% to 48% had “equivocal” ratings, 10% to 61% had stage 1 scores and one study reports a prevalence of 7% of stage 2 scores. Three other studies report a prevalence of HAD ranging from 22.4% to 61% [9,48].

### **Neuropsychological test batteries**

In the studies included in this review the neuropsychological test batteries vary widely. In general terms, half of the studies include formal tests of verbal learning, with three using the California Auditory Verbal Learning Test, three the Rey Auditory

Verbal Learning Test, and one an unspecified verbal learning test. Psychomotor speed is tested using the Trail-Making Test B (TMT B) in seven instances, and the Color Trails 2 in two instances. In addition, the Grooved Pegboard (either dominant hand or non-dominant, or both) is used in eight of the batteries. Executive functions are assessed using a variety of tests, including the Stroop Color-Word Test (n=4), and components of the trail-making tests (TMT B or Color Trails 2). The Digit Symbol Substitution Test, a test of attention and speed of processing, is used in ten batteries.

### **Use of HAART**

A wide range of HAART regimens are reported and there was no emergent trend. Studies where these are specified note regimens based on non-nucleotide reverse transcriptase inhibitors (NNRTI's) (efavirenz in 2 cases, nevirapine in 2 cases), while in others protease inhibitor-based regimens are reported (n=3). The duration of use ranges from eight weeks to two years, but most (n=8) utilise an average study period of six months. In studies reporting non-significant neuropsychological improvement, the HAART regimen is noted in two of three cases: in the one study a combination of AZT, 3TC and EFV was used, while in the other an unspecified combination of NNRTI's was used. These regimens both rank CPE >2.

### **Neuropsychological outcomes**

Studies included in this review report on neuropsychological outcomes in a number of different ways (see table 2). In some instances, neuropsychological data are compared to an HIV-negative group. Most of the included studies (n=11) note a significant improvement in neuropsychological function of individuals following HAART initiation. In most instances, investigators make use of z-scores based on population norms (seven of the eleven studies), with most using a composite z-score based on combinations of neuropsychological tests (NPZ). It was not possible to generate a pooled effect due to the variability in the number of tests used for calculating the NPZ scores in different studies (for example, NPZ4 or NPZ6). In two studies where composite z-scores pre-and post-HAART are reported, the scores improved from -0.74 to -0.52 [49] and -0.62 to 0.29 [50] respectively. There were three studies that do not find significant improvements in neuropsychological function following initiation of HAART. In one, the authors report that this may be explained by the fact that HAART may improve more severe HAND (such as HAD), as opposed to milder forms, and that their sample size was small (14 participants) [51]. In another study, the specific focus was on hepatitis C co-infection, and while there was a trend to improvement on HAART, it does not reach significance [52]. The remaining study

reports separately on HAART responders and non-responders; there were more non-responders than responders (39 vs 19). No reason for this disparity is provided [9].

### **Quality of studies**

When we conducted a review of the quality of the studies, using the method described above, we found that most are high quality studies (n=8), with only one study rating less highly (should this be referenced?). This particular study was published as a brief report, and could have excluded certain clinical parameters for the sake of brevity. What is striking is that most studies do not report on or address all of those factors which may be considered as comprising a detailed and high quality study of HAND. For example, four studies do not report on any assessment of substance misuse and only two note any neuro-imaging findings. In addition, only four studies formally report on functional assessment. It may be suggested that more formal reporting of these issues are needed to fully appreciate the complexity of the diagnostic issues in HAND.

### **DISCUSSION**

To our knowledge this is the first systematic review examining the effect of initiating HAART in a prospective treatment effect cohort of people with HIV. Our findings support the existing literature that, in general, the initiation of HAART results in improvement of neuropsychological function. Although the duration of treatment most often reported was only six months, improvement was neither full nor universal. Factors contributing to the variety of treatment responses include genetic vulnerabilities, co-morbid substance abuse, viral resistance and neurovirulence factors, and host immune and inflammatory responses. These have only been explored to a limited extent in these prospective cohort studies. In addition, these studies vary greatly in their methodologies, leading to difficulties in interpreting and collating these findings.

In this review, a significant improvement in either neurocognitive status or neuropsychological profile was reported in eleven of the 15 studies. It was not possible, due to the variability of reporting, to conduct formal meta-analysis, although this would clearly be desirable. In most instances these improvements were noted across a number of different neuropsychological domains. It may be possible in subsequent reviews or meta-analyses to identify whether particular domains improve more than others and over what period of time. Deficits reflective of a loss of cortical

neurons, for example, may be more persistent than ones suggesting white matter damage, which may be reversible. Such studies may best employ specific neuro-imaging techniques coupled to neurocognitive assessment. In studies that did not find significant improvement in neuropsychological function, it is suggested that the nature of HAND studied may have been a factor. In particular, it is now known that while HAD occurs less commonly in the HAART era, milder forms are becoming more frequent [5]. In this way, it is possible that HAART has a greater mitigating effect on severe HAND, and less so on milder neurocognitive disorders.

We also note that different investigators report on this field very differently. In the first instance, there is little uniformity in the selection of neuropsychological tests. Whilst there is general agreement about the nature of HIV-related neurocognitive impairment, test batteries vary both in length and structure. It could be argued that a greater uniformity would lead to improved comparability across not only different regions (and viral clades), but also between different study questions (for example, some studies examine co-infections while others examine CSF viral loads). In this regard, investigators across studies may consider using an agreed upon minimum number of tests- for example six tests measuring three domains which must include memory, psychomotor and executive functions. Specific tests may also be agreed upon, particularly where language and cultural effects may be regarded as less influential. Examples include the grooved pegboard test, the digit symbol test, and the color trails test. The adoption of such an approach does not, however, remove the need for age and language appropriate norms. In addition to test batteries, few studies include a description or classification of HAND. While it is understandable that the key to understanding the mechanism of neuropsychological change requires that such scores are reported in detail, the provision of diagnostic categories and prevalence would again aid in the understanding of regional differences in severity and course. Other challenges identified in this review are that sample sizes were mostly small, with most studies including less than 100 participants, although follow-up rates are generally very high. These cohorts do allow for intra-individual comparison, but they are limited in their ability to report on categories of HAND and the associated factors or predictors of severe types of cognitive disorder.

A key issue that has emerged in the understanding of neurocognitive change or improvement over time is the duration of treatment and the point at which follow-up

assessment is made. A recent study has highlighted the variability of this improvement, noting that in some individuals improvement occurs within the first weeks of HAART, while for the majority of individuals this may occur after up to a year [53]. These findings seem to contribute to the notion of the variable course of HIV-D, as noted by Nath and others [5,6]. The “early improvement” group might be associated with a greater degree of baseline impairment, and with clinical and immunological features suggestive of an inflammatory process, as well as with good viral suppression on HAART [54]. Discriminating between those with active disease and those with “burnt out” or stable deficits may not only provide indicators as to factors driving disease activity, but may also be relevant to developing targeted or adjuvant treatments. Given that the majority of people living with HIV/AIDS (PLWHA) continue to improve from six months, the importance of following these individuals up to one year and probably beyond is critical to understanding further the effects of HAART in the long term.

Research into the predictors of response to HAART is essential to informing the clinical practice likely to produce the best outcomes. The measurement of peripheral blood CD4 count and viral load is considered standard practice before and during HAART. In addition to a low baseline CD4 nadir being predictive of HIV-D, the suppression of peripheral viral load has consistently been linked to better neurocognitive outcomes in the face of good adherence to HAART [55]. The role of CSF analysis is less clear, with good CNS penetrating HAART being associated with suppression of CSF viral load and good neurocognitive outcomes in some studies, but with either failure to suppress or adverse neuropsychological performance in others [10,56,57]. In this review, of the studies reporting neurocognitive improvement on HAART, only three reported on baseline and follow-up peripheral viral loads. In all three there was significant improvement in this parameter, and in all cases suppression of peripheral viral load was directly associated with suppression of CSF viral load. Corresponding measures of CNS inflammation were examined only in one study using MRS [47]. In one other study, peripheral monocyte HIV DNA was associated with poorer neuropsychological performance at 48 weeks, suggesting that the peripheral pool of infected monocytes may stimulate ongoing CNS inflammation [58]. Further cohort studies wherein other measures of immune and inflammatory response (such as cytokines), inflammatory protein (for example amyloid) and imaging markers (such as diffusion tensor imaging) are addressed, should be conducted. Other baseline characteristics, such as anti-retroviral drug resistance,

may predict neurocognitive outcome. In a recent study it was reported that the presence of anti-retroviral resistance mutations may be associated with diminished neurovirulence [59].

The importance of obtaining locally derived population normative data is central to the neuropsychological characterisation of impairment. In general the approach has been to generate these data from either matched or similar groups of individuals within the population under study. The degree to which these groups are regarded as similar is often based on the cultural and language expression of the group in question, and it is these parameters which drives the development of new normative datasets [60]. While other demographic factors such as education are well known to exert significant effects on test performance, there may be other difficult-to-measure group variables which also do so and which may result in the over-diagnosis of HAND [4]. So, while good normative data represent an essential starting point for conducting research into HAND, the deconstruction of cultural and linguistic variables may be needed to make a test battery truly relevant to the group under study [61].

Much of the literature examining neurocognitive disorders could not be included in this review, mainly due to the absence of cohort-type studies. In many instances, studies comparing HAART-naïve and HAART-using groups were made, but not in the same individuals. Many studies we initially found were excluded because they did not make use of formal neuropsychological measures. This has been emphasised by many as the key to the diagnosis of HAND [62]. This analysis was limited by the small number of included studies, but we believe that our findings remain important and valid. In particular, the variability among studies and their differing approaches was evident.

There is clearly then a need for further studies examining the effect of HAART in a cohort of well-characterised individuals, and preferably making use of tools that allow for comparison with other studies. Given the constraints of resource-limited settings in terms of time and skills, we would recommend that a neuropsychological battery for use in international settings might include tests of the following domains: attention and concentration, verbal memory, psychomotor function and executive function.

Tests selected for use require age and educational appropriate norms, and should be properly translated into the local language. A longer battery has been used successfully in cross-cultural settings (see [63] and [64]). In addition, we recommend the use of a brief activities of daily living scale - we have adapted the Lawton Brody Scale for this purpose [65]. An assessment of neurologic status is a pre-requisite, and should be structured to examine neurological functions affected in HIV/AIDS. Structured or semi-structured clinical interviews are needed to establish substance use history and psychiatric disorder status. Together, this approach will allow at least for some standardisation across studies and regions. In order to address the issue of treatment effect, it is suggested that studies clearly report on HAART regimen used, duration, and possibly CPE rank. The reporting of neuropsychological test means and standard deviations would allow for potential meta-analytic approaches.

In the next chapter, the baseline neurocognitive characteristics of individuals commencing HAART are described, using an international neuropsychological battery outlined above- and adapted for use in South Africa. The assessment included functional and neurologic assessment. This would be the first study in South Africa using a detailed battery to assess neurocognitive function across a number of domains. In addition, the next chapter reports on whether a number of demographic and clinical variables are associated with HIV-associated dementia (HAD)- the most severe form of HAND. The inclusion of apolipoprotein E (APOE) genotyping at this assessment (as reported in chapter 5), adds to knowledge regarding the putative role of APOE as a vulnerability factor for HAD. In chapter 7, I present data from the re-evaluation of neuropsychological function at one-year using the same detailed battery, in order to address the question raised by this review- whether the use of HAART is associated with neurocognitive improvement in individuals infected with clade C HIV; and whether there are associated or predictor variables of this change.

Figure 1: Results of search strategy

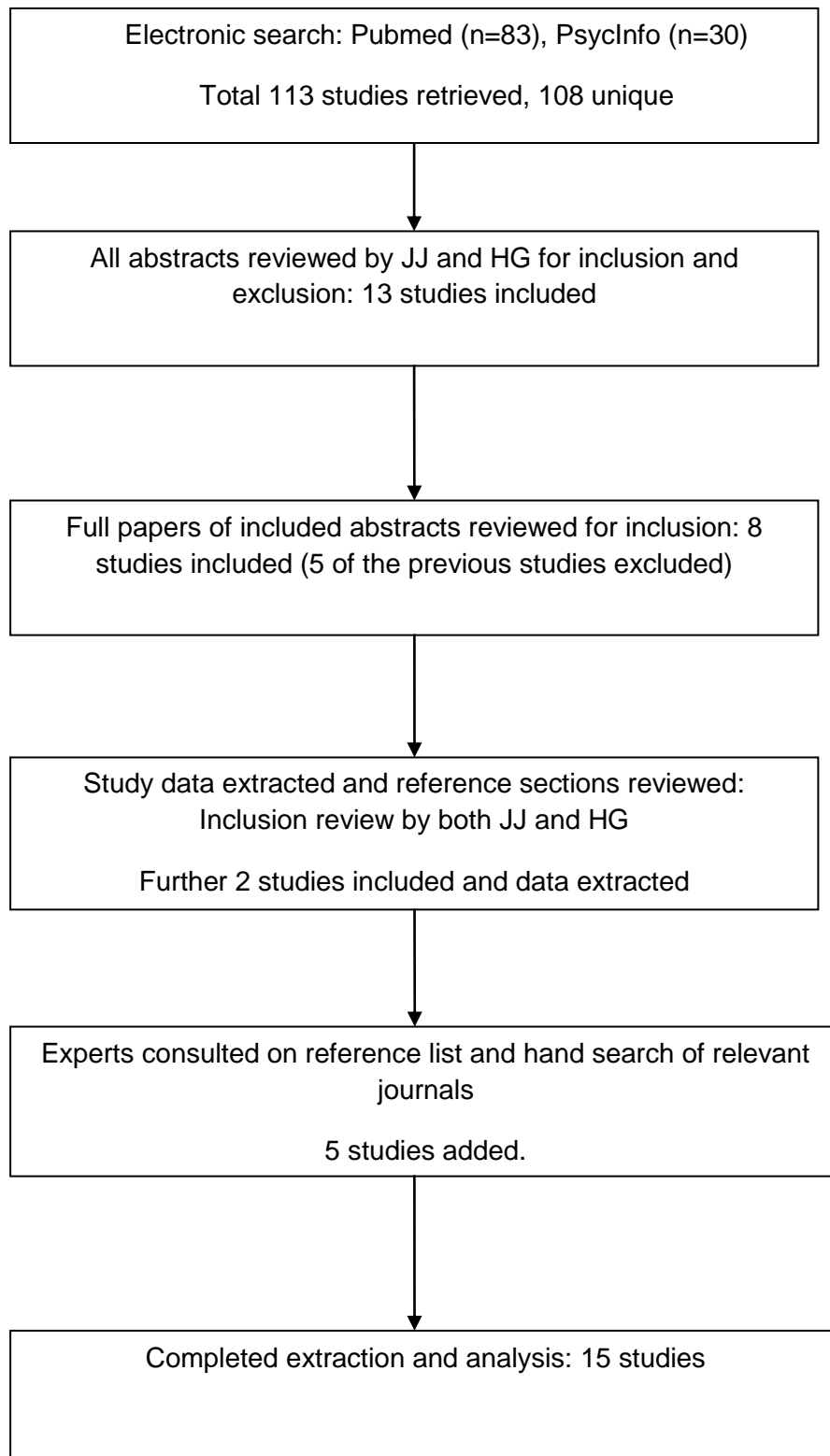


Table 1. Descriptive variables of studies examining neurocognitive function before and after initiating HAART

Author	Country	Setting	Sample size	Follow-up rate	Age	Education in years	Men %	Pre-HAART CD4	Post HAART CD4	Pre-HAART viral load	Post-HAART viral load
Baldewicz 2004	USA	Research clinic	59 HIV+ and 55 HIV-	91.2	29.7	14.2	100	392.2	Not reported	Not reported	Not reported
Carvalho 2006	Brazil	Infectious diseases centres	14	71.4	35.5	8.4	57	134.6	239.1	4.56	<80 to 25 000
Chang 1999	USA	Infectious diseases centres	16 HIV+ and 15 HIV-	100	44.3	Not reported	88	163	274	Not reported	Not reported
Clifford 2005	USA	Infectious diseases centres	303	93.4	37	Not reported	81	219	Not reported	4.74	Not reported
Cohen 2001	USA	Infectious diseases centres	126 (55 received HAART)	100	33.2	12.2	0	64.9	119.4	77978	48226
Cysique 2009	USA	Research clinic	37	18	39.7	13.6	86.5	195.6	Not reported	4.9	50% LDL at week 12
Marra 2003	USA	Not specified	Total 25- 13 HAART naïve	88	34.5	13	92.9	207	Not reported	4.73	Not reported
Marra 2009	USA	Research clinic	79 (44 naïve)	60	39	13	83.5	111	Not reported	4.86	Not reported
Parsons 2006	USA	Health Care system	65	67.7	41.4	12.3	64	239.8	316.95	4.25	3.13
Robertson 2004	USA	Infectious diseases centres	48	100	38.77	12.54	62.5	225.81	310.52	4.56	2.64
Sacktor 2000	USA	MACS cohort infectious diseases clinic	33	100	38.5	13	88.5	Not reported	Improved by 60	Not reported	<1 in responders

Sacktor 2003	USA	MACS cohort infectious diseases clinic	49	100	45.2	% college: 57- 70	100	281	337	4.35	Responder: 2.4
Sacktor 2006	Uganda	Infectious diseases Clinic, Mulago Hospital	23	91	32.8	8.7	23	71	176	Not reported	Not reported
Sacktor 2009	Uganda	Infectious diseases clinic, Kampala	102 HIV+and 25 HIV-	92	34.2	HIV+ 9.1 (4.3); HIV- 10.3 (4.2)	29	129	272	Not reported	Not reported
Valcour 2009	Thailand	Infectious diseases/ neurology clinics, HIV testing centres	30	93.3	32	6	33	23	190	5.3	All LDL, but 1

Table 2. Clinical and neuropsychological characteristics

Author	Substances	Psychiatric	Neurologic exam	Neuro-imaging	Function	Neuro-cognitive prevalence	HAART Used and Duration	Test Battery	NP Baseline	NP outcome
Baldewicz 2004	Clinical interview, but not reported	Hamilton Depression Rating Scale; SCID depression	Clinical examination	Not reported	Not reported	Asymptomatic at baseline	Included NRTI's but not specified; duration not specified	FT, Ruff 2 and 7 Selective Attention Test, CVLT, TMT B, SCWT, DSS-W-R	All domains reported as F-scores and significantly different from HIV-controls	Average z-score by domain over time: AIDS fine motor 0.2, attention 0.4, memory 0.55, executive 0.4, speed of processing 0.3
Carvalho 2006	N/A	Not reported	Clinical examination	Not reported	Not reported	Not reported	AZT, 3TC, EFV; duration 6 months	Verbal Fluency Test <sup>1</sup> , Logical Memory, Visual Recognition Test, Word Span, SCWT	NPZ6 0.05	NPZ6 0.075 (ns)
Chang 1999*	Urine toxicology negative	Not specified-excluded on history	Neurologic examination-exclusion	No MRS changes except NA/CR elevated in BG at baseline	Karnofsky 80.6 (50-100)	All patients recruited had Cognitive Motor Complex	Various; duration 9.1 (3-14) months	HDS plus neuropsychological evaluation not reported, providing Karnofsky and ADC staging	HDS 10.3	HDS 12.2
Clifford 2005*	On history, IDU 1 current, 29 previous	CES-D (median score 12), State-Trait Anxiety Inventory (median 55)	Not reported	Not reported	Not reported	Not reported	EFV and non-EFV groups; duration 24 weeks	TMT A/B, DSC-W-III, NPZ3	-0.09 (EFV) and -0.03 (non-EFV) Z-score	0.51 and 0.61 medians in groups (sig)
Cohen 2001*	interview for alcohol: 46% alc, 20.6% IDU and 38.9% illicit subs within last 6 months	CES-D 22.6	Not reported	Not reported	Not reported	None HIV-D, high prevalence of impairment but figures of NCD status not reported	PI, plus NRTI/NNRTI; duration 28.4 (15.3) weeks	GP D, CTM 1/2, COWAT, FWL	HAART SD: CT1 49.8 (19.9), CT2 106.5 (31.8), COWAT 24.2 (8.4), GP D 87.0 (36.5), FWL 3.1 (1.1)	HAART SD (change): CT1 41.3 (11.0-sig), CT2 89.6 (19.0-sig), COWAT 22.5 (-1.0), GP D 81.0 (8.6-sig), FWL 3.4 (0.3)

Cysique 2009	Clinical interview	Clinical Interview for Psychotic Disorder	Neurologic history only	Not done	Not reported	All impaired at baseline with average GDS 1.44 (0.93)	Not specified; CPE rank mean 1.4, duration 48 weeks	GP D and ND, PASAT, TMT A and B, Letter Fluency (F,A,S)	GDS 1.44 (0.93)	13.5% improvement at 12 weeks, 40.9% at 36 weeks, and 33.3% at 48 weeks respectively
Marra 2003*	Not reported	Not reported	Standard neurologic examination	Not reported	Not reported	Only NPZ reported	Indinivir, AZT and others; duration 8 weeks	TG, GP D, FT ND, Digit Symbol Tests <sup>1</sup>	NPZ4= -0.31 (-0.83-1.01)	NPZ not specifically reported, but improved significantly
Marra 2009	Not reported	Medical history	Standard neurologic examination	Not reported	Not reported	Only NPZ reported: 43/79 impaired	NNRTI regimens at 39% of visits, duration 52 weeks	NPZ4: timed gait, GP dominant, digit symbol, FT non-dominant; NPZ8: add RAVLT, GP non-dominant, TMT A and B, FT dominant, CalCAP	NPZ4=-0.29 (-0.96-0.14); NPZ8= -0.24 (-0.70-0.15)	NPZ4 in those on 3 ARVs was 0.36 (s); those on 4 was -0.58 (ns)
Parsons 2006	Current substance abuse- alcohol 16 drinks per month, cocaine 0.85 days per month, cannabis 0.85 days per month	1/3 current depression, 34.5% current anxiety on history	Assessed on history	Not reported	Not reported	Not specified	Not specified; duration 6 months	Ruff 2 and 7 SAT, PASAT, Computerised Reaction Time Tasks, DS <sup>1</sup> , TMT A and B, SW, SCWT, AVLT <sup>1</sup> , Complex Figure Test-IM and DR <sup>1</sup> , GP <sup>2</sup> , FT, TG	z-score -0.78	z-score -0.55 (ns)
Robertson 2004*	Not reported	Not specified	ACTG full evaluation using a weighted scoring approach	Not reported	Quality of life scale/ MSK scale	MSK scores- 69% normal, 21% equivocal, 10% stage 1	Not specified; duration 6 months	Ruff 2 and 7 SAT, PASAT, Computerised Reaction Time Tasks, DS <sup>1</sup> , TMT A/B, SCWA, COWAT, RAVLT, ROCF, GP <sup>2</sup> , FT, TG, Vocabulary -WAIS-R	Total z-score -0.74	Total z-score -0.52
Sacktor 2000*	Not reported	Not reported	Clinical examination	Not reported	Not reported	HAD 61% at baseline	Various including PI; duration 2 years	GP D/ND	23/30 had GP ND z-score <-1.0	23/33 GP ND improved
Sacktor 2003	Screened on history but not reported	Not reported	Screened on history but not reported	Not reported	Not reported	11/49 probable HAD, 34/49 possible HAD	Not specified; duration not specified, minimum 6 months	DSMT and TMT B	Symbol digit -1.45; TMT B -0.825	Symbol digit --1.025; TMT B -0.225
Sacktor 2006*	Clinical interview, but not reported	Psychiatric history	Standard neurologic examination	Not reported	Karnofsky score, mean 66	MSK scores- 4% normal, 35% equivocal, 61% stage 1	3TC/D4T/NVP (n=18), AZT/3TC/TEN (n=5); duration 6 months	WHO-UCLA AVLT, GP D/ND,DSMT, Timed Gait, CT 1/2, DS F/B, Karnofsky Performance Scale, MSK	z-scores: WHO-UCLA AVLT total -1.7, GP D - 0.4, GP ND -0.2, CT1 - 1.2, CT2 -1.5, DSF -0.7, DSB -0.7	z-scores: AVLT -0.1, GP D 0.2, GP ND 0.3, CT1 -0.1, CT2 -0.3, DSF -0.2, DSB -0.2

Sacktor 2009*	Clinical interview, but not reported	Psychiatric history	Standard neurologic examination	Not reported	Karnofsky HIV+ 84 (8.5); HIV- 98 (4.1)	MSK scores- 12% normal, 48% equivocal, 33% stage 1, 7% stage 2	Triimmune (stavudine, lamivudine, and nevirapine); duration 6 months	WHO-UCLA AVLT, Timed Gait, Finger Tapping, GP D/ND, DSMT, , CT 1/2, DS F/B, Category Naming <sup>1</sup> , Karnofsky Performance Scale, MSK	z scores: AVLT total -1.2, CT1 -1.7, CT2 -2.8 SDMT -0.8, GP D 0, GP ND -0.7, FT -1.0, TG 2.7, Verbal Fluency -0.4	z scores: AVLT total - 0.1, CT1 -0.4, CT2 - 1.3 SDMT -0.3, GP D 0.4, GP ND 0.3, FT - 0.5, TG -1.8, Verbal Fluency 0
Valcour 2009*	Interview and urine screen-	Thai depression inventory 19.5	Clinical examination	MRI brain	Not reported	12/27 (44%) HAD	NNRTI based; D4T/3TC/NVP (n=24); duration 48 weeks	IHDS, RAVLT, timed gait, DSMT, NPZ	IHDS 10.2; NPZ composite score -0.62	NPZ comp 0.29

\*denotes studies in which there was significant improvement in NP outcome after initiating HAART

Key to abbreviations used for neuropsychological tests:

ADC = AIDS Dementia Complex; AVLT = Auditory Verbal Learning Test; COWAT = Controlled Oral Word Association Test; CTM 1/2 = Colour Trails 1 and 2; CVLT = California Verbal Learning Test; DSMT = Digit Symbol Modalities Test; DSS-W-R = Digit Symbol Subtest WAIS-R; DSC-W-III = Digit Symbol-Coding Subtest – WAIS III; DS = Digit Symbol; DSS-WR = Digit Symbol Subtest – WAIS R; DS F/B = Digit Span Forwards and Backwards;

FT= Finger Tapping; FWL = Four Word Learning; GP D/ND = Grooved Pegboard dominant hand/ non-dominant hand; HDS = HIV Dementia Scale; IHDA = International HIV Dementia scale; Complex Figure Test –IM and DR = Complex Figure Test – Immediate memory and delayed recall; MSK = Memorial Sloan Kettering Dementia Stage; NPZ = Neuropsychological Z-score (composite); RAVLT = Rey Auditory-Verbal Learning Test; ROCF = Rey-Osterrieth Complex Figure, SAT= Selective Attention Test; SCWT = Stroop Color-Word Test; SW = Stroop Word; TMT A/B = Trail Making Test A/B; TG= Timed gait; WHO-UCLA AVLT = World Health Organization-University of California-Los Angeles Auditory Verbal Learning Test

<sup>1</sup> Indicates test not specified <sup>2</sup> Indicates dominance not specified

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## **Chapter 4.**

### **Characterisation of HIV- Associated Neurocognitive Disorders among individuals starting antiretroviral therapy in South Africa**

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**Abstract**

HIV- Associated Neurocognitive Disorders (HAND) exert an impact on everyday functions, including adherence. The prevalence of and risk factors for HAND in patients commencing anti-retroviral therapy in Southern Africa are unknown. Participants from primary care clinics in Cape Town, South Africa underwent detailed neuropsychological, neuropsychiatric and neuromedical evaluation. Using the updated American Academy of Neurology criteria, participants were classified into categories of HAND, and demographic and clinical risk factors for HIV-dementia (HIV-D) were assessed. The prevalence of mild neurocognitive disorder (MND) and HIV-D were 42.4% and 25.4% respectively. There were significant associations between lower levels of education and older age with HIV-D, and a trend to association with HIV-D and lower CD4 count. In a regression model, a lower level of education and male gender were predictive of HIV-D. These findings suggest that HAND are highly prevalent in primary care settings in South Africa where clade C HIV is predominant.

## Introduction

Data on HIV-associated neurocognitive disorders come mainly from high-income countries. While some data exist for Uganda, there have been none conducted in South Africa using the detailed approach outlined previously. This standard approach is necessary for comparison across regions and to formally document the impact that clade C HIV might have on neurocognitive function. In addition, there are other important regional differences, which could be ascribed to social and demographic factors- namely, that our HIV epidemic is marked by a majority of women; that individuals enter care late (and by inference may have higher levels of immune-compromise and greater levels of neurocognitive impairment); and that resources for assessment and treatment are few. In this chapter, I document these factors and report on the extent to which they may be associated with HIV-associated neurocognitive disorders or specifically, HIV-associated dementia (HAD). I included the International HIV Dementia Scale in this baseline assessment and compared it to our research diagnostic categories, in order to establish its utility- these findings are reported in chapter 6.

HIV associated neurocognitive disorders (HAND) remain prevalent in the era of HAART. Rates of HAND of up to 50% have been reported [1]. Such disorders impact negatively on social and occupational functioning. In addition, they may be associated with increased risk behaviors and decreased adherence to medication [1-3]. Although the prevalence of HAND is well-established in some regions, there is less data on prevalence and risk factors in areas where clade C HIV predominates, such as South Africa,

The diagnosis of HAND rests on neuropsychological, as well as psychiatric and medical evaluation [4]. In busy primary care settings, clinicians usually do not have access to detailed neuropsychology, and therefore need to make use of clinical assessment or brief screening tools [5]. A number of diagnostic research approaches have been proposed, including the updated criteria of the American Academy of Neurology (AAN), and the Memorial Sloan Kettering staging (MSK) of HIV Dementia (HIV-D) [6,7]. The AAN system proposes four categories: “normal”, “asymptomatic neurocognitive impairment (ANI)”, “mild neurocognitive disorder (MND) and “HIV-dementia (HIV-D)”. The ANI, MND and HIV-D categories are used when an individual's performance on a range of neuropsychological tests falls below age and education-defined norms in at least two domains of function. In the absence of everyday functional impairment, the ANI category is used, while the MND and HIV-D

categories are used when everyday impairment is mild to moderate or severe, respectively.

Given that HAND are both common and exert deleterious effects on everyday function including adherence to medication, it is important to identify and address potential risk factors. To date, several risk factors for HIV-D have been established, including lower CD4 count [8], advancing age [9], lower levels of education [10], and drug and alcohol abuse [11]. Depression has also commonly been reported to both co-exist with HAND as well be associated with severity [12,13]. In South Africa, high rates of alcohol and substance abuse have been reported in HIV clinic attendees [14]. The role of nutritional factors such as vitamin B12 and folic acid in the development of cognitive impairment is well known, but less clear in HAND. They may have particular relevance in regions where poverty and malnutrition are common. Other factors such as HIV subtype (clade) are now thought to be significant [8,15]. While clade B has been proposed to be more neurotoxic, clinical studies in India, and a study utilizing a brief cognitive screen in South Africa, suggested that individuals infected with clade C may be at equal risk of developing HAND [16,17].

We undertook a detailed evaluation of neurocognitive disorder status and possible risk factors among HIV-infected individuals awaiting HAART in South Africa.

## **METHODS**

### **Subjects**

All participants who met study criteria and agreed to participate provided written informed consent. Approval to conduct the study was obtained from the research ethics committee of the Faculty of Health Sciences, University of Cape Town, and from the relevant health authorities.

A total of 283 HIV-infected individuals were invited to participate at three primary health care centres in Cape Town, South Africa from February 2008 through August 2009. All potential participants were ambulant and able to attend out-patient visits. A study nurse screened clinic attendees randomly drawn from the day's list on assigned days. To be included, participants were (1) HAART naïve and in a pre-treatment phase of counseling, (2) aged 18-35 years, (3) had a positive diagnosis of HIV infection made within the last six months (includes initial and confirmatory tests) and (4) at least seven years of formal education. In order to diagnose HAND, it is necessary to exclude other potential causes of neurocognitive problems. We elected

to restrict participants to a younger age in order to reduce the impact of age-related illness, and the education criteria to reduce the potential effect of poor education and intellectual disability. We also excluded participants if they had a history of severe mental illness, such as schizophrenia and bipolar disorder (assessed using the Mini International Neuropsychiatric Interview- MINI [18]). We also excluded those with active major depression (assessed with the Centers for Epidemiological Study-Depression scale [19]), a recent (6 month) substance abuse history (assessed with the Alcohol Use Disorders Identification Test-AUDIT [20]- and a history of head injury with loss of consciousness exceeding 30 minutes. Participants completed a series of assessments after screening, and were then given appointments to attend a second study visit at Groote Schuur Hospital. HIV negative control participants (n=51) were recruited by invitation from Voluntary Counseling and Testing services at the same community clinics. Other than being HIV negative, as confirmed by a recent rapid HIV test and confirmatory serological test, inclusion and exclusion criteria were identical in the HIV positive and the control groups. Of the originally screened 283 participants, 170 completed the full assessment. Reasons for not attending the second assessment included financial constraints, could not get away from work, and travel out of Cape Town. We felt that this group was reflective of the clinic sample in question because we noted that the above logistical problems in tracking participants into the second visit most likely accounted for this rate of completion (that is, factors independent of participant variables). In addition, participants not attending due to possible neurocognitive factors would lead to an under-estimation of the prevalence.

The lowest pre-treatment CD4 cell count was obtained from the clinic records. This was assumed to be the nadir count, as all participants were entering treatment. Clade sequencing was not available on this sample at this time but 89% of infected individuals in Cape Town area are infected with clade C virus [21]. Hepatitis sero-status was not established but the prevalence of hepatitis C in South Africa is extremely low [22].

### **Procedures**

Once they had signed informed consent, participants completed a series of psychiatric and demographic questionnaires, which included measures of depression and substance abuse status (as above), as well as reported function and quality of life scales (the Patient's Assessment of Own Functioning- PAOFI- and the Quality of Life and Satisfaction Scale- QLESQ). All instruments were forward and back-translated into the first language of the participants.

### **Neuropsychological test battery**

A neuropsychological test battery was administered to all participants to assess specific domains neurocognitive function. Our rationale for selecting the particular battery was first, that the battery represented measures of domains typically affected by HIV [23]; second, the battery made use of tests commonly used in international settings so as to be able to make findings comparable; and thirdly, the battery needed to be adapted to be locally suitable. We based the battery on that used by the HIV Neurobehavioural Research Center (located at the University of California, San Diego) [24]. We sought advice on translation and applicability from three local expert neuropsychologists. Changes to word lists to reflect local language and idiom were made. All instruments had their instructions and content translated into isiXhosa and Afrikaans- instructions were also back-translated for fidelity. The battery comprised tests of the following domains: Attention (the Mental Alternation Test and the Mental Control Test), learning and memory (the Hopkins Verbal Learning Test and the Brief Visuospatial Memory Test), motor (Finger tapping and Grooved Pegboard- both dominant and non-dominant hands), psychomotor speed (Trail-Making part A, Color Trails 1 and Digit Symbol coding) executive function (Colour Trails 2, the Stroop Colour Word test, the Wisconsin Card-Sorting Test and the Rey Complex Figure), and language (Category fluency animals and Category fluency fruit and vegetables).

Data from the 51 HIV negative controls were used to generate Z-scores for establishing the degree of impairment. No published norms are currently available in South Africa, and as most participants spoke isiXhosa, we elected to generate control data from similar community participants.

### **Determination of neurocognitive disorder status**

We used the above neuropsychological test battery, together with scores from a neuromedical assessment and an evaluation of functional assessment to classify participants into one of four HAND categories, based on the updated American Academy of Neurology criteria [25]: no impairment, ANI, MND and HIV-D. We used z-score cut-offs of  $>2$  SD and  $1-2$  SD in order to classify participants into categories of neuropsychological impairment.

In order to establish the presence and extent of functional impairment, we reviewed data from the PAOFI and QLESQ, as well as neurologic examination. The advantage of including a neurologic assessment is that it allows for a more objective measure of

impairment status. The neurologic examination included measures of peripheral neuropathy using both a visual analogue scale and assessment of vibration sense, ratings of motor tone and power changes, involuntary movements, primitive reflexes and the timed gait test. The presence of neurological findings was recorded using a standardized assessment, based on a previously defined tool [26]. Findings were then used to generate a neurologic raw score using a semi-quantitative scale. A final rank of 0, 1 or 2 was then assigned depending on the range of score on this scale. Functional impairment was recorded using the PAOFI as above. Similarly, ratings on these scales were used to generate a rank score of 0, 1, or 2. When assigning a HAND category, we reviewed scores for both methods of assessing functional impairment. From the neurologic scale, the presence of peripheral neuropathy was included as a potential correlate of HIV-D. We included this due to consistent reports of neuropathy co-occurring with HAND [27]. This was coded as either present or absent. The final classification was conducted by a consensus panel comprising two HIV neuropsychiatrists (JJ, JH) and a neurologist (MC).

### **Statistical analysis**

Analysis was conducted using STATA 10.0 (Stata Corporation, College Station, Texas, USA). Demographic, clinical and biochemical variables were compared across AAN-defined HAND categories using Fisher exact and Kruskal Wallis tests as appropriate. Variables which appeared to be associated with HAND categories in bivariate analysis were included in multiple logistic regression analysis comparing HIV-D to normal participants; variables were retained in the model if they demonstrated persistent independent association with HIV-D, or if their removal altered associations involving other covariates. All statistical tests are 2-sided at  $\alpha=0.05$ .

### **RESULTS**

A total of 170 HIV+ participants were evaluated. The majority were women (n=126, 74%), isiXhosa speaking (n=151, 89%) and had a median CD4 cell count of 168 (IQR 115-199). The mean age was 29.5 years (SD = 3.65) and mean number of years schooling was 10.0 years (SD 1.85) (see table 1). Scores on the AUDIT and CES-D were low, ranging from 0-3 on both. More than half of patients had at least mild peripheral neuropathy (n=94). A range of other variables intended to establish the contribution of nutritional factors to neurocognitive impairment is presented in Table 2.

Utilising the AAN criteria, 43 of the 170 individuals (25%) evaluated met criteria for HIV-D, while 72 of 170 (42%) had mild neurocognitive disorder, and 15 of 170 (9%) met criteria for asymptomatic neuropsychological impairment; 40 individuals (24%) were assessed as being neurocognitively normal.

In bivariate analysis, only age and level of education differed significantly between the groups (table 2). Patients with HIV-D tended to be older and less well educated. The CD4 cell count tended to be lower in those with HIV-D ( $p=0.051$ ). Similarly, there were more men in the HIV-D category, although this did not achieve statistical significance across groups ( $P=0.055$ ). There were no significant differences in any clinical or laboratory parameters associated with nutritional or systemic disease processes, including peripheral neuropathy.

In a multiple logistic regression model comparing normal patients to those with AAN-defined HIV-D status, level of education ( $p=0.001$ , odds ratio= 0.529) and male gender ( $p=0.048$ , odds ratio= 3.989) were predictive of HIV-D. In the model used, CD4 cell count was not associated with HIV-D status ( $p=0.718$ , odds ratio=0.999).

## **DISCUSSION**

We report on the first detailed evaluation of HIV-associated neurocognitive disorders in patients attending primary care health facilities in South Africa where clade C HIV virus is prevalent. Using the updated American Academy of Neurology (AAN) criteria, we found a high prevalence of HAND. In particular, we noted rates of HIV-D of 25.3% and of MND of 42.4%. Furthermore, in this study population, level of education and older age were associated with AAN criteria for HIV-D in bivariate analysis, while both clinical and laboratory markers of nutritional and systemic disease process were not. The large number of individuals with MND has implications for possible progression to HIV-D, as does the related impairment in everyday function on various behavioural outcomes.

The prevalence of HAND in this study is in keeping with other reports from the developing world. In Uganda 31% of individuals in ambulatory care met criteria for MSK-defined HIV-D, while in India 51% of individuals demonstrated significant neuropsychological impairment in at least two domains of function [26,28]. Studies in the developed world have reported similar rates of up to 27% [29]. In the study in India, the neuropsychological cut-off for impairment was 1.5 SD. This was lower than the standard cut-off employed in our analysis. While no other detailed research

approaches have been used in South Africa, there have been some reports using simple screening tools, wherein 24% of individuals demonstrated cognitive impairment using the HIV Dementia Scale [17].

High rates of HAND may be explained by the fact that individuals in the public sector in South Africa access HAART very late, with a median CD4 count in our study of 168 cells/ml. In this care system, access to HAART is provided to individuals with CD4 cell counts <200 cells/ml, or to those with a diagnosis of “HIV encephalopathy”, according to World Health Organization criteria. Accordingly individuals with CD4 cell counts >200 who have “HIV encephalopathy” would qualify for HAART, potentially reducing this burden of disease. Further work to improve screening for HAND in primary health care is needed. In addition to this primary prevention, whereby those with clinical disorder at higher CD4 counts could access HAART, there are implications for secondary prevention. In particular, individuals with HAND have some degree of impairment in everyday function, and so require substantial treatment support in order to improve adherence to medication [30].

We found that level of education and age was associated with differences in HAND category, with participants with HIV-D being older and having a lower level of education. There was a trend to those with HIV-D having a lower CD4 cell count. In a parsimonious regression model, only lower level of education and male gender predicted HIV-D. While others have noted an association with CD4 count nadir and HIV-D [8], it is possible that the restricted range of CD4 count pre-HAART in this sample may limit this association. In addition, the small sample size may have limited power to detect associations. Both educational level and age are well known to affect neuropsychological performance [31]. In addition, the quality of education, insofar as it improves paper-and-pencil tasks, test attitudes and performance confidence may also affect test outcomes [32]. The effects of older age are now also known to be associated with an increased risk for neurocognitive disorder [9]. However, in this study, the differences in age and level of education between controls and participants were small and not likely to be clinically significant. We purposefully included only participants under the age of 45 to control for the effects of ageing. Study participants in this sample were generally drawn from a lower socio-economic group, and that this might be associated with poor nutritional status (reflected by anaemia or low albumin) that may contribute to the aetiology of HIV-D. In our study, this was not the case, and while haemoglobin values were in the low normal range, medians across AAN

categories did not differ. The impact of systemic disease, as measured by the proxy marker of low body mass index also was not significant.

The category of MND also requires further study in prospective cohort studies. Firstly, there is growing evidence that HIV-D is a diverse clinical category, with a range of possible outcomes from improvement to deterioration [33]. Secondly, while the AAN categorical approach is useful to group individuals with similar types of neuropsychological impairment and problems with everyday function, it is likely that in reality, individuals with HAND fall on a clinical disease spectrum. Thirdly, while by definition, the MND category proposes that impairment of everyday function is mild to moderate, it remains a clinically measurable and significant outcome. In particular, these impairments have been linked to reduced rates of gaining and sustaining employment, impaired ability to manage finances and reduced driving ability [30]. The MND group in our study comprised 42.4% of the sample. Further research elucidating how this group changes over time is needed. This group may represent a precursor to HIV-D, in which case early treatment with HAART could be justified.

Limitations of this study include the small control group size and the fact that normative data for isiXhosa speakers do not exist. However, efforts were made to recruit HIV-negative individuals from the same community and clinics as the HIV+ patients. While we attempted to remove potential confounding causes of neurocognitive disorder, we did not perform routine brain imaging or lumbar punctures on participants, due to resource and ethic constraints. We therefore could not ascertain with complete certainty that all neurocognitive disorder was due to HIV, despite careful clinical assessment. In addition, we noted that of the 283 participants recruited at site, 170 attended the full assessment visit. This could have introduced a selection type bias. We felt that early logistic problems, such as financial constraints and accessibility- which were late addressed- most likely accounted for this drop-out. In addition, we noted that if participant factors such as neurocognitive disorder were at issue, then our findings would under-report the prevalence of these disorders.

In summary, this study found that a high frequency of HAND exists in a region where clade C HIV is predominant. Nearly two-thirds of patients attending a primary health clinic were suffering from either MND or HIV-D according to rigorous AAN criteria. Correlates of HIV-D included lower education level and male gender. Further research into the impact of HAART on neurocognitive function utilising prospective

cohort studies in this region is needed, as well as more detailed investigation into the impact of HAND on everyday function in this population.

Given the high rates of HAND, and indeed, HAD- the most severe form- it is key to better understand mechanisms of disease. In addition to the demographic, clinical and laboratory measures reported in this chapter, an investigation into other possible associated factors may shed light on the very high prevalences we found. The apolipoprotein E (APOE) allelic variants- in particular the E4 variant- have been well-documented to occur more frequently in people who develop Alzheimer's disease- a common dementia of old-age. Studies of APOE to date in people living with HIV (PLWH) have yielded some inconsistent findings. The relevance to South Africa, is that given our high rates of HAND (and HAD), should a higher frequency of the APOE4 variant be found in PLWH with HAD, it affords an opportunity to investigate disease mechanisms related to APOE4 (for example, vascular and metabolic factors), and to consider adjuvant treatments related to protein products of APOE4. Screening for APOE4 has not been regarded as a cost-effective strategy in Alzheimer's disease, as the E4 variant is not predictive of dementia, rather it is a vulnerability factor. The exploration offered in the next chapter therefore builds on a model of neurocognitive disease vulnerability, involving genetic, socio-demographic and disease-related variables.

Table 1. Demographic and clinical characteristics of the sample

<i>characteristic</i>	<b>HIV+ participants</b>	<b>HIV-negative participants</b>
Mean age (SD)	29.5 (3.65)	25.28(5.58)
Women (%)	126 (74)	32 (64)
Years of education (SD)	10.06 (1.85)	10.82 (1.64)
Speak isiXhosa (%)	151 (88.8)	42 (84)
CD4 count	181 (118)	--
Body Mass Index, mean (range)	25.13 (48.04-17.64)	--
Haemoglobin, mean (range)	11.39 (7.7-14.6)	--
Serum iron, mean (range)	11.40 (3.6-26)	--
Total protein, mean (range)	93.61 (5.9-120)	--
Albumin, mean (range)	38.74 (24-49)	--
B12, mean (range)	333.68 (34-994)	--
Serum folate, mean (range)	1587.04 (191.4-3676.4)	--

Table 2. Demographic and clinical variables stratified by AAN category

	Neurocognitive disorder category				statistical value
	Normal	ANI	MND	HIV-D	
No (%)	40 (23.5)	15 (8.8)	72 (42.4)	43 (25.3)	
<b>Demographics</b>					
Women, no (%)	33 (82.5)	11 (73.3)	53 (73.6)	29 (69.1)	Fisher's exact= 0.551
Left handed, No (%)	35 (87.5)	14 (93.3)	66 (91.7)	39 (90.7)	Fisher's exact= 0.903
Language isiXhosa, no (%)	34 (85)	14 (93.3)	63 (87.5)	40 (93)	Fisher's exact= 0.203
Age, median (IQR)	30.5 (27.5-32)	28 (25-31)	28.5 (26-32)	31 (28-33)	chi-sq=8.420, p=0.038
Education, median (IQR)	11 (11-12)	9 (9-11)	10 (9-11)	10 (8-11)	chi-sq=18.215, p=0.0003
<b>Medical</b>					
CD4, median (IQR)	172 (126-190)	205 (148-235)	174.5 (116.5-236.5)	139 (97-182)	chi sq=7.76, p=0.0512
Peripheral neuropathy, no (%)	37 (64.86)	15 (26.67)	69 (60.87)	39 (61.54)	Fisher's exact= 0.072
AUDIT	0 (0)	1 (0)	2 (0)	3 (0)	chi sq=0.925, p=0.815

CES-D	0 (0)	1 (0)	2 (0)	3 (0)	chi sq=1.000, p=0.815
BMI, median (IQR)	24.69 (22.68-28.72)	19.37 (18.8-21.3)	23.81 (21.81-26.44)	24.44 (21.03-28.12)	chi sq=6.179, p=0.103
HB, median (IQR)	10.9 (10.5-12.1)	9.05 (7.7-10.04)	11.7 (10.9-12.1)	11.2 (9.05-13.05)	chi sq=3.727, p=0.291
serum iron, median (IQR)	13 (8-18.6)	9.3 (9.3-9.3)	8.35 (5.9-10.2)	9.65 (9.5-15.3)	chi sq=3.980, p=0.263
total protein, median (IQR)	91 (87.5-97)	98 (98-98)	92.5 (88-100)	94.5 (84.5-109.5)	chi sq=1.127, p=0.77
Albumin, median (IQR)	40 (38-44)	42 (42-42)	40 (35-42)	37 (32-42)	chi- sq=3.204, p=0.369
serum B12, median (IQR)	288 (223-377)	306 (244-324)	299 (202-391)	306 (227-455)	chi sq=1.058, p=0.787
Red cell folate, median (IQR)	1290 (1125-1548.3)	1700.55 (1255.5-2145.6)	1803.5 (1091-2283.4)	1449.85 (1161.85-1919.55)	chi sq=2.448, p=0.485

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Key: AUDIT- Alcohol Use Disorders Identification Test; CES-D- Centers for Epidemiological Study- Depression scale; BMI- body mass index; HB- haemoglobin;

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## **Chapter 5.**

**Association between apolipoprotein E4 genotype and human immunodeficiency virus-associated dementia in younger adults starting antiretroviral therapy in South Africa.**

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## **ABSTRACT**

It is not known whether apolipoprotein  $\epsilon 4$  is associated with HIV-associated dementia (HAD) in a South African population, where HIV clade C is predominant. ApoE genotyping was performed on 144 participants in a larger study of HIV associated neurocognitive disorders (HAND). There was a lower frequency of the  $\epsilon 2$  and  $\epsilon 3$  alleles in the HIV positive group, compared to a group of 300 community-based newborn infants. There were no differences in ApoE genotype across different categories of HAND. The  $\epsilon 4$  allelic variant was less common in individuals with HAD, than in those without HAD. Our findings suggest that the  $\epsilon 4$  allelic variant in HIV positive individuals is not associated with the development of HAD in Southern Africa.

## INTRODUCTION

In this chapter, I present data on the allelic frequency of apolipoprotein (APOE) allelic variants in a community sample of infants from the Western Cape, and then compare these to those found in our HIV positive sample. In a model of neurocognitive disease, it would be expected – as in Alzheimer's disease- that older persons are more vulnerable to developing dementia. In chapter 4, we found that older age was associated with higher rates of HIV-associated dementia (HAD). However, the clinical significance of this is unclear, given that our sample was purposively selected as being younger than 35 years of age in order to exclude potential neurodegenerative diseases of old age. It is possible, however, that APOE4 represents a vulnerability factor, that together with HIV infection could lead to higher rates of HAD.

Apolipoprotein E (ApoE) is a protein involved in lipid metabolism in both peripheral tissue, as well as the central nervous system. It has three major allelic variants:  $\epsilon$ 2, 3, 4. ApoE allelic variants occur with different frequencies across different ethnicities, in particular between individuals of European and African descent [1]. The ApoE4 allelic variant is thought to be commonest in African populations. In one epidemiologic review, the frequency of  $\epsilon$ 4 in Caucasian samples (defined as the number of  $\epsilon$ 4 alleles in the total sample) ranged from 0.082 to 0.194, while in a Nigerian sample, the reported frequency was 0.310 [2]. In a study of the Khoi San population of Southern Africa, an allelic frequency of 0.37 was reported [2,3]. The higher frequency of ApoE4 in individuals of African ethnicity may have substantial implications for the frequency of neurodegenerative conditions in this setting.

The  $\epsilon$ 4 allelic variant has been shown to increase the risk of developing both Alzheimer's disease (AD) and certain brain disorders [4,5]. ApoE4 is thought to be involved in the consequences of abnormal lipid metabolism (such as atherosclerosis), as well as in several inflammatory pathways in the central nervous system (CNS) [2]. The latter pathways include increased microglial responses, oxidative stress and the production of nitric oxide [6-8] In AD, the  $\epsilon$ 4 allelic variant is associated with an increased deposition of amyloid plaques [9] Amyloid production is in part mediated by cytokines secreted from activated microglia, a process which may be accelerated in carriers of the  $\epsilon$ 4 allele [10]. Microglial activation may also occur in infections of the CNS infections, such as HIV. The combined effect of certain ApoE allelic variants and HIV infection may result in an additive neurodegenerative effect [11].

There are similarities between neurodegeneration in HIV-associated dementia (HAD) and that of AD. For example, the amyloid precursor protein has been reported in those with HIV encephalitis [12]. Other clinical-pathological studies among older subjects with HIV infection have identified an association with CNS plaques and elevations of CSF amyloid beta [13,14]. In one report it was noted that a genetic variant of tumour necrosis factor alpha is associated with HAD (reported as AIDS dementia complex) [11]. Clinical studies of the associations between ApoE and HAD have produced conflicting results, with some reporting increased risk for HAD and peripheral neuropathy and others describing no association [10,15]. Some have noted that the ApoE4 genotype together with advancing age confers an increased risk for HAD [16,17].

To date most studies of ApoE and HIV-associated neurocognitive disorders have been conducted in high income countries where HIV clade B predominates. It has been proposed that the different HIV clades exert different neurotoxic effects via a functional difference in the transactivator protein (tat) [18]. Clinical reports from India and South Africa suggest that HAND may be as common in regions where clade C predominates [19,20]. In these populations, HAND may then involve mechanisms of neurotoxicity other than those mediated by tat. The possible role of other correlates of HAND, such as ApoE4 requires further study. Evidence for the interactive effect of age with ApoE4 on neurodegeneration suggests that this variant is associated with an accelerated disease course and progression to death [17,21] These data are supported by the fact that while HAD has been linked to a higher frequency of  $\epsilon 4$  in some reports, this genotype occurs less often in older individuals, even when controlling for ethnicity [21]

In this study, we sought to establish the distribution and frequency of ApoE allelic variants in a South African HIV positive clinic population well characterized with respect to HAND status. In particular we evaluate potential associations between ApoE4 and HAD. We hypothesized that the ApoE4 allelic variant would occur at higher frequencies similar to that reported in other African populations, and that this allelic variant would be associated with HAD status

## **METHODS**

### **Subjects**

From a larger primary study, we invited 283 HIV-infected individuals at three primary health care centres in poor communities in Cape Town, South Africa to participate from February 2008 through August 2009 [22]. Of these, 144 attended two full study visits during which detailed a socio-demographic, medical and neuropsychological assessment were conducted, and laboratory measures (including genotyping) were completed. Primary reasons for this loss to follow-up included financial constraints, casual employment on clinic days, migration between cities and the need to attend other clinic appointments. Individuals included in this study ranged from 18 through 40 years in age. All were naïve to highly active anti-retroviral therapy (HAART). Cases were excluded if they had a severe psychiatric disorder, recent history of substance (including recent alcohol) abuse or other significant neurological disorder. Control data for neuropsychological testing was obtained from 50 HIV negative participants. These were recruited from Voluntary Counseling and Testing services at the same primary care clinics. Other than being HIV negative, as confirmed by a recent rapid HIV test and confirmatory serological test, inclusion and exclusion criteria were identical to HIV positive participants.

Participant CD4 cell counts were obtained from the clinic records. Although clade sequencing was not available on this sample, previous reports have noted that 89% of infected individuals in Cape Town area are infected with clade C virus [23]. Furthermore, although hepatitis C status was not tested, the prevalence of hepatitis C in South Africa is extremely low [24].

Background population prevalence of ApoE genotype was obtained from a sample of 300 infants born to isiXhosa speaking mothers in an area of Cape Town where the majority of the clinical sample above was drawn. The blood from these infants was drawn from 2002 through 2004 and stored in accordance with a previously approved Research Ethics Committee submission, in order to allow for analysis of background prevalence of genes under study. The HIV serostatus of this group was not known. It was assumed to reflect the background population prevalence in newborns at the time. According to local surveys, the seroprevalence amongst mothers in this community in 2006 was 15% [25]. With untreated mother-to-child transmission rates of 10-30%, we estimated the seroprevalence in this infant sample to be between 1.5-5%. This rate was felt to be unlikely to affect this data.

We obtained written informed consent from all included participants, including separate consent for genomic analysis. Approval to conduct the study was obtained

from the Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, and from the relevant health authorities.

### **Neuropsychological test battery**

A neuropsychological testing battery assessed specific domains of attention, concentration, language, learning, memory, psychomotor speed and executive function. We translated the instructions and content of all instruments into local languages- isiXhosa and Afrikaans. The instructions were back-translated for fidelity. The battery comprised the following tests: Attention (the Mental Alternation Test and the Mental Control Test), learning and memory (the Hopkins Verbal Learning Test and the Brief Visuospatial Memory Test), motor (Finger tapping and Grooved Pegboard- both dominant and non-dominant hands), psychomotor speed (Trail-Making part A, Color Trails 1 and Digit Symbol coding) executive function (Colour Trails 2, the Stroop Colour Word test, the Wisconsin Card-Sorting Test and the Rey Complex Figure), and language (Category fluency animals and Category fluency fruit and vegetables). Information regarding selection and adaption of the battery is contained in the "Introduction" to the thesis on page 15.

### **Determination of neurocognitive disorder status**

We used the above neuropsychological test battery, together with scores from the medical assessment and self-reported functional assessment to classify participants into one of four HAND categories, based on the updated American Academy of Neurology criteria [26]: no impairment, asymptomatic neuropsychological impairment (ANI), mild neurocognitive disorder (MND) and HIV-associated dementia (HIV-D). Using the modified AAN classification, participants who scored  $>2$  SD below the control-derived means cut-offs on at least two domains of function were rated as having HAD; those who scored between 1.0 and 2 SD on two domains of function, or  $>2$  SD and 1-2 SD, were rated MND or ANI, depending on their loss of function. The final classification was conducted by a consensus panel comprising two HIV neuropsychiatrists (JJ, JH) and a neurologist (MC).

### **Genotyping**

DNA was isolated by standard procedures and the method of Hixson and Vernier was used for Apolipoprotein E (ApoE) genotyping [27]. We did not include genotyping data of the HIV negative control adults, as this was available on only 10 participants.

### **Statistical analysis**

Data were analyzed using STATA 10.0 (Stata Corporation, College Station, Texas, USA). In order to establish whether the allelic frequency was randomly distributed in the sample of control newborns and the HIV positive participants, we conducted an analysis of the Hardy-Weinberg equilibrium (HWE). This principle asserts that mating is random, there is no migration or inbreeding and no selective survivorship [28]. Calculation of the HWE allows the investigator to report with some confidence that external factors have not altered the gene frequency in the population to any significant degree, and therefore that the observed frequency is not an artifact of these external factors. The allelic distributions between the newborn and HIV positive adult groups were compared using exact statistics, while allelic frequencies were calculated to include 95% binomial confidence intervals in addition to chi-squared tests. Similarly, we compared allelic distributions across HAND categories. Finally, we compared HAD and non-HAD groups with respect to the presence of the  $\epsilon 4$  allelic variant using a table exact statistic.

## **RESULTS**

A total of 144 HIV positive participants were included in the analysis. The majority were women (74%), isiXhosa speaking (88%) and had a median CD4 cell count of 168 (IQR 115-199). The median CD4 counts for the categories of HAND were 182 (IQR 148-191) for non-impaired, 181 (IQR 146-235) for ANI, 180 (IQR 128-241) for MND and 139 (IQR 75-184) for HAD. The mean age was 29.5 (SD 3.65) and level of education was 10 years (SD 1.85) (table 1).

The distribution of ApoE alleles were found to be in Hardy-Weinberg equilibrium (HWE) for both the group of controls, as well as the HIV+ participants. Using an Exact statistic the probability of the two groups not being in HWE was 0.68 and 0.55 respectively.

### **Apolipoprotein $\epsilon$ in HIV positive group and the general population**

The distribution of ApoE allelic combinations for HIV positive study participants and a newborn population-based sample for comparison is presented in table 2. When we compared the overall genotype distributions between 2 groups, we found significant differences between the genotypes (two-sided exact p-value = 0.042). The  $\epsilon 2/\epsilon 2$  allelic combination was significantly less frequent in the HIV positive group than the newborn controls.

The allelic frequencies between the HIV positive and the newborn groups are presented in table 3. We found that the  $\epsilon 2$  and  $\epsilon 3$  alleles differed significantly (chi-squared = 12.2 df = 2,  $p = 0.002$ ), with a lower frequency of the  $\epsilon 2$  and  $\epsilon 3$  alleles in HIV positive adults than newborn controls.

### **Apolipoprotein $\epsilon$ and HAND**

There were no differences in the allelic distributions of ApoE across different categories of HAND (overall exact  $p$ -value=0.66), nor were there differences in CD4 count for the ApoE genotypes ( $p=0.14$ ). The relationship between  $\epsilon 4$  status (having one or more  $\epsilon 4$  allele) was compared to participants with and without HAD (table 5). In this analysis, there were significantly fewer individuals with  $\epsilon 4$  in the HAD group (chi-squared,  $df = 1$ ,  $p=0.03$ , odds ratio with 95% C.I. 0.41 (0.17-0.99)).

### **DISCUSSION**

We report the first study, to our knowledge, evaluating the relationship between ApoE genotype and HAND in southern Africa. In this study, the allelic frequency of the  $\epsilon 4$  allelic variant in both the community-based newborns and the HIV positive adults was similar to reports of other African populations. The frequency of the  $\epsilon 2$  allelic variant was significantly lower in the HIV positive adults compared to newborns. There were no differences in ApoE genotype and category of HAND. We did note a significantly lower frequency of the  $\epsilon 4$  allelic variant in adults with HAD, compared with those without HAD.

In this sample, among isiXhosa speakers, we found a high frequency of the  $\epsilon 4$  allelic variant amongst newborns, as well as HIV positive adults (0.302 and 0.297 respectively). The relatively high frequency of the  $\epsilon 4$  variant is in keeping with other studies of African populations, where frequencies of Apo $\epsilon 4$  of 0.31 and 0.20 have been found, and confirms that Apo $\epsilon 4$  is more common in this ethnic group than in non-African groups [17,29]. In a study of the indigenous Khoi San population in Southern Africa, a slightly higher frequency was found (0.37) [3]. A similar frequency of Apo $\epsilon 4$  was found also in the control sample (0.30). The implications of this high frequency of  $\epsilon 4$  are for increased rates of  $\epsilon 4$ -related neurodegenerative diseases, including HAD. This is of especial relevance in a regions with a very high HIV seroprevalence and an ageing population [17,30]

In addition, we found that the  $\epsilon 2/\epsilon 2$  genotype occurred significantly less frequently among HIV positive adults compared with community-based newborns, while the individual  $\epsilon 2$  and  $\epsilon 3$  allelic variants specifically were less frequent in this group. We noted also the distribution of ApoE alleles compared with other studies suggests higher frequencies of the 2/3 and 2/4 combination, and a lower frequency of the 3/3 combination [2,3,16]. Some have proposed that the  $\epsilon 4$  variant is associated with increased rates of HIV acquisition [31]. Should this be the case, then a putative “biological protective” factor may be able to be identified in  $\epsilon 2/\epsilon 2$  carriers. In the absence of ApoE genotype of the adult HIV negative controls, and survival data on our HIV positive group, we are unable to draw conclusions regarding the role that ApoE allelic variants might have on acquisition of HIV infection in our population. Larger cross-sectional and sero-conversion studies are needed to provide evidence for this link.

In this sample of young adults entering HIV care, we report that there were no differences in allelic distributions across HAND categories. However, we did find a significantly lower frequency of the  $\epsilon 4$  allelic variant in individuals diagnosed with HAD, compared with those without HAD. Our sample size was likely too small to detect differences in genotype across the four groups. In our two-by-two analysis, we found a significantly lower frequency of  $\epsilon 4$  status among HIV positive adults with HAD. This finding is in contrast to that of Corder and colleagues who found that in a sample of 44 HIV+ participants followed prospectively from around the time of sero-conversion, mild suspected dementia was nearly twice as frequent in the  $\epsilon 4$  bearing individuals [32]. Our sample was too small to separate  $\epsilon 4$  status in hetero- and homozygous groups, although others have noted that a dose-dependent relationship of  $\epsilon 4$  with the development of AD has been shown [17,30]. Should a larger study confirm non-association, as opposed to non-survival, then it might suggest that the pursuit of APOE as a vulnerability factor in the development of HAND would not be a worthwhile one.

Our findings are consistent with the report of Dunlop and the younger group in the Valcour study [15,16]. Our finding that the  $\epsilon 4$  allelic variant was negatively associated with HAD could be explained by the relatively small sample size, or it could suggest a non-association between  $\epsilon 4$  status and the development of HAD. In addition, while there is controversy regarding  $\epsilon 4$  as a risk factor for Alzheimer’s disease in African Americans, our findings suggest that it is not associated with HAD in black Africans. Further conclusions regarding the effect of  $\epsilon 4$  on survival require follow-up survival

data. The negative impact of  $\epsilon 4$  has been proposed by the Valcour group. A longitudinal study design would be needed to provide data for this explanation.

Our study was limited by relatively small numbers of participants and by the absence of other data on vascular risk factors. Nonetheless, we believe that our findings warrant scrutiny, given the burden of disease of infection with clade C HIV and the frequency of ApoE4. Many of these issues would be clarified by larger prospective cohort studies where recently diagnosed isiXhosa speakers are genotyped. To our knowledge, this is the first study of ApoE genotype in a well-characterised group of HIV+ individuals with predominantly clade C HIV about to commence HAART. These findings are of relevance given the high prevalence of HIV in Southern Africa, the growing evidence that clade C HIV may be as neurotoxic as other clades, and the changing phenotype of HAND in the era of HAART. In addition, we noted that of the 283 participants recruited at site, 170 attended the full assessment visit. This could have introduced a selection type bias. We felt that early logistic problems, such as financial constraints and accessibility- which were late addressed- most likely accounted for this drop-out. In addition, we noted that if participant factors such as neurocognitive disorder were at issue, then our findings would under-report the prevalence of these disorders.

The investigation into the APOE genotype represents one possible genetic pathway of disease development; others were not explored in this thesis. In addition to exploring possible biologic and socio-demographic vulnerability factors as reported in this and the previous chapter, clinicians in busy settings need tools with which to uncover the problem of HIV-associated neurocognitive disorders (HAND). This addresses the need for treatment. In the next chapter, I turn to establish whether a brief screening tool, validated in Uganda, performs adequately in South Africa. Such tools may empower non-neurologists or psychologists to quickly establish whether severe HAND is present, and to offer the best-evidence treatment to date: HAART.

Table 1. Demographic characteristics of HIV positive participants and HIV negative adult neuropsychology controls

<i>characteristic</i>	HIV+ participants (n=144)	HIV-negative participants (n=50)	p-value
Mean age (SD)	29.5 (3.65)	25.28(5.58)	<0.001
Women (%)	106 (74)	32 (64)	
Years of education (SD)	10.02 (1.85)	10.82 (1.64)	0.642
Speak isiXhosa (%)	127 (88.2)	42 (84)	
CD4 count	188 (118)	--	--
AAN-defined HIV-D (%)	41 (28.4%)	--	--

Table 2. Allelic distributions for newborns and participants.

Alleles	HIV positive n (%)	Control newborns n (%)	Exact (2-sided)
22	2 (1)	18 (6)	0.028
23	21 (15)	52 (17)	0.497
24	8 (6)	33 (11)	0.079
33	50 (35)	78 (26)	0.073
34	49 (34)	90 (30)	0.444
44	14 (10)	29 (10)	0.999
Total	144	300	

Table p-value (exact) 0.042

Table 3. Allelic frequencies for control newborns and HIV positive participants

alleles	300 newborns		144 HIV positive adults		p value
	n	frequency (95% CI)	n	frequency (95% CI)	
ε 2	121	0.202 (0.170 - 0.236)	35	0.114 (0.081 - 0.155)	0.001
ε 3	298	0.497 (0.455 - 0.537)	180	0.588 (0.531 - 0.643)	0.01
ε 4	181	0.302 (0.265 - 0.340)	91	0.297 (0.247 - 0.352)	0.876
total	600		288		

Table 4. Allelic frequencies of HIV positive participants across neurocognitive disorders

Genotype	Neurocognitive disorder category				Exact (2-sided)
	Non impaired (n= 29)	ANI (n=18)	MND (n=56)	HIV-D (n=41)	
22	1 (50%)	0	0	1 (50%)	0.47
23	2 (9.5%)	2 (9.5%)	8 (38.1%)	9 (42.9%)	0.381
24	2 (25%)	1 (12.5%)	3 (37.5%)	2 (25%)	0.999
33	7 (14%)	9 (18%)	21 (42%)	13 (26%)	0.31
34	12 (24.49%)	4 (8.2%)	19 (38.9%)	14 (28.6%)	0.627
44	5 (35.7%)	2 (14.3%)	5 (35.7%)	2 (14.3%)	0.351

Exact p-value for table, 0.66

Table 5. Comparison between  $\epsilon 4$  status with HAD and non-HAD clinical groups.

	HIV positive n = 102		
	with $\epsilon 4$ allele	without $\epsilon 4$ allele	total
HAD	18	33	51
No neurocognitive disorder	29	22	51
total	47	55	102

Exact p-value for table, 0.029 (ie, 18/51 is significantly lower than 29/51)

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## **Chapter 6.**

### **Validity of the International HIV Dementia Scale in South Africa**

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## **ABSTRACT**

HIV-associated neurocognitive disorders (HAND) remain prevalent, especially in regions like South Africa where HIV prevalence is high but access to anti-retroviral treatment (ART) is limited. The incidence of HIV dementia (HAD) has been halved with the use of ART, but the prevalence remains high. Appropriate brief screening tools to screen for HAD are needed in order to facilitate treatment initiation. The validity of the International HIV Dementia Scale has not been established in a region where infection with HIV clade C is predominant. The International HIV Dementia Scale (IHDS) was administered together with a detailed neuropsychological test battery to 163 HIV positive individuals who had not received ART and who were attending primary care HIV clinics. The validity of the IHDS was established using a receiver operating characteristic (ROC) analysis. HIV+ individuals performed worse than HIV- controls on the IHDS and a range of neuropsychological tests. Neuropsychological tests discriminated well across HAND categories for HIV+ individuals. In ROC analysis, the IHDS returned an area under the curve of 0.73, with a sensitivity of 85% and specificity of 54% at a cut-off score of 10. Individuals with HAD, who screened negative on the IHDS, performed poorly on some tests of executive function. These data suggest that the IHDS adequately screens for HAD in South Africans infected with predominantly clade C HIV. Variable performance in neuropsychological testing may account for false negative screens. The inclusion of brief tests of executive function in a screening battery should be considered.

## **BACKGROUND**

Clinicians in busy clinic settings, especially in South Africa, where throughput of patients is enormous, seldom consider neurocognitive issues in their patients unless they are overt. In chapter 4, I presented data based on detailed neuropsychological and clinical assessment of this patient group. This approach is not feasible in practice, and so in this chapter, I present data supporting the potential use of the International HIV Dementia Scale. Then, even patients with CD4 cell counts >200 cells per ml would be eligible for HAART under current South African treatment guidelines.

HIV associated neurocognitive disorders (HAND) remain highly prevalent, occurring in an estimated 50% of people living with HIV/AIDS, depending on disease stage [1-3]. HAND remain common in both the developed and developing world. The one-year incidence of HIV dementia (HAD), the severest and most debilitating form was reported in 25% of individuals in two separate North American cohorts, irrespective of the use of anti-retroviral treatment (ART) [4]. Detailed studies of HAND globally are few, but similar figures have been reported in India, China and South Africa [5-7]. The advent of ART has substantially altered the nature of these disorders, although they are now known to persist despite its widespread use [3,8]. HAD results in a number of important adverse outcomes, including increased mortality, decreased adherence to ART, and decreased employment [4,9].

Formal neuropsychological testing remains the gold standard of diagnosis of HAND [10]. While most studies report a typical pattern of neuropsychological impairment, involving executive functions, motor functions, speed of processing and impaired recall, there is evidence to suggest a wider variability of deficits [11].

Neuropsychological deficits in HAND are thought to track the brain regions primarily involved affected by HIV, namely the sub-cortex and striatum [12]. Accordingly, impairments of executive dysfunction, motor slowing, impaired speed of processing and impaired memory recall are regarded as characteristic of HAND [10].

Neuropsychological test batteries need to sample across a number of domains in order to detect impairment. Furthermore, the application of neuropsychological testing is well known to be dependent on a range of factors including age, culture, language, level of education, and the presence of co-morbid neurologic problems, such as substance abuse [3,13].

A number of brief screening tools have been proposed for use in primary health care and resource limited settings. These include the HIV Dementia Assessment, HIV Dementia Scale (HDS) and the International HIV Dementia Scale (IHDS) [14-16]. The IHDS has been validated in both the USA and Uganda [15]. Both the HDS and IHDS were found to be useful tools in a smaller study in Canada [18]. The development of an appropriate screening tool for HAND first requires detailed neuropsychological characterisation of impairments which may be affected by regional differences, such as culture, language or clade differences [3,17]. Differences in the neurotoxic effects of the different HIV clades have been proposed, although early clinical studies suggest that clade C, common in South Africa, may produce high rates of HAND [19]. The validity of a brief screening tool would then need to be validated across different settings.

A number of potential issues need to be considered when making use of screening tools. These include the need to be brief, easy for non-specialists to administer, and adaptable for use in cross-cultural settings. Also, a screening tool should be both as sensitive and specific to HAND, taking into account neuropsychological functions commonly thought to be affected by HIV. 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 an American and Ugandan sample, and was found to have a sensitivity and specificity in the Uganda sample of 80% and 55% respectively [15]. More recently, the IHDS was compared to a brief neuropsychological battery in Canada, where a receiver operating characteristic (ROC) analysis generated an area under the curve of 0.74 [18]. The authors reported that the HDS and IHDS were relatively efficient in diagnosing HAND, with the IHDS displaying a sensitivity and specificity of 76.9% and 65% respectively [18]. Further studies in regions of high HIV prevalence and unique clade sequencing are needed.

In the present study we aimed to describe the performance of the IHDS against categories of HAND defined by the updated American Academy of Neurology criteria. We examined scores from a detailed neuropsychological battery in a sample of clade C HIV positive individuals, compared to a group of HIV negative controls.

## **METHODS**

### **Subjects**

This study formed part of a larger investigation of HIV-associated neurocognitive disorders in Cape Town, South Africa. In summary, 283 HIV-infected individuals at three primary health care centres were invited to participate from February 2008 through August 2009. Of these, 163 attended two full study visits during which detailed socio-demographic, neuromedical, neuropsychological, and laboratory measures were administered. The evaluations took between two and three hours. Details of this characterization have been previously published [19]. Of the 163 individuals characterized, 97 received the IHDS in addition to the above assessments, and these were included in this analysis. Included individuals ranged from 18 through 40 years of age, and were assessed prior to commencing treatment with ART. Potential participants were excluded if they had a severe psychiatric disorder (such as schizophrenia or bipolar disorder), recent history of substance abuse or significant neurological disorder (such as epilepsy or significant head injury with loss of consciousness more than 30 minutes).

All participants who met study criteria and agreed to participate provided written informed consent. Approval to conduct the study was obtained from the Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, and from the relevant clinic authorities.

### **Neuropsychological test battery**

A neuropsychological test battery was administered to all participants to assess specific domains of neurocognitive function. The battery comprised tests of attention (the Mental Alternation Test (MAT) and the Mental Control Test (MCT)), learning and memory (the Hopkins Verbal Learning Test (HVLT) and the Brief Visuospatial Memory Test (BVMT)), motor (Finger Tapping (FT) and the Grooved Pegboard Test (GPT)), psychomotor speed (Trail Making Test part A (TMTA), Color Trails Test 1 (CT1) and Digit Symbol-Coding (DSC)), executive function (Color Trails Test 2 (CT2), Stroop Colour-Word test (SCW), Wisconsin Card-Sorting Test (WCST), and category fluency- including an animal list and a fruit and vegetable list).

Control data for neuropsychological testing was obtained from 93 HIV-negative participants. These participants were recruited from Voluntary Counseling and Testing services at the same primary care clinics. We liaised with lay counsellors at clinics to obtain suitable participants who had been both counselled and tested, and who would be willing to be approached for the research study. Other than being HIV negative, as confirmed by a recent rapid HIV test and confirmatory serological test

done at the study visit, inclusion and exclusion criteria were identical to those applied to HIV-positive participants. The CD4 cell count was obtained from the clinic records. Data from the 93 HIV-negative controls were used to generate z-scores for establishing the degree of impairment for each test. No published norms are currently available in South Africa, and as most participants spoke isiXhosa, we elected to generate control data from similar community participants.

The IHDS was administered by medical officers trained in HIV neuropsychiatry. A uniform approach was ensured through the study leader. The IHDS was administered during the same visit as the study neuromedical assessment.

### **Determination of neurocognitive disorder status**

We used the above neuropsychological test battery, and an evaluation of functional assessment (the Patients' Assessment of Own Function (PAOFI) and the Quality of Life and Enjoyment Satisfaction Questionnaire (QLESQ)), to classify participants into one of four HAND categories, based on the updated criteria published by Antinori and colleagues [20]: no impairment, asymptomatic neuropsychological impairment (ANI), mild neurocognitive disorder (MND), and HAD. Individuals who scored more than two standard deviations (SD's) below the mean on at least two domains of function, and were noted to have significant functional impairment on self-report, were classified as having HAD. Those who displayed impairment between one and two SD's, were classified either as MND or ANI, depending on the presence or absence of functional impairment. The remaining participants were classified as non-impaired. The final classification was conducted by a consensus panel comprising two HIV neuropsychiatrists and a neurologist.

### **Statistical analysis**

Data were analysed using STATA 11 (Stata Corporation, Texas, USA). The HIV-positive and HIV-negative groups were compared with respect to age, level of education and neuropsychological test characteristics using unpaired t-tests, and with respect to gender distribution using a Fisher exact test. The HAND categories of HIV-positive participants were compared using Kruskal-Wallis tests for differences between medians of multiple groups. The difference between the means of the HAD group and the remaining participants were compared using an unpaired t-test. The ability of the IHDS to predict HAD was established using a receiver operating characteristic curve, and cut-off scores calculated from this. Correlations between the

IHDS total score and sub-test scores were calculated for each neuropsychological test, and r-values reported. P-values were regarded as significant at the 5% level.

## RESULTS

The demographic characteristics of the HIV-negative and HIV-positive groups are shown in Table 1. There were significant between-group differences in terms of age and level of education, but not gender distribution. There were also significant between-group differences in performance on a number of neuropsychological tests within each domain. Regarding the screening test (IHDS), the only test for which there was no significant between-group difference was the Luria sequence non-dominant hand ( $p=0.94$ ). HIV-positive participants performed significantly more poorly than HIV-negative controls on all other neuropsychological tests, other than the MAT ( $p=0.75$ ), CT1 ( $p=0.61$ ) and CT2 ( $p=0.35$ ).

The neuropsychological test performance characteristics of the HIV-positive participants classified into HAND categories is shown in Table 2. In this analysis, we found significant between-group differences on all neuropsychological tests, except for the FT non-dominant sub-score of the IHDS ( $p=0.10$ ) and the MAT ( $p=0.06$ ). Participants with HAD had lower mean total IHDS scores than the remaining participants, with means of 9.69 (SD=1.97) and 10.67 (SD=1.11) respectively ( $p=0.00$ ).

Correlations between the IHDS total and sub-test scores, on the one hand, and each neuropsychological test, on the other, are shown in Table 3. We identified significant correlations between the IHDS total score and the FT-ND, HVLT, BVMT, MC, CT1, CT2 and category fluency (fruit and vegetables ) z-scores. The CT1 was significantly correlated with all components of the IHDS.

The receiver operating characteristic curve of the IHDS using the HAND categories of HAD together with MND versus other categories as gold standard is shown in Figure 1. The area under the curve was 0.71. The varying cut-off scores for the IHDS are presented in Table 4. At a cut-off score of  $\leq 10$ , the sensitivity and specificity of the IHDS was 53 % (95% CI 41-66) and 80 % (95% CI 63-93) respectively. Using a cut-off of  $\leq 11$ , the sensitivity and specificity of the IHDS was 86 % (95% CI 75-93) and 32% (95% CI 17-51) respectively.

## DISCUSSION

In South Africa where HIV is highly prevalent, and rates of severe HAND are in excess of 50%, access to a screening tool for cognitive impairment has both clinical and research relevance. In this study we identified that the IHDS performs adequately when used to diagnose severe HAND (including HAD and MND). We utilized a detailed neuropsychological battery which was sensitive to differences between HIV negative and HIV positive controls. When we compared IHDS and neuropsychological test performance in the HIV positive participants across categories of HAND, we observed that the battery discriminated well across different groups, and for a number of test domains. In a receiver operating curve analysis using a cut-off score of  $\leq 11$ , the IHDS was 53% sensitive and 80% specific. The IHDS correlated moderately with individual neuropsychological tests.

The ROC analysis revealed that the IHDS performed reasonably well, although a cut-off score of  $\leq 11$  provided a better sensitivity. When we used a cut-off score of  $\leq 10$ , the sensitivity and specificity was 53% and 80% respectively. It may be more beneficial to retain the higher sensitivity and therefore use the cut-off of  $\leq 11$  rather than  $\leq 10$ , in order not to miss cases. In the original description of the IHDS, cut-offs of  $\leq 10$  yielded sensitivities of more than 80%, with moderate specificity [15]. The performance of the grooved pegboard test non-dominant has previously been reported to yield a sensitivity and specificity of 71% and 46% respectively, using a cut-off of 1.5 SD below adjusted means. Others have reported sensitivity and specificity of 77% and 65% respectively. The IHDS therefore could be useful tool, although clinicians in South Africa may need to consider using the higher cut-off score of 11.

HIV-positive individuals in this study performed worse than their HIV-negative counterparts on most tests of neuropsychological function, including the IHDS. Of note, there were no differences in performances on the hand sequence component of the IHDS, the MAT, CT1 and CT2 tests. Overall, then, our battery was sensitive to HIV-associated neuropsychological impairments. Of interest is that both groups of participants in our study performed better on CT1 and 2 than participants in Uganda [15]. This may be explained by lower levels of education in that study's participants (9.7 for controls and 8.7 years for HIV-positive participants). In addition, in the Ugandan study, no differences in motor performance were reported. These included the GPT and timed gait tests, while in our study, we did find differences in this domain [15]. One possible explanation is that regional and clade differences account for these domain-specific differences. The HIV epidemic in Uganda is known to be

predominantly made up of clades A, D, and D, while in South Africa clade C is predominant [21]. Previous studies of participants with clade C HIV have also reported abnormalities on GP [23].

The IHDS, together with individual neuropsychological tests, discriminated well between HAND categories. This suggests that these tests are suitable in differentiating different forms of HAND. The mean total IHDS score for individuals with HAD was 9.67, compared to 10.67 for other participants. In the group analysis, the finger-tapping test of the IHDS did not discriminate well across all categories, though the performance in the HAD group was worse than the other three groups (mean of 3.19, versus 3.64, 3.72 and 3.63 respectively;  $p=0.10$ ). The finger-tapping test used in the battery, however, did discriminate well between the groups. An adapted brief screening tool might therefore include a more detailed finger-tapping test.

The correlation between the IHDS total score and sub-test scores, on the one hand, and individual neuropsychological test scores, on the other, varied from weak to moderate. We expected the total IHDS score to correlate more strongly with non-dominant hand performance on the GP-non dominant test, as reported by Sacktor and colleagues [15]. However, as noted above, this difference might be explained by regional and clade-specific differences in test performance. We also expected the IHDS finger tapping test to correlate more strongly with the similar neuropsychological test, but the results were weak. The IHDS recall test correlated moderately with the HVLT test. The weaker correlations that we found might be explained by differences in test internal construct validity and ecology being different. To date there has been little or no research describing construct validity of these neuropsychological tests in South Africa; and in isiXhosa speakers in particular. Further comments regarding the selection of the test battery may be found on page 15 of the "Introduction". Suggestions for further research are made in the "Conclusions" chapter.

This study had several limitations. First, our sample size was not very large. We did not believe this is a significant weakness as we were able to identify statistically significant differences between groups in terms of cognitive status. Secondly, while our group has taken care in translating and adapting the neuropsychological test battery to ensure its suitability for South Africans, we have not undertaken to establish construct validity. Thirdly, while we took care to train and supervise medical

officers administering the IHDS, we did not conduct inter-rater reliability assessments, and this might have influenced our results. The administrator of the IHDS and the full neuropsychological battery was not the same rater.

We believe this study contributes to our knowledge about HAND in Southern Africa. Specifically, that neuropsychological impairment is frequent and severe in South Africa. Furthermore, the IHDS may be a useful screening tool, though efforts to understand the modest level of specificity requires further study. Individuals with HAD clearly perform worse on the IHDS, as well as on most tests included in our detailed battery as expected since performance on cognitive testing is utilized in the diagnostic classification. Nonetheless, as a brief tool, the IHDS performed well in comparison to the detailed battery and discriminated across different severities of HAND. Further research into refining the IHDS and incorporating it as a brief screening tool in to primary care is needed.

In this chapter, I have reported on the performance of the IHDS. The use of this tool could improve detection through its use, as well as through awareness of the problem of severe HAND. As mentioned previously, the detection of HIV-associated dementia, even at higher CD4 counts affords the patient the possibility of initiating HAART. In the next chapter, I report on findings of a one-year follow-up of individuals who had initiated HAART in the Western Cape of South Africa.

Table 1. Demographic and neuropsychological characteristics of HIV- and HIV+ participants

	HIV negative controls (n=93)	HIV positive participants (n=96)	P value
Age	25.16 (5.15)	29.75 (3.67)	0.00
Education	10.88 (1.28)	10.05 (1.77)	0.00
Gender female (%)	58 (62.4)	76 (79.2)	0.79
CD4 cell count		218.09 (150.57)	
Years since diagnosis		3.35 (2.04)	
IHDS total	10.89 (1.10)	10.29 (1.55)	0.01
IHDS FT subscore	3.88 (0.36)	3.52 (0.79)	0.00
IHDS hand sequence subscore	3.21 (0.88)	3.22 (0.92)	0.94
IHDS 4-word recall	3.78 (0.59)	3.35 (0.91)	0.00
FT non-dom	6.77 (1.71)	8.87 (2.08)	0.00
GP non-dom	78.36 (12.54)	87.05 (25.46)	0.00
HVLT recall	8.07 (2.07)	7.03 (2.09)	0.00
BVMT recall	8.97 (2.92)	6.52 (3.50)	0.00
MAT	16.47 (6.43)	16.76 (5.20)	0.75

Mental control	23.67 (5.93)	19.48 (5.62)	0.00
Digit symbol	46.83 (14.62)	40.72 (13.18)	0.00
TMTA	40.18 (16.31)	60.56 (32.41)	0.00
Colour I	54.66 (21.10)	53.21 (16.93)	0.61
Colour II	115.48 (50.98)	122.27 (48.47)	0.35
Stroop C/W	33.66 (9.57)	27.91 (9.24)	0.00
WCST per errors	31.92 (19.68)	45.34 (25.41)	0.00
animal	15.80 (4.64)	13.27 (4.67)	0.00
fruit & veg	15.11 (3.61)	13.70 (4.07)	0.01

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Table 2. Screening and Neuropsychological Test performance according to AAN classification

	HAND Category				Kruskal Wallis p-value
	Normal (n=18)	ANI (n=13)	MND (n=30)	HIV-D (n=35)	
IHDS total	11.11 (1.08)	10.92 (0.76)	10.23 (1.17)	9.69 (1.97)	0.01
IHDS FT subscore	3.50 (0.68)	3.78 (0.44)	3.78 (0.52)	3.25 (1.00)	0.10
IHDS hand sequence subscore	3.83 (0.39)	3.55 (0.73)	3.09 (0.77)	2.96 (1.10)	0.02
IHDS 4-word recall	3.92 (0.29)	3.55 (0.73)	3.30 (0.87)	3.09 (1.07)	0.03
FT non-dom	7.54 (1.34)	8.68 (1.62)	8.93 (1.98)	9.59 (2.34)	0.01
GP non-dom	77.80 (10.09)	88.49 (9.15)	78.35 (11.06)	99.07 (37.28)	0.00
HVLT recall	8.17 (1.38)	7.06 (1.80)	7.40 (1.95)	6.11 (2.30)	0.01
BVMT recall	8.72 (2.11)	7.31 (3.15)	7.37 (3.22)	4.37 (3.38)	0.00
MAT	18.56 (6.16)	16.92 (4.44)	17.50 (5.35)	14.79 (4.24)	0.06

Mental control	22.22 (5.39)	21.23 (4.49)	20.57 (5.76)	16.41 (4.74)	0.00
Digit symbol	51.06 (18.03)	50.83 (9.58)	52.91 (17.06)	32.46 (9.44)	0.00
TMTA	46.96 (11.0)	52.34 (14.55)	58.73 (21.20)	77.29 (44.47)	0.00
CT1	39.40 (10.11)	49.40 (15.92)	53.51 (14.63)	61.72 (17.30)	0.00
CT2	101.17 (24.85)	115.96 (36.28)	105.79 (32.87)	150.39 (60.21)	0.00
Stroop C/W	31.24 (9.46)	30.17 (7.87)	29.86 (8.54)	23.59 (8.94)	0.00
WCST per errors	28.47 (12.10)	41.15 (11.58)	43.57 (23.61)	56.94 (30.24)	0.01
Category fluency: animal	16.17 (4.11)	12.77 (4.11)	13.40 (4.70)	11.86 (4.60)	0.03
Category fluency: fruit & veg	16.17 (4.09)	13.31 (3.84)	14.60 (3.49)	11.80 (3.81)	0.00

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Table 3. Correlation coefficients of the IHDS total, finger-tapping, hand-sequence and recall subtests with the battery of neuropsychological tests with p-values

	IHDS total	IHDS FT	IHDS Luria	IHDS recall
FT non-dom	0.26 (0.01)*	0.26 (0.03)*	0.26 (0.03)*	0.17 (0.15)
GP non-dom	-0.03 (0.78)	-0.01 (0.97)	-0.06 (0.60)	-0.06 (0.59)
HVLT recall	0.29 (0.00)*	0.30 (0.01)*	0.12 (0.31)	0.30 (0.01)*
BVMT recall	0.35 (0.00)*	0.18 (0.12)	0.35 (0.00)*	0.27 (0.02)*
MAT	0.09 (0.43)	0.09 (0.45)	0.10 (0.42)	0.11 (0.36)
Mental control	0.22 (0.03)*	0.33 (0.00)*	0.12 (0.31)	0.18 (0.14)
Digit symbol	0.19 (0.06)	0.14(0.24)	0.15 (0.20)	0.14 (0.23)
TMTA	0.03 (0.78)	0.07 (0.58)	-0.03 (0.83)	-0.05 (0.66)
CT1	0.35 (0.00)*	0.24 (0.04)*	0.37 (0.00)*	0.36 (0.00)*
CT2	0.21 (0.04)*	0.34 (0.00)*	0.03 (0.78)	0.27 (0.02)*
Stroop C/W	0.10 (0.38)	0.23 (0.06)	0.09 (0.48)	-0.11 (0.35)
WCST per errors	0.19 (0.06)	0.10 (0.27)	0.08 (0.53)	0.11 (0.36)

Category fluency: animal	0.13 (0.22)	0.19 (0.00)*	0.06 (0.61)	0.09 (0.46)
Category fluency: fruit & veg	0.26 (0.01)*	0.18 (0.00)*	0.23 (0.05)	0.22 (0.06)

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Figure 1. ROC Curve of Total IHDS compared to HAND categories as gold standard (AAN disorder categories vs asymptomatic groups)

(SE=0.051; 95% confidence interval 0.607- 0.808)

Using a cut-off score of 10 or less, the sensitivity of the IHDS was 81% and the specificity was 54%.

Table 4. Cut-off points of the IHDS against the HAND classification

Cutpoint	Sensitivity	Specificity	Classified
( $\leq 4$ )	1%	100%	33%
( $\leq 6$ )	3%	100%	34%
( $\leq 7$ )	9%	100%	38%
( $\leq 8$ )	16%	96%	42%
( $\leq 9$ )	33%	93%	53%
( $\leq 10$ )	53%	80%	62%
( $\leq 11$ )	86%	32%	68%
( $\leq 12$ )	100%	0%	67%

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**Chapter 7.**

**Neuropsychological Outcomes in Young Adults Commencing Combination  
Anti-Retroviral Treatment in South Africa**

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## ABSTRACT

The impact of highly active anti-retroviral therapy (HAART) on individuals initiating therapy in South Africa where clade C HIV is predominant is unknown. In this study we sought to describe the change in neuropsychological function in a cohort of participants initiating HAART over a one year period. Participants included a group of individuals commencing HAART (n=82) and a group who did not (n=22). Baseline and one-year neuropsychological function was assessed using a detailed neuropsychological battery. Between-group comparisons, as well as unadjusted and adjusted linear associations were evaluated. A total of 105 participants were assessed at two time points a year apart. Retained participants had a median CD4 cell count of 165 at baseline and 350 at follow-up. There was no difference in baseline or follow-up summary neuropsychological score between the HAART - initiating and non-initiating groups ( $p=0.54$  and  $p=0.17$  respectively). There was a trend for the group initiating HAART to show a greater degree of neuropsychological change ( $p=0.08$ ). There was an association between worse baseline neuropsychological score and greater neuropsychological change for both groups ( $p<0.01$ ). This association remained significant in a model that adjusted for baseline CD4 cell count and HAART use as additional independent variables ( $p=0.03$ ). All included participants improved over one year, although individuals initiating HAART showed a trend to greater neuropsychological improvement than those not initiating. The most impaired individuals seemed to benefit most. Studies with larger comparison groups, and where HIV disease characteristics are needed to establish whether the trends we identified are clinically meaningful.

## **BACKGROUND**

In this chapter, I describe the impact of HAART on neuropsychological function at one year in the cohort. As I have outlined previously, it is not known whether HAART exerts the same effects in individuals infected with clade C HIV; whether particular domains benefit more than others; and whether there are certain predictors or associated variables of change. People living with HIV in South Africa frequently commence HAART at a late disease stage; it is therefore important to document the extent to which HAART can reverse pre-treatment impairment. This would allow for planning of treatment support, tailoring of treatment regimens or providing adjuvant therapies, and motivating for earlier treatment initiation in cases where significant impairment exists at higher CD4 cell counts. Neuropsychological impairments due to HIV infection of the CNS are detectable across all disease stages, but are more prevalent and marked in individuals with more severe HIV disease [1,2]. Although the use of HAART has halved the incidence of HIV-associated dementia (HAD), the prevalence remains significant [3].

Several published studies have reported on neuropsychological improvement following HAART initiation [4-6]. To date, this improvement has been ascribed to effective peripheral viral load suppression, CNS penetration effectiveness (CPE) of HAART regimens, and has been associated with severity of baseline (or study entry) neuropsychological function, and possibly also practice effects in cohort studies [6-9].

More recently attention has also been drawn to the potential for highly penetrating HAART regimens to exert neurotoxic effects. In one recent study, individuals with low CSF viral loads and high CPE ranked regimens had more impaired neuropsychological function than individuals with higher CSF viral loads [10]; and in another cohort study, immunologically reconstituted individuals who interrupted CART performed better over a two year period than those who continued treatment [9].

The impact of HAART in a prospective study in South Africa where clade C HIV is predominant has not been reported. In this study, we hypothesized that individuals initiating HAART would show improved neuropsychological function over one year. Moreover, we hypothesized that individuals with more severe disease at baseline would have worse neuropsychological outcomes at one year.

## **METHODS**

### **Subjects**

This study was conducted as part of a larger investigation of HIV-associated neurocognitive disorders (HAND) in Cape Town, South Africa previously described [11]. In summary, we conducted detailed neuropsychological assessments on 165 participants recruited from three primary health care centres. At each visit, detailed socio-demographic and neuromedical measures were also administered and laboratory tests completed. Included individuals ranged from 18 through 40 years, and were assessed prior to commencing treatment with HAART. They were excluded if they had a severe psychiatric disorder (such as schizophrenia or bipolar disorder), recent (within the last three months) history of substance abuse, or significant neurological disorder (such as epilepsy or significant head injury defined as a loss of consciousness for more than 30mins). We were able to retain 105 participants at one year study follow-up assessment. Of this group, 22 were deemed not to have initiated HAART - one participant had one month of treatment before being imprisoned and defaulting. Of the remaining 21, 15 had CD4 cell counts above the guideline for initiation and were not enrolled onto HAART. Their CD4 cell counts were unknown at the time of initial study recruitment. The other six participants qualified for HAART, and were included in the study on an intention to treat basis, but had not attended clinic appointments during the one-year period and so were not initiated. Neuropsychological technicians were blinded to the use of HAART at one year assessment.

Normative data for neuropsychological testing was obtained from 94 HIV- negative participants. These participants were recruited from Voluntary Counseling and Testing services at the same primary care clinics. We liaised with lay counsellors conducting testing at the clinics to obtain lists of potential participants who had been both counselled and tested. We approached potential participants drawn from these lists. Other than being HIV negative, as confirmed by a recent rapid HIV test and confirmatory serological test, inclusion and exclusion criteria were identical to the HIV-positive participants.

The CD4 cell count and viral load were extracted from the laboratory records, linked by the participants' clinic numbers. Clade sequencing was not available on this sample but 89% of infected individuals in the Cape Town area are infected with clade C virus [12]. Hepatitis sero-status was not established but the prevalence of hepatitis

C in South Africa is extremely low [13]. The use of HAART was also extracted post-hoc from clinical records, and the CPE rank for each was calculated using previously published criteria [7].

All participants who met study criteria and agreed to participate provided written informed consent. Approval to conduct the study was obtained from the Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, and from the relevant health authorities.

### **Neuropsychological test battery**

A neuropsychological test battery was administered to all participants to assess specific domains of neurocognitive function. The battery comprised tests of the domains: attention (the Mental Alternation Test (MAT) and the Mental Control Test (MCT)), learning and memory (the Hopkins Verbal Learning Test (HVLT) and the Brief Visuospatial Memory Test (BVMT)), psychomotor speed (Finger tapping (FT) and the Grooved Pegboard Test (GP)), psychomotor speed (Trail Making Test part A (TMTA), Color Trails Test 1 (CT1) and Digit Symbol-Coding (DSC)), executive function (Color Trails Test 2 (CT2), Stroop Colour-Word test (SCW), Wisconsin Card-Sorting Test (WCST), and language (category fluency)).

### **Determination of global neuropsychological function**

We used the above neuropsychological test battery to generate global neuropsychological performance scores for each participant. For each neuropsychological test, we generated a z-score by using the formula: (raw score-control mean)/control standard deviation. We then summed the z-scores for each participant and divided by the number of tests included to arrive at a global neuropsychological score.

### **Statistical analysis**

Data were analysed using STATA 11 (Stata Corporation, Texas, USA). As data were not normally distributed across all variables, we calculated medians and interquartile ratios for the one- and two-visit groups, as well as the HAART and non-HAART groups. Comparisons were made using Wilcoxon rank sum tests or Fisher Exact tests where appropriate. We generated box-and-whisker plots for the two time-points of the HAART and non-HAART groups, and compared mean summary scores using paired t-tests. We then evaluated linear trends first using simple models across all retained participants, HAART-initiators and non-initiators, with change in summary

score between follow-up and baseline as the dependent variable. We included independent variables in a final multiple linear regression, if the simple model rendered a p-value of less than 0.1, or the variable was known to be strongly associated with neuropsychological test performance. The final model included baseline CD4 cell count, baseline neuropsychological test summary score and HAART use.

## **RESULTS**

Of the total recruited cohort, 109 participants were retained and completed neuropsychological (NP) batteries at the two time points, while 56 were not retained. Of these 56 participants, nine had deceased before the follow-up period, six had migrated out of the district, 33 were lost to follow-up (had not attended routine clinic for at least 3 visits and could not be tracked after three telephone calls), and the remaining eight were not retained for unknown reasons.

Demographic, disease and neuropsychological characteristics of the retained and non-retained groups are presented in table 1. The retained participants performed better than those not retained on their baseline neuropsychological summary score ( $p=0.02$ ), as well as on a number of individual neuropsychological tests, including GP non-dominant ( $p=0.02$ ), HVLT recall ( $p=0.04$ ), BVMT recall ( $p=0.02$ ), TMTA ( $p=0.01$ ) and category fluency (fruit and vegetables) ( $p=0.01$ ). Retained participants were seen again after a mean of 12.9 months (SD 3.13), while their CD4 cell count had improved from a median of 176 (IQR 121-221) to 330 (IQR 241-464).

Of the 109 participants who were retained, full data were available for 104. Of these, 82 had initiated HAART (mean duration of use 11.6, SD=6.04), and 22 had not. When these two groups were compared, the HAART group had a significantly lower CD4 cell count at baseline (medians 183 vs 195;  $p<0.01$ ) and had a longer period since being diagnosed with HIV (medians 3 years vs 1 year;  $p=0.01$ ). Of note, there was no significant difference between HAART -initiators and non-initiators with respect to baseline summary NP scores (medians -0.42 vs -0.58;  $p=0.54$ ), but they did differ with regard to CT1, SCW and category fluency (animals). There was again no significant difference at one-year follow-up when participants on HAART were compared to those not, regarding summary NP score (medians -0.12 and -0.39;  $p=0.17$ ); however there was a trend for those using HAART to have shown a greater degree of improvement (median NP change 0.28 vs 0.13;  $p=0.08$ ). The majority of

the participants (n=73) initiating HAART group had a CNS penetration effectiveness rank of 1.5 or more.

When we compared the global neuropsychological test performance of the two groups at baseline and follow-up, we note that both had improved significantly (means from -0.42 to -0.13 for the HAART group,  $p < 0.01$ ; and means -0.51 to -0.36 for the non-HAART group  $p = 0.04$ ). The box and whisker plots of their global neuropsychological test performance are shown in Figure 1.

The unadjusted linear associations between a number of independent variables and change in global NP performance were then examined for all retained participants, those initiating HAART, and those not initiating (Table 3). The independent variables considered were HAART use, age, gender, level of education, years since diagnosis, baseline CD4 cell count, baseline NP summary score, months between testing, CPE rank and viral load at follow-up. There was a negative association between baseline NP summary score in the unadjusted associations for the whole participant and HAART-initiating groups ( $p < 0.001$  CI -0.27 to -0.08;  $p < 0.001$  CI -0.31 to 0.10). In those initiating HAART, male gender was associated with greater change in NP score ( $p = 0.01$ , CI 0.04 to 0.35).

In the adjusted model for all participants, we included baseline CD4 count, baseline NP summary scores and HAART use as predictors. The overall model was significant ( $p < 0.00$ ), with only baseline NP score remaining significantly predictive of NP change ( $p < 0.00$ ). The use of CART was not significant in this model ( $p = 0.21$ ). In a separate model including only participants initiating HAART, the overall model was significant ( $p = 0.003$ ), with baseline NP score again significant ( $p = 0.04$ ), as well as baseline CD4 cell count was 0.04.

## **DISCUSSION**

We report on the first follow-up study conducted among a dominant clade C group of patients utilizing a detailed neuropsychological battery to establish the effects of HAART. The majority of participants (66%) was retained and completed the study one-year later. Of these, 74% (n=82) had commenced HAART, while 26% (n=22) had not, allowing us to compare outcomes with a group exhibiting similar disease characteristics. While both initiators and non-initiators improved significantly on global NP summary score, there was a trend for the HAART -initiating group to show

a greater degree of NP change at one year. Using unadjusted and adjusted linear models, we found that the degree of baseline neuropsychological performance was most strongly associated with neuropsychological change. A trend was noted between use of HAART and neuropsychological improvement in the unadjusted model.

The use of HAART has frequently been reported as improving neuropsychological outcomes in prospective cohorts [3,6,14,15]. However, few studies have been able to include a control group with similar demographic and disease characteristics. This has been pointed out to be an important need in the literature [9]. Our control group had significantly higher baseline CD4 cell count (medians 172 and 329 respectively;  $p < 0.01$ ). While both groups represent substantial immunocompromise, it is not possible to draw clear conclusions. It is not possible to obtain a fully matched untreated control group. Both HAART initiating and non-initiating groups improved significantly over one year ( $p < 0.01$  and  $p = 0.02$  respectively). Several possible explanations for neuropsychological change over time exist, including real change (in this case due to HAART), baseline performance, participant variables (such as age, neuropsychological "competence"), test profile, time between testing, and mental state at the time of testing. The issue of practice effect has frequently been raised as a confound to measuring real change in neuropsychological test performance over time. Strategies to deal with re-testing include statistical prediction of follow-up testing and comparing this to performance to see whether real change has occurred. A number of possible contributors to change may then be included in the model to control for these confounds. The strongest predictor of follow-up performance is baseline performance: worse performance at baseline predicts worse performance at follow-up. Other factors such as older age and lower education also predict poorer repeat performance [16]. We found the opposite effect with regard to baseline performance. For this reason, and the absence of a "norming" dataset, we did not perform predictive scoring to control for repeat testing. Also, it can be argued that the one year interval between testing is sufficiently long to reduce practice effects. It is possible, however, that the previous experience of being tested does improve "neuropsychological competency" - a factor recognised in the literature to improve performance [16].

Neuropsychological improvement was significantly associated with a greater degree of baseline neuropsychological impairment, in unadjusted models including all retained participants, as well as HAART -initiators ( $p < 0.01$  and  $p < 0.01$  respectively).

This association remained significant in a final adjusted linear model, which included baseline CD4 count and HAART use as independent variables (overall significance  $p=0.01$ ). This finding is not surprising, given that HAART is known to reduce the incidence but not the prevalence of severe forms of HAND [3]; and that HAART has been reported to exert a limited impact on individuals with less severe impairment, or who were on stable regimens [17]. In addition, our participants had late stage disease by virtue of their low CD4 cell counts. This finding stands in contrast to the neuropsychology literature, which asserts that worse baseline performance predicts worse repeat performance [16]. This lends support to the conclusion that the improvement seen was real change. When we included HAART use in an unadjusted linear model, we a trend association with neuropsychological improvement ( $p=0.07$ ). This effect become non-significant in the final adjusted model, most likely due to the relatively small non-HAART comparison group and the strong effect of severity of baseline NP impairment. The improvement of both groups of participants may also be ascribed to a “neuropsychological competency effect”.

We did not detect an effect of different CPE ranks, as first-line regimens, used by the majority of participants in our study have high CPE ranks (73 of 82 participants had a CPE rank  $\geq 1.5$ ). Also, almost all participants achieved peripheral viral load suppression during the first year of treatment, and therefore were adequately immune reconstituted. This suggests that they were also adherent to treatment.

There were a number of limitations to our study. First, a significant number of participants were not retained, despite our best efforts at retention. These individuals performed significantly worse on neuropsychological summary score ( $p=0.02$ ). Also, the sample size of the non-initiating group was restricted, and this could have impacted on our findings. Although the two comparison groups differed with respect to CD4 cell count, this was the result of anti-retroviral guidelines in South Africa, as those with CD4 cell counts  $>200$  cells/ml do not qualify for HAART. We were not able to obtain CSF in order to characterize intra-theal viral load or inflammatory markers, again for ethical reasons.

We believe that this study makes an important contribution to our understanding of the effect of HAART on neuropsychologically impaired individuals. It highlights the fact that individuals initiating HAART in South Africa, where clade C HIV is highly prevalent, may benefit neuropsychologically. This benefit seemed to accrue most to individuals with more severe baseline impairment. Studies with larger comparison

groups, and where HIV disease characteristics are similar, are needed to establish whether the trends we identified are clinically meaningful or specific to HAART itself.

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Table 1. Demographic, disease and neuropsychological characteristics of participants

	Retained participants (n=109)	Not retained (n= 56)	p-value
Age: median (IQR)	29.55 (27-32)	29.54 (27-32)	0.63
Level of education: median (IQR)	10 (9-12)	10 (8-11)	0.35
Home language isiXhosa: n (%)	99 (91)	47 (84)	0.05
Baseline CD4: median (IQR)	182 (136-240)	165 (88-224.5)	0.47
Years since diagnosis: mean (SD)	3.29 (2.43)		
Months between visits: mean (SD)	12.93 (3.13)		
Follow-up CD4	330 (241-464)		
Follow-up viral load	1.69		
Baseline neuropsychological z-scores: median (IQR)			
Summary score	-0.51 (-0.9- 0.3)	-0.62 (-1.26- -0.12 )	0.02*

FT non-dom	-0.7 (-1.63- -0.12)	-1.1 (-2.11- -0.46)	0.08
GP non-dom	-0.18 (-1.2- -0.62)	-0.53 (-1.43- 0.19)	0.02*
HVLT recall	-0.04 (-1- 0.45)	-0.52 (-1.49- -0.04)	0.04*
BVMT recall	-0.33 (-1.7- 0.35)	-1.01 (-2.38- 0.01)	0.02*
MAT	-0.07 (-0.37- 0.53)	-0.07 (-0.52- 0.23)	0.44
Mental control	-0.62 (-1.29- -0.11)	-0.96 (-1.63- -0.28)	0.09
Digit symbol	-0.47 (-0.98- 0.29)	-0.54 (-1.01- 0.01)	0.19
TMTA	-0.59 (-1.49- -0.12)	-1.09 (-2.38- -0.44)	0.01*
Colour I	0.365 (-0.2- 0.78)	0.06 (-0.5- 0.59)	0.05
Colour II	-0.08 (-0.43- 0.45)	0.2 (-0.7- 0.42)	0.69
Stroop C/W	-0.65 (-1.32- 0.04)	-0.59 (-1.32- -0.07)	0.89
WCST per errors	-0.19 (-1.32- 0.35)	-0.46 (-1.02- 0.2)	0.39
animal	-0.39 (-1.25- 0.26)	-0.6 (-1.47- 0.26)	0.25
fruit & veg	-0.03 (-0.86- 0.52)	-0.58 (-1.42- 0.25)	0.01*

Table 2. Demographic, disease and neuropsychological characteristics of participants at baseline and follow-up in HAART and non-HAART groups

	Initiating HAART (n=82)	Not initiating HAART (n=22)	p-value
Age: median (IQR)	30 (27-32)	30 (28-34)	0.12
Level of education	10 (9-12)	10.5 (9-11)	0.95
Home language isiXhosa: n (%)	80 (97.5)	20 (91)	0.84
Baseline CD4: median (IQR)	172 (121-214)	329 (321-602)	<0.00
Baseline neuropsychological z-scores: median (IQR)			
Summary score	-0.42 (-0.9- 0.03)	-0.58 (-0.93- -0.39)	0.54
FT non-dom	-.066 (-1.64- -0.12)	-0.9 (-1.65- 0.11)	0.7
GP non-dom	-0.27 (-1.26- 0.67)	0.02 (-1.05- 0.56)	0.85
HVLT recall	-0.04 (-1- 0.45)	-0.52 (-1- -0.04)	0.46
BVMT recall	-0.33 (-1.36- 0.35)	-1.01 (-1.7- 0.35)	0.36

MAT	-0.7 (-0.37- 0.53)	-0.7 (-0.37- 0.53)	0.94
Mental control	-0.79 (-1.46- 0.22)	-0.54 (-1.29- -0.11)	0.82
Digit symbol	-0.33 (-1.01- 0.42)	-0.74 (-0.95- 0.33)	0.26
TMTA	-0.53 (-1.38- -0.02)	-1.07 (-2.13- -0.27)	0.05
Colour I	0.44 (-0.04- 0.8)	0.01 (-0.49- 0.36)	0.02*
Colour II	-0.1 (-0.52- 0.45)	-0.18 (-0.4- 0.4)	0.85
Stroop C/W	-0.49 (-1.32- 0.14)	-1.06- -1.43)	0.03*
WCST per errors	-0.11 (-1.58- 0.35)	-0.39 (-1.07- 0.35)	0.92
animal	-0.6 (-1.25- 0.04)	-0.07 (-0.6- 0.69)	0.04*
fruit & veg	-0.03 (-0.86- 0.8)	-0.31 (-1.14- -0.03)	0.05
Years since diagnosis: median (IQR)	3 (2-5)	1 (1-2)	0.00*
Months between visits: median (IQR)	12 (11-14)	12 (11-15)	0.68
Follow-up CD4: median (IQR)	324 (231-451.5)	430 (241-560)	0.35
Follow-up viral load: median	1.69		
Follow-up neuropsychological z-scores: median (IQR)			

Summary score	-0.12 (-0.56- 0.22)	-0.39 (-0.75- 0.17)	0.17
Neuropsychological change	0.28 (0.04- 0.51)	0.13 (-0.12- 0.39)	0.08
FT non-dom	-0.18 (-0.73- 0.28)	-0.18 (-1.11- 0.32)	0.89
GP non-dom	0.21 (-0.79- 0.66)	0.45 (-0.04- 0.93)	0.18
HVLT recall	-0.04 (-0.52- 0.93)	-0.86- -2.02- 0.56)	0.30
BVMT recall	-0.67 (-1.7- 0.35)	-1.36 (-2.38- 0.01)	0.06
MAT	-0.7 (-0.52- 0.53)	0.23 (-0.52- 0.82)	0.52
Mental control	-0.45 (-1.13- 0.73)	-0.20 (-1.13- 0.39)	0.88
Digit symbol	-0.33 (-0.95- 0.49)	-0.78 (-1.29- 0.35)	0.37
TMTA	-0.18 (-1.01- 0.31)	-0.65 (-1.92- -0.39)	0.01*
Colour I	0.47 (-0.13- 0.9)	0.49 (-1.1- 0.68)	0.38
Colour II	0.28 (-0.27- 0.54)	-0.27 (-0.86- 0.64)	0.29
Stroop C/W	-0.44 (-1.32- 0.32)	-0.8 (-1.11- -0.38)	0.27
WCST per errors	-0.1 (-0.46- 0.56)	-0.21 (-0.87- 0.35)	0.06
animal	0.26 (-0.6- -.69)	-0.17 (-0.82- 0.69)	0.42

fruit & veg		-0.03 (-0.58- 0.8)	-0.31 (-0.86- 0.8)	0.23
Duration of HAART in months: mean (SD)		11.56 (10-12)		
CPE rank:	1	7		
	1.5 - 2	47		
	>2	26		

Figure 1. Global neuropsychological performance of participants initiating HAART (left) (n=82) and not initiating HAART (right) (n=22)

HAART group:  $p=0.000$ ; non-HAART group:  $p= 0.02$

Table 3. Unadjusted regression models for predictors of change in global neuropsychological test performance

	All participants (n=105)			HAART initiated (n=85)			HAART not initiated (n=22)		
	Regression coefficient	95% CI	p-value	Regression coefficient	95% CI	p-value	Regression coefficient	95% CI	p-value
HAART	-0.13	-0.28- 0.01	0.07						
Age	-0.01	-0.02- 0.01	0.45	-0.01	-0.03- 0.01	0.41	0.01	-0.04- 0.05	0.75
Male gender	0.09	-0.05- 0.22	0.22	0.19	0.04- 0.35	0.01*	-0.13	-0.46- 0.20	0.41
Education	-0.02	-0.05- 0.02	0.37	-0.03	-0.07- 0.01	0.11	0.05	-0.12- 0.03	0.15
Years since diagnosis	0.01	-0.02- 0.04	0.67	-0.00	-0.03- 0.03	0.98	0.26	-0.37- 0.89	0.34
Baseline CD4	-0.00	-0.00- 0.00	0.04*	-0.00	-0.00- 0.00	0.03*	-0.00	-0.00- 0.00	0.83
Baseline Global NP	-0.18	-0.27- -0.08	0.00*	-0.20	-0.31- 0.10	0.00*	-0.03	-0.33- 0.27	0.84
Months between testing	-0.00	-0.02- 0.02	0.79	-0.00	-0.03- 0.02	0.75	0.03	-0.02- 0.07	0.20
CPE				0.00	-0.14- 0.14	0.95			
Viral load at follow-up	0.04	-.016- 0.13	0.46						

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## **Chapter 8.**

### **Summary and Conclusions**

As summarized in earlier chapters, the need for further investigation into HIV-associated neurocognitive disorders (HAND) in South Africa is underscored by five key problems: (1) that HIV infection is epidemic in southern Africa, with the highest number of infected people in the world living in South Africa, and that there may be key differences in the epidemic in this region as opposed to others [1]; (2) that HIV infects the brain early in infection, exerts neurotoxic effects throughout the long asymptomatic phase of the illness, and that these effects are most pronounced with more severe immune-compromise [2,3]; (3) that adverse neurotoxicity of HIV manifests commonly as neuropsychological impairments across a number of neurocognitive domains [4]; (4) that HAND have a dramatic effect on a range of behavioural and health outcomes, such as employment status and function, impaired activities of daily living such as driving, and poor medication adherence (with consequent viral resistance and adverse health outcomes) [5]; and (5) that despite the impact that highly active anti-retroviral therapy (HAART) has had on reducing severe forms of HAND, mild forms remain prevalent [6]. Indeed these milder forms may also exert deleterious effects on functional abilities.

Despite the fact that HIV is most prevalent in Southern Africa, most of the empiric research has been conducted in North America and to a lesser extent in Europe. The epidemic in these high-income countries (HICs) differs from that in South Africa- a middle-income country (MIC)- in several important psycho-social and biological ways: (1) spread- in HICs, HIV transmission is primarily driven by intra-venous drug abuse and by homosexual contact, while in MICs, spread has been driven by heterosexual contact, and indeed by the phenomenon of sexual concurrency [1]; (2) Size and resources- the size of the epidemic and the availability of resources in MICs is not only vastly different, but has an effect on prevention and treatment of HIV-related disorders; (3) Poverty and socio-demographic factors- people infected with HIV in MICs are more likely to be poor, have lower levels of education, and have a higher burden of co-morbid physical disease, than those in HICs [1]; (4) HIV-1 sub-type- the HI virus frequently mutates and recombines to form different variants. Of the main group of HIV-1 sub-types or clades, sub-types A, B, C and D are commonest, but they are not equally distributed across the globe. HICs have a predominance of sub-type B, while Southern Africa has a predominance of sub-type C [7]. Genetic differences in HIV-1 clades may be relevant in two ways- first, that there may be putative differences in neurotoxicity of the different clades; second, that people infected with different clades of HIV may respond to HAART differently [8].

In vitro work has suggested that the clade C viral tat protein exerts a lesser neurotoxic effect than that of clade B tat protein [8]. In the first locally published clinical study, using a brief cognitive screening tool, which in large part challenged this hypothesis came from our Cape Town group [9] - the second, which provided detailed characterisation of neurocognitive disorders, is part of this thesis (see chapter 4). Further studies which investigate the association between neuropsychological impairments and the genetic make-up of the HIV-1 sub-type producing HAND in individuals in Cape Town are needed. The second important aspect of understanding HAND in our population- that of response to HAART- was also addressed by this thesis. We have been able to show that individuals with severe HAND benefitted significantly over the course of one year of treatment, although it is likely that neuropsychological performance is likely to be affected by practice effects or “test experience familiarity”.

In chapter 5, we were able to demonstrate that apolipoprotein E4 (APOE4) is a more common variant in Cape Town than other regions [14], but that it was (a) not over-represented in individuals with HIV-associated dementia, and (b) that the APOE2 variant appeared to be under-represented in the HIV-positive cohort. Further work to explain these findings by including an adult HIV negative control group is needed. In addition to APOE, other gene candidates and protein products which predispose to the development of HAND have been proposed, including monocyte-chemoattractant protein-1 (MCP-1) [10,11]. The theoretical basis of APOE lies in its association with the development of a range of neurocognitive disorders, such as dementia following traumatic brain injury and Alzheimer’s disease. In these conditions, the APOE4 variant occurs more frequently in people with dementia, probably as the result of defective lipid metabolism and consequent vasculopathy [12]. A few studies linking APOE4 to adverse outcomes in HIV have been done, though with diverse results[11,13]. Longer prospective studies are needed to identify the role that APOE4, HAART use and ageing may have on neurocognitive outcomes. All are associated with an increased in vascular risk factors.

Further acquired and socio-demographic factors may impact on the development of HAND. These include intellectual disability, low level and quality of education, gender, the presence of substance abuse, co-morbid infections (such as hepatitis C), history of traumatic brain injury and advancing age [6,15]. In this thesis screened individuals in order to control for their effects. Other factors, such as nutritional

status, we reported on in chapter 4. These were not associated with the development of HAND.

Viral factors, and host-viral interactions are also part of a model of neurodegeneration, though poorly understood. Clues to these mechanisms must be sought across different modalities of study, and synthesised into a coherent model. These include clinical studies, imaging studies, investigation into neuro-inflammation, and as mentioned above, correlations between viral genotype and neuropsychological outcomes. More recent studies have begun to focus on early HIV infection and the sequence of ensuing lympho- and neuro-invasion [16]. These suggest that much of what occurs later in HIV disease is determined by early host-viral factors.

### **Summary of findings**

It is now well-established that HAND are both common and disabling. In developed countries, rates of HAND (including milder forms) has been estimated to be between 30-60%. In the face of questions being raised in the literature concerning possible differences between the neurotoxicity of the different HIV clades, it has become important to document the extent to which HIV causes neuropsychological impairment in South Africans. In this work, the first to utilise a detailed neuropsychological test battery in adults attending HIV clinics in South Africa, we were able to show that HAND are common in the Western Cape of South Africa- with a prevalence of HIV-associated dementia (HAD) of 25.4% of individuals entering care. Nearly two-thirds of all individuals assessed had some form of neurocognitive disorder. These patients tended to be older and less well educated, with a trend to lower CD4 cell counts. When we examined anthropomorphical and nutritional and measures, these were found to bear no associated with the development of HAND. The relevance of these findings is not only to confirm that clade C is indeed neurotoxic and produces high rates of HAND, but to underline the importance of understanding mechanisms of disease, improve detection, and therefore to improve treatment outcomes.

In a genetic analysis, as discussed above, we investigated the putative role that APOE might play in the development of HAD. We found that the E4 variant was not associated with HAD in our sample. While other investigators have raised the possibility that individuals with the E4 variant may not survive into older age [15], our participants were younger, and therefore we could not explore this hypothesis. In the

absence of a group of adult controls, we are also unable to draw clear conclusions about the lower allelic frequency of the E2 allele in the HIV positive participants. An hypothesis which requires further exploration is that the E2 variant may be protective against HIV infection.

With the high prevalence of HAND noted, we proceeded in chapter 6 to establish whether or not the International HIV Dementia Scale (IHDS) would be a useful screening tool in our setting. It has previously been validated in Uganda, with some preliminary work being published from Botswana. We found that when we combined individuals with HIV-associated dementia and those with mild neurocognitive disorder, the IHDS performed satisfactorily, using a cut-off score of  $\leq 10$  on the scale. However, the sensitivity was much improved when we raised this to  $\leq 11$ , although the specificity was low. Clinicians may need to consider using this higher cut-off in our setting. It is not clear whether differences in neuropsychological effects of HIV across different countries might account for this.

In chapter 7, we reported significant improvements in neuropsychological performance in both groups (HAART initiators and non-initiators), with the effect of HAART offering a trend to significance in an unadjusted linear model. This is likely explained by a range of testing variables, not least the “test experience familiarity” mentioned above. The group initiating HAART showed a trend towards a greater degree of neuropsychological improvement, as reflected in a summary change score. A greater degree of baseline neuropsychological impairment was predictive of improvement at one year in unadjusted and adjusted models of neuropsychological improvement. Studies of the effect of HAART on neuropsychological outcomes are limited by a range of factors. First, studies differ in the extent of the batteries employed, with some using as few as three or four test measures. Second, cohorts across studies differ with respect to disease characteristics- many such studies have reported on individuals already on HAART, as opposed to pre- and post-treatment. Thirdly, with repeated measurements being conducted, there is the problem of practice effects in testing. These are clearly exacerbated with shorter periods between tests, even when different forms are used. Our view is that even using a one-year test-retest interval, participants who are not well-educated are more familiar with the experience of being tested. In attempting to control for this practice effect, and to establish the effect of HAART, a control group of untreated individuals would be best. In our study, we were able to include a small sub-sample of individuals not treated, but who completed 2 assessments over one year.

## **Overall conclusions**

We have been able to confirm using a detailed neuropsychological test battery, and medical and functional assessment, that HIV-associated neurocognitive disorders are highly prevalent in young adults commencing HAART in the Western Cape. This confirms the single earlier report from Cape Town, using only a brief cognitive screening tool, that HAND are indeed prevalent in individuals infected with clade C HIV. While this finding is in keeping with international studies, especially of individuals at this severe disease stage (that is, with CD4 cell counts <200 cells/ml), our high rates suggest that these patients might be developing HAND much earlier, and therefore should be screened. With the large number of individuals needing to initiate treatment, it is likely that clinicians are being guided mainly by CD4 cell counts, rather than clinical indications, unless a life-threatening illness presents itself. We would argue that with more active screening at higher CD4 cell counts, individuals may be referred for more formal testing, and if found to have a HAND, should be initiated onto HAART. Tools such as the IHDS may be used for this purpose, followed by focussed neuropsychological batteries.

Individuals with severe neuropsychological impairment demonstrate an improvement in neuropsychological scores after HAART. Some of this effect may be due to practice effects. The presence of residual deficits (individuals initiating HAART had a summary neuropsychological deficit score of -0.12), suggests that either certain neurodegeneration is irreversible, or that further improvement could occur over a longer period of time. Implications include the possibility of adding adjuvant neuroprotective treatments, re-assessing patients over time, and the impact of impaired functional abilities for this duration.

## **Limitations of this study**

This study has a number of limitations. First, we noted along with other investigators, that our measures of activities of daily living and functional ability were inadequate. This occurred for a number of reasons, including that many participants are unemployed, and therefore this aspect of life cannot be measured. Also, we propose that a significant amount of executive function needs to be lost in order to be measure by a “crude” self-report scale. Lastly, we hypothesise that many individuals under-report the extent of their functional deficits, either through shame or loss of insight. The need for a broad-based, culturally-appropriate and yet sensitive tool to measure functional ability is great.

Another limitation was the lack of randomisation of participants into a non-HAART control group. The importance of this sub-group only become clear once the study had complete recruitment. A prospective, controlled study comparing similar disease groups of important key measures would add greatly to knowledge of the impact (or not) of HAART, and whether adjuvant treatments should be considered. As it would not be ethical to withhold treatment from participants who would normally qualify for treatment, it would be necessary to utilise a comparison group with similar disease characteristics, but who did not qualify for HAART under current guidelines. Such a group in South Africa may be constituted of participants with CD4 cell counts from 200-350 cells/ml. In my study, though, we were able to identify only 22 treatment-free participants, which may have limited our ability to make draw firm conclusions about this group.

We did not conduct clade sequencing of our participants at this time. This would have enabled to us to more definitively describe the relationship between the respective clade and viral genome, and the extent and nature of neuropsychological impairment. Previous studies of HIV clade in Cape Town have confirmed that the “C” sub-type is predominant [17].

The further detailing of neuropathology of HAND through the use of magnetic resonance imaging (MRI) could have added a useful component to the study. While we were able to image a small sub-set of participants, these data were included in this clinical analysis. Larger samples of imaged participants are needed to better understand the association between neuropsychological performance and brain structure. Modalities such as diffusion tensor imaging may be useful in detailing white matter damage induced by HIV neurotoxicity [18]. In addition, careful viral characterisation would be beneficial, including viral loads, as well as viral sub-type and genetics.

### **Recommendations**

A number of important clinical issues should be considered as a result of this research. These include the importance of screening for HAND in patients at HIV “wellness clinics”, monitoring neuropsychological progress of patients initiating HAART with a view to providing adjuvant treatments, and considering additional psycho-social support to patients with functional disability as a result of HAND.

As previously outlined, HAND are common, and may occur at CD4 cell counts above 200 cells/ml. It is current practice in the Western Cape that this group of patients are seen 3-6 monthly by nurse practitioners. They are referred into pre-HAART counselling once their CD4 count falls to below 200 cells/ml, or they develop stage 3 or 4 clinical conditions. It is our experience that these patients are not routinely screened for neurocognitive problems. Individuals who score  $\leq 11$  on the IHDS should be referred for further confirmatory assessment, and then a motivation for inclusion in to HAART provided. Further end-organ brain damage might be prevented if this practice were implemented.

To date, there have been relatively few clinical trials of adjuvant treatments for persistent HAND in the context of HAART. These include agents currently registered in South Africa for other conditions- namely, memantine and lithium [19,20]. Brief trials of these agents have suggested benefit, but further work with our local population is needed before they can be used in routine clinical practice. Nonetheless, the need for a cost-effective, well-tolerated and clinically effective agent is pressing.

The need for studies to address neuropsychological issues is also urgent. In this regard, our group has commenced a study of normative data in the Western Cape. Other related studies should include closer examination of the internal properties of a battery- that is, whether certain tests correlate more or less strongly with global effects. Also, concerted efforts to understand and develop both culture and language-appropriate tests are needed. For example, can isi-Xhosa speakers understand blue-green differences, as in the Stroop test.

Finally, despite limited resources, clinic teams must consider strategies to support patients with HAND, especially if they are on HAART. This may take the form of more focussed family or treatment support partner counselling, regarding the needs for support; or lay counsellor/home-based carer interventions. Individuals who are employed may benefit from occupational therapy assessment, support or job description re-assignment. Further work is needed to translate the neuropsychological impact of HAND into occupational disability across a range of occupations in South Africa- not least mining, driving, or machine operation.

### **Future research**

A number of further studies should be considered following this work. First, larger prospective studies using comparison groups of participants with similar disease-stage initiated and not initiated on HAART would allow for clearer delineation of the effects of HAART on neurocognitive outcomes. Second, such studies would also allow for understanding the correlations between neuropsychological impairment, viral genetics and neuro-imaging findings. This may provided clearer links between viral clade, specific neuropsychological impairments and brain regions affected. Third, prospective treatment studies should investigate the effects of CNS penetrating and non-penetrating regimens of HAART in order to describe not only the potential benefits of better-penetrating regimens, but also the potential neurotoxic effects of HAART. In this respect, preliminary work in our group has suggested that magnetic resonance spectroscopy (MRS) might detect lipid or protein peak signals in CSF that distinguish between HAART-induced neurotoxicity.

Finally, further research into adjuvant treatments for HAND is needed in our population. Putative molecules have been suggested above, but all are problematic for reason of either cost or toxicity. Clinical studies using more cost-effective agents thought, might lead to proof-of-principle findings could in turn result in larger scale investigation of affordable molecules.

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