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MPHil (Neuropsychiatry) Dissertation

University of Cape Town

‘The relationship between neurocognitive disorders, prospective memory impairment and white matter damage in Clade C HIV-positive subjects’

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Declaration

I hereby declare that the work contained in this dissertation is my original work and that I have not previously submitted it, in its entirety or in part, at any other university for a degree neither has it been published *prior to registration* for the abovementioned degree

The dissertation will have the following format:

Part A: *The protocol*

Part B: *A structured literature review*

Part C: *Two manuscripts*

Part D: *Supporting documents*

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**Relationship between prospective memory and neuroimaging
among HIV positive individuals in South Africa**

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AIMS:

To examine the relationship between prospective memory, cognitive function and Diffusion tensor imaging (DTI)/ White matter integrity of human immunodeficiency virus (HIV) positive individuals in the Western Cape. We hypothesize that:

1. Individuals infected with HIV will exhibit significantly poorer microstructural integrity of the white matter than HIV negative individuals, as determined by in vivo diffusion tensor imaging. We expect that values of fractional anisotropy (FA) - a measure of directional water diffusion- in the frontal white matter will be significantly lower among HIV patients compared to controls
2. Lower FA measured in the frontal white matter will correlate significantly with impaired performance on tests of prospective memory

INTRODUCTION

Neuropsychological impairment in HIV

Neuropsychological impairment is evident in 30 to 50% of persons with HIV-1 disease, with the characteristic pattern of deficits thought to reflect a preferential disruption of prefronto-striatal circuits (Reger, 2002). Deficits in motor coordination, information processing speed, working memory, and executive functions are commonly associated with HIV-1 (e.g., Heaton et al., 1995).

Deficient episodic (i.e., retrospective) learning and retrieval are common in individuals with HIV-1 infection (Murji et al., 2003). Group studies of HIV-1 disease reveal evidence of limited free recall, diminished use of organizational strategies (e.g., semantic clustering), interference effects, inconsistent recall across learning trials, and high rates of repetition errors (Murji et al., 2003). By way of comparison, retention (i.e., consolidation) and recognition discrimination are less commonly affected in persons with HIV-1 disease (e.g., Delis et al., 1995). Taken together, the memory profile of HIV-1-infection is

most consistent with dysfunction in the strategic (i.e., executive) aspects of encoding and retrieval as seen in prototypical “subcortical” disorders, such as Parkinson’s and Huntington’s diseases (Murji et al., 2003).

The neuropsychological features of HIV associated dementia (HAD) are indicative of the neuro-pathological changes. Therefore, HIV-associated degeneration of sub-cortical striatal and fronto-striatal structures leads to motor/psychomotor problems, memory dysfunction and frontal/executive dysfunction (Grant et al 2005). More specifically, motor problems are seen on the timed gait and grooved pegboard tests; memory is impaired mainly in its recall domain, and less in recognition; while frontal/executive problems are observed during tests of verbal fluency, set-shifting (trail making) and slowed information processing.

It is unclear how neuropsychological impairment relates to neuro-degeneration. A likely summary is that HIV continues to replicate within the central nervous system (CNS) during the clinically asymptomatic phase, with periods of enhanced replication and consequent CD4 cell loss. These effects may be attenuated by highly active antiretroviral therapy (HAART). Lastly, there is a predictably strong association between neuropsychological impairment and post-mortem evidence for HIV-encephalitis (which is defined by myelin pallor, multi-nucleated giant cells and microglial nodules) (Cherner et al 2002).

One cognitive construct with obvious relevance to medication adherence is prospective memory (ProM). ProM, or “remembering to remember”, is an aspect of declarative memory that refers to the execution of a future intention (e.g., remembering to pay one’s rent at the beginning of the month).

Recent research shows that individuals infected with HIV report experiencing more frequent prospective memory (ProM) failures as compared to demographically similar seronegative persons, especially on self-cued daily tasks (Carey, et al., 2007). Consistent with the largely frontal systems neuropathophysiology of HIV the profile of HIV-associated ProM impairment is

characterized by deficient self-initiated cue detection and retrieval (Carey et al., 2006).

ProM generally involves a series of cognitive processes that includes: (1) the formation of an intention that is paired with a specific retrieval cue (i.e., a specific time or event); (2) maintenance of the intention-cue pairing over a delay interval while concurrently engaged in a foreground task, during which time both active (e.g., strategic) and automatic (i.e., spontaneous) monitoring may occur (McDaniel 2007); (3) detection and recognition of the cue; (4) search and retrieval from retrospective memory (RetM) for the content of the intention; and (5) successful execution of the intention. At a neural systems level, ProM is most heavily reliant upon prefrontal systems (e.g., Brodmann's area 10; Simons, 2006), but is also dependent upon the RetM contribution of medial temporal (e.g., hippocampal) networks (e.g., Martin, McDaniel, et al., 2007).

An important distinction is often made between *event-based* (EB) and *time-based* (TB) ProM tasks (Einstein & McDaniel, 1990). The difference between these two tasks concerns the type of cues that initiate retrieval of the intention. For example, an external stimulus (sometimes embedded in an ongoing activity) provides the cue for action in an EB task (e.g., a mailbox cues the mailing of a letter), whereas in TB tasks, the intended action is performed after a specified time interval (e.g., taking a medication every four hours). It has been hypothesized that TB tasks require slightly different cognitive processes than EB tasks (i.e., a greater emphasis on self-initiated monitoring and retrieval), and empirical evidence suggests that the former are generally more sensitive in older adults (Henry, MacLeod, Phillips, & Crawford, 2004) and traumatic brain injury samples (e.g., Cockburn, 1996).

From a more practical perspective, ProM is hypothesized to play a unique and influential role in numerous aspects of daily life, including household chores (e.g., cooking; Fortin, 2002) and employment (e.g., Sellen, 2007).

Why prospective memory is important?

ProM is critical in order to properly adhere to a medication regimen; that is, one must first form the intention to take the medication at some point in the future (e.g., take two pills of medication X after dinner), maintain the intention-cue pairing throughout the day despite the distraction of normal daily activities, detect and recognize the cue when it occurs (e.g., clearing the dinner table), recall the specific medication and directions, and finally, take the medication as instructed. (Woods 2008)

Prior studies in HIV (e.g., [Hinkin et al., 2002](#)), standard clinical measures of executive functions (i.e., planning and divided attention), attention, episodic retrospective memory, and verbal fluency were also predictive of medication management. HIV-associated ProM impairment emerged as a significant predictor of medication management, even after considering the contributions of these established cognitive domains.

Strict adherence to prescribed medication regimens (i.e., HAART) is essential to the long-term clinical management of HIV disease. Infected individuals who are highly adherent (i.e., taking over 90% of their prescribed doses) evidence considerably better disease outcomes, including lower rates of virologic failures ([Perno et al., 2002](#)), treatment resistant viral strains ([Harrigan et al., 2005](#)), and mortality ([Lima et al., 2007](#)). Mild-to-moderate deficits in numerous areas of neuropsychological functioning (e.g., memory, executive functions, information processing speed) remain prevalent in the era of HAART, despite the effectiveness of such treatments on immune health.

As many as one half of individuals with HIV associated neurocognitive impairment experience problems with independently managing their instrumental activities of daily living (IADLS) ([Heaton et al., 2004](#)). These findings converge with a recent study showing that ProM demonstrates

incremental ecological validity as a predictor of general IADL dependence in HIV (Woods et al, 2008).

Neuropsychological impairment contributes to decrements in medication management, automobile driving and vocational functioning even after one considers the effects of HIV disease severity and psychiatric distress (van Gorp et al., 1999). Woods et al. (2008) showed that Prospective memory plays an important and unique role in successful daily functioning among individuals infected with HIV.

What is known about HIV and the brain?

At present the neurobiological substrates of HIV cognitive impairment are not well described. This is particularly the case for the genetic form of HIV known as clade C, since nearly all studies of cognitive function in HIV have been conducted in North America where the dominant genetic strain of the virus is clade B. Early studies suggested that individuals infected with clade C may be less likely to develop cognitive impairments due to a biological defect in the tat protein present in clade C (Ranga et al., 2004). Tat protein is thought to be an important mediator of the neuro-toxic effects of HIV.

In recently published data from India, it was shown that individuals infected with clade C exhibit significant cognitive impairment, suggesting that the brain is, in fact, vulnerable among individuals with clade C HIV (Yeptomhi et al., 2006). These findings are notable because clade C is the most common form of HIV in the world, as well as the dominant strain in South Africa.

The importance of this distinction is that the two strains of the virus differ in terms of specific protein binding sites and binding characteristics, replicative capacity (Centlivre et al., 2005), and possibly in the development of treatment resistance (Grossman et al., 2001; Kantor et al., 2002), all of which suggests a potentially different outcome associated with the clade C viral strain.

HIV-associated neuropathologies predominantly affect the frontostriatalthalamocortical circuits which are known to support normal ProM

functioning. Two of the principal components of prospective memory (i.e., remembering to carry out delayed intentions) are recognizing the appropriate context to act ("cue identification") and remembering the action to be performed ("intention retrieval"). In a functional MRI (fMRI) study, it was found that a consistent pattern of hemodynamic changes occurred in both prospective memory conditions/components in anterior prefrontal cortex (Brodmann area 10- BA 10), with lateral BA 10 activation accompanied by medial BA 10 deactivation. These effects were more pronounced when demands on intention retrieval were high. This is consistent with the hypothesis that anterior prefrontal cortex (area 10) supports the biasing of attention between external events (e.g., identifying the cue amid distracting stimuli) and internal thought processes (i.e., maintaining the intention and remembering the intended actions). The results suggest that cue identification and intention retrieval share some common neural basis in anterior prefrontal cortex. (Simons et al 2006). Recent studies implicate the contribution of hippocampal neuropathology to HIV-associated episodic memory deficits. (Moore et al 2006) and demonstrate that ProM is impaired in conditions with prominent involvement of the medial temporal lobe, such as Alzheimer's disease (Duchek et al 2006). Prior studies of ProM among individuals with HIV (Carey et al 2006) postulated that HIV associated neural injury disrupts the executive aspects of encoding and retrieval of future intentions.

White matter abnormalities have been described in HIV, but structural MRI may lack the sensitivity to detect pathological changes (i.e., normal appearing white matter). A more sensitive approach is diffusion tensor imaging (DTI). DTI measures the random thermal motion of water in brain tissue (Tuch et al 2003). Water diffusion along directional pathways (e.g., white matter) occurs preferentially along the axis of the pathway (anisotropic diffusion) compared to equally distributed movement (isotropic diffusion) in nondirectional regions (e.g., grey matter). Alterations in myelin (e.g., Multiple Sclerosis) or changes to the microstructure of axonal projections reduce fractional anisotropy (Beaulieu 2002) and therefore DTI metrics are useful biomarkers of white matter integrity. DTI in HIV has not been extensively researched to date, and the correlates between DTI and neuropsychiatry/neuropsychological

impairments is not known. Patients with HIV had abnormalities despite normal-appearing white matter on MR images and nonfocal neurologic examinations. Patients with the highest diffusion constant elevations and largest anisotropy decreases had the most advanced HIV disease. (Filippi 2001)

A Cross-University Brain-Behaviour Initiative has recently been established between the Universities in the Cape, has taken delivery of a 3T Siemens MRI, which is one of the few such machines in the developing world, and is focused on attempting to develop capacity in MRI research that is relevant to local problems such as HIV.

Conclusion

Cognitive impairment in HIV is a common and debilitating condition. It impairs both the quality of life as well the adherence of affected individuals.

Identifying salient risk factors for nonadherence and dependence in IALS is therefore of considerable public health importance, both in terms of facilitating early detection and informing targeted cognitive and behavioral interventions to maximize adherence.

OBJECTIVES:

1. To establish the prevalence of deficits in Prospective memory in a clinic population of HIV positive individuals attending ARV sites in the Western Cape
 - i. to perform a detailed assessment of the presence of neurocognitive disorders including assessment of prospective memory in patients using the neuropsychological test battery, an assessment of functional status using the HNRC Activities of Daily Living Scale and the Patients' Assessment of Own Functioning scale,.
2. To establish the influence of selected other factors on ProM deficits.

- i. to collect data from each patient with regard to age, gender, obstetric history, current adversity/life events, level of education (including pre-schooling), substance use, risk behaviour, medical history, childhood psychiatric history and socioeconomic status
 - ii. to obtain data on CD4 count, viral load, concurrent medical treatment, presence of neurological symptoms and signs
3. To determine neuro-radiological correlates of neurocognitive disorders in particular ProM
 - i. Obtain brain imaging data on subjects in order to delineate abnormalities of white matter, and correlates of change in cognitive function

METHODS:

Patients will be recruited from an existing study investigating neurocognitive deficits in patients infected with HIV. The principal investigator on this project is Dr John Joska

1. Subjects and sampling

- a. Patients will be recruited from Infectious Diseases clinics Khayelitsha Site C, Woodstock and Mitchells Plain Community Health Centres. Provincial approval has also been obtained from the Groote Schuur Hospital and GF Jooste Hospital ID clinics.
- b. It is intended to obtain a moderately representative sample of young adults attending public sector services in the Western Cape over the study period. Once the subject has been drawn by the study coordinator, he or she will be invited to participate in the study; should they decline, another subject will be drawn from the list. Eligible subjects are those who have attended the ARV clinic at least once, and are being counseled for commencement of ARV's, and who meet inclusion criteria (as ascertained by a brief chart review and brief screening interview).

- c. Subjects need to be able to provide full informed consent to participate in the study; they will sign full informed consent once it is confirmed that they are eligible and have agreed to participate. An arrangement for the study visits will then be made by the study coordinator.
- d. It is intended to include 45 patients in the study.

2. Controls

- a. This study will use 10 cases as controls-Subjects tested as being HIV negative who attended the same ARV clinic as the subjects

3. Inclusion criteria

- a. Young adults between the ages of 18 and 35 years will be included. In an older study population, age-consequent disease, such as neurodegenerative or cerebrovascular disease may have developed.
- b. Positive diagnosis of HIV infection made within the last six months (includes initial and confirmatory tests).
- c. Have not previously used anti-retroviral medications (“HAART naïve”) AND who being enrolled into anti-retroviral treatment.
- d. Patients who attend an out-patient clinic.
- e. Patients able to read and write to a grade 7 (std 5 level)

4. Exclusion criteria

- a. Schizophrenia or bipolar disorder.
- b. Presence of uncontrolled medical condition, such as poorly controlled diabetes mellitus, epilepsy, active tuberculosis requiring admission or non-standard treatment (e.g. regimen 2).
- c. Presence of an identified central nervous system neurological condition, such as lymphoma, or untreated neuro-syphilis or cryptococcal infection. Patients who have had such conditions and who are deemed to have been fully treated are eligible for inclusion.
- d. Patients who have abused alcohol or other psycho-active substances within the preceding six months.
- e. History of a head injury with a duration of loss of consciousness of >30 minutes, AND/OR requiring overnight admission to hospital.

- f. Contra-indications to MRI- such as pregnancy, metal or claustrophobia.
- g. Patients who refuse to sign informed consent.

5. Study design

This is a two-group cross-sectional study of ProM and neuroimaging associated with clade C HIV. Laboratory indices of HIV disease burden, neuroimaging and cognitive function will be collected from 45 seropositive individuals infected with clade C HIV and 10 healthy seronegative controls matched for age, education, and sex. Neurocognitive function will be measured with tests sensitive to cognitive impairment

6. Instruments and measures

The following measures have been selected. They include a range of both categorical (for example- the Miniature International Neuropsychiatric Interview Plus version (MINI+)) and continuous (International HIV/AIDS Dementia Rating Scale (IHDRS)) measures. The selected instruments meet criteria of validity, reliability and feasibility. See appendix 1 for summary of scales and instruments. Note: Neuropsychological test battery- This battery is based on a battery used in a previous local study and on a battery used by an international research group in HIV neuropsychology, together with input from local neuropsychologists. It includes tests of intelligence, attention, executive functions, and speed of processing and psychomotor function. These are areas mostly affected by HIV. A summary sheet is attached for information (appendix 2).

Prospective memory assessment

ProM will be measured using a subjective self report measure as well as objective measures. The objective measure will consist of both a time and an event based task. The event based task will be a measure previously used by Huppert et al., 2000 (High Prevalence of Prospective Memory Impairment in the Elderly and in Early-stage Dementia: Findings from a Population-based Study, APPLIED COGNITIVE PSYCHOLOGY, 14: S63±S81). The time based task will be one of the items from the MIST scale.

Objective event based prospective

Prospective memory instructions were 'Later on, I am going to give you a name and address to write on the envelope. When you have finished doing this, I would like you to do the following: turn it over, seal it and write your initials on the back. Could you remember to do this then without me reminding you'. This was followed by a delay interval of around 10 minutes which was filled with other cognitive tasks. Then the envelope was shown and the interviewer said 'Please write the following name and address on this envelope. The interviewer dictated the name and address slowly once, pausing at the end of the name, the house number, the street and the city, to keep pace with the respondent's writing speed, then said 'Please go on remembering this name and address and I will ask you about it later'. The interviewer then observed whether the respondent carried out the prospective memory task. If the respondent did not do so within about 5 ± 10 seconds (the longer times were for slower respondents), the interviewer said 'Were you going to do something else with the envelope' If only one correct action was carried out (i.e. just the envelope sealed or just initial written on the back), the interviewer said 'Was there something else you were going to do' If the respondent wrote their name rather than their initials on the back of the envelope, this was scored as a correct response.

Time based prospective memory

Taken from the Memory for Intentions Screening Test (MIST; [Raskin, 2004](#)), which is a standardized laboratory measure of ProM with published evidence of its reliability and construct validity ([Carey et al., 2006](#)).

'In 2 minutes ask me when this session ends today

Self-reported ProM is assessed using the ProM Scale from the Prospective and Retrospective Memory Questionnaire (PRMQ; [Smith, Della Sala, Logie, & Maylor, 2000](#)), which consists of eight ProM-specific items that are rated on a five-point Likert-type scale that ranges from 1 ("never") to 5 ("very often"). The

PRMQ ProM complaints are separated into four self-cued (e.g., “*How often do you forget appointments if you are not prompted by someone else or by a reminder, such as a diary or a calendar?*”) and four environmentally cued (e.g., “*How often do you forget to buy something you planned to buy, like a birthday card, even when you see the shop?*”) Total scores on the PRMQ ProM Scale can range from 8 to 40. These instruments will be forwards and back-translated into Afrikaans and isiXhosa, in order to be completed in the patient’s home language.

In addition to these instruments, a detailed collection of socio-demographic data and personal history will be taken.

Determination of dementia

Each HIV individual will have his or her demographics, medical history, neurologic symptoms, functional status, neuropsychological test scores, and neurologic examination assessed by the primary examiners. Using all this information, a Memorial Sloan-Kettering (MSK) severity score of 0, 0.5, or 1.0 will be assigned to each HIV individual.

An MSK severity score of 0 represents no cognitive impairment, 0.5 represents mild cognitive impairment not sufficient to meet criteria for dementia (subclinical), and a score of 1.0 represents dementia. An HIV positive subject is determined to be demented and given an MSK severity score of 1.0 if he or she has (scores less than 10 on the IHDS, and confirmed on neuropsychological testing, wherein two or more neuropsychological tests on which he or she scores 2 SD below the locally determined mean for their normative age and education group, and has symptomatic and/or functional complaints consistent with dementia or physical findings on neurologic exam consistent with HIV dementia such as extrapyramidal signs.. An HIV positive subject who scores 10-11 on the IHDS with abnormalities on only one or two neuropsychological tests in which they score from 1.0 to 2 SD below the locally determined mean for their normative age and education group, or an HIV positive subject with greater neuropsychological impairment but with no symptomatic or functional complaints and a normal neurologic examination will be given an MSK severity score of 0.5 (Wong et al 2007)

Brain Imaging Protocol: Cognitive decline may present early in the course of HIV disease and be asymptomatic for some time. It is accompanied by changes in brain structure, function, blood-brain barrier integrity, and brain metabolites. In fact some of these changes are evident before clinical symptoms and cognitive deficits manifest themselves and are different prospectively from HIV-negative controls. Structural MRI will be performed to exclude intracerebral pathology.

Imaging sequence is listed below. The total proposed imaging protocol duration is approximately 60 minutes.

- *3D T1 – MPRAGE* – High resolution 3D-T1 image to be used as a basis for examining morphometric change in grey and white matter regional volumes prospectively. Imaging parameters include; 3D sagittal plane acquisition, TR 2300ms, TE 3.93ms, FOV 220mm and voxel size of 1mmx0.9x1mm. Sequence duration 6.53 min. This sequence will be repeated after gadolinium contrast enhancement adding another 6.53mins to the protocol.
- *3D T2 and FLARE* sequences are most suited to examination of white matter signal changes from a variety of causes. TR 3500, TE 354, with an FOV of 256mm and slice thickness of 1mm and duration 9.25min. The heavily T2-weighted FLARE with TR 9000ms, TE 96ms, FOV 220mm and slice thickness of 5mm. Sequence duration 2.26min.
- *A multi-direction diffusion weighted imaging (MDDW)* sequence will be acquired to more specifically quantify the specific apparent diffusion coefficient (ADC) and fractional anisotropy (FA) of white matter in frontal, sub-cortical and callosal white matter fibres. This acquisition will also enable us to compute diffusion tensor from which directionality and density of white matter tracts can be defined in each group. Correlations with ProM deficits will be calculated. TR 5000ms, TE 88ms, B= 0, 1000 and 30 diffusion directions for a sequence of

2.40mins in duration. Average of four repeats of this sequence will be used in analysis.

7. Study procedure

- a. Clinical staff at the Khayelitsha site C, Woodstock and Mitchells Plain primary care ARV clinics will be informed about the study. Other sites may be added, with permission from the Provincial Health Department.
- b. Patients attending these services will be screened by attending clinical staff for inclusion and exclusion criteria. The investigator will be notified of a potential subject.
- c. Potential subjects will then be properly screened, and if suitable, will sign informed consent to enter the study. Subjects between the ages of 18 and 21 will require the consent of a parent or legal guardian.
- d. At screening, patients will be asked about:
 - their medical (including head injuries) and HIV treatment history
 - their alcohol and substance abuse history (the AUDIT and the SAMISS will be used to aid in this enquiry. Significant overlap in these instruments)
 - their psychiatric history

Following completion of informed consent- *at the same clinic contact-* subjects will complete the following self-report questionnaires:

- Beck Depression Inventory
 - Life events questionnaire of Bruga
 - Childhood Trauma Questionnaire
- e. Subjects will then be referred to the Groote Schuur Hospital psychiatry out-patients department or the GF Jooste Hospital out-patients department (whichever is closest). The assessment will include of: MINI, MADRS, HDS, HADS, CTQ, LEQ, and MSSS. A second visit (#2) will be scheduled within 2 weeks to complete: neurocognitive profile and prospective memory assessment, service utilization, CAN, SDS and risk behaviour questionnaire. Data regarding medical and neurological problems will be retrieved from the patient notes. A neuromedical assessment will be performed according to a standardized brief scale. *Patients will receive R30 per visit to*

compensate for transport costs, plus an additional R26 rand to pay for out-patient fees.

- f. Brain imaging will be performed at the Cape Universities Brain Imaging Centre located at the Tygerberg Campus of Stellenbosch University. The imaging session will take approximately 1 hour to complete.
- g. All subjects will follow up with routine clinic services. Any who are diagnosed with psychiatric conditions will be treated by the study team, in the first instance, or by local mental health services, if the patient is unable to attend the study site for this care.
- h. Data on viral load\CD4 count and use of ARV's will be obtained from the files at the hospital treating the subject.

8. Data management and analysis

Data will be captured directly into a study generated Case Report Form (CRF). This will include forms to capture demographic, clinical and serological data, as well as instruments. These data will then be entered into a Microsoft Excel Spreadsheet and cleaned. Once the data is clean, it will be analysed using SPSS statistical software package. Prevalence's of disorders, symptom severity and impairment will be described using frequencies and percentages. Univariate analysis will be used to compare the frequencies of cognitive disorders in HIV positive subjects and controls, as well the frequencies of other neuropsychiatric disorders and possible contributory factors. A number of regression models will be fitted, whereby the presence of HIV-associated ProM disorder will be entered as the dependent variable, and a number of socio-demographic, clinical, and biochemical factors will be included as independent variables- these will include age, gender, level of education, history of head trauma, alcohol abuse, presence of neurological impairment, CD4 count and history of intra-cranial pathology in preceding 12 months. A biostatistician will be consulted for this.

Regarding sample size and power analysis, it is assumed that significance tests will be performed at the 0.05 levels, and power to 80%. The primary outcome is the prevalence of ProM disorder in the HIV positive population compared to controls.

Pomara in 2001 DTI was performed in six HIV-1 patients and nine controls. The two groups were similar in age. Abnormal fractional anisotropy was found in the white matter of the frontal lobes and internal capsules of the HIV-1 patients (Pomara 2001).

Time frame

Study commencement: June 2009

(Recruitment rate 4 per week= 64 subjects in 4 months subjects in one year)

Field work complete: October 2009

Data analysis: October 09-Nov09

Study write-up: December 09-Jan 2010

9. Ethical considerations

This study will adhere to the principles laid down in the Helsinki Declaration (2002). Accordingly, this research is intended to “improve prophylactic, diagnostic and therapeutic procedures and the understanding of the etiology and pathogenesis of disease”, as it pertains to cognitive disorders in HIV/AIDS. The study has been approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. REC REF: 263/2007

a. Potential benefits and harms- patients included in this study will receive a detailed assessment of psychiatric, cognitive and other biopsychosocial parameters. Individuals with identified conditions will be referred to appropriate mental health services for care and treatment. Some patients may become distressed about re-discussing issues or may feel these were inadequately dealt with. These problems will be dealt with in a sensitive and professional manner within the study team. Other potential benefits include a greater awareness of mental health among community teams where the study will be conducted, and the reporting of results to both local role players and in the scientific community.

b. Equity and justice- The access to specialised mental health services, and particularly tertiary services, is limited within the public sector. It is hoped that this study will afford eligible patients the opportunity to undergo a comprehensive evaluation, while raising the profile of the problems that these patients have for all sufferers. In this way, it may be possible to offer a more comprehensive service once the need has been demonstrated.

c. Informed consent- All patients will be provided with a patient information sheet *in their first language* which describes the scope and aims of this study (Xhosa and Afrikaans versions will be supplied to REC later, but prior to the study commencing). Patients who decide not to partake in this study will be reassured that their decision will not bias them or be held against them at any time in the future. Clinicians at other points of service will not be made aware of patients not wanting to sign consent, or who do not meet inclusion criteria. Patients who have been fully informed about the study will then be invited to sign consent, which will be valid for the duration of the current protocol. Any amendments to this protocol, insofar as they affect patient care will be re-negotiated with them, and a new consent form signed. Patients will sign two copies of the consent form and will keep one copy for themselves. Patients who, in the opinion of the investigator, are deemed to be too unwell to sign informed consent, will be asked to sign, together with a member of his/her immediate family or next of kin.

d. Confidentiality- Patients numbers will be used in order to manage patient records and special investigations. All information pertaining to named patients will be kept within the rules and norms of confidentiality at both the GF Jooste and Groote Schuur Hospitals and the service in general. Where data is reported on outside of this context, no reference will be made to the patient's name or personal information. Only the study team will be aware of the full identity and clinical data of patients included in the study. Completed forms and case report material will be stored in a locked cabinet in a locked office. Where disclosure is necessary, such as where treatment or referrals are needed, patient's consent will be sought. Any written or verbal reports or

presentations of this information will retain the patient's anonymity. Third party information will also be held confidentially. As such, patients will not have direct access to their clinical records, but may, in consultation with the study team, have access to information regarding them.

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World Health Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. October 2000 with amendments 2002

Appendix 1: Schedule of measures

Completed at screening (at primary care clinic)

Informed consent for Interview and MRI	ICF:IVMRI	<input type="checkbox"/>
		<input type="checkbox"/>
Beck Depression Inventory	BDI	<input type="checkbox"/>
The Alcohol Use Disorders Inventory	AUDIT	<input type="checkbox"/>
Substance Abuse and Mental Illness Screener	SAMISS	<input type="checkbox"/>
Life Events Questionnaire of Brugha	LEQ	<input type="checkbox"/>
The Childhood Trauma Questionnaire	CTQ	<input type="checkbox"/>
Patient's Assessment of Own Functioning	PAOF	<input type="checkbox"/>
Activities of Daily Living	ADL	<input type="checkbox"/>

Completed at Visit #1: Interview and Neuromedical Assessment

Sociodemographic questionnaire		<input type="checkbox"/>
Neuropsychological test Battery		<input type="checkbox"/>

Prospective and Retrospective memory	PRMQ and MIST	
Break		

Miniature International Neuropsychiatric Interview plus	MINI+	<input type="checkbox"/>
International HIV Dementia Rating Scale	IHDRS	<input type="checkbox"/>
The Neuropsychiatric Inventory	NPI	<input type="checkbox"/>
The UCT/GSH Neuromedical Assessment Form	UNAF	<input type="checkbox"/>
Cape Town HIV Consortium risk behaviour scale	RBS	<input type="checkbox"/>
Karnoksky Performance Scale	KPS	<input type="checkbox"/>
Camberwell Assessment of Need	CAN	<input type="checkbox"/>
Sheehan Disability Scale	SDS	<input type="checkbox"/>

Structural Diffusion Weighted Sequence

Appendix 2: Summary score sheet

Sticker:

CD4

J

HIV-associated neurocognitive disorders study: Neuropsychological Test Summary Sheet

Name

Gender: M F

Date of examination (DD.MM.YYYY)

Years of education:

Date of birth (DD.MM.YYYY)

Age:

Examiner: JL JX JJ

Handedness:

Examined in: English

Xhosa

A: Motor function

A1: Successive Finger Taps	Raw Score	Mean	SD
D(sec):		()	()
ND(sec):		()	()
A2: Grooved pegboard			
D(sec):		()	()
ND(sec):		()	()

B: Learning and memory

B1: Hopkins Verbal Learning Test

	Raw Score	Mean	SD
Trial 1			
Trial 2			
Trial 3			
Total			
Learning			
DR	(%)		
Recognition: true positives		Discrimination index	
false positive errors:	related ()	unrelated ()	

B2: Rey Complex Figure raw score mean SD

Copy

3 min recall

delayed recall

B3: Brief Visuospatial Memory Test

Form	1	2	3	4	5	6	Raw Score	T-score	Percentile
Trial 1									
Trial 2									
Trial 3									
Total recall									
Learning									
Delayed recall									
Percent retained									
Recognition hits									
Recognition false alarms									
Recognition Discriminatin Index									
Recognition response bias									

B: Learning and memory

B4: Prospective Memory Test

C: Attention

C1: Mental alternation test

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Raw Score	Mean											
ii.alphabet										raw score					s errors					score												
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	mean		s										
										11 12 13 14 15 16					17 18 19 20					21 22 23												
1	2	3	4	5	6	7	8	9	10	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W

C2: WMS III Mental Control

raw score

SS

C3: WMS III- spatial span

Raw Scores (SS)

Forward:	())	Total	()
Backward	())		

D: Speed of processing Raw Score (Scaled Score)

D1: WAIS III: Digit symbol coding ()

D2: WAIS III: Symbol search ()

E: Executive function

E1: Trail-making RS	Percentile	T-score	SD
Test A			

E2: Color Trail-Making

i.part 1

ii.part 2

E3: Stroop Colour and Word Test

expected

residual

T (table IV)

Word

Colour

colour word

Interference

E4: Wisconsin Card Sorting Test

Categories completed

Trials to first category

Failure to maintain set

Learning to learn

F: Language

G1: Category fluency test

Raw Score

Mean

SD

i. animals

ii. Fruit and vegetables

G: Intelligence

WASI

Raw score

SS

Vocabulary test

Block design

Similarities

Matrix reasoning

Cognition and White matter damage in HIV: a Literature Review

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ABSTRACT

Infection with Human immunodeficiency virus (HIV) is associated with central nervous system (CNS) changes that may affect cerebral blood flow (CBF), metabolism, structure, and diffusion. Diffusion Tensor Imaging (DTI) is a neuroimaging technique offering unique insight into the neural mechanisms underlying HIV, as well as a potential means of monitoring disease progression and treatment response. The purpose of this review is to provide a review of experimental studies evaluating changes related to HIV with DTI

INTRODUCTION

Human immunodeficiency virus (HIV) is associated with a high prevalence of neuropsychiatric disorders, including depression, mania, anxiety and cognitive disorders⁽¹⁾. These problems contribute to the morbidity and mortality associated with HIV⁽¹⁾. While Highly active antiretroviral therapy (HAART) has been shown to be effective in reducing the incidence of severe HIV-associated neurocognitive disorders (HAND), evidence from functional Magnetic resonance imaging (fMRI) studies suggests that many will develop these disorders earlier than current treatment regimes would sanction initiation of treatment⁽²⁾. It is also not known which individuals in the earlier phases of the disease will go on to develop HAND, nor who will respond to treatment⁽³⁾. Preliminary evidence suggests that the neurotoxic effects of HIV results in damage to white matter tracts in the brain⁽⁴⁾. Once damage is established and HAND ensue, the

ability of HAART to reverse existing dysfunction is probably limited ⁽⁵⁾. Earlier treatment with HAART in at-risk or minimally symptomatic patients may prevent further decline in cognition and delay the course of HIV disease. Identification of reliable early imaging correlates of risk for subsequent cognitive dysfunction, such as early changes to white matter integrity, become all the more crucial to delineate.

Nearly two-thirds of patients infected with HIV go on to develop neurologic involvement, including cognitive deterioration ⁽¹⁾. Autopsy studies of HIV associated dementia (HAD) patients indicate prominent injury, including damage to the deep white matter⁽⁶⁾. Studies of acute HIV infection suggest that virus enters the brain during acute infection but it is not clear whether virus that enters the brain during acute infection persists there for life or whether this is cleared by the host and HIV encephalopathy (HIVE) develops as a result of later introduction of new virus into the brain. It is crucial to understand the events that lead to establishment of virus in the brain and the eventual development of HIVE⁽⁶⁾.

On both Computed Tomography (CT) and structural Magnetic Resonance Imaging (sMRI), diffuse atrophy with ventricular dilatation ⁽⁷⁾ and white matter lesions have been noted to be associated with HAD. A correlation between declining cognitive function and the loss of volume in certain brain structures, including the basal ganglia and caudate nucleus, has also been reported ⁽⁸⁾.

Dynamic contrast-enhanced MRI has identified sub-cortical grey and frontal white matter as the principal sites of early metabolic abnormalities ⁽⁹⁾. Both increased regional cerebral blood volume (rCBV) and post-contrast enhancement have been reported in the basal ganglia in moderate and advanced HAD, reflecting increased vascularity and blood-brain-barrier (BBB) permeability. These findings are consistent with the characteristics of the early neurological deficits, and the known predilection of HIV for the basal ganglia ⁽¹⁰⁾. The degree of neurocognitive impairment is correlated both with the degree of BBB breakdown in the basal ganglia and with viral load ⁽¹¹⁾.

Both post mortem studies and a number of imaging studies in different modalities have established that HIV causes damage to the CNS, particularly to the subcortical regions and the frontal white matter. In HIV infection, white matter changes are unreliably detected by sMRI. Diffusion tensor imaging (DTI), an MRI method, is uniquely suited to the study of subtle white matter abnormalities. DTI studies have revealed CNS abnormalities in asymptomatic HIV positive patients with no cognitive impairment and normal structural MRI studies. DTI can be used to quantify the magnitude and directionality of tissue water mobility (i.e. self-diffusion). Barriers, such as myelin sheaths, membranes, or white matter tracts, result in greater self-diffusion along the axis of the barrier and reduced diffusion out of the tract. This type of restricted self-diffusion is termed 'anisotropic.' Fractional anisotropy (FA) is a measure derived from the diffusion tensor imaging that assesses the degree of anisotropic self-diffusion, that is, the integrity of the white matter tract⁽¹²⁾. The higher the FA (closer to 1) the healthier the tract, the lower FA (closer to 0) indicates damage to the integrity of the white matter tract. A second measure commonly reported in DTI is mean diffusion (MD), which measures the diffusion of water out of a tract. The higher the MD (closer to 1), the greater the damage to the tract. FA indices from DTI measurements reflect the amount of coherently restricted diffusion (imposed by the presence of myelin) of free water. These coherently restricted diffusion pathways are most prominent in axonal bundles. DTI voxels are several orders of magnitude larger than cellular dimensions, so that the computed anisotropy indices reflect the cumulative effect of the underlying microstructure. While there is still controversy regarding the source of the anisotropy such as the contribution of the intracellular versus extracellular water to the diffusion signal⁽¹³⁾, one can argue from a physical point of view that a reduced anisotropy can be the result of one of the following phenomenon: loss of myelin leading to reduced restricted diffusion, intact fibers but not coherently oriented and loss of fibers. Diffusion anisotropy thus carries microscopic (cellular level) anatomical information. However, the microscopic information is averaged over the large voxel volume. If there are multiple fiber populations with different fiber orientations, their contributions to the signal could be averaged. As a matter of fact, the cortex has low anisotropy ($FA < 0.2$), not because there are no fibers, but because axon and dendrite orientations are not normally aligned

within the large voxels in human cortex. If we can improve image resolution, we are likely to see higher anisotropy in the cortex. We observe diffusion anisotropy only when there are microscopic sources of diffusion anisotropy AND there is macroscopic homogeneity of the structures within a pixel. If we find changes in diffusion anisotropy, we cannot immediately conclude that the source of abnormalities lies in cellular level structures, such as myelin and axons; it could be due to the reorganization of axons at macroscopic levels. In summary DTI could provide us with information about the large-scale networks that are made up of long tracts connecting distant relay stations in the brain. These networks are important for the development of higher brain functions such as language, praxis, social behavior and emotion. Lesions affecting white matter connections lead to dysfunction, and cognitive disorders are sometimes better explained by a disconnection mechanism between distant cerebral regions than by primary damage of those regions themselves⁽¹⁴⁾.

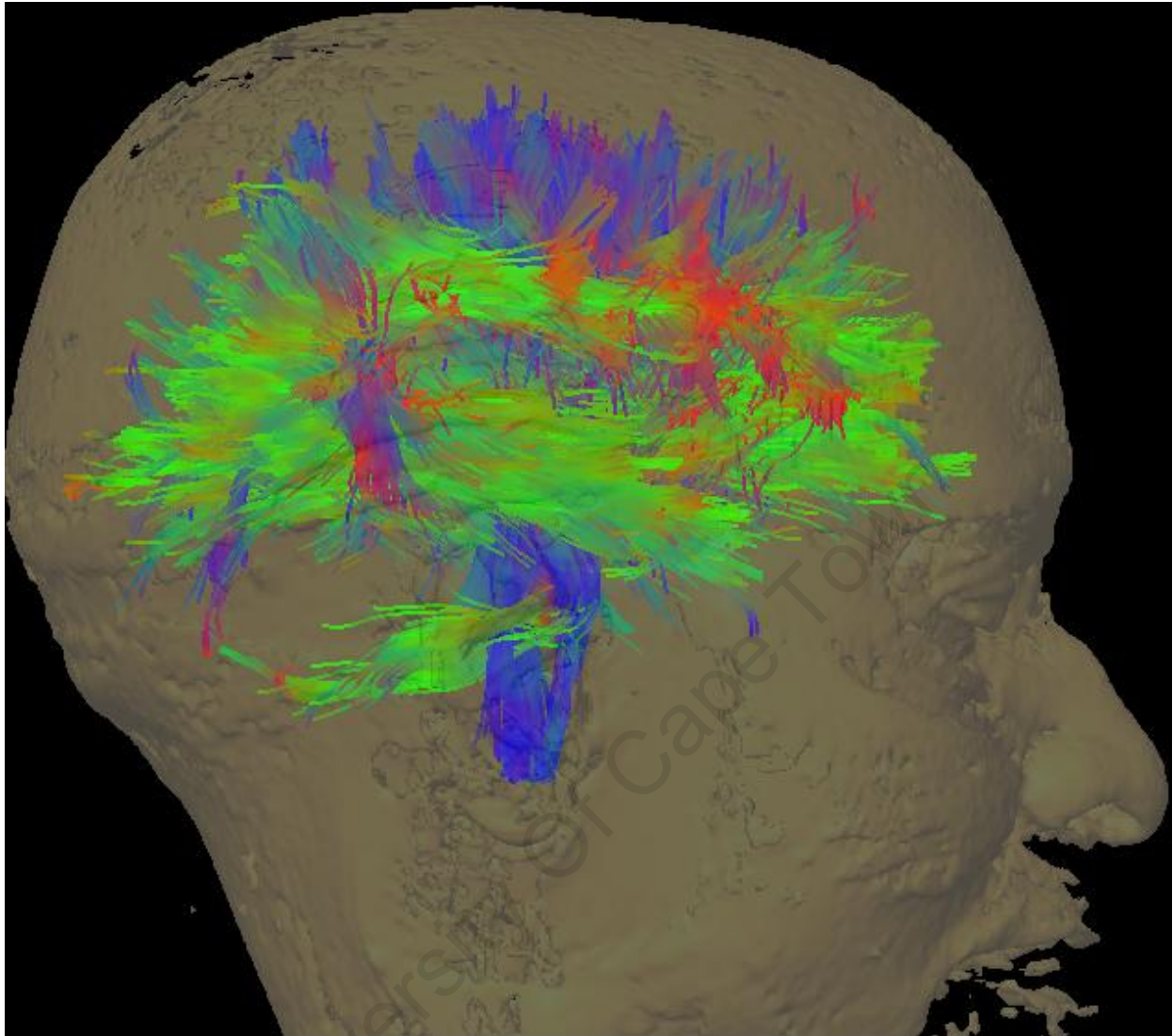


Figure 1

An example of DTI tractography. Sagittal view. Different colors indicate different directions of the white matter tracts. Green shows tract moving anterior-posterior, blue shows superior-inferior and red shows left to right. Tractography image was created on our Brainlab computer at CUBIC

DTI has been used to identify age-related brain changes⁽¹⁵⁾ and white matter alterations in a number of neurodegenerative disorders including Alzheimer's disease⁽¹⁶⁾. Although loss of white matter is prominent in later stages of the neurodegenerative process, preliminary DTI studies in Alzheimer's disease found fractional anisotropy reduction in

vulnerable white matter regions even at preclinical stages. For example DTI of the corpus colosum and medial temporal lobe revealed that an increased genetic risk for developing Alzheimer disease (APOE epsilon4 carriers) is associated with reduced fractional anisotropy well before the onset of dementia ⁽¹⁷⁾.

In this review, I will describe the known imaging and neuropathological correlates of HIV disease. Then novel MRI techniques such as diffusion tensor imaging will be discussed, with a view to elucidating how these techniques can be correlated with clinical and neuropsychological findings. Specific brain regions previously implicated in DTI studies will be explored, in order to develop hypotheses regarding which regions are likely to be affected in late stage HIV.

METHODS

Search strategy

The search for studies was conducted using two 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 (DTI, Diffusion Tensor Imaging [mh]) AND (Cognition* Disorders [mh] OR memory [mh] OR memory* [tw] OR cognition [mh] OR cognition* [tw] OR AIDS Dementia Complex [mh] OR Neuropsychological Tests [mh]). We applied the following limits: Humans, Clinical Trial, Letter, and All Adult: 19+ years.
- ii) PsycINFO: HIV and DTI and Cognition

2) Reviewing the reference sections of articles found in this way and searching for relevant publications.

Inclusion and exclusion criteria

We included peer-reviewed published studies in which a clinical sample received a neuropsychological and/or neuromedical assessment in addition to DTI. A clear categorisation of EITHER a neurocognitive disorder OR of global/overall neuropsychological status in patients OR Centre for Disease Control (CDC) staging needed to be reported. Included studies were written in English and had experimental designs with adult human subjects with HIV.

We excluded studies of children, or studies in which patients had acute medical illness (e.g. Tuberculosis), current HIV related neurologic disease (e.g. Tuberculosis meningitis), current DSM-IV Axis I diagnoses (depression) and current alcohol or substance abuse.

Study sorting

All articles retrieved on electronic search were loaded into a single Reference Manager™ database. Duplicates were removed. Using the criteria set out above, the database was reviewed to ascertain reliability of inclusion and exclusion. After this stage, 14 studies were identified and included.

RESULTS

Nature of studies

The majority of studies identified were conducted in the USA, where clade B is predominant (n=13), with one being completed in Austria. 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 ranged from 6 to 60. Most studies were cross-sectional, with only one study following up patients after one year. Most studies reported the range of CD4 cell count

and these ranged from a significant degree of immune suppression to CD4 counts of 600+. A summary of the studies is presented in table 1

Measures

The clinical assessment of neurocognitive disorders and white matter damage in HIV requires the exclusion of confounding causes. Most studies used psychiatric history or made use of rating scales to exclude participants who suffered from psychiatric disorders. Of those that used rating scales, one used the Centers for Epidemiology rating scale for Depression (CES-D), and one each used the Hamilton Depression Rating Scale. Patients with current psychiatric disorders are generally not included in studies of HAND. Many did not formally report on the screening of substance use disorders. Of those that did, used a combination of self-report and clinician-interview, with only one using formal drug testing procedures. Most utilized some type of standardized clinical or neurologic examination. Most did not report on of functional assessment.

DTI measures

FA and apparent diffusion coefficient or MD (ADC, equivalent to MD) were utilized in most of the studies to explore neurological dysfunction resulting from HIV infection (n=10). One used FA only, with another using MD alone. DTI analysis using Statistical Parametric Mapping (SPM) was utilized in one recent study, while two have used fiber tracking and only one with axial and radial diffusion.

White matter damage

Diffuse damage to cerebral white matter is one of the most frequent neuropathological features of HIV-1 infection and was reported to be particularly prominent in the advanced stages of the disease. DTI abnormalities were most commonly reported in the frontal white matter (FWM), the corpus collosum, the internal capsule and the superior longitudinal fasciculus.

Neuropsychological test batteries

In the studies included in this review, the neuropsychological test batteries varied widely. The prevalence of neurocognitive disorder was noted in 11 studies, with two utilizing the Memorial Sloane Kettering (MSK) score ⁽¹⁾. Three utilized the American Academy of Neurology criteria (AAN) ⁽¹⁸⁾. Three used a global deficit score and one a NPZ-8 score. The MMSE was used to establish the presence or absence of dementia in 3 studies. The IHDS was utilized in one study

Use of HAART

A wide range of HAART regimens were reported. One did not mention whether the patients were receiving HAART. In five of the studies all of the patients were on HAART. A number of the studies did not mention which regimens the patients were receiving. One study assessed the effect of the CNS penetration of the HAART regimens on white matter integrity

Quality of studies

Most were high quality studies. One particular study was published as a brief report within a larger review of neuroimaging in HIV. What is striking is that most studies do not report on, or address all of those factors which might be considered to comprise a detailed and high quality study of the effects of HIV on white matter. For example, many did not report on any assessment of substance misuse and only one used urine tests to exclude substance use. One of the studies included participants with a previous history of substance abuse. Axis I diagnosis were often excluded on history only. In addition most of the studies did not formally report on functional assessment, which should be part of an assessment of neurocognitive dysfunction in HIV. One study included 11 participants who tested antibody +ve for Hep C. It may be suggested that more formal reporting of this issues is needed to fully appreciate the role of HIV in causing loss of white matter integrity.

Table 1: Summary of Literature review

Subjects	Major study findings	Investigators	Title
10 HIV+ (viral load <400 to >400 K) All on HAART	As viral load increased, anisotropy in the splenium and Genu decreased. Patients with greater viral loads had elevated diffusion constants in subcortical white matter.	Filippi et al., 2001 ⁽¹⁹⁾ USA	Diffusion tensor imaging of patients with HIV and normal-appearing white matter on MR images of the brain
6 HIV+, 9 controls. CD4 mean=289 One patient not on HAART	FA was decreased in the frontal lobes and increased in the internal capsule among HIV+ patients compared to controls. No group differences were found in mean diffusivity	Polari et al., 2001 ⁽²⁰⁾ USA	White matter abnormalities in HIV-1 infection: a diffusion tensor imaging study
6 HIV+, 8 controls CD4 range 10–187 All on HAART	Whole brain FA was decreased in HIV+ group, and significantly related to severity of dementia. Diffusion coefficient measures did not differ among groups. FA was decreased in all patient groups, indicating diffuse white matter disease. White matter abnormalities in asymptomatic patients may be detected before clinical findings.	Ragin et al., 2004 ⁽²¹⁾ USA	Whole brain diffusion tensor imaging in HIV-associated cognitive impairment
11 HIV seropositive	The apparent diffusion coefficient (ADC) was	Cloak et al., 2004 ⁽²²⁾	Increased frontal white matter diffusion is

<p>patients were compared to 14 seronegative subjects Mean CD4 191</p> <p>6 patients on HAART</p>	<p>significantly increased in the HIV patients, primarily in the frontal white matter</p>	<p>USA</p>	<p>associated with glial metabolites and psychomotor slowing in HIV</p>
<p>25 HIV+ (normal cognitive, MCMD, HAD)</p>	<p>FA was decreased in all patient groups, indicating diffuse white matter disease. White matter abnormalities in asymptomatic patients may be detected before clinical findings.</p>	<p>Hall et al., 2004⁽⁸⁾</p> <p>USA</p>	<p>Neuroimaging in HIV infection</p>
<p>11HIV+ and 11 controls</p> <p>CD4 24-427</p> <p>All on HAART</p>	<p>Lower FA values correlated with specific cognitive deficits in the putamen, caudate and centrum semiovale</p>	<p>Ragin et al., 2005⁽²³⁾</p> <p>USA</p>	<p>Diffusion tensor imaging of subcortical brain injury in patients infected with human immunodeficiency virus.</p>
<p>60 HIV + patients and 30 controls</p> <p>Compared CD4<250 to CD4>250</p> <p>HAART not mentioned</p>	<p>reduction in FA in the frontal white matter, corpus callosum and hippocampus of HIV positive individuals compared to controls</p> <p>No difference between the high and low CD4 count groups</p>	<p>Thurnher et al 2005⁽²⁴⁾</p> <p>Austria</p>	<p>Diffusion-Tensor Imaging of the brain in Human Immunodeficiency virus-positive patients.</p>
<p>11 HIV and 11 control participants</p> <p>CD4 ranged from 24 to 427</p>	<p>FA values for the splenium were significantly reduced in the patients infected with HIV and correlated with dementia severity and deficits in motor speed</p>	<p>Wu et al., 2006⁽²⁵⁾</p> <p>USA</p>	<p>Diffusion Alterations in Corpus Callosum of Patients with HIV</p>

On HAART for 5yrs			
30 HIV + and 30 seronegative controls Mean CD4 612 13 on HAART	MD increases in normal appearing white matter in the +ve group	Stebbins et al., 2007 ⁽²⁶⁾ USA	HIV-associated alterations in normal-appearing white matter: a voxel-wise diffusion tensor imaging study
39 HIV + and 32 seronegative controls Mean CD4 -461 Some on HAART	After one year +ve group showed greater increase in MD in frontal and parietal white matter and Genu than seronegative controls	Chang et al 2008 ⁽²⁷⁾ USA	Greater than age-related changes in brain diffusion of HIV patients after 1 year
21 HIV-1 infected and 19 healthy control men and women	Poorer fiber integrity of the corpus callosum in HIV-1 than controls that was more pronounced in posterior than anterior regions. component processes of Visuospatial perception are compromised in HIV-1 infection attributable, at least in part, to degraded callosal microstructural integrity	Muller-Oehring et al 2009 ⁽²⁸⁾ USA	Callosal Degradation in HIV-1 Infection Predicts Hierarchical Perception: A DTI study
39 HIV-infected individuals (49% with acquired immunodeficiency syndrome [AIDS]; mean CD4 = 529) and 25	Cognitive impairment in the HIV-infected group was related to white matter injury in the internal capsule, corpus callosum, and superior longitudinal fasciculus.	Gongvatana et al 2009 ⁽²⁹⁾ USA	White matter tract injury and cognitive impairment in human immunodeficiency virus-infected individuals

seronegative subjects. On HAART			
29 men and 13 women with HIV infection and 88 healthy, age-matched controls Some on HAART Patients without dementia	Unmedicated patients had notably high transverse diffusivity, indicative of myelin compromise, in the occipital forceps, inferior cingulate bundle, and superior longitudinal fasciculus.	Pfefferbaum et al 2009 ⁽³⁰⁾ USA	Frontostriatal fiber bundle compromise in HIV infection without dementia.
18 healthy controls, 21 HND and 8 HAD patients. 18 on HAART	HAD patients exhibited significantly elevated MD and RD (radial diffusion) in the parietal white matter when compared to HND patients. RD was affected to a much greater extent than AD (axial diffusion) by HIV infection, which may suggest that demyelination is the prominent disease progression in white matter.	Chen et al 2009 ⁽³¹⁾ USA	White matter abnormalities revealed by diffusion tensor imaging in non-demented and demented HIV+ patients

DISCUSSION

Studies have used DTI to explore the integrity and orientation of brain tissue in vivo by measuring tissue water diffusion. These studies have measured fractional anisotropy (FA), considered to be an index of white matter integrity, by either using a region of interest analysis or by exploring whole brain differences in FA and MD with voxel-based analysis. More recent work has used tractography or radial and axial diffusion. DTI tractography can delineate the core of large white matter tracts and is expected to be a powerful tool to investigate white matter anatomy and disease in situ⁽³²⁾. FA and MD have been utilized to explore neurological dysfunction resulting from HIV infection. Studies included in this review have reported that white matter damage in the CNS in HIV correlates with severity of disease and cognitive impairment. These studies also suggest that damage to white matter tracts can occur early in the disease process in HIV and that some of this damage may be modified by HAART. In fact white matter abnormalities in asymptomatic patients may be detected before clinical findings.

A correlation between disease burden markers such as increased viral load and reduction of FA in the splenium and Genu of the corpus callosum has been found in HIV infected subjects⁽¹⁹⁾. FA has been found to be decreased in white matter of the frontal lobes, corpus callosum and the internal capsule in non-demented HIV+ patients when compared to age-matched controls^(20, 24). Based on their results Wu et al 2006 suggested the in vivo assessment of callosal integrity, using neuroimaging as a potential biomarker of brain injury in patients infected with HIV⁽²⁵⁾. Formulations of callosal function emphasize multiple routes for transfer of information between hemispheres. Injury may be reflected in slowed response initiation and longer reaction times on tasks involving hemispheric transfer or integration between regions.

A whole-brain DTI histogram analysis demonstrated that a reduction of the mean FA was significantly associated with the severity of dementia⁽²¹⁾. DTI measures in the sub-

cortical regions have been significantly correlated with a loss of function in specific cognitive domains^(21, 23). Increased frontal white matter ADC has been associated with increased glial metabolites in HIV-infected subjects when compared to seronegative subjects. ADC was correlated positively with the glial marker myoinositol, suggesting that the increased brain water diffusion may reflect increased glial activation or inflammation⁽²²⁾. However a study with regions of interest (ROIs) placed in the splenium and Genu of the corpus callosum, frontal white matter, and hippocampus in patients grouped with the viral loads and CD4 counts, reported significantly reduced FA and increased ADC only in Genu of HIV+ patients and no statistically significant correlation existed between FA (and ADC) values and CD4 counts⁽²⁴⁾. More recently, voxel-based DTI analysis using Statistical Parametric Mapping (SPM) has demonstrated significantly increased MD in the seropositive population when compared to seronegative subjects⁽²⁶⁾. A prospective DTI study showed that after one year the HIV+ve group showed a greater increase in MD in frontal and parietal white matter and Genu than seronegative controls⁽²⁷⁾.

Unmedicated patients were shown to have notably high transverse diffusivity, indicative of myelin compromise, in the occipital forceps, inferior cingulate bundle, and superior longitudinal fasciculus compared to those receiving HAART⁽³³⁾. However a recent study found that white matter injury was not found to be associated with estimated CNS penetration of antiretroviral⁽²⁹⁾.

While these reported findings clearly demonstrate the potential clinical utility of DTI in discerning white matter abnormalities resulting from HIV viral infection, most of the studies to date have only focused on comparing HIV+ patients with healthy controls in predominantly Clade B regions. To our knowledge there have been no investigations of how white matter abnormalities may be associated with a range of HAND in HIV+ populations or the effect of Clade C on white matter.

The development of a neuroimaging biomarker for early CNS disease in HIV positive patients, may allow for the earliest possible detection of cognitive impairment. The principal advantage of DTI is quantitative sensitivity to changes that may not be

detected with conventional T1-, T2-weighted magnetic resonance imaging (MRI). Postmortem studies have found that conventional MR imaging is insensitive to microscopic brain injury in HIV patients ⁽³⁴⁾

I hypothesize that:

(1) White matter damage as measured by alterations in FA and MD using DTI will be found in HIV positive subjects infected with Clade C HIV

(2) Both FA and MD will correlate with severity of HAND

(3) Poor Prospective memory will be correlated with decreased FA in HIV positive patients.

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White matter damage in Clade C HIV-positive subjects: A diffusion tensor imaging study

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ABSTRACT

The relationship between cognitive impairment and white matter integrity in human immunodeficiency virus (HIV) remains poorly understood, particularly in clade C. We utilized diffusion tensor imaging (DTI) and a comprehensive neuropsychological evaluation to investigate the relationship between cognitive impairment and white matter integrity in HIV-positive subjects with clade C HIV. 44 HIV-infected individuals and 10 seronegative subjects were compared using a voxel-based approach to define fractional anisotropy (FA) and mean diffusion (MD). Compared to healthy controls the HIV-infected group exhibited decreased FA in the corpus callosum, superior longitudinal fasciculus, cingulum and sagittal stratum. Within the HIV group, those with HIV associated Dementia (HAD) (n=14) had more widespread damage, including decreased FA in the inferior frontal occipital fasciculus. Poor performance in executive function in the HIV-infected group was associated with reduced white matter integrity in the sagittal stratum. Abnormalities in MD were seen in both early and late dementia. Compared with HIV negative controls, HIV patients with no cognitive impairment had white matter changes (decreased FA and increased MD). White matter changes precede cognitive changes, and although there are few associations between changes in FA/MD and neuropsychological impairment, white matter changes were more widespread in more severely cognitively impaired patients. This study provides evidence that white matter integrity is compromised in individuals infected with clade C HIV.

INTRODUCTION

HIV-associated neurocognitive disorders (HAND) remain important even in the era of highly active antiretroviral therapy (HAART). Although a decline has been noted in the incidence of HIV-associated dementia (HAD) in the developed world since the roll out of HAART, HIV associated neurocognitive disorders (HAND) remain prevalent (1).

Despite the relative efficacy of HAART in controlling HIV disease, there is no treatment which specifically targets the cause of HAD nor which prevents neuronal damage caused by the virus. Although first recognized two decades ago as a subacute, subcortical dementia, the clinical syndrome has evolved, in part due to the use of antiretroviral drugs(2). In South Africa the roll out of HAART has not reached levels seen in the developing world.

Early studies suggested that individuals infected with clade C may be less likely to develop cognitive impairments due to a biological defect in the tat protein present in clade C (3). However in recently published data from India, it was shown that individuals infected with clade C exhibit significant cognitive impairment, suggesting that the brain is, in fact, vulnerable among individuals with clade C HIV(4). These findings are notable because clade C is the most common form of HIV in the world, as well as the dominant strain in South Africa (5). In support of this, a study using the HIV Dementia scale (HDS) reported HAND in 23, 5% of patients attending HIV clinics in South Africa(6)

Although much work has been done to elucidate the complex mechanisms underlying HIV associated neurotoxicity, the relationship between cognitive impairment and white matter integrity in HIV remains incompletely understood. This is particularly the case for the genetic form of HIV known as clade C, since nearly all studies of cognitive function in HIV have been conducted in North America where the dominant genetic strain of the virus is clade B. Autopsy studies of HAD patients indicate prominent injury, including damage to the deep white matter (7) A range of neuroimaging modalities have been used to study the relationship between white matter damage and neuropsychological impairment, which has identified subcortical grey and frontal white

matter as the principal sites of early abnormalities (8). HAD is associated with diffuse atrophy with ventricular dilatation (9) and white matter lesions. A correlation between impaired cognitive function and the loss of volume in certain brain structures, including the basal ganglia and caudate nucleus, has also been reported (10). These findings are consistent with the characteristics of the early neurological deficits, and the known predilection of HIV for the basal ganglia (11).

There are limitations to these methods. In HIV infection white matter changes are unreliably detected by structural Magnetic Resonance Imaging (sMRI). Fortunately, diffusion tensor imaging (DTI), which quantifies the magnitude and directionality of tissue water mobility, is able to provide information about the micro structural integrity of the white matter. DTI provides two indices, fractional anisotropy (FA), which reflects the orientation specificity of water diffusion, and which is lower when white matter damage is present and mean diffusivity (MD), which reflects the degree of water diffusion within an imaging voxel, and which is higher when white matter damage is present (12). DTI studies in HIV have shown diffuse damage to cerebral white matter (i.e., decreased FA or increased MD) and these effects have been found to be particularly prominent in the advanced stages of the disease. Studies conducted in countries where clade B is predominant have reported subcortical white matter injury mainly in the regions of the corpus callosum, frontal lobes and internal capsule (13-15). Furthermore, diffusion abnormalities in the corpus callosum in HIV patients have been associated with dementia severity and motor speed losses (16).

The present study examined the relationship of DTI indices to global neurocognitive impairment among HAART naïve patients infected with clade C HIV. We hypothesized that (i) Clade C HIV infection would be associated with reduced white matter integrity in the regions of the corpus callosum, frontal lobes and internal capsule; (ii) that more white matter injury would be evident among individuals with HAD.

METHODS

Subjects

Participants (n=46) with HIV and healthy non-HIV controls (n=10), aged 18-35 years were recruited from the Infectious Diseases Clinics within the Groote Schuur Hospital recruitment area. To be eligible HIV participants were required to have a positive diagnosis of HIV infection made within the last six months (based on serologic testing by ELISA and Western blot), and have not previously used anti-retroviral medications (“HAART naïve”) In addition, patients were required to have at least 7 years of formal education.

Patients were excluded if they were 1) using psychotropic medication, 2) had a recent (6 month) substance abuse history as defined by responses to the Alcohol Use Disorders Inventory (AUDIT) and the Substance Abuse and Mental Illness Screener (SAMISS) 3) had a score greater than 16 on the Beck Depression Inventory (II), 4) had other current DSM-IV disorders as determined by the MINI , or 5) a lifetime history of head injury with loss of consciousness exceeding 30 minutes.

Procedure

Participants completed clinical assessments in a single testing session. Neuroimaging was conducted within one week of the initial evaluation. All measures were administered and scored according to standard procedures. Written informed consent was obtained prior to enrollment and exposure to any study related procedure. The protocol was approved by the Committee for Human Research of the University of Cape Town.

A neuropsychological test battery (Table 1) was administered to all participants to assess specific domains of attention, concentration, learning, memory psychomotor speed and executive function. The battery was based on that used by the HIV Neurobehavioral Research Centre(17), and included tests were adapted in discussion with three local 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. A full

neuromedical examination was conducted on all participants. Blood for CD4 cell counts was taken in the infectious diseases clinic.

Table 1: Neuropsychological test Battery

Domain	Test
<u>A: Motor function</u>	Successive Finger Taps, Grooved pegboard
<u>B: Learning and memory</u>	Hopkins Verbal Learning Test, Rey Complex Figure, Brief Visuospatial Memory Test (BVMT)
<u>C: Attention</u>	Mental alternation test, WMS III Mental Control, WMS III- spatial span
<u>D: Speed of processing</u>	WAIS III: Digit symbol coding, WAIS III: Symbol search
<u>E: Executive function</u>	Trail-making test A(TMTA), Colour trails part 1 and 2, Stroop Colour and Word test, Wisconsin Card Sorting Test
<u>F: Language</u>	Category fluency test i) animals ii) fruit and vegetables
<u>G: Intelligence</u>	WASI

Determination of dementia

The neuropsychological test battery, together with scores from a neuromedical assessment and reported functional assessment was used to classify participants into one of four HAND categories, based on the updated American Academy of Neurology criteria (18): 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 z-score cut-offs on at least two domains of function was rated NP2; those who scored between 1.0 and

2 SD on two domains of function, or >2 SD and 1-2 SD, were rated NP1. In order to allow for comparability with other studies, we also categorized participants into the corresponding Memorial Sloan Kettering (MSK) scores. An MSK severity score of 0 represents no cognitive impairment, 0.5 represents mild cognitive impairment not sufficient to meet criteria for dementia, and a score of 1.0 represents dementia(19). The final classification was conducted by a consensus panel comprising two HIV neuropsychiatrists (JJ, JH) and an experienced neurologist (MC).

Data from 51 HIV negative controls were used to generate Z-scores for establishing local norms.

Brain Imaging

Imaging was performed on a Siemens Magnetom 3T Allegra within 7 days of the screening and neuropsychological assessment. We acquired a multi-directional diffusion weighted sequence with 30 diffusion directions, 1 $b=0$ sec/mm^2 direction, $\text{TR}=8800$ ms, $\text{TE}=88$ ms and a $b\text{-value}=1000$ sec/mm^2 . The images were acquired as a mosaic resulting in a 960×960 matrix with 60 slices per volume, and a corresponding in-plane spatial resolution and slice thickness of 2×2 mm^2 and 2.2 mm, respectively. The 2.30 minute sequence was repeated three times and averages were derived.

Image processing

Eddy current correction was performed separately in each of the three acquisitions using affine transformations in FSL (Oxford Center for MRI of the Brain, Oxford, UK). The data were then imported to MATLAB (The Mathworks Inc, Natick, MA) for further processing. Co registration was performed across the three acquisitions using affine transformations with the unweighted image of the first average as a reference. For each of the three acquisitions, diffusion tensors were calculated and outliers were rejected by first calculating Z-scores based on 25 and 75 percentile limits, and then discarding data points more than 3 standard deviations away from the mean. The three acquisitions were then averaged and mean diffusivity (MD) maps were calculated. Diffusion tensors and fractional anisotropy (FA) images were then derived. An affine as well as a non-linear registration of the B_0 images to each subject's structural T1 image (intra-subject)

was performed. The warps were applied to the FA images. A mean FA template was then created from all the subjects followed by a final registration and warp of all the FA images to the mean template. The same registration transforms were applied to the MD images. DTI analysis based on voxel-based morphometry methods was performed group-wise for every voxel. And this was true for each t-test comparison (ie Controls vs Dementia, Controls vs NAD etc.). A general linear model ANOVA was performed on the FA and MD voxels of each cohort to determine significant differences between the groups. This was performed at a $p < 0.01$ uncorrected threshold with a FA mask of 0.25. Significant clusters were defined by the analysis ($p < 0.01$). Mean FA and MD values for each significant voxel cluster was calculated and tabulated. A DTI white-matter atlas (reference Mori et al. 2005, MRI atlas of human white matter, published 2005 by Elsevier, authors are Susumu Mori, Setsu Wakana, Lidia M. Nagae-Poetscher and Peter C.M van Zijl from John Hopkins University) was used to determine the anatomical locations by manually inspecting and comparing the mean FA color map with the atlas labels.

Statistical analysis

Multivariate tests of overall differences between HIV-positive groups and healthy seronegative control (HC) group were conducted for motor function, learning and memory, attention, speed of processing or executive function domains. Additionally, three linear regression models were created to determine the independent associations between the individual neuropsychiatric tests and FA values in the regions of the CC rostrum, sagittal stratum and the cingulum. Years of education was controlled for in all analyses, as it was significantly different between cases and controls. Age and gender were not significantly different between cases and controls.

Contrasts between HIV-positive groups (0, 0.5 and 1) and the healthy seronegative control (HC) group were then drawn to examine the associations between HIV infection, cognitive impairment and white matter FA. Whole brain voxel analysis of FA and MD in the HC group was also compared to the subgroups of HIV+ (no cognitive impairment, mild cognitive impairment and dementia). Protection from type I error was maintained by lowering the p-value to $p < 0.01$.

RESULTS

Of the 56 subjects originally enrolled, 54 participants (HIV+ n=44 and seronegative controls n=10) were included in the final analysis. The imaging data from two subjects in the HIV group were discarded due to movement artifacts. The mean age of the HIV+ participants and the control group was 29.1 (SD3.61) and 25.8 years (SD 4.78) respectively (see Table 2). At 11.6 years the average level of education of the seronegative control cohort was higher than the HIV cohort (p=0.0287). The mean CD4 count of the HIV positive group was 331.

Table 2: Demographic characteristics of sample

	Seronegative Controls Means(SD)	HIV+ Means(SD)	T-test 2 tailed, unequal variance
Gender			P=0.1174
Age	25.8(4.78)	29.08(3.61)	P=0.06
Level of education	11.55(2.13)	9.63(1.85)	P=0.0287

The multivariate test of overall differences among groups were not statistically significant for the motor function, learning and memory, attention, speed of processing or executive function domains, when controlling for years of education completed.

DTI

Individual FA values were found to be significantly lower in HIV+ patients in the corpus callosum (CC) rostrum (p=<.001) the sagittal stratum (p=<.001) and the cingulum (p=0.003) compared to healthy controls. Other comparisons to the seronegative controls were made using a NAD group (n=4), a group with mild cognitive impairment (n=26)

and a HAD group (n=14). A final comparison was made between those with HAD and the rest of the HIV cohort. The FA results are summarized in Table 3.

Table 3: Summary of FA results

Analysis	Anatomy	Hemisphere	Fractional anisotropy	P value	Mean FA
Control vs. NAD	Superior longitudinal fasciculus	Left	Decrease in FA	p = 0.002	Control - 0.425 (0.035) NAD - 0.376 (0.033)
	Sagittal stratum	Left	Decrease in FA	p = 0.001	Control - 0.411(0.081) NAD - 0.482 (0.077)
	Inferior fronto-occipital fasciculus	Right	Decrease in FA	p = 0.003	Control - 0.391(0.058) NAD - 0.323 (0.049)
	Inferior longitudinal fasciculus	Right	Decrease in FA	p = 0.003	Control - 0.409 (0.07) NAD - 0.320 (0.07)
Control vs. mild cog impairment	Superior corona radiata	Right	Decrease in FA	p = 0.0039	Control - 0.377 (0.05) Mild - 0.330 (0.051)
	Anterior thalamic	Left	Decrease	p =	Control - 0.329

	radiation		in FA	0.0025	(0.039) Mild – 0.280 (0.04)
	Posterior limb of internal capsule	Right	Decrease in FA	p = 0.0076	Control – 0.401 (0.091) Mild – 0.370 (0.084)
	Genu of the corpus callosum	Bilateral	Decrease in FA	p = 0.0072	Control – 0.452 (0.106) Mild – 0.361 (0.094)
	Inferior longitudinal fasciculus	Bilateral	Decrease in FA	p = 0.0034	Control – 0.379 (0.067) Mild – 0.312 (0.06)
Control vs. HAD	Genu of the corpus callosum	Bilateral	Decrease in FA	p = 0.007	Control – 0.382 (0.073) Dementia – 0.299 (0.053)
Control vs. HIV	CC rostrum	Bilateral	Decrease in FA	p = 0.003	Control – 0.423 (0.105) HIV – 0.347 (0.0894)
	Superior longitudinal fasciculus	Left	Decrease in FA	p = 0.005	Control – 0.394 (0.0328) HIV – 0.343 (0.0319)
	Sagittal stratum	Left	Decrease	p =	Control – 0.395

			in FA	0.001	(0.0845) HIV – 0.336 (0.0805)
	Cingulum	Right	Decrease in FA	p = 0.007	Control – 0.522 (0.0427) HIV – 0.457 (0.0270)
HIV vs. HAD	Inferior frontal occipital fasciculus	Right	Decrease in FA	p = 0.003	HIV – 0.352 (0.037) HAD – 0.308 (0.031)
	Superior longitudinal fasciculus/Posterior limb of internal capsule	Bilateral	Increase in FA	p = 0.0007	HIV – 0.360 (0.085) HAD – 0.403 (0.09)

Three linear regression models were created to determine the independent associations between the individual neuropsychiatric tests and FA values in the regions of the CC rostrum, sagittal stratum and the cingulum. The overall model predicting FA values in the sagittal stratum was significant, $F(13, 26) = 2.29$, $p = 0.04$, adjusted $R^2 = .30$. Increased impairment in Colourtrails 1 ($b = -.36$, $t = 2.16$, $p = .040$), was a significant predictor of lower FA scores in the sagittal stratum region. Although the overall model predicting FA values in the cingulum region was statistically significant ($F(13, 26) = 2.75$, $p < .014$, adjusted $R^2 = .067$), only lower level of education was a significant predictor of lower FA scores ($b = -0.46$, $t = 2.73$, $p = .011$).

Fig. 1 Axial view: Control cohort vs. HIV dementia cohort. The blue blobs indicate a FA decrease in the genu of the corpus callosum in the dementia group when compared to controls. Cluster threshold = 10 voxels and $p < 0.01$.

Fig. 2 Sagittal view: Control cohort vs. HIV cohort. The blue blobs indicate an FA decrease in the sagittal stratum of the HIV cohort compared to controls. Cluster threshold = 10 voxels and $p < 0.01$.

The same group comparisons were made measuring MD (see table 4). No significant differences were found between controls and HIV+participants, or between controls and those with mild cognitive impairment. However, significant increases in MD were found in the HIV+ group with no cognitive impairment and the dementia group when compared to the control group. This may suggest that inflammation is greatest in the very early and late stages of HAD (a biphasic inflammatory model). When comparing patients with HAD to the rest of the HIV cohort MD was increased in the inferior fronto-occipital fasciculus.

Table 4: Summary of MD results

Analysis	Anatomy	Hemisphere	Mean diffusion	Significance value
Control vs. NAD	Superior longitudinal fasciculus	Right	Increase in MD	$p = 0.00085$
	Internal capsule	Right	Increase in MD	$p = 0.0024$
	Anterior thalamic radiation	Left	Increase in MD	$p = 0.0013$
	Splenium of	Right	Increase in MD	$p = 0.0016$

	corpus callosum			
Control vs. HAD	Superior longitudinal fasciculus	Left	Increase in MD	p = 0.00045
	Inferior fronto-occipital fasciculus	Right	Increase in MD	p = 0.00044
	Cingulum (hippocampus)	Bilateral	Increase in MD	p = 0.0012
HIV vs. HAD	Inferior fronto-occipital fasciculus	Right	Increase in MD	p = 0.0019

Fig. 3 Coronal view: Control cohort vs. NAD cohort. The orange blobs indicate an increase in mean diffusion of the NAD cohort compared to controls in the internal capsule. Cluster threshold = 10 voxels and $p < 0.01$.

DISCUSSION

This study confirms that DTI-detected micro structural white matter abnormalities are present in clade C HIV when compared with healthy controls. These changes were more extensive in those with HAD. These include MD and FA changes in the inferior fronto-occipital fasciculus. We were able to detect white matter abnormalities in cognitively normal individuals and HIV+ participants with mild cognitive impairment, suggesting loss to white matter integrity early in the disease process. Although the overall model of neuropsychological tests predicted lower FA in the sagittal stratum and cingulum, there were few associations between individual neuropsychological tests and lower FA in the regions identified. Increased MD detected in the cognitively normal

(NAD) group and they HAD groups only, suggests a biphasic inflammatory process in HIV dementia.

Our study indicates that, in keeping with previous work in Clade B, individuals infected with clade C HIV have white matter abnormalities. The white matter damage has been found to be more prevalent and severe in patients with HIV-1 associated dementia (20). Consistent with a previous DTI study we demonstrated preferential injury to the corona radiate, corpus callosum and superior and inferior longitudinal fasciculus (21). Involvement of the corpus callosum in HIV encephalitis has been reported in postmortem findings (22). A number of DTI studies in HIV have reported white matter damage to the corpus callosum (23-25).

We found that HIV+ individuals without HAND also demonstrated white matter changes. Thus HIV related neurotoxicity occurs in the absence of clinical features of cognitive impairment. DTI abnormalities have previously been reported in the FWM of cognitively asymptomatic HIV+ patients with normal structural MR studies (13):

We also found increases in FA and MD in the HAD group when compared to the rest of the HIV cohort in the superior longitudinal fasciculus. The standard interpretation of these results are that increased FA in the areas represent increased white matter integrity in HAD participants compared to participants with mild cognitive impairment. However Stebbins et al (26) found similar increases in FA and suggested that increased FA could be due to loss of the complexity of the white matter matrix in those regions. If a voxel contains only parallel white matter fibers, FA can be high. Damage to the matrix would result in loss of crossing and non-parallel fibers with sparing of the parallel fibers and a paradoxical increase in FA. One indicator of such a process would be an increased MD, which suggests general tissue damage, together with the increased FA (27). This relationship is seen in the superior longitudinal fasciculus in this study.

Colour trails 1 predicted reduced FA in the sagittal stratum, suggesting that this neuropsychological test may be sensitive to white matter damage in clade C HIV. It is possible that this may be a clade-specific difference, 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 (28, 29). Previous DTI studies in clade B found correlations between motor speed losses and poor white matter integrity of the corpus callosum(30)

Data from Alzheimer's research show that data show that maximal immunoreactivity in neurons occurs during the early disease stages prior to the maximal activation of astrocytes and microglia (31) and very late stages of the disease. The increases in MD which we detected follow a similar biphasic pattern.

The neurocognitive impact of the clade C HIV has not been determined. A study in southern India among individuals with the clade C virus with advanced HIV. The results suggested that cognitive difficulties are present among individuals with the clade C virus in India, with more than half of the patients with advanced HIV meeting the criterion for impairment in two cognitive domains(32).However investigation of the neuropathogenesis induced by HIV-1 clades using an animal model, revealed greater astrogliosis and increased loss of neuronal network integrity and an increased number of memory errors in clade B compared to clade C(33). Additional study is needed to determine if clade C HIV infection is more or less prone to cause neurocognitive deficit than the clade B virus. Furthermore, the impact of antiretroviral therapy on neurocognitive dysfunction in clade C viral infection needs to be determined.

Limitations of our study include the relatively small samples, and the cross sectional design. Previous reviews of cross-cultural influences on neuropsychological testing in South Africa indicate that normative studies should take account of the influential variable of quality of education, in addition to level of education. (34). Normative neuropsychological data was generated for this study from 51 individuals from the same socio-economic group, however level of education was higher in the control group. The data here are not able to address the question of whether white matter changes noted in the asymptomatic and mild cognitive disorder groups represent an early marker of subsequent cognitive decline.

Despite these limitations, findings here revealed lower FA and increased MD in the HIV positive groups in the corpus callosum, and other regions. Future work could usefully focus on anisotropy measurements as quantitative imaging biomarker in the setting of HANDs using larger longitudinal studies. Identification of a reliable early imaging correlates of risk for subsequent cognitive dysfunction, such early changes to white matter integrity, may allow for earlier treatment with HAART and delay the course of HIV disease. Studies examining response to HAART treatment in patients infected with HIV will be important to determine whether DTI abnormalities observed in the corpus callosum and other regions reflect reversible or more advanced irreversible injury. Correlates of white matter damage and neurocognitive decline need to be sought, including whether measures of central viral load correlate with poor FA values, illness duration, age, treatment exposure and treatment adherence. These factors are almost certainly critical in determining the overall impact of HIV on brain function, and in particular on white matter integrity. In the interim these findings point to the potential value of FA and MD as a measure of central nervous system damage in HIV positive patients.

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Prospective memory impairment in HIV: a Diffusion Tensor Imaging

Study

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ABSTRACT

HIV-associated prospective memory (ProM) impairment has emerged as a significant predictor of medication management and independence in activities of daily living. The relationship between ProM and white matter integrity in HIV has not previously been investigated. Participants including 128 HIV-infected individuals and 32 seronegative subjects were assessed using a comprehensive neuropsychological evaluation and both objective and subjective measures of ProM. We utilized diffusion tensor imaging (DTI) to investigate the relationship of white matter integrity to ProM in 37 HIV-positive subjects using a voxel-based approach to define fractional anisotropy (FA) and mean diffusion (MD). Compared to healthy controls the HIV-infected group experienced difficulty with the complex cognitive processes involved in ProM. Poor ProM correlated with performance on neuropsychological tests of executive functioning, information processing speed, learning, and working memory and retention or semantic memory. The prospective retrospective memory questionnaire (PRMQ) did not correlate with objectively measured ProM. The study also provides evidence that CNS injury is evident among individuals with poor ProM infected with HIV. Those with poor ProM had significantly decreased FA in the regions of superior corona radiata, the corpus callosum and the cingulum. This study reinforces importance of ProM in HIV.

| INTRODUCTION

Neuropsychological impairment is seen in up to 50% of people infected with HIV-1, with the characteristic pattern of deficits reflecting a preferential disruption of prefronto-striatal circuits (1). Deficient episodic memory and retrieval are common in individuals with HIV-1 infection (2). The memory profile of HIV-1-infection is most consistent with dysfunction in executive aspects of encoding and retrieval as seen in other “subcortical” disorders, such as Parkinson’s and Huntington’s diseases (2).

Prospective memory (ProM), or “remembering to remember”, is an aspect of declarative memory that refers to the execution of a future intention (e.g., remembering to take one’s medication). Recent research shows that individuals infected with HIV report experiencing more frequent ProM failures as compared to demographically similar seronegative persons(3). A recent study showing that ProM demonstrates incremental ecological validity as a predictor of general IADL dependence in HIV (4).

ProM generally involves a series of cognitive processes that includes: (a) the formation of an intention that is paired with a time or event; (b) maintenance of the intention-cue pairing over a delay interval while concurrently engaged in a other tasks, (c) detection and recognition of the cue; (d) search and retrieval from retrospective memory (RM) for the content of the intention; and (e) successful execution of the intention. (5)

A distinction is often made between *event-based* (EB) and *time-based* (TB) ProM tasks (6) The difference between these two tasks concerns the type of cues that initiate retrieval of the intention. An external stimulus provides the cue for action in an EB task (e.g., grocery store cues the buying of bread), whereas in TB tasks, the intended action is performed after a specified time interval (e.g., taking a medication every six hours).

Intact ProM is critical in order to properly adhere to a medication regimen; that is, one must first form the intention to take the medication at some point in the future then maintain the intention-cue pairing throughout the day despite the distraction of normal daily activities, detect and recognize the cue when it occurs (e.g., making dinner), recall the specific medication and directions, and finally, take the medication as instructed. (7)

HIV-associated ProM impairment has emerged as a significant predictor of medication management, even after considering the contributions of executive functions, attention, episodic retrospective memory, and verbal fluency(8).

Neuroimaging studies have been conducted to investigate ProM at a neural systems level. During a ProM task, there was activation of the anterior prefrontal cortex (BA 10) on fMRI(9), and the left frontal pole (BA 10), as well as right dorsolateral and ventrolateral prefrontal cortex (BA 8/9/47) and anterior cingulate (BA 24) in an earlier PET study. This is consistent with the hypothesis that anterior prefrontal cortex (area 10) supports the biasing of attention between external events and internal cues.

Given that white tracts from prefrontal cortex are involved in HIV(10), it might be hypothesized that in HIV damage to such tracts leads to ProM impairment. Diffusion weighted imaging used to derive measures of fractional anisotropy (FA) in white matter tracts provides an indirect indication of the structural integrity of the regional white matter(11). Previous studies using DTI, arguably a more sensitive measure of white matter integrity than conventional structural MR sequences, have demonstrated frontal and internal capsular pallor in clinical studies of HIV-1 positive patients(10). As such, preliminary evidence suggests that there is white matter involvement in HIV associated neurocognitive disorder (HANDS), but as yet, no study of which we are aware has investigated the correlation with changes in white matter and ProM.

We hypothesized that persons with HIV-1 infection would demonstrate impaired ProM relative to demographically comparable seronegative comparison subjects. We further hypothesized that ProM functioning in HIV would correlate with performance on neuropsychological tests of executive functioning, information processing speed, learning, and working memory, but not with measures of retention or semantic memory. Finally, we hypothesized that HIV-1-infected individuals with poor ProM would demonstrate white matter changes reflected as lower FA using DTI in anterior prefrontal cortical white matter regions.

METHODS

Subjects

Participants (n=128) with HIV and healthy non-HIV controls (n=32), all aged 18-35 years were recruited from the Infectious Diseases Clinics within the Groote Schuur Hospital recruitment area. To be eligible HIV participants were required to have a positive diagnosis of HIV infection made within the last six months (based on serologic testing by ELISA and Western blot), to have not previously used anti-retroviral medications ("HAART naïve, and have at least 7 years of formal education.

The presence of other current DSM-IV disorders determined through semi-structured interviews using the MINI was an exclusion criterion. Patients were also excluded if they were using psychotropic medication or had a recent (6 month) substance abuse history as defined by responses to the Alcohol Use Disorders Inventory (AUDIT) and the Substance Abuse and Mental Illness Screener (SAMISS) Individuals with a score greater than 16 on the Beck Depression Inventory (II) were excluded from the study. Patients were also excluded if they reported a lifetime history of head injury with loss of consciousness exceeding 30 minutes or a current neurological disease or infection. Controls, who attended the same ARV clinics as the subjects, had the same inclusion and exclusion criteria although they tested as being HIV negative.

Procedure

Participants completed clinical assessments in a single testing session. Neuroimaging was conducted within one week of the initial evaluation on 37 patients in the HIV positive cohort and on 10 controls. All measures were administered and scored according to standard procedures. Written informed consent was obtained prior to enrolment and exposure to any study related procedure. The protocol was approved in writing by the Committee for Human Research of the University of Cape Town.

A neuropsychological test battery was administered to all participants to assess specific domains of attention, concentration, learning, memory psychomotor speed and

executive function. The battery was based on that used by the HIV Neurobehavioral Research Centre. The neuropsychological test battery consisted of the following instruments: Verbal Fluency tests (animal; fruit & vegetable); Digit Symbol and Symbol Search subtests from the Wechsler Adult Intelligence Scale – third edition (WAIS-III, Psychological Corporation, 1997); Trail making test Part A (TMT, Reitan Neuropsychology Laboratory, 1992); Hopkins Verbal Learning Test – revised (HVLT-R; Brandt & Benedict, 2001); Brief Visuospatial Memory Test – revised (BVM-T-R, Benedict, 1997); Wisconsin Card Sorting Test (WCST 128-item version; Grant & Berg, 2000); Stroop Colour and Word Test (Golden, 1978); Grooved Pegboard Test (Lafayette Instrument Company, 1989); Finger Tapping Test; Rey-Osterrieth Complex Figure (Rey, 1941 & Osterrieth, 1944); Wechsler Memory Scale - Mental Control (WMS; The Psychological Corporation, Wechsler, 1997); Mental Alternation Test (Jones, Teng, Folstein & Harrison, 1993). 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 WCST perseverative error score was taken from the published norms generated through the report. The Stroop Interference scores were generated using Golden's (1978) methodology. For the remainder of the test scores, raw scores were used given that published norms are not suitable for this population.

A full neuromedical examination was conducted on all participants. Blood for CD4 cell counts was taken in the infectious diseases clinic.

Prospective memory assessment

ProM was assessed using a subjective self-report measure as well as objective measures. Self-reported ProM was assessed using the ProM Scale from the Prospective and Retrospective Memory Questionnaire (PRMQ; Smith, Della, Sala, Logie, & Maylor, 2000). The objective measure consisted of both a time and an event based task. The event based task was a measure previously used by Huppert et al., 2000. The instructions were 'Later on, I am going to give you a name and address to write on the envelope. When you have finished doing this, I would like you to do the following: turn it over, seal it and write your initials on the back. 'Could you remember to

do this then without me reminding you?' This was followed by a delay interval of around 10 minutes which was filled with other cognitive tasks. The interviewer then observed whether the respondent carried out the prospective memory task. If the respondent did not do so within about 10 seconds, the interviewer prompted 'Were you going to do something else with the envelope' If only one correct action was carried out (i.e. just the envelope sealed or just initial written on the back), the interviewer said 'Was there something else you were going to do'. The time based task was taken from the Memory for Intentions Screening Test (MIST) (Raskin, 2004), which is a standardized laboratory measure of ProM with published evidence of its reliability and construct validity (3). The instruction was: 'In 2 minutes ask me when this session ends today'. Prospective memory scores were totaled across the tasks. The scores of the tasks completed without prompt were doubled in order to form a Total Prospective Memory Index.

Brain Imaging

Imaging was performed on a Siemens Magnetom 3T Allegra and occurred within 7 days of the screening and neuropsychological assessment. We acquired a multi-directional diffusion weighted sequence with 30 diffusion directions, 1 $b=0$ sec/mm^2 direction, $\text{TR}=8800$ ms, $\text{TE}=88$ ms and a $b\text{-value}=1000$ sec/mm^2 . The images were acquired as a mosaic resulting in a 960×960 matrix with 60 slices per volume, and a corresponding in-plane spatial resolution and slice thickness of 2×2 mm^2 and 2.2 mm, respectively. The 2.30 minute sequence was repeated three times and averages were derived.

Image processing:

Eddy current correction was performed separately in each of the three acquisitions using affine transformations in FSL (Oxford Center for MRI of the Brain, Oxford, UK). The data were then imported to MATLAB (The Mathworks Inc, Natick, MA) for further processing. Co registration was performed across the three acquisitions using affine transformations with the unweighted image of the first average as a reference. For each

of the three acquisitions, diffusion tensors were calculated and outliers were rejected by first calculating Z-scores based on 25 and 75 percentile limits, and then discarding data points more than 3 standard deviations away from the mean. The three acquisitions were then averaged and mean diffusivity (MD) maps were calculated. Diffusion tensors and fractional anisotropy (FA) images were then derived. An affine as well as a non-linear registration of the B0 images to each subject's structural T1 image (intra-subject) was performed. The warps were applied to the FA images. A mean FA template was then created from all the subjects followed by a final registration and warp of all the FA images to the mean template. The same registration transforms were applied to the MD images. A general linear model ANOVA was performed on the FA and MD voxels of each cohort to determine significant differences between the groups. This was performed at a $p < 0.01$ uncorrected threshold with a FA mask of 0.25.

Comparisons between HIV-positive groups (good and poor ProM) and the healthy seronegative control (HC) group were then drawn to examine the associations between HIV infection, ProM and white matter FA. Whole brain voxel analysis of FA and MD in the control group was also compared to the HIV+ subgroups (good and poor ProM).

Statistical Analysis

Descriptive statistics, t-tests and Chi-square analyses, were performed to examine between-group differences on demographic variables. Because the assumptions of normality for ProM data were not met for this sample, non-parametric tests were performed. The Mann-Whitney test was used to assess between-group differences on measures of ProM. The self-report measure of the PRMQ was correlated with the objective measures of prospective memory. In order to indicate which other cognitive domains bore the strongest relationship prospective memory in the current sample, Spearman's rho correlation coefficients were calculated to explore associations between the prospective memory total score and scores on the rest of the neuropsychological battery. Linear regression was performed in order to see which tests from the remainder of the battery were the best predictors of poor prospective memory.

RESULTS

One hundred and sixty participants (HIV+ n=128 and seronegative controls n=32) were included in the final analysis. DTI was performed on a subsample of 40 HIV+ participants and 10 controls. The imaging data from 3 subjects in the HIV group were discarded due to movement artefacts. Age, education and gender in the HIV+ and control groups were tabulated (Table 1); the groups were not significantly different in terms of education or gender, but that the HIV group was significantly older.

Table 1: Demographic variables **Mean/SD**

Variable	HIV+ (n=128)	HIV- (n=32)	Significance
Age	29.62 (3.59)	24.53 (5.99)	p = 0.000049
Education	10.07 (1.94)	10.66 (1.45)	p = 0.114
Gender (M:F)	32:95	9:23	p = 0.833

Total prospective memory was significantly poorer in HIV+ than in controls ($p=0.023$). Time-based ProM was decreased in the HIV compared to controls (($p=0.00004$ without prompts, $p=0.001$) with prompts). Neither event-based ProM without nor with prompt differed between HIV+ and control groups.

Good prospective memory was defined as those participants who were able to perform the ProM tasks without prompting. Using the good/poor prospective memory distinction, 39.8% of HIV+ group had good prospective memory while 71.9% of the controls did. Similarly 60.2% of the HIV+ group had poor prospective memory while 28.1% of the controls did.

Table 2**Good and Poor Prospective Memory**

		Prospective Memory		
		Good	Poor	Total
HIV+	Count	51	77	128
	% within HIVStatus	39.8%	60.2%	100.0%
Controls	Count	23	9	32
	% within HIVStatus	71.9%	28.1%	100.0%

The associations between the Total Prospective Memory Summary score and the neuropsychological tests from the remainder of the battery are shown in Table 3. There were a greater number of significant correlations with Total Prospective Memory score for the HIV+ group compared to controls. Tests in motor function, learning, working memory, processing speed, executive function and word fluency domains had significant correlations with Total Prospective Memory Summary score. Correlations that were not significant for both groups were those for Finger Tapping Test (dominant and non-dominant hands), HVLTL Learning, Stroop Interference and WCST Perseverative Errors. None of the tests of motor function in the control group correlated with the Total Prospective Memory Summary score.

Table 3: Correlation between Total ProM summary score and neuropsychological tests

Tests in their Domains	HIV+	Controls
Motor		
Finger Tapping Dominant	-.140	-.314
Finger Tapping Non-Dominant	-.035	-.312
Grooved Pegboard Dominant	-.193*	-.028
Grooved Pegboard Non-Dominant	-.253**	.076
Learning		
HVLT-R Total	.376**	-.075
HVLT-R Learning	-.015	.132
HVLT-R Recall	.419**	.236
HVLT-R Recognition	.308**	.170
BVMT-R Total	.405**	.367*
BVMT-R Learning	.197*	.210
BVMT-R Recall	.429**	.280
BVMT-R Recognition	.279**	.081
Working Memory		
MAT	.153	.407*
WMS-III Mental Control	.381**	.384*
Processing Speed		
WAIS- III Digit Symbol	.434**	.214
WAIS- III Symbol Search	.243**	.342
TMTA	-.179*	-.279
Colour Trails I	-.163	-.383*
Executive Functions		
ROCF Copy	.319**	.273
Colour Trails II	-.218*	-.303
Stroop Word	.329**	.518**
Stroop Colour	.297**	.618**
Stroop Colour-Word	.220*	.474**
Stroop Interference	.007	.096
WCST Perseverative Errors	-.104	-.157
Word Fluency		
Animal Fluency	.543**	.423*
Fruit & Vegetable Fluency	.378**	.119

*p<0.05

**p<0.001

As poor retention and verbal fluency were associated with impaired ProM we assessed whether the results obtained here differ from those in previous research. Previous studies have shown that retention and recognition are less commonly affected in persons with HIV-1 disease(12). T-tests found no significant differences between HIV+ and control participants in tests of word fluency ($p=.717$), verbal retention ($p=.083$) and recognition($p=.077$). This remained the case even when test language was controlled for and when only those tested in their first language were included.

Stepwise linear regression models were used to examine the neuropsychological predictors of poor ProM. The overall model predicting poor ProM was significant $F(3,95) = 18.387$, $p = 0.00003$, adjusted $r^2 = .347$. Impaired performance on Semantic fluency (animals) ($\beta = -.378$, $t = -4.332$, $p = .00003$), BVMT recall ($\beta = -.274$, $t = -3.159$, $p = .002$), BVMT recognition ($\beta = .254$, $t = 3.139$, $p = .002$), HVLT recall ($\beta = -.2$, $t = -2.359$, $p = .02$), and Colour trails I ($\beta = .182$, $t = 2.310$, $p = .023$) were significant predictors of poor ProM.

The Kruskal-Wallis test revealed that the time-based task without prompt was weaker in the HIV associated dementia (HAD) group than the asymptomatic group ($p=.022$) It suggests that the event-based task without prompt similarly distinguishes between dementia category groups ($p=.028$) as defined by the updated American Academy of Neurology criteria (13).. Both the time and event-based tasks with prompt did not distinguish between dementia category groups.

Total Prospective Memory score correlated poorly with the self -report PRMQ (Spearman's rho $R=.073$)

DTI

Comparisons were performed to examine the effect of ProM on FA and MD. The seronegative control group was compared to HIV+ participants with good ProM and then again to those with poor ProM. The final comparison was between the 2 HIV positive groups (ie. good vs poor ProM). Those HIV+ participants with poor ProM had significantly decreased FA in the regions of superior corona radiata, the corpus

collosum and the cingulum when compared to those who were HIV+ with good ProM. We found increased FA in the Superior longitudinal fasciculus (left) in the HIV+ good ProM group compared to controls ($p = 0.0024$). When comparing controls to HIV+ participants with poor ProM we found significant decreases in FA in the regions of the corpus collosum, the sagittal stratum and the superior longitudinal fasciculus. Complete FA results are summarized in table 4. No differences were found in MD in the 3 group comparisons.

Table 4: Significant FA decreases

Poor ProM HIV+ vs HC ($p < 0.01$ uncorrected)	Area	P value
	Genu of the CC (bilateral)	$p = 0.0029$
	Superior longitudinal fasciculus (right)	$p = 0.0062$
	Sagittal stratum (left)	$p = 0.00063$
Good ProM HIV+ vs poor ProM HIV+ ($p < 0.01$ uncorrected)	Superior corona radiata right	$p = 0.0035$
	Genu of the corpus callosum bilateral	$p = 0.006$
	Cingulum near hippocampus (right)	$p = 0.0052$
	Cingulum near hippocampus (left)	$p = 0.0033$
	Posterior thalamic radiation (bilateral)	$p = 0.0067$

Figures 1-2 show the output from the voxel-based analysis with the colour blobs indicating significant FA differences ($p < 0.01$ uncorrected).

Figure1: **Good ProM HIV+ vs. poor ProM HIV+ ($p < 0.01$ uncorrected):** Genu of the corpus callosum bilateral ($p = 0.006$)

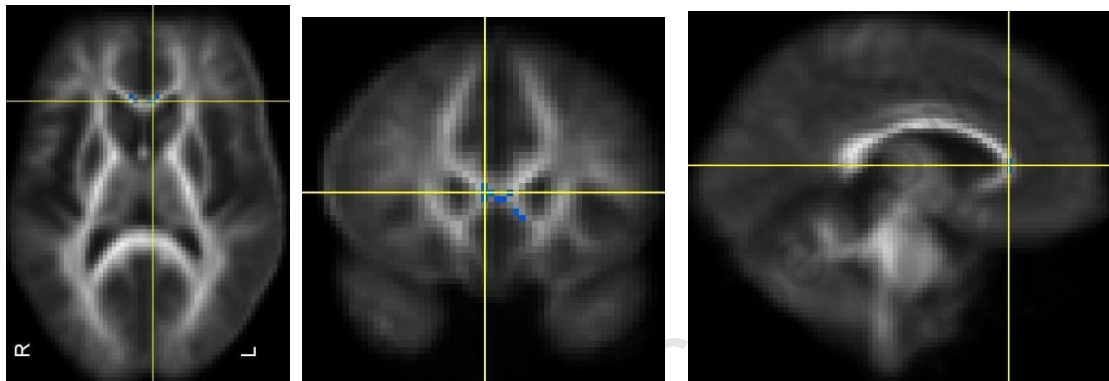
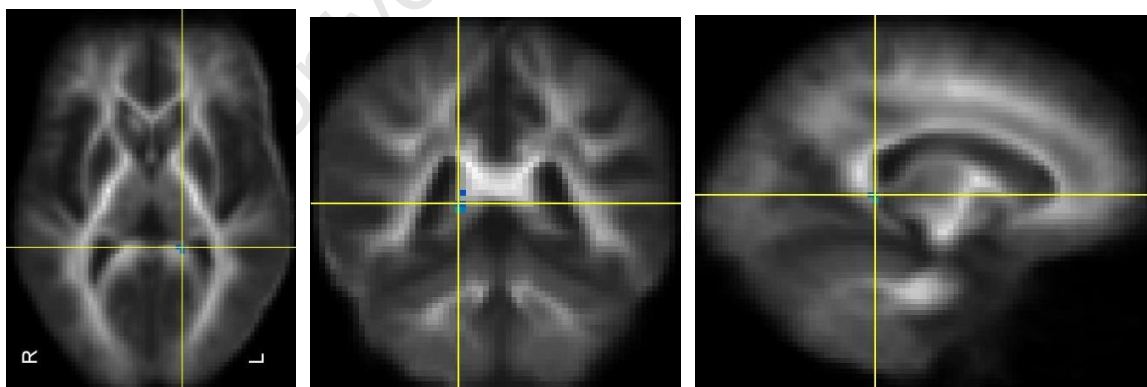


Figure2: **Good ProM HIV+ vs. poor ProM HIV+ ($p < 0.01$ uncorrected):** Cingulum near hippocampus on left side ($p = 0.0033$)



Linear regression models were created to determine the neuropsychological predictors of FA in the HIV+ cohort ($n=37$). Given the significant difference in age between the

controls and the HIV+ participants and the role that age plays in changes in the white matter, age was entered as a potential predictor in all of these models. However, it was not significant for any of the models. Colour trails II ($\beta = .305$, $t = 2.151$, $p = .044$), HVLT learning ($\beta = .425$, $t = 3.458$, $p = .002$), and the Digit Symbol test ($\beta = .415$, $t = 3.254$, $p = .004$) were significant predictors of FA scores in the superior corona radiata. Semantic fluency (animals) ($\beta = .493$, $t = 2.717$, $p = .012$) was a significant predictor of FA scores in the genu of the CC and in the cingulum ($\beta = .432$, $t = 2.295$, $p = .031$)

DISCUSSION

Consistent with our original hypotheses, first, results from the current study provide evidence of ProM impairment in HIV+ patients. Furthermore ProM was worse for those with HAD. Second, ProM functioning in HIV+ patients correlated with performance on neuropsychological tests of executive functioning, information processing speed, learning, and working memory, but also with measures of retention or semantic memory. Third, HIV+ participants with poor scores of ProM correlated significantly with lower FA values when contrasted with subjects who were HIV positive, but did not have ProM impairment. Although age was statistically different between the groups, it did not predict low FA in the HIV + cohort.

The data here confirm previous work on ProM impairment in HIV. It is unlikely that findings from the current study are confounded by depressive symptoms or demographic factors such as level of education, as the HIV + and seronegative groups were comparable years of education, and sex. However, although in a previous study the HIV+ve sample demonstrated deficits in time-and event-based ProM, we found that only time-based and total ProM, not event based ProM, discriminated between HIV+ and control participants. The sample size in our study is larger, or this may be due to differences in assessment of ProM. It has been hypothesized that time-based tasks require slightly different cognitive processes than event-based tasks (i.e., a greater emphasis on self-initiated monitoring and retrieval), and empirical evidence suggests

that the former are generally more sensitive in older adults (14) and traumatic brain injury samples (15).

Specific correlations were observed between the Total Prospective Memory score and tests of executive functioning, information processing speed and verbal working memory within the HIV+ve sample. This is not surprising given either previous literature, or the relationship ProM has to these cognitive domains. People infected with HIV often exhibit dysfunction in the executive aspects memory tasks(3) Difficulties in executive function include a disorganized plan for retrieving the correct information from episodic memory stores and would manifest in poor ProM performance(3) Nevertheless, a previous study of ProM in HIV ,with a smaller sample size, found that ProM performance did not correlate with measures of semantic memory, retention, or recognition discrimination . However consistent with previous literature verbal fluency and HVLT recognition and recall did not discriminate well between HIV+participants and controls(16).

Previous findings on the relationship between subjective and objective measures of cognitive performance suggest a relatively weak association in healthy volunteers(17). In our study PRMQ scores correlated poorly with Total Prospective Memory score. The underlying relationship between awareness of prospective memory impairment in HIV infection is unknown.

Results of the present study using DTI to contrast HIV + participants with poor ProM to HIV+ participants with good ProM, confirm our hypothesis in which we anticipated changes associated with poor ProM in white matter tracts that relay through the anterior prefrontal cortical regions including the corpus callosum, the cingulum (hippocampus) and the superior corona radiata. These findings provide evidence that the poor ProM is associated with neural changes induced by HIV. ProM requires retrospective memory (and thereby medial temporal and limbic structures such as the hippocampus). This is consistent with the neuropsychological findings that failures in retrospective recall (i.e., forgetting the content of an intention) will result in ProM failure (18). Colour trails 1&2, HVLT learning, the Digit Symbol test and semantic fluency predicted reduced FA in the

superior corona radiata and semantic fluency in the corpus callosum and cingulum, suggesting that these neuropsychological tests may be sensitive to white matter damage in clade C HIV. It is possible that this may be a clade-specific difference, wherein we found that HIV+ participants performed as well as sero negative controls on the Grooved Pegboard Test, a measure consistently used to ascertain whether HIV-associated subcortical neuropathology exists (19;20). Previous DTI studies in clade B found correlations between motor speed losses and poor white matter integrity of the corpus callosum(21)

Limitations of our study are that our HIV negative control group is small. Our scanned sample size is also small and our data are cross-sectional and as such are not able to address the question of whether white matter changes noted in participants with poor ProM represent an early marker of subsequent cognitive decline. A more comprehensive objective assessment of ProM such as the MIST may be useful in future work.

Despite these limitations this is the first study of which we are aware which has shown changes in white matter associated with ProM in HIV + participants. The prevalence of ProM impairment in our HIV+ve sample potentially carries profound clinical implications in terms of their possible impact on independent living skills(8) and the risk of nonadherence to complex antiretroviral medication regimens(7). Such findings imply that assessment and consideration of the ProM deficits associated with HIV infection might be useful in the day-to-day management of the disease. For example medication adherence efforts might also be enhanced by keeping to a minimum the number and complexity of tasks to be completed. Future research could focus on intervention strategies for persons infected with HIV who have poor ProM, and on longitudinal study designs that may identify ProM as a reliable early correlate of risk for subsequent cognitive dysfunction in HIV

Acknowledgements

JH-has received support from the Medical Research Foundation of South Africa and the Discovery Foundation Academic Award of South Africa

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Journal of Neurology, Neurosurgery & Psychiatry:

Instructions for authors

Papers

Full papers must present important and substantial new material. Articles should be of direct relevance to clinical practise. Thus we do not generally publish research based on animal experiments nor studies of normal nervous system function.

Word count: 3500 words maximum.

Abstract: 250 words.

Tables/Illustrations: should not normally exceed 8.

References: 40

Title page

The title page **must** contain the following information:

1. The title.
2. The name, postal address, e-mail, telephone and fax numbers of the corresponding author.
3. The full names, institutions, city and country of all co-authors.
4. Up to five keywords or phrases suitable for use in an index (it is recommended to use [MeSH](#) terms).
5. Word count - excluding title page, abstract, references, figures and tables.

Manuscript format

The manuscript format must be presented in the following order:

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3. Main text (tables should be in the same format as your article and embedded into the document where the table should be cited; images must be uploaded as separate files)
4. Acknowledgments, Competing interests, Funding
5. Copyright licence statement
6. References
7. Appendices



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Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
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07 August 2007

REC REF: 263/2007

Dr J Joska
Psychiatry & Mental Health
J Block

Dear Dr Joska

PROJECT TITLE: NEUROCOGNITIVE DISORDERS IN YOUNG ADULTS WITH HIV/AIDS COMMENCING ANTI-RETRO-VIRAL TREATMENT IN THE WESTERN CAPE

Thank you for your letter to the Research Ethics Committee dated 02 August 2007.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study including the following documentation:

- Study Protocol.
- Mini International Neuropsychiatric Interview English Version 5.0.0 dated 01 July 2006.
- Participant Information Leaflet and Consent Form Version 1, dated 03 May 2007.

Your comments to the queries raised are noted with thanks.

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Please note that the ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM: *INTERVIEW AND NEUROPSYCHOLOGICAL ASSESSMENT: CONTROLS*

TITLE OF THE RESEARCH PROJECT: Neurocognitive disorders in young adults with HIV/AIDS commencing anti-retro-viral treatment in the Western Cape

PRINCIPAL INVESTIGATOR: Dr John A. Joska

ADDRESS: Department of Psychiatry and Mental Health, J-block, Groote Schuur Hospital, Anzio Road, Observatory, 7925

CONTACT NUMBER: 021- 404 2164/021- 4042151

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- *The study will be conducted at the primary care antiretroviral clinics in Khayalitsha site C, Woodstock and Mitchells Plain. The study aims to include 200 HIV positive people and 50 HIV negative people.*
- *This study will perform a detailed interview and neuropsychological assessment when people start taking anti-retrovirals and again at one year, to find out if there are any problems in thinking or moving in people with HIV/AIDS. This is in order to understand why certain people with HIV/AIDS develop these problems. We will also do these assessments on the 50 HIV negative people.*
- *You will also be asked for a sample of blood. This will be used to look at your body's response to infection with HIV. Tests of inflammation will be done. This will help us to understand if inflammation is important in the way that problems in thinking and memory happen in people with HIV/AIDS. The study will require about 30 mls (two tablespoons) for this purpose. This will involve minor discomfort at the time taking blood and may cause some reddening and bruising*

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of your arm in this area. You may choose not to participate in this part of the study.

- *Some people will be asked to have a type of brain scan which will be done at the Cape Universities Brain Imaging Centre at Tygerberg Hospital. This scan is called an MRI (magnetic resonance imaging) scan. The scan will require you to lie on your back on a table that will move into the scanning machine for the 85 minutes it will take for the scan. During this time you will be able to close your eyes and rest. You will also be able to talk to the study doctor/assistant at all times during the scan if you should experience any discomfort. The scan is a safe procedure if you have been screened correctly for the presence of any magnetic material on or inside you such as pace-makers, surgical clips and metal objects in the eyes. A formal screen for this will be done at the screening visit by a member of the study team. As the scan is done in a relatively confined space, occasionally people become anxious. This does not happen often, and if you feel anxious, we will spend time allowing you to get used to the surroundings before we begin. When the magnet in the machine is switched on, it will make some loud banging noises, but you will be clearly warned when this will take place. At this time you will feel nothing and the noise is not harmful to you in any way. To minimise the possible discomfort associated with this, we will give you some soft earplugs and will also put earphones on so that you can listen to music if you so choose*
- *Apart from these tests, the study will not offer special treatment or medication. If a mental health problem is found, you will be referred for treatment at your nearest clinic.*

Why have you been invited to participate?

- *You have been invited to participate, because memory and thinking problems in HIV/AIDS are not properly understood by medical science. We also need to compare these problems in people with and without HIV to see if there are differences.*

What will your responsibilities be?

- *You will be required to attend the study visit on time and to participate as fully as possible. This means that you will answer questions as fully and honestly as possible. If there are questions you do not want to or cannot answer, you should say so.*

Will you benefit from taking part in this research?

- *You will receive little benefit directly from the study. If you do have a mental health problem, we will be able to refer you to someone who may help. Second, if any memory or thinking problems are identified, we will be able to explain these to you. In addition, information from this study may allow us to understand these problems better, and to develop studies which will help us to treat them better.*

Are there in risks involved in your taking part in this research?

- *This study may make you feel uncomfortable as you talk about mental health problems. You may feel embarrassed or shy. Also, some people feel that is it better not to know about memory or thinking problems.*
- *These are the main risks. You should feel free to mention your feelings or concerns to any member of the study team*

If you do not agree to take part, what alternatives do you have?

- *You are free not to participate in the study or to refuse parts of the study.*

Who will have access to your medical records?

- *The information collected about you will be treated as confidential and protected. If it is used in a publication or thesis, your identity will remain anonymous. Only the direct study team will have full access to the information. If we need to refer you to a clinic for treatment, we will provide them with the relevant information needed to treat your condition.*

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to take part in the study but your transport costs will be covered for the study visit- The study nurse will give you R150 for this. She will also provide the money it costs to attend the clinic. There will be no costs involved for you, if you do take part.

Is there any thing else that you should know or do?

- You should inform your family practitioner or usual doctor that you are taking part in a research study.
- You can contact Dr John Joska at tel 021-4042164 if you have any further queries or encounter any problems.
- You can contact the Research Ethics Committee of the Health Sciences Faculty of the University of Cape Town 021-4066338 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- If you would like a copy of this information and consent form for your own records, please ask a member of the study team to give you one.

Declaration by participant/guardian/treatment partner (circle)

By signing below, I agree/agree on behalf of..... to take part in a research study entitled: "Neurocognitive disorders in young adults with HIV/AIDS commencing anti-retro-viral treatment in the Western Cape".

I declare that (delete whichever is NOT applicable):

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that my taking part/my relative or friend's participation in this study is **voluntary** and I/we have not been pressurised to take part.
- I/my relative or friend may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I/my relative or friend may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) on (*date*) 200_.

.....
Signature of participant/guardian/treatment partner

.....
Signature of witness

Declaration by investigator

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above

Signed at (*place*) on (*date*) 2005.

.....
Signature of investigator

.....
Signature of witness

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Initials _____

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM: *INTERVIEW AND MRI*

TITLE OF THE RESEARCH PROJECT: Neurocognitive disorders in young adults with HIV/AIDS commencing anti-retro-viral treatment in the Western Cape

PRINCIPAL INVESTIGATOR: Dr John A. Joska

ADDRESS: Department of Psychiatry and Mental Health, J-block, Groote Schuur Hospital, Anzio Road, Observatory, 7925

CONTACT NUMBER: 021-404 2164/021-4042151

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- *The study will be conducted at the primary care antiretroviral clinics in Khayalitsha site C, Woodstock and Mitchells Plain. The study aims to include 200 HIV positive people and 50 HIV negative people.*
- *This study will perform a detailed interview when people start taking anti-retrovirals and again at one year, to find out if there are any problems in thinking or moving in people with HIV/AIDS. This is in order to understand why certain people with HIV/AIDS develop these problems. You will also be asked to provide a sample of blood- you will sign a **separate form** to provide this blood sample. You can decide not to give this sample if you wish, without it affecting any treatment you may receive. This blood sample will help us to understand HIV better in the future. Some people will be asked to undergo a brain scan.*
- *Patients who are eligible to enter to the study will be asked to sign this form. They will then have 2 interviews on one day of about 2 hours each, where they will be asked questions about themselves and their mental health. You will be given a break during these interviews and given refreshments. You will perform*

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certain tests, like memory tests and movement tests. The interviews (without blood tests) will be repeated at one year.

- *Not everyone who comes to the clinic will be asked to participate. We will choose people who are eligible, depending on if they have other mental problems or not..*
- *Apart from the interviews and tests, the study will not offer special treatment or medication. If a mental health problem is found, you will be referred for treatment at your nearest clinic. Any treatment related to HIV/AIDS you will also receive at your normal clinic.*

Why have you been invited to participate?

- *You have been invited to participate, because memory and thinking problems in HIV/AIDS are not properly understood by medical science. Younger people with these problems may demonstrate more clearly why they develop, in order for us to detect and treat these problems better in the future.*

What will your responsibilities be?

- *You will be required to attend the study visits on time and to participate as fully as possible. This means that you will answer questions as fully and honestly as possible. If there are questions you do not want to or cannot answer, you should say so.*

Will you benefit from taking part in this research?

- *You will benefit directly from the study in 2 main ways- first, a detailed mental health interview will be conducted, which will allow us to diagnose and treat any problems you may have. Second, any memory or thinking problems will be diagnosed, which will allow us to treat them if possible, but also to provide you with the assistance you need to manage with HIV/AIDS. In addition, information from this study may allow us to develop possible treatments for these problems, and to develop studies which will help us to understand these problems better.*

Are there in risks involved in your taking part in this research?

- *This study may make you feel uncomfortable as you talk about mental health problems. You may feel embarrassed or shy. Sometimes painful information is shared. Also, some people feel that is it better not to know about memory or thinking problems.*
- *During the second visit in this study you will have a type of brain scan which will be done at the Cape Universities Brain Imaging Centre at Tygerberg Hospital. This scan is called an MRI (magnetic resonance imaging) scan. The scan will require you to lie on your back on a table that will move into the scanning machine for the 85 minutes it will take for the scan. During this time you will be able to close your eyes and rest. You will also be able to talk to the study doctor/assistant at all times during the scan if you should experience any discomfort. The scan is a safe procedure if you have been screened correctly for the presence of any magnetic material on or inside you such as pace-makers, surgical clips and metal objects in the eyes. A formal screen for this will be done*

at the screening visit by a member of the study team. As the scan is done in a relatively confined space, occasionally people become anxious. This does not happen often, and if you feel anxious, we will spend time allowing you to get used to the surroundings before we begin. When the magnet in the machine is switched on, it will make some loud banging noises, but you will be clearly warned when this will take place. At this time you will feel nothing and the noise is not harmful to you in any way. To minimise the possible discomfort associated with this, we will give you some soft earplugs and will also put earphones on so that you can listen to music if you so choose.

- *These are the main risks. You should feel free to mention your feelings or concerns to any member of the study team*

If you do not agree to take part, what alternatives do you have?

- *You are free not to participate or to withdraw at any time during the study. Your treatment will not be affected in any way. You may continue to attend your clinic. It would be helpful for the study team to let us know why you have decided not to take part, but you are free to not give a reason.*

Who will have access to your medical records?

- *The information collected about you will be treated as confidential and protected. If it is used in a publication or thesis, your identity will remain anonymous. Only the direct study team will have full access to the information. If we need to refer you to a clinic for treatment, we will provide them with the relevant information needed to treat your condition.*

Will you be paid to take part in this study and are there any costs involved?

You will not be paid to take part in the study but your transport costs will be covered for each study visit- The study nurse will give you R150 for this. She will also provide the money it costs to attend the clinic. There will be no costs involved for you, if you do take part.

Is there any thing else that you should know or do?

- You should inform your family practitioner or usual doctor that you are taking part in a research study.
- You can contact Dr John Joska at tel 021-4042164 if you have any further queries or encounter any problems.
- You can contact the Research Ethics Committee of the Health Sciences Faculty of the University of Cape Town 021-4066338 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- If you would like a copy of this information and consent form for your own records, please ask a member of the study team to give you one.

Declaration by participant/guardian/treatment partner (circle)

By signing below, I agree/agree on behalf of..... to take part in a research study entitled: "Neurocognitive disorders in young adults with HIV/AIDS commencing anti-retro-viral treatment in the Western Cape".

I declare that (delete whichever is NOT applicable):

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that my taking part/my relative or friend's participation in this study is **voluntary** and I/we have not been pressurised to take part.
- I/my relative or friend may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I/my relative or friend may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) on (*date*) 200_.

.....
Signature of participant/guardian/treatment partner

.....
Signature of witness

Declaration by investigator

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above

Signed at (*place*) on (*date*) 200_.

.....
Signature of investigator

.....
Signature of witness

Informed Consent, Version 1, dated 06 Feb 2009

Initials _____

Sticker:				
CD4			J	Scoresheet
HIV-associated neurocognitive disorders study: Neuropsychological Test Summary Sheet				
Name		Gender:		M F
Date of examination (DD.MM.YYYY)		Years of education:		
Date of birth (DD.MM.YYYY)		Age:		
Examiner: JL JX JJ		Handedness:		R L
Examined in:		English	Xhosa	Afrikaans
A: Motor function				
				Z = (x-X)/SD
A1: Successive Finger Taps	Raw Score	Mean	SD	Deviation from mean
D(sec):		()	()	
ND(sec):		()	()	
A2: Grooved pegboard				
D(sec):		()	()	
ND(sec):		()	()	
B: Learning and memory				
B1: Hopkins Verbal Learning Test				
	Raw Score	Mean	SD	Deviation from mean
Trial 1				
Trial 2				
Trial 3				
Total				
Learning				
DR	(%)			
Recognition: true positives		Discrimination index		(True positives - false positives)
false positive errors:	related ()	unrelated ()		
B2: Rey Complex Figure	raw score	mean	SD	Deviation from mean
Copy				
3 min recall				
delayed recall				
B3: Brief Visuospatial Memory Test				
Form 1 2 3 4 5 6	Raw Score	T-score	Percentile	Deviation from mean
Trial 1				
Trial 2				
Trial 3				
Total recall				
Learning				
Delayed recall				
Percent retained				
Recognition hits				
Recognition false alarms				
Recognition Discriminatin Index				
Recognition response bias				

B: Learning and memory				
B4: Prospective Memory Test				
C: Attention				
C1: Mental alternation test				
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20	Raw Score	Mean	SD	
ii.alphabet	raw score	s	errors	score
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z	mean	s	sd	s
1 2 3 4 5 6 7 8 9 10	11 12 13 14 15 16 17	18 19 20	21 22 23	24 25 26
A B C D E F G H I J	K L M N O P Q	R S T	U V W	X Y Z
C2: WMS III Mental Control	raw score	SS		Deviation from mean
C3: WMS III- spatial span	Raw Scores (SS)			Deviation from mean
Forward: ()	Backward ()	Total	()	
D: Speed of processing		Raw Score (Scaled Score)		
D1: WAIS III: Digit symbol coding		()		
D2: WAIS III: Symbol search		()		
E: Executive function				
E1: Trail-making RS	Percentile	T-score	SD	Deviation from mean
Test A				
E2: Color Trail-Making				
i.part 1				
ii.part 2				
E3: Stroop Colour and Word Test	expected	residual	T (table IV)	
Word				
Colour				
colour word				
Interference				
E4: Wisconsin Card Sorting Test				
Categories completed				
Trials to first category				
Failure to maintain set				
Learning to learn				
F: Language				
G1: Category fluency test	Raw Score	Mean	SD	Deviation from mean
i.animals				
ii. Fruit and vegetables				
G: Intelligence				
WASI	Raw score	SS		Deviation from mean
Vocabulary test				
Block design				
Similarities				
Matrix reasoning				