HIV-Associated Neurocognitive Disorders:
Biomarkers and the Response to Antiretroviral Therapy

Thesis submitted to the University of Cape Town in fulfilment of the requirements for the degree of Master of Science in Medicine – Neuroscience (MM095)

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Abstract

Background: The human immunodeficiency virus (HIV) affects the central nervous system (CNS) causing a spectrum of neuropsychological impairment known as HIV-Associated Neurocognitive Disorders (HAND). Although highly active antiretroviral therapy (HAART) has decreased the incidence of HIV dementia, the milder forms of HAND have increased in prevalence. This suggests incomplete CNS viral control and potential drug toxicity. The CNS penetration-effectiveness (CPE) of HAART regimens may be an important factor. Additionally, there is need for biomarkers of HAND, to identify patients before the onset of functionally significant disease, for earlier treatment, and to monitor response.

Aims: This study aimed to determine whether HAART improved cognitive function in HIV positive people in South Africa, and whether this effect differed according to the CPE of the regimen used. I also investigated potential HAND biomarkers (serum neopterin, osteopontin and neurofilament H) to determine their relationship to the severity of cognitive impairment at baseline in HAART-naïve patients, and whether initial levels of these biomarkers related to the change in cognitive function a year later.

Methods: I reviewed 125 HIV positive patients with varying degrees of cognitive function, who were assessed initially (pre-HAART) and were followed up a year later. A neuropsychological test battery was administered at both visits to derive two sets of global deficit scores (GDS). Some of the participants remained HAART-naïve throughout this period, thereby providing a comparison for the effects of HAART. I measured biomarker levels, using immuno-assays, on stored serum samples from the baseline assessment.

Results: More HAART recipients maintained or improved cognitive function, and had greater beneficial change in GDS after one year, than those not on HAART. No significant difference in cognitive response was found between the higher and lower CPE HAART regimen groups. Neopterin levels were higher in the HIV positive group than in the HIV negative controls. Neopterin did not relate to baseline cognitive function in the HIV positive group. However, higher baseline neopterin levels were associated with maintenance of, or improvement in, cognitive function after one year. Osteopontin levels did not differ significantly between the HIV positive and negative groups, but did relate to cognitive function in the HIV positive group, with highest levels found in the severely impaired. Baseline osteopontin was not associated with change in cognitive function. Neurofilament H was largely undetectable in the serum.

Conclusions: This study showed that HAART was beneficial for cognitive function, and that regimen CPE did not affect cognitive outcomes after one year. This is a promising finding in the protocol-bound South African context, where the current first-line HAART regimen has a low CPE rating. Serum neopterin and osteopontin levels may have clinical use; however, further research is required to identify biomarkers for HAND in South Africa.

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Declaration

I, Helen Margot Cross, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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List of abbreviations

AAN	American Academy of Neurology
AD	Alzheimer's disease
ADRDA	Alzheimer's disease and Related Disorders Association
AIDS	Acquired Immunodeficiency Syndrome
ANI	Asymptomatic Neuropsychological Impairment
ANOVA	analysis of variance
APO E	apolipoprotein E
ART	antiretroviral therapy
ARV	antiretroviral
BBB	blood-brain barrier
BVMT	Brief Visuospatial Memory Test
CCR5	chemokine co-receptor five
CD4	cluster of differentiation four
CD4 count	CD4+ T cell count
CDC	Centers for Disease Control
CNS	central nervous system
CPE	central nervous system penetration-effectiveness
CPGR	Centre for Proteomic and Genomic Research
CRP	C-reactive protein
CSF	cerebrospinal fluid
CT	computerised tomography
CUBIC	Cape Universities Brain Imaging Centre
CXCR4	C-X-C chemokine receptor type four
df	degrees of freedom
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay

FTA – Abs	fluorescent treponemal antibody-absorption test
GDS	Global Deficit Score
GSH	Groote Schuur Hospital
gp120	glycoprotein 120
HAART	highly active antiretroviral therapy
HAD	HIV-Associated Dementia
HIV	human immunodeficiency virus
HAND	HIV-Associated Neurocognitive Disorders
HCV	hepatitis C virus
HDS	HIV Dementia Scale
HIVE	HIV encephalitis
HVLT	Hopkins Verbal Learning Test
IHDS	International HIV Dementia Scale
IL 1	interleukin one
IQR	interquartile range
MAT	Mental Alternation Test
MCP 1	monocyte chemotactic protein one
MMSE	Mini Mental State Examination
MND	Mild Neurocognitive Disorder
MRI	magnetic resonance imaging
NFH	neurofilament heavy chain
NFL	neurofilament light chain
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
NMDA	n-methyl-d-aspartate
NFκB	nuclear factor kappa B
OPN	Osteopontin

PMTCT	prevention of mother-to-child transmission
RCF	Rey (Osterrieth) Complex Figure
RNA	ribonucleic acid
RPR	rapid plasma reagin
S100β	S 100 beta
SCWT	Stroop Colour Word Test
SD	standard deviation
Tat	transactivator of transcription
TMTA	Trail Making Test A
TNF	tumour necrosis factor
UCT	University of Cape Town
VL	viral load
Vpr	viral protein R
WCST	Wisconsin Card Sorting Test

Chapter 1 – Introduction

The human immunodeficiency virus (HIV) is a retrovirus, belonging to the subfamily of lentiviruses, which are well known for their neurovirulence (Grant 2008; Haase 1986). The primary effects of HIV on the nervous system were noted early on in our experience with the disease (Grant *et al.* 1987; Snider *et al.* 1983; Ho *et al.* 1985, Gabuzda *et al.* 1987), yet the exact pathogenic mechanisms remain unclear. It seems that the central nervous system (CNS) is invaded early by HIV particles, although clinical effects are generally only seen later with advanced immunosuppression (Grant *et al.* 1987). The viral invasion of the CNS has both direct and indirect effects, yet most of the neurotoxicity seems to result from the indirect effects, namely, ongoing neuro-inflammation (Hult *et al.* 2008). The result of this neurotoxicity is a clinical spectrum of impairment, collectively known as HIV-associated neurocognitive disorders (HAND).

Highly active antiretroviral therapy (HAART) has had a dramatic effect on HIV infection worldwide, allowing for the systemic control of viral replication, which in turn allows for at least partial regeneration of the immune system. HAART has been less successful in terms of CNS disease. Although there has been a decrease in the incidence of the most severe form of HIV CNS disease, HIV dementia (HAD), the milder forms of cognitive impairment have actually increased in prevalence (McArthur 2004; Heaton *et al.* 2010). This is in part attributable to longer lifespan secondary to treatment with HAART, and potentially increased awareness and diagnosis of the problem. However, it also suggests incomplete control of the virus and its effects within the CNS, and potentially, neurotoxicity of the antiretrovirals (ARVs) themselves (Robertson *et al.* 2010).

The issue of treatment of HAND is therefore currently an important research area. Questions include the optimal choice of ARV agents, the timing of ARV initiation, as well as whether additional agents are required, perhaps targeting some of the other proposed pathogenic mechanisms.

In this regard, there is a need for biomarkers of HAND, which can identify susceptible patients before the onset of functionally significant disease, to be able to target them for earlier or additional treatment. Studies of such biomarkers could also help to elucidate the pathogenesis of this spectrum of disorders.

I therefore undertook to test a few potential biomarkers for HAND, in our setting, to assess whether they had any associative or predictive qualities. A largely retrospective review was undertaken of a cohort of HIV positive patients with varying degrees of cognitive impairment, who were seen initially at baseline, pre-HAART, and were then followed up after a period of one year. Some of these patients remained ARV-naïve throughout this period and therefore provided a useful group for comparison to determine the effects of HAART. Biomarker levels were measured on stored serum samples from the baseline assessment. I planned to assess the effect of HAART on cognitive status, and whether this status and its potential change showed any association with the initial measured biomarker levels. Furthermore, I wished to examine whether the CNS penetration-effectiveness of the antiretroviral regimen used, had effect on cognitive outcomes.

Chapter 2 – Rationale and motivation:

Background to the study with review of the literature

2.1 Introduction to chapter

This chapter aims to provide the reader with a background understanding of the HIV-Associated Neurocognitive Disorders, according to the current literature. It covers pathogenesis, clinical features, risk factors, diagnosis, and management of HAND in general, before focussing on HAND in the South African context. Finally the concept of biomarkers is introduced, followed by a discussion of current HAND biomarker research.

Most of this HAND literature has been based on studies conducted in Europe, North America and Australia. This is important as these regions represent developed world contexts, with a much lower prevalence and a different predominant subtype of HIV-1 to that found in South Africa. This could mean that our experience with HAND is different.

2.2 HIV-Associated Neurocognitive Disorders

2.2.1 Neuropathogenesis

2.2.1.1 Mechanisms of injury

The pathogenesis of HAND is still not yet fully understood. It is commonly accepted that HIV causes neuronal injury through direct viral effects, but also to a greater extent, through the inflammatory cascade which is created as a result of its presence within the central nervous system (Hult *et al.* 2008).

The process is thought to start with the early invasion of HIV particles into the CNS (Ho *et al.* 1985; Brew *et al.* 1989) typically arriving inside infected monocyte-derived macrophages ("Trojan Horse Theory") (Anthony *et al.* 2008; Gonzalez-Scarano *et al.* 2005; Haase 1986). These cells traverse the blood-brain barrier (BBB) as part of routine immune surveillance, yet there may be increased influx during HIV infection due to the effects of chemokines, and the

structural and functional disruption of the blood-brain barrier (BBB). This disruption of the BBB is mediated by inflammatory cytokines and the viral proteins gp120 (glycoprotein 120) and Tat (Transactivator of Transcription) (Hult *et al.* 2008; de Vries *et al.* 1997; Kanmogne *et al.* 2002).

It has also been suggested that transcytosis and endothelial cell infection are mechanisms of entry into the CNS, yet probably occurring to a much smaller degree (Bomsel 1997; Banks *et al.* 2001; Liu *et al.* 2002; Bobardt *et al.* 2004; Edinger *et al.* 1997; Gonzalez-Scarano *et al.* 2005).

Once inside the brain parenchyma, the HIV particles infect perivascular macrophages and tissue microglia, via interaction with their CD4 and CCR5 receptors (Hult *et al.* 2008). This infection may initially be latent, until advanced immunosuppression occurs. These cells then become productive of HIV particles in their own right, and also begin to secrete a variety of inflammatory cytokines. These chemicals in turn, cause neuronal injury, dendritic damage and, ultimately, apoptosis of neurons. The latter process occurs primarily through excitotoxicity with over-activation of n-methyl-d-aspartate (NMDA)-coupled ion channels, excess influx of calcium and oxidative stress (Hult *et al.* 2008).

Astrocytes may also be infected by HIV, via unclear mechanisms. They do not have CD4 receptors, but they do have the chemokine co-receptors CCR5 and CXCR4. This infection however, is restricted, in that it does not lead to production of new virions. The important supportive functions of the astrocytes, namely buffering of neurotransmitter levels and the production of neurotrophic factors, do however become disrupted and there may be arachidonic acid and nitric oxide release (Anthony *et al.* 2008).

Many of the HIV proteins have toxic effects (Ghafouri *et al.* 2006). Tat promotes an increase in neuronal intracellular calcium, the development of reactive oxygen species and caspase activation, leading to apoptosis. It also affects endothelial permeability causing blood-brain barrier dysfunction, and stimulates further cytokine production by macrophages and microglia (Ghafouri *et al.* 2006). Viral protein R (Vpr) causes cell cycle arrest at the G2/M phase, which leads to cell death. It may also alter mitochondrial permeability (Ghafouri *et al.* 2006). Gp120 has direct neurotoxic effects by interacting with the NMDA receptor and disrupting neuronal calcium homeostasis (Ghafouri *et al.* 2006). It also has indirect effects by stimulating cytokine production from macrophages and arachidonic acid from astrocytes (Ghafouri *et al.* 2006). (See figure 2.1, p6).

Although the virus and its products have direct neurotoxic effects, the inflammatory cascade that is initiated appears to cause the majority of the CNS damage (Hult *et al.* 2008). This concept is supported by a study by Glass *et al.* (1995) which showed that the number of activated microglia and macrophages is a better correlate for HIV-associated dementia than the number of HIV-infected cells in the brain. It has also been suggested that this mechanism of inflammatory injury can be self-sustaining in a "hit and run" type phenomenon (Nath *et al.* 1999; Brew *et al.* 2008). This means that once initiated by the presence and effect of HIV particles, the inflammatory cascade within the CNS could remain activated, even if the HIV particles are largely removed by the effects of HAART. This could have implications for the treatment of HAND.

An alternative model has been suggested more recently. Gartner (2000) proposes that abnormal systemic inflammation in late stage AIDS leads to the production of increased macrophage-colony stimulating factor, which causes altered monocyte production in the bone marrow, with an increase in the CD14+CD16+ subset. This subset usually makes up about 6% of monocytes, but in late AIDS has been found to be about 16%, and in patients with HAD, about 37%. These cells are extremely neuro-invasive and release high levels of pro-inflammatory cytokines (especially tumour necrosis factor alpha and interleukin 1-beta). It is thought that it is the invasion of these cells into the CNS late in the course of the disease, and the resultant neuro-inflammation that is induced, that leads to the damage associated with clinical HAND.

2.2.1.2 Pathological correlates

Many pathological changes have been identified in the brains of HIV infected persons; most commonly HIV encephalitis (HIVE). The characteristic features of HIVE are multinucleated giant cells, perivascular macrophage accumulation, astrocytic hyperplasia, and microglial nodules (Anthony *et al.* 2008). Other findings include generalised atrophy (secondary to neuronal apoptosis and white matter rarefaction), myelin pallor, and dendritic, synaptic and axonal damage (Anthony *et al.* 2008). Of these changes, synapto-dendritic damage correlates best with cognitive impairment (Everall *et al.* 1999), and may be reversible with effective treatment.

2.2.1.3 Sites of involvement

Any area of the brain could be affected in theory, however the sites predominantly affected appear to be the basal ganglia, the central white matter and the frontal cortex (Anthony *et al.* 2008; Langford *et al.* 2003). These sites correlate with the presentation of a sub-cortical dementia. Morphometric studies have shown a 40% reduction in brain tissue density in the frontal and temporal regions of the brain (Ketzler *et al.* 1990; Masliah *et al.* 1992a), and a 50-90% reduction in the hippocampus (Masliah *et al.* 1992b). Immunochemistry studies have shown that the areas worst affected in terms of synapto-dendritic damage are the striatum and hippocampus (Moore *et al.* 2006).

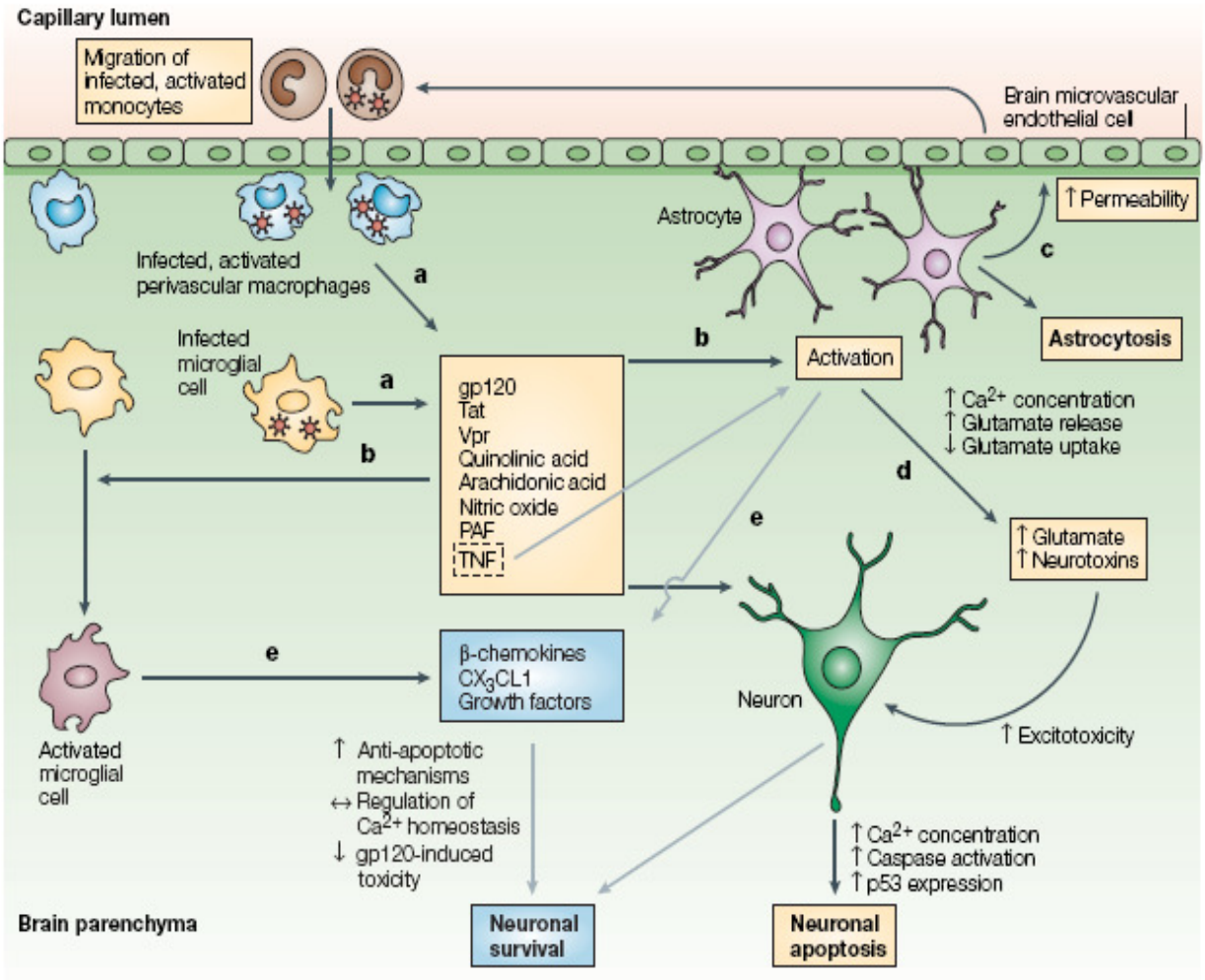

Figure 2.1: *Mechanisms of neurodegeneration and neuroprotection in AIDS*

From: Gonzalez-Scarano F, Martin-Garcia J. The Neuropathogenesis of AIDS. *Nature Reviews Immunology* 2005; **5**: 69-78.

2.2.2 Clinical features

2.2.2.1 Typical clinical picture

HIV-associated CNS dysfunction is a clinical spectrum which presents typically with cognitive, motor and behavioural features (McArthur *et al.* 2005). In its initial stages the impairments may be subtle, and are often overlooked or misdiagnosed as depression (Grant 2008).

Early features may include "short-term" (episodic) memory loss, mental slowing, comprehension difficulties and apathy. Signs that may be detectable include mild gait disturbance, tremor, impairment of fine manual dexterity, impairment of rapid eye and limb movements, hyperreflexia, hypertonia and frontal release signs. Of note, apraxias and aphasias are typically absent (Grant 2008; Ghafouri *et al.* 2006). In terms of neuropsychological impairment, psychomotor speed, memory and executive function are usually worst affected in the initial stages.

Later on the cognitive deficits become more widespread, with the overall clinical picture that of a sub-cortical dementia. This is often accompanied by features of a symmetrical distal sensory polyneuropathy, also due to the effects of HIV, on the peripheral nervous system (McArthur *et al.* 2005).

HIV infection can also result in other pathology in the nervous system, through its indirect effects such as immune suppression or immune dysfunction, which allows for opportunistic infections, neoplasia and autoimmune disorders (see Figure 2.2).

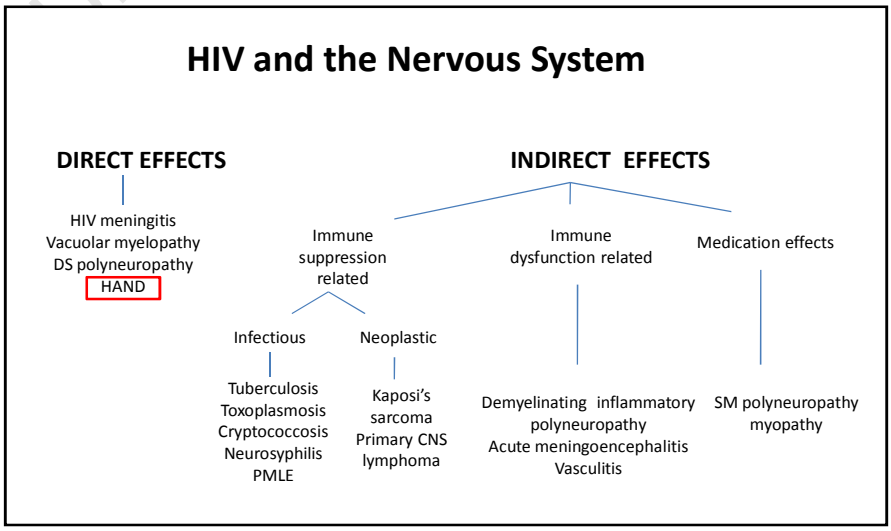

Figure 2.2: *The effects of HIV on the nervous system*

2.2.2.2 Clinical course

The clinical course of this disorder has changed with the introduction of HAART therapy (Woods *et al.* 2009). In the pre-HAART era, the condition typically took the form of a sub-acute progressive dementia, often leading to death within six months (Bouwman *et al.* 1998). This pattern would still be true for untreated patients.

This contrasts with the situation in HAART-treated patients. Firstly, the life expectancy after a diagnosis of HAD is now considerable longer; 39.6 months in one study in Australia (Dore *et al.* 2003). Secondly, milder forms of cognitive impairment are now much more prevalent than actual dementia, and thirdly, different forms of HAD are now recognisable (Nath *et al.* 2008). A more slowly progressive dementia may be found in patients on HAART who have incomplete virological control, this could be termed chronic active dementia. Patients with virological suppression on HAART, with stable neurological signs and symptoms and possibly even some recovery, could be said to have chronic inactive dementia. Patients on effective HAART therapy may experience a major improvement in terms of cognitive function, and could even be said to have had reversible dementia. This classification is entirely clinical and can only be applied retrospectively.

Probably the most significant change in the HAART era has been the increase in prevalence of milder forms of HAND, which may or may not indicate increased risk of progression to dementia.

2.2.2.3 Classification

As mentioned above, HIV-induced neurotoxicity within the CNS results not in a single clinical disorder but rather, a spectrum of cognitive impairment. Creating clinical definitions has been a challenge since the mid 1980s, when it was recognised that HIV-related CNS disorders were not limited to opportunistic infections, and that the virus itself was able to enter the CNS and cause pathology. At this stage it was noted to result in a syndrome of cognitive and motor dysfunction - a sub-cortical dementia - that generally progressed fairly rapidly and often led to death (Navia *et al.* 1986). This was termed the AIDS dementia complex or HIV Dementia, and became included as a Centers for Disease Control (CDC) AIDS defining condition in 1987 (Rackstraw 2011; Grant 2008).

In 1991 the classification expanded to incorporate a seemingly similar, but far milder form of dysfunction. This was compiled by the American Academy of Neurology (AAN), and defined

HIV-associated dementia complex and minor cognitive motor disorder. HIV-associated dementia complex implied severe cognitive, motor and or emotional or personality disturbances with marked impact on everyday life, whilst in minor cognitive motor disorder the symptoms were far milder and had less functional impact. The major criticisms of this classification were that it was too loosely defined (meaning a diagnosis of dementia could be made without severe cognitive symptoms) and that it did not recognise an entity of cognitive impairment which was subtle enough not to impair everyday function.

Therefore the classification was updated by an AAN working group assembled in Italy in 2005, with the support of the United States National Institutes of Health. The results of this meeting were published by Antinori *et al.* in 2007, and detail the new widely accepted clinical definitions for HIV-associated Neurocognitive Disorders (Antinori *et al.* 2007) (See Table 2.1).

Asymptomatic Neurocognitive Impairment (ANI) is defined as "acquired impairment in cognitive functioning, involving at least two ability domains, documented by performance of at least 1.0 SD below the mean for age-education-appropriate norms on standardised neuropsychological tests." (Antinori *et al.* 2007). This testing should cover language ability, attention/working memory, abstraction/executive function, memory (learning and recall), speed of information processing, sensory-perceptual and motor skills. Importantly, in this category, the cognitive impairment has no noticeable impact on everyday functioning. This contrasts with Mild Neurocognitive Disorder (MND) which is also an impairment of at least 1.0 SD below the mean, but produces at least mild interference with daily functioning.

HIV-1-associated Dementia (HAD) is a "marked acquired impairment in cognitive functioning, involving at least two ability domains; typically the impairment is in multiple domains, especially in learning of new information, slowed information processing, and defective attention/concentration." The impairment is objectively measured as at least 2 SD below the demographically corrected mean. The impairment produces marked interference with daily functioning (Antinori *et al.* 2007).

Across all of the categories, there must be no pre-existing cause or other explanation for the cognitive impairments, other than HIV, and no delirium (Antinori *et al.* 2007). Motor difficulties were initially considered to be cardinal in the diagnosis of HIV-associated dysfunction, but more recently it has been decided that the cognitive features are more central, hence the change in the diagnostic criteria (Grant 2008).

HIV-associated asymptomatic neurocognitive impairment (ANI)*
1. Acquired impairment in cognitive functioning, involving at least two ability domains, documented by performance of at least 1.0 SD below the mean for age-education-appropriate norms on standardized neuropsychological tests. The neuropsychological assessment must survey at least the following abilities: verbal/language, attention/working memory, abstraction/executive, memory (learning, recall), speed of information processing, sensory-perceptual, motor skills
2. The cognitive impairment does not interfere with everyday functioning.
3. The cognitive impairment does not meet criteria for delirium or dementia.
4. There is no evidence of another pre-existing cause for the ANI*.
** If there is a prior diagnosis of ANI, but currently the individual does not meet criteria, the diagnosis of ANI in remission can be made.*
** If the individual with suspected ANI also satisfies criteria for a major depressive episode or substance dependence, the diagnosis of ANI should be deferred to a subsequent examination conducted at a time when the major depression has remitted or at least 1 month after cessation of substance use.*
HIV-associated mild neurocognitive disorder (MND)**
1. Acquired impairment in cognitive functioning, involving at least two ability domains, documented by performance of at least 1.0 SD below the mean for age-education-appropriate norms on standardized neuropsychological tests. The neuropsychological assessment must survey at least the following abilities: verbal/language, attention/working memory, abstraction/executive, memory (learning, recall), speed of information processing, sensory-perceptual, motor skills. Typically this would correspond to an MSK scale stage of 0.5 to 1.0.
2. The cognitive impairment produces at least mild interference in daily functioning (at least one of the following): a) Self-report of reduced mental acuity, inefficiency in work, homemaking, or social functioning. b) Observation by knowledgeable others that the individual has undergone at least mild decline in mental acuity with resultant inefficiency in work, homemaking, or social functioning.
3. The cognitive impairment does not meet criteria for delirium or dementia.
4. There is no evidence of another pre-existing cause for the MND**.
*** If there is a prior diagnosis of MND, but currently the individual does not meet criteria, the diagnosis of MND in remission can be made.*
*** If the individual with suspected MND also satisfies criteria for a severe episode of major depression with significant functional limitations or psychotic features, or substance dependence, the diagnosis of MND should be deferred to a subsequent examination conducted at a time when the major depression has remitted or at least 1 month after cessation of substance use.*
HIV-associated dementia (HAD) * **
1. Marked acquired impairment in cognitive functioning, involving at least two ability domains, typically the impairment is in multiple domains, especially in learning of new information, slowed information processing, and defective attention/concentration. The cognitive impairment must be ascertained by neuropsychological testing with at least two domains 2 SD or greater than demographically corrected means. (Note that where neuropsychological testing is not available, standard neurological evaluation and simple bedside testing may be used, but this should be done as indicated in algorithm, see below) Typically this would correspond to an MSK scale stage of 2.0 or greater.
2. The cognitive impairment produces marked interference with day-to-day functioning (work, home life, social activities).
3. The pattern of cognitive impairment does not meet criteria for delirium (e.g. clouding of consciousness is not a prominent feature); or, if delirium is present, criteria for dementia need to have been met on a prior examination when delirium was not present.
4. There is no evidence of another pre-existing cause for the dementia (e.g. other CNS infection, CNS neoplasm, cerebrovascular disease, pre-existing neurologic disease, or severe substance abuse compatible with CNS disorder). ***
****If there is a prior diagnosis of HAD, but currently the individual does not meet criteria, the diagnosis of HAD in remission can be made.*
**** If the individual with suspected HAD also satisfies criteria for a severe episode of major depression with significant functional limitations or psychotic features, or substance dependence, the diagnosis of HAD should be deferred to a subsequent examination conducted at a time when the major depression has remitted or at least 1 month has elapsed following cessation of substance use. Note that the consensus was that even when major depression and HAD occurred together, there is little evidence that pseudodementia exists and the cognitive deficits do not generally improve with the treatment of depression.*

Table 2.1: Revised research criteria for HIV-associated neurocognitive disorders (HAND) (modified from HIV Neurobehavioural Research Center criteria)

From: Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* 2007; **69**: 1789-1799.

2.2.2.4 Neuropsychological Impairments

It was accepted by the early 1980s that there were primary CNS complications associated with HIV. However, the idea of cognitive impairment which was directly due to the effects of HIV, including during the medically asymptomatic stages, remained controversial until 1987 when a comprehensive study on HIV-associated neurocognitive impairments was published by Grant *et al.* This study was conducted on ambulatory participants, in whom there was no clinical suspicion of an underlying neurological disease, and it provided evidence of multiple domains of cognitive impairment, across the spectrum of HIV disease. The most prominent abnormalities were in executive function, episodic memory and information processing speed (Grant *et al.* 1987).

The neuropsychological impairments found in HIV disease have subsequently been well studied (Heaton *et al.* 1995 – see figure 2.3; Reger *et al.* 2002). It is now acknowledged that almost any cognitive ability could be compromised, but that there is a more typical pattern which tends to occur (Grant 2008). This pattern fits with the classification of HAND as an entity due predominantly to sub-cortical dysfunction.

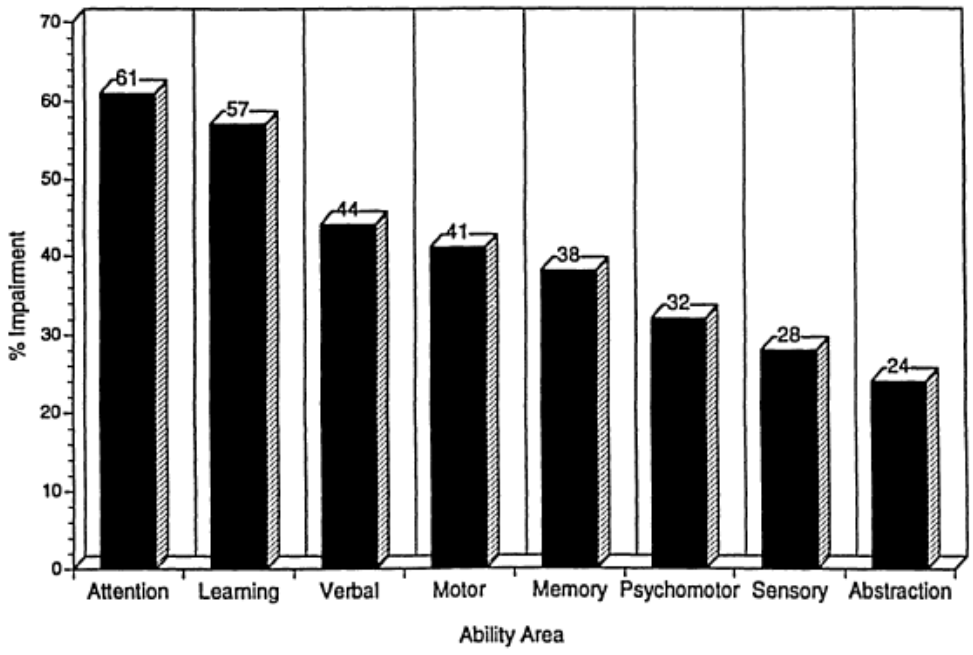

Figure 2.3*: Prevalence of neuropsychological deficits in HIV positive subjects (percentage of subjects with impairments in these domains)*

From: Heaton RK, Grant I, Butters N, White D, Kirson D, Hampton Atkinson J *et al.* The HNRC 500 – Neuropsychology of HIV infection at different disease stages. *Journal of the International Neuropsychological Society* 1995; **1**: 231-251.

Attention embraces a variety of functions, including focussing on a particular stimulus, whilst filtering out others, orientating to that stimulus and maintaining focus on it. Assessing one's surroundings and focussing in on a stimulus needs to happen quite quickly, and is linked therefore to speed of information processing. There is also an overlap with working memory, as information needs to be maintained and manipulated to complete a task. Therefore tests of attention often have overlap with these two domains. Simple attention tasks (such as Trail Making Test A) can normally be quite easily completed by HIV positive individuals, whilst more complex attention tasks (such as the Digit Symbol Test), especially under time pressure, often present difficulty (Grant 2008; Vally 2011).

Memory disturbance is prominent, especially episodic memory (Vally 2011). This is seen in difficulty with learning and the retrieval of new information (Grant 2008). Learning is the process by which new information is moved from immediate storage (working memory) to intermediate storage. There are two different kinds of memories at this stage: explicit and implicit memories.

Explicit (also known as declarative) memory involves people, things, events or ideas whereas implicit (or procedural) memory relates to the learning of skills or procedures. Explicit memory in particular is found to be defective in HIV (Grant 2008). This can be evaluated with tests of logical memory such as the learning of stories, which is very sensitive to early HIV deficits (Grant 2008).

From intermediate storage, information often needs to be consolidated before it can be moved to long term storage (semantic memory). In HIV, semantic memory is usually well preserved or only very mildly impaired, with no temporal gradient such as that typically found in Alzheimer's disease (Grant 2008). If there is difficulty in recalling long term memories, it is more likely to be due to problems with search and retrieval, indicating executive dysfunction. Thus, in summary, the problem with regards to memory in HAND is with the learning and retrieval of new information.

Language is not typically affected by HIV, except for reduced fluency, which is slowed generation of words, or speech in general. There may also be poor word choice and inappropriate interruptions during conversations (Vally 2011), although these may reflect executive dysfunction rather than language dysfunction. This contrasts with cortical dementias, where aphasia is common (Grant 2008).

Motor speed and co-ordination difficulties are often prominent in the later stages of HIV. Impairments include bradykinesia, hypomimia, hand tremor and bradyphrenia (Vally 2011). They are easily detected through tasks such as the Finger Tapping Test which requires rapid movements, or the Grooved Pegboard Test which tests speed as well as fine motor co-ordination (Grant 2008).

Deficits in executive function are common in HIV. These include difficulties with tasks such as abstraction, set shifting, response inhibition and decision making (Grant 2008), which are associated with impairment in everyday functioning (Vally 2011).

Earlier studies suggested that visuospatial function is largely unaffected by HIV, however newer evidence suggests that there may be subtle impairments (Vally 2011).

Except in cases of frank dementia, the above deficits are often subtle and may vary according to the individual. Therefore a structured approach is required to create an overall assessment of a person's neuropsychological function. This is done by testing with a battery of validated neuropsychological tests, to cover all the relevant domains, and then through the creation of a summary score. The reference standard is considered by most to be clinical ratings by suitably trained, experienced neuropsychologists (Carey *et al.* 2004). A more computational approach which is far less labour intensive, is however, usually required. A well validated method is the Global Deficit Score approach (Heaton *et al.* 1995; Carey *et al.* 2004).

An overall T score is calculated (from the individual's performance in comparison to the demographically adjusted normative values) for each ability domain. This T score is then assigned a deficit score, according to the schema devised to by Heaton *et al.* (1995), which is displayed in Table 2.2.

T scores	Deficit Score
≥ 40	0
39-35	1
34-30	2
29-25	3
24-20	4
≤19	5

Table 2.2: *T scores and deficit scores*

From: Heaton RK, Grant I, Butters N, White D, Kirson D, Hampton Atkinson J *et al.* The HNRC 500 – Neuropsychology of HIV infection at different disease stages. *Journal of the International Neuropsychological Society* 1995; **1**: 231-251.

The Global Deficit Score is then calculated by adding the Deficit Scores from each domain, and dividing by the total number of added scores. As T scores of 40 or more (which indicate performance within normal limits) are assigned a deficit score of 0, whilst T scores in the impaired range are assigned progressively higher scores, this method of summarising the results from a neuropsychological test battery emphasises poorer performance. This allows for the detection of more subtle impairment which may otherwise be lost in simple mean calculations across test results.

A cut point (for determination of global impairment) of ≥0.50 has been shown to have very good diagnostic power: sensitivity 0.77, specificity 0.92, positive predictive value 0.88, negative predictive value of 0.83 and overall diagnostic accuracy rate, as compared to clinical ratings by experienced neuropsychologists, of 0.85. The likelihood ratio of detecting neuropsychological impairment is 9.38. (Carey *et al.* 2004).

In the same study by Carey *et al.* (2004), it was shown that 93% of the participants who had a global deficit score of ≥0.50, also had impairment in at least two ability domains as determined by clinical ratings (Carey *et al.* 2004). This is important as the diagnostic criteria for HAND require diagnosis of impairment in at least two ability domains (Antinori *et al.* 2007).

The global deficit score approach has advantages in that it provides a continuous measure of impairment as opposed to a categorical measure. It also avoids the need to assess and score activities of daily living and related functional impairment, which is always a challenge.

2.2.2.5 Functional Significance

HAND is common, with 30-60% of HIV positive individuals being affected (Grant 2008), and can have multiple adverse effects on the patients' lives, including difficulties with social and occupational functioning and potentially medication adherence (Heaton *et al.* 2004). With the success of HAART therapy and now much longer life expectancy for HIV infected persons, management of HIV has shifted from palliation to chronic disease management, and quality of life concerns such as the above have become all the more important (Rackstraw 2011).

2.2.2.6 Risk factors / modulators

Disease stage

Risk for HAD increases with advanced immunosuppression (Childs *et al.* 1999). This is evidenced by the much higher rates of dementia in the pre-HAART era, when CD4+ T cell (CD4) counts routinely fell below 200 cells/ul, and most HIV patients reached end stage AIDS. It was also recently confirmed in a large 15 year study in Italy looking at HAND prevalence and risk factors (Balestra *et al.* 2011) and in another study in Uganda (Wong *et al.* 2007). Now, with HAART, we have an effective treatment option which can halt disease progression in the majority and allow for immune recovery. However, relatively high rates of HAND remain even with HAART. It has been shown that the nadir rather the current CD4 count (which may be high due to immune recovery on HAART) is important in terms of predicting risk of cognitive impairment (Ellis *et al.* 2011; Balestra *et al.* 2011). This suggests that the nadir CD4 count represents a "legacy event", at which a critical level of immunosuppression was reached causing permanent neural damage (Ellis *et al.* 2011). This study also showed the converse, that HIV positive patients who never experienced low CD4 counts were relatively protected from cognitive dysfunction. This suggests that we should be initiating HAART at an earlier stage. Although most international (and South African) guidelines currently recommend a CD4 count of 350 cells/ul to be the cut-point for HAART initiation, much debate continues as to whether a cut-point of CD4 count of 500 cells/ul might not be better. Studies are currently underway to investigate this possibility.

Viral clade

It has been suggested that there may be a difference in the neurotoxic potential amongst the different viral clades (subtypes). HIV-1 is the viral strain most commonly found globally. It is subdivided into 3 classes M (main), O (outlying) and N (new). More than 90% of the global HIV-1 burden is class M. Class M is then further subdivided into 9 clades, according to viral envelope diversity (A-D, F-H, J and K) (Sacktor *et al.* 2007).

About 50% of the world's HIV positive population is infected with clade C virus; making it the most common subtype worldwide (Gannon *et al.* 2011). This is probably related to the fact that it is the predominant clade in sub-Saharan Africa and parts of Asia, including India and China (Gannon *et al.* 2011). However clade B is the predominant form found in Europe, North

America and Australia, and thus most published data for HIV-1 relates to clade B (see figure 2.4).

This differing neurotoxic potential has been attributed to differences in the viral protein Tat amongst the subtypes (Mishra *et al.* 2008). Clade B was initially thought to be more neurotoxic (Rao *et al.* 2008) and clade C less so, due to a defect in the dicysteine motif in tat in clade C virus (Mishra *et al.* 2008). However this idea has not been supported by clinical studies in India (Gupta *et al.* 2007) and South Africa (Joska *et al.* 2010b), where rates of HAND in these clade C regions have been found to be equivalent to those in Clade B regions.

A study from Uganda (Sacktor *et al.* 2009) found clade D to cause worse cognitive outcomes than clade A. A different study in Uganda (Laeyendecker *et al.* 2006) and another in Tanzania (Vasan *et al.* 2006) found clade D to be more rapidly progressive in general. More rapid disease progression and therefore immunosuppression, would most likely impact on cognitive function given the link between nadir CD4 count and HAND.

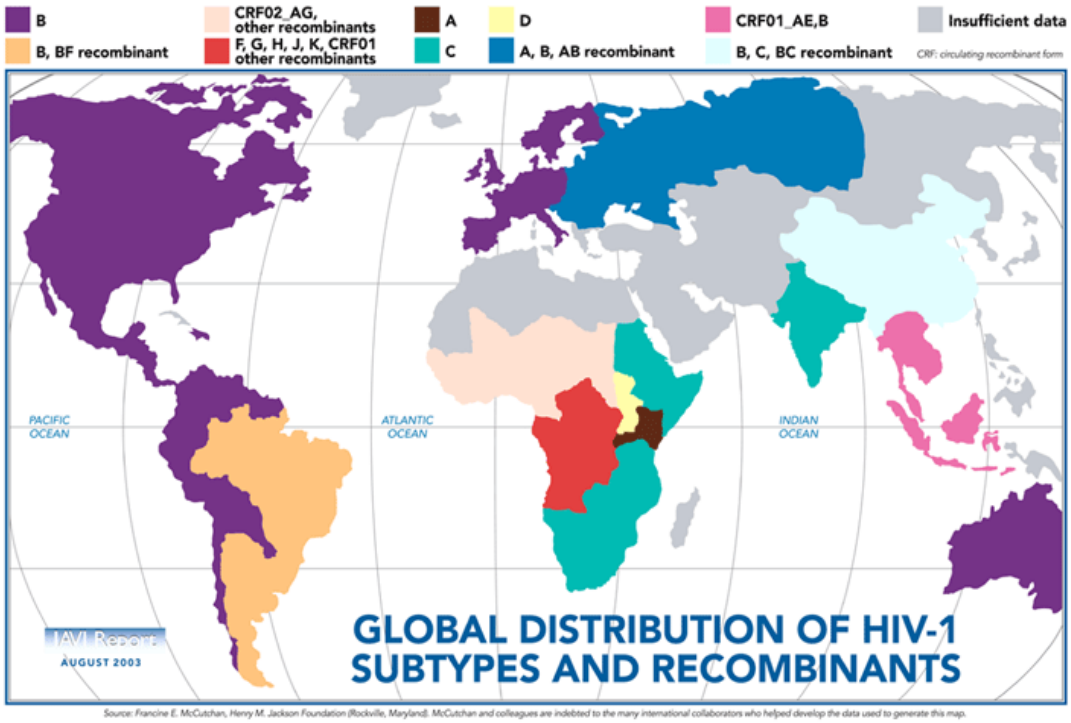

Figure 2.4: *Global distribution of HIV-1 subtypes (clades) and recombinants*

From: International AIDS Vaccine Initiative (IAVI) Report, August 2003 (vol 7)
http://www.iavireport.org/archives/2003/Pages/default2.aspx

Host genotype

Differences in host genotype may play a role in increasing susceptibility to HAND. Some studies have shown the E4 allele of apolipoprotein E (APOE) to be associated with the severity of cognitive impairment in HIV (Corder *et al.* 1998; Spector *et al.* 2010), possibly by increasing vulnerability to oxidative stress. The APOE E4 allele has been shown to be strongly associated with an increased risk for Alzheimer 's disease (Corder *et al.* 1993; Saunders *et al.* 1993; Poirier *et al.* 1993). However, other studies have not supported the idea that the E4 allele confers increased risk of HAD (Joska *et al.* 2010d; Burt *et al.* 2008; Dunlop *et al.* 1997).

Genetic polymorphisms in monocyte chemoattractant protein-1 (MCP-1) or its receptor (CCR2) may influence the risk of HAD by altering CNS inflammatory responses (McArthur *et al.* 2005; Singh *et al.* 2004; Gonzalez *et al.* 2002).

Polymorphisms of the tumour necrosis factor alpha (TNF-α) promoter gene may also increase the risk of HAD by increasing production of the neurotoxic TNF-α in response to inflammatory stimuli (McArthur *et al.* 2005; Quasney *et al.* 2001; Nath *et al.* 2008).

Co-morbidities

Anaemia has been suggested to be a risk factor for HAD (McArthur *et al.* 1993), but given that in the HIV positive population it tends to occur with the severe weight loss that occurs with advancing suppression, it is uncertain whether it can be considered a stand-alone risk factor.

In the developed world there is a high rate of hepatitis C virus (HCV) and HIV co-infection (15-30%; increasing to 80% in IV drug abusers) (Nath *et al.* 2008). HCV has been shown to be neurotropic and can cause significant neuropsychological impairment (even in the absence of liver dysfunction) (Kramer *et al.* 2002), yet rarely severe enough to cause dementia. Some studies have shown an additive effect in terms of cognitive dysfunction in co-infected patients (Letendre *et al.* 2002; Ryan *et al.* 2004), but other studies have not supported this idea (Clifford *et al.* 2009). The prevalence of HCV infection in South Africa is reported to be very low (Firnhaber C *et al.* 2008).

Substance abuse

HIV and substance abuse often occur together, especially in the developed world where intravenous drug abuse and sharing of needles is one of the major forms of HIV transmission (Nath *et al.* 2008). It has been shown that HIV positive people who abuse drugs have more rapid progression to more severe forms of cognitive dysfunction (Bouwman *et al.* 1998; Nath *et al.* 2001). Alcohol abuse has also been shown to increase the risk of HAND (De Ronchi *et al.* 2002; Joska *et al.* 2010c). This may be due to the additive effects of neurotoxicity, as well as the psychosocial implications of drug abuse, such as poor medication adherence (Nath *et al.* 2008). It has also been suggested that intravenous drug abuse increases the risk of cognitive impairment through systemic immune activation (Ancuta *et al.* 2008).

Socio-demographic factors

Lower level of education has been linked to increased risk of cognitive impairment in HIV in a number of studies (Joska *et al.* 2010b; Balestra *et al.* 2011).

There has been a dramatic increase in the number of older people living with HIV. This is related mostly to longer lifespan due to HAART therapy, but also reflects the fact that older people are now more commonly becoming infected with HIV. Older age has been shown to be a risk factor for HAND (Balestra *et al.* 2011; Joska *et al.* 2010b; Wong *et al.* 2007; Valcour *et al.* 2004). There is evidence to suggest that the ageing brain is more vulnerable to HIV-associated neurotoxicity (Gannon *et al.* 2011; Ernst *et al.* 2004; Cherner *et al.* 2004). However, it is still uncertain whether the resultant increased capacity for cognitive impairment is due to acceleration of the effects of HIV neuropathogenesis, or due to other factors such as the effect of HIV on other neurodegenerative processes, or the effect of age-related co-morbidities (Gannon *et al.* 2011). The long term metabolic effects of ART, especially the protease inhibitors, may also play a role in cognitive dysfunction, by increasing vascular risk factors and probably cerebrovascular disease.

The interplay of HIV and other neurodegenerative conditions has raised much interest. There is growing evidence for the accumulation of abnormal proteins in HIV positive brains, including hyperphosphorylated tau, amyloid and alpha-synuclein (Valcour *et al.* 2011a; Gannon *et al.* 2011). The accumulation of these proteins is linked to disorders such as Alzheimer's disease and Parkinson's disease. The HIV protein tat has been implicated in cerebral amyloid aggregation, in that it inhibits neprilysin, an amyloid-beta degrading enzyme (Hult *et al.* 2008).

2.2.3 Management of HAND

2.2.3.1 Screening and diagnosis

It is important to be aware of the possibility of cognitive impairment in all patients seen with HIV, especially considering that the early features of HAND may be quite non-specific. Forgetfulness, mental slowing, poor concentration, low mood or even just poor medication adherence should alert the practitioner that further investigation is required (Rackstraw 2011). A thorough psycho-social history is imperative to detect possible dysfunction not otherwise mentioned by the patient. This should cover occupation, managing finances, meal preparation and driving abilities (Rackstraw 2011). The European AIDS Clinical Society Guidelines 2009 advise that any cognitive complaint in an HIV positive person should be assessed with a full neurological examination, neuropsychological testing, CSF examination and CNS imaging. It also advises further investigation for cognitive dysfunction in all patients with a detectable plasma viral load, a CD4 count nadir of less than 200 cells/ul, an ARV regimen with limited CNS penetration or ongoing depression (Rackstraw 2011). A large proportion of the HIV positive people in South Africa fall into these categories, suggesting that we need more stringent screening guidelines.

The gold standard for assessing cognitive function remains a full neuropsychological test battery, covering multiple domains of function, administered by an experienced neuropsychologist (Anthony *et al.* 2008; Cherner *et al.* 2007). According to the recommendations of the National Institute of Mental Health workgroup in 1990, optimal sensitivity requires an extensive battery, taking seven to nine hours to administer. Ideally this battery should include two to four tests per cognitive domain, with the focus on domains known to be affected by HIV (Butters *et al.* 1990). With increased experience in HIV neuropsychological testing, more focussed batteries have been devised, usually taking only one to two hours to administer. There has also been emphasis on making tests locally applicable in terms of language and cultural norms, for optimal results.

Even a focussed neuropsychological test battery is however generally not feasible in most settings, especially resource-limited South Africa. Several screening tools have been developed to assist in this regard, yet while some have been shown to be valuable in detecting HAD, detecting the more subtle forms of HAND using bedside tests remains challenging.

The Mini Mental State Examination (MMSE) is not a good screening tool for HAD, as it focuses on detecting cortical rather than sub-cortical deficits (Sacktor *et al.* 2005). Its validity as a psychometric test is also dependent on educational level and cultural background (Vally 2011).

The HIV Dementia Scale (HDS) was developed by Power *et al.* in 1995. It consists of 4 subtests which examine memory (four word recall), psychomotor speed (timed written alphabet test), construction ability (cube copying) and executive function (anti-saccadic error test). It is scored out of 16 with a score of 10 or less indicating possible HAD. Used in this way, it has been shown to have a sensitivity of 80%, specificity of 91% and a positive predictive value of 78% for identifying HAD (Power *et al.* 1995) as compared to formal neuropsychological assessment for HAD.

Many recent studies have looked at the utility of the HDS in detecting the milder forms of HAND (Bottiggi *et al.* 2007; Smith *et al.* 2003; Simioni *et al.* 2010). Smith *et al.* and Bottiggi *et al.* found the tool to be not sensitive enough, except in frank dementia, whilst Simioni *et al.* found that by increasing the cut-off to 14 points out of 16, the detection of HAND as a whole can be greatly increased. For HIV positive patients with cognitive complaints and a score of $\leq$ 14, Simioni *et al.* (2010) found that a possible HAND diagnosis can be detected with sensitivity of 83%, specificity of 63% and a positive predictive value of 92%. The criticisms of the HDS are that it may be unsuitable for patients of non-Western education, and that the anti-saccadic error subtest is difficult for non-neurologists to administer (Sacktor *et al.* 2005).

The International HIV Dementia Scale (IHDS) was developed by Sacktor *et al.* (2005) as a cross-cultural test which aimed to address the limitations of the HDS. It emphasises motor function and speed. There are three subtests: a four word recall, a timed finger tapping test and a timed alternating hand sequence test (based on the Luria Motor Sequence). The test is scored out of 12, with a score of $\leq$ 10 indicating possible HAD, requiring further assessment (Sacktor *et al.* 2005). The screening tool was validated by Sacktor *et al.* in the United States and in Uganda, where they found similar rates of sensitivity (80%) and specificity (55-57%) for detecting possible HAD. A study assessing the validity of this tool in South Africa was performed by Joska *et al.* (Joska *et al.* 2011b), who found it to be less useful overall, with a sensitivity of only 45% and a specificity of 79%.

A new screening algorithm has been proposed by Cysique *et al.* (2010) which utilises non-cognitive clinical information (recognised HAND risk factors) in a computer-based

mathematical equation to determine risk of neuropsychological impairment (Cysique *et al.* 2010). Although shown to have sensitivity of 70% and specificity of 78% for detecting HAD, it is unlikely to be a feasible option in the South African public sector in the near future given the requirement of a computer for use during the patient consultation.

2.2.3.2 General management approach

The only current effective therapy for HAND is HAART. HAART aims for complete virological suppression, which in turn allows for immune recovery. The optimal timing of initiation is still under investigation. Earlier initiation to avoid immunosuppression appears beneficial in terms of cognitive protection. However, this must be balanced with the fact that HAART is life-long therapy that has several potential side-effects, and for which resistance may develop over the long term. Most current international guidelines recommend initiation of HAART when the CD4 count falls below 350 cells/ul.

Part of the management of HAND also involves optimising the patient's general condition. Co-morbidities should be aggressively managed, as should risk factors for cerebrovascular disease. Good medication adherence must be encouraged and the patient should engage in physical and social activities.

2.2.3.3 HAART: The effect of HAART in HAND

Since the introduction of HAART, clear improvements have been seen in terms of CNS disease. Most notably, the incidence of HAD has decreased in the developed world from approximately 16% to less than 5% of AIDS patients (Heaton *et al.* 2011; McArthur *et al.* 1993; Heaton *et al.* 2010). A recent systematic review revealed that studies largely agree: HAART induces a significant improvement in neurocognitive status, typically within 6 months of treatment (Joska *et al.* 2010a). This does not, however, imply a complete recovery in all cases, and in fact incident cases of cognitive dysfunction can arise even whilst on suppressive ARV regimens. Unfortunately, high levels of the milder forms of HAND (prevalence approximately 50%) do remain even in the era of HAART (Heaton *et al.* 2010; Robertson *et al.* 2007; Simioni *et al.* 2010).

Causes for the continually high rate of HAND are uncertain, yet several theories have been proposed.

Irreversible brain injury may occur prior to the introduction of HAART (Heaton *et al.* 2011). According to current local and international guidelines, HAART is initiated only once the CD4 count falls below 350 cells/ul, unless there are special circumstances (SA DOH 2010, 2011; WHO 2010). In most cases this will allow many years of untreated HIV infection. Additionally, recent work looking at the effects of acute HIV infection on the CNS suggests that damage is done as early as a few days after initial infection (Ragin *et al.* 2011; Valcour *et al.* 2011). Optimal management of acute HIV infection is still uncertain, but perhaps early initiation of HAART may be warranted (McPhail *et al.* 2011).

The blood-brain barrier (BBB) is selectively permeable, allowing passage of molecules according to factors such as size, protein-binding and lipophilicity. Additionally it has other mechanisms such as the P-glycoprotein membrane efflux transporter, which makes it difficult for substances to pass into the central nervous system. In this way it restricts the access of antiretroviral agents, and also creates a compartment which is quite secluded from the rest of the body.

Antiretroviral drugs differ in their ability to cross the BBB, and in some cases this may result in inadequate levels for viral suppression. This observation has led to the development of the CNS penetration-effectiveness (CPE) ranking system, published initially by Letendre *et al.* in 2008 and revised in 2010. This system was devised after extensive pharmacodynamic and pharmacokinetic investigations which showed the differing abilities of ARV agents to cross the BBB and establish viral suppression in the CSF. According to the ranking system, each agent is assigned a score according to its chemical properties, CSF-plasma concentrations and effectiveness in achieving CNS HIV control (1 = below average; 2 = average; 3 = above average and 4 = much above average).

A regimen score is calculated by the sum of the individual agent scores. A regimen with a total score of seven or less is considered to have low penetration within the CNS, whilst a score of more than seven is considered to have a high penetration (Letendre *et al.* 2011). There is convincing evidence that this system correctly identifies regimens which will achieve control of HIV replication within the CNS, that is, those regimens with higher CPE have been shown to be associated with CSF viral load (VL) suppression (Letendre *et al.* 2008). However studies looking

at the correlation between cognitive outcomes and higher CPE regimens, have shown mixed results (Tozzi *et al.* 2009; Rourke *et al.* 2011; Letendre *et al.* 2004; Marra *et al.* 2009). In this regard it is important to remember that CSF viral load is not necessarily equivalent to brain viral load. Post-mortem studies have shown higher levels of HIV in brain tissue than in the CSF of the same patients (Kumar *et al.* 2007).

This phenomenon of differing CSF concentrations of ARV agents in the CNS compartment could also have implications in terms of the development of resistance. Suboptimal suppression could allow resistant strains to emerge, which could then continue to cause neurotoxicity despite ART, and which could create a risk of systemic reseeding later on (Hult *et al.* 2008). The seclusion of the CNS compartment from systemic immune responses also allows for a reservoir of latently infected cells which cannot be targeted by ART and which could act as a driver for ongoing neurodegeneration.

Given that the CNS damage associated with HIV infection is largely immune-mediated, HAART may simply be inadequate treatment (Heaton *et al.* 2011). Although the control of ongoing viral replication (which HAART targets) is undoubtedly important in stemming the neuro-inflammatory response to some extent, more focussed anti-inflammatory therapy may be required. Unfortunately, to date, studies examining adjunctive therapies have mostly had disappointing results (see table 2.3).

HAART itself may have CNS neurotoxic properties, especially considering the well described toxic effects on peripheral nerves (Heaton *et al.* 2011). A magnetic resonance spectroscopy study found evidence of brain mitochondrial damage associated with the use of nucleoside reverse transcriptase inhibitors such as stavudine and didanosine (Schweinsburg *et al.* 2005). Another basic science study examined the effect of different ARVs (across the differing mechanistic classes), on neuronal integrity and function (Liner *et al.* 2009). Almost all the ARV agents induced some degree of neuronal dysfunction.

Additionally, clinical studies have also indicated potential ART neurotoxicity. In an important study by Robertson *et al.*, a cohort of 167 patients who were on successful ARV regimens in terms of viral suppression and immune recovery (all had CD4 count above 350 cells/ul), had their treatment stopped for a period of 96 weeks. There was a statistically significant improvement in the mean neuropsychological test scores, indicating an improvement in cognitive function off ART (Robertson *et al.* 2010). The discrepancy in study findings with

regards to cognitive outcomes and higher CPE regimens, could be related to neurotoxicity of the ARVs. Marra *et al.* (2009) found higher CPE regimens to be associated with poorer cognitive outcomes.

Finally, other factors must also be considered, such as the effect of co-morbidities and ageing in the now longer-living HIV positive population (Joska *et al.* 2010a). These factors may be important in increasing susceptibility to, or worsening already existing, cognitive dysfunction. This longer-living HIV positive population may also be a source for the increased prevalence of HAND, even with effective HAART therapy.

2.2.3.4 Other treatment strategies

Methods of circumventing the problem of drug access across the BBB are being investigated, which include the use of nanotechnology for the delivery of ART (Nowacek *et al.* 2009).

Additionally, given the largely immune-driven pathogenesis and the poorer than expected cognitive response to HAART, many other treatment approaches are being investigated (see table 2.3). Unfortunately none has yet shown strong enough evidence of efficacy (Simioni *et al.* 2011). It has been suggested that perhaps this is related to short follow-up time and the lack of biomarkers to allow early identification of HIV positive individuals at increased risk of cognitive decline, which would allow for more appropriate trial samples (Simioni *et al.* 2011). Additionally, perhaps, a combination of these agents and HAART would have the best result (Simioni *et al.* 2011).

Review of neuroprotective agents studied for use in HAND	
Antioxidants	
OPC-14117	Trend towards cognitive improvement shown in small phase II trial (Dana Consortium 1997)
Selegiline (oral and tranSDermal routes)	Initial trial showed significant efficacy in HAND, but effect has not been reproduced in larger trials. (Sacktor *et al.* 2000; Schifitto *et al.* 2007; Evans *et al.* 2007; Schifitto *et al.* 2009a)
Anti-apoptotic drugs	
Lithium	No clear neuropsychological benefit (Letendre *et al.* 2006; Schifitto *et al.* 2009b), but an MRS study showed an improvement in CNS injury (Schifitto *et al.* 2009b)
Calcium channel blockers	
Nimodipine	No significant cognitive change, but a trend toward cognitive improvement with higher doses (Navia *et al.* 1998)
CCR5 Antagonist	
Peptide T	No significant cognitive benefit, but trend toward improvement in the more severely impaired and those with higher CD4 counts (Heseltine *et al.* 1998)
PAF antagonist	
Lexipafant	No significant cognitive benefit. Suggested trend towards improvement. (Schifitto *et al.* 2002)
TNF antagonist	
CPI-1189	No cognitive benefit (Clifford *et al.* 2002)
NMDA antagonist	
Memantine	No cognitive benefit, although potential neuroprotective effects shown with MRS study

Table 2.3: *Review of neuroprotective agents studied for use in HAND*

Adapted from Table 2 in: Simioni S, Cavassini M, Annoni J, Hirschel B, Du Pasquier R. HIV- associated neurocognitive disorders: a changing pattern. *Future Neurology* 2011; **6** (1): 81-95.

2.3 HAND in South Africa

The majority of the worldwide HIV burden lies within sub-Saharan Africa. The 2010 edition of the UNAIDS Report on the global AIDS epidemic states that there are approximately 33.3 million people living with HIV infection worldwide. Of these, 22.5 million are in sub-Saharan Africa (UNAIDS 2010). However the prevalence of HAND in this region is poorly defined due to the paucity of studies conducted here, compared with Europe and the United States.

The HIV literature from the developed world may not be applicable in South Africa given the difference in viral clades. These different clades (or subtypes) are due to the genetic diversity of the HIV-1 virus, mostly secondary to the high error rate of the reverse transcriptase enzyme and lack of exonuclease proof reading activity. There are 9 different clades (A-D, G-H, J and K), which are found in different parts of the world (Sacktor *et al.* 2007). Clade B predominates in Europe and the US, whereas in sub-Saharan Africa, clade A, D and C are found.

Clade C is the predominant HIV-1 subtype in South Africa. Studies have been done in the Cape Town region which show a high proportion of its HIV positive population is infected with HIV-1 clade C virus (Orrell *et al.* 2009; van Harmelen 1999). Orrell *et al.* (2009) in fact found 98% of the HIV positive study participants from Cape Town to be infected with clade C virus.

A few studies have been done in distinct Clade C regions, suggesting a high risk for moderate to severe HAND (Joska *et al.* 2010b; Yepthomi *et al.* 2006; Lawler *et al.* 2010).

HAART only became readily available within South Africa's public sector in 2003 (as opposed to 1996 in the developed world), and access remains poor in some areas. Additionally, until very recently, national guidelines only allowed that the majority of patients be started on HAART when their CD4 count fell below 200 cells/ul (SA DOH 2010). This is in contrast to the 350 cells/ul which is advised by the World Health Organisation (WHO) (WHO 2010), and to which South African guidelines were amended in August 2011 (SA DOH 2011). If a diagnosis of HIV-associated dementia is made, the patient can be classified as WHO Disease Stage IV, and will then be considered for HAART regardless of CD4 count (SA DOH 2010). However no provision is currently made for the milder forms of HAND. The fact that the South African HAART threshold was CD4 count less than 200 cells/ul for so long, could allow for a higher prevalence

of HAND in our setting, given that more advanced immunosuppression has been related to increased risk of HAND (Heaton *et al.* 2010).

In addition to the heavy burden of HIV disease, South Africa is a resource-poor setting. This equates to busy, often under-resourced clinics, where there is minimal time for lengthy patient consultations which may be necessary to detect the more subtle forms of HAND. Furthermore, we are bound by protocol in the public sector, which means limited scope for individualised ARV choices.

Of note, however, is the change in the South African Antiretroviral Guidelines in April 2010. This change was largely aimed at discontinuing the use of the agent Stavudine, which has a very unsatisfactory side-effect profile. Therefore the first line regimen changed from Stavudine, Lamivudine and either Efavirenz or Nevirapine, to Tenofovir, Lamivudine and either Efavirenz or Nevirapine. Although this new regimen is largely much better tolerated, it has a lower CPE ranking (7 or 8 previously, compared to 6 or 7 currently) which may be significant in terms of cognitive outcomes in HIV. Also of interest is that the first line regimen for pregnant women, Zidovudine, Lamivudine and Nevirapine, has a CPE ranking of 10. There is a significant proportion of South African women using this regimen, as pregnancy is a time when many women undergo testing and initiation of ART, if necessary, as part of the National Prevention of Mother-to-Child Transmission (PMTCT) Programme. The regimen would then be continued after pregnancy unless unacceptable side-effects or treatment failure necessitated a change.

Further research on HAND in South Africa is needed to determine our unique disease profile and response to HAART therapy, as well as our greater management needs. Clinically useful biomarkers with the ability to identify patients with increased risk of cognitive decline are also urgently needed, and could provide evidence to support a HAART protocol change which would cater for patients with the milder forms of HAND. Additionally, further research into the CPE implications of our local regimens could provide evidence necessary for considering individualised ART.

2.4 Biomarkers

2.4.1 Introduction

At a theoretical level, a biomarker (biological marker) is an indicator of a biological state. Most often the interest in biomarkers lies in the potential for diagnostic or predictive value. They offer the possibility for an objective measure in a condition which may otherwise be a purely clinical diagnosis. They could also be used to identify people at increased risk for a given condition, which could allow for more focussed and possibly earlier treatment for these people. This ability would also allow for the optimal choice of participants for clinical trial populations for potential new therapies.

Biomarkers could really be any measurable entity relating to a biological state; however chemical and radiological biomarkers are generally most studied.

Chemical markers are substances found in body fluids. In HAND, that would usually be either blood or cerebrospinal fluid (CSF). CSF probably offers a closer reflection of what is occurring in brain tissue, yet requires a much more invasive procedure to acquire. Alternatively, blood is more accessible and is far more frequently sampled. Given that HIV is a multisystem disease, and that the pathogenesis of HAND is linked to systemic immune function, examining substances in the peripheral circulation can still be extremely informative in terms of HAND. I therefore believe that the investigation of serum markers deserves further attention.

The two main biomarkers of HIV disease already thoroughly incorporated into clinical practice are the HIV RNA level which indicates the viral load in the measured body fluid, and the CD4+ T lymphocyte count, which gives an indication of immunodeficiency (Mildvan *et al.* 2005). Further potential biomarkers are being investigated.

There has been much recent work in the developed world around biomarkers of HIV CNS disease in particular, which could help with the identification and categorisation of HAND (McArthur *et al.* 2005). It is hoped that biomarkers might be able to help not only with diagnosis and prediction of prognosis of HAND, but also be able to assist in management options. This might include being able to identify patients with active versus inactive HIV CNS

disease, and to assist with choosing optimal ARV regimens and in the assessment of response to treatment (Brew *et al.* 2009).

The biomarkers under investigation are largely drawn from three important areas in the pathogenesis of HAND, namely viral markers, markers of immune activation and markers of neurodegeneration. Most work has been done on cerebrospinal fluid. However, there are also important markers which can be measured in the peripheral blood.

2.4.2 Viral biomarkers

Viral markers include HIV RNA, HIV DNA and specific viral proteins. HIV DNA is created through the reverse transcription of viral RNA, and is then incorporated into the host cell genome. Plasma levels (measured from peripheral monocytes) therefore reflect latent infection. A correlation with HAND has been proposed, as levels were found to be increased in patients with clinical features of HAND (Shiramizu *et al.* 2005; Shiramizu *et al.* 2007). A correlation was also found between the level of HIV DNA in monocytes and cognitive function, in a group of patients prior to ART initiation and after 48 weeks on treatment (Valcour *et al.* 2009).

2.4.3 Immunological biomarkers

The markers of immune activation that have been most studied are neopterin, beta-2-microglobulin, quinolinic acid and monocyte chemotactic protein 1 (MCP1/CCL2) (Price *et al.* 2007). There are however multiple other potential immunological biomarkers.

2.4.3.1 Neopterin

Neopterin is a product of guanosine triphosphate metabolism, which is produced by macrophages which have been stimulated by interferon gamma. It can therefore be considered as a marker for cellular immune activation (Huber *et al.* 1984). It is found in human body fluids during times of cellular immune responses, for example during viral, bacterial and parasitic infections, and with autoimmune disease, malignancy and allograft rejection (Wirleitner *et al.* 2005).

Neopterin levels are raised in the body fluids of HIV positive individuals throughout the course of their illness. Levels are high around the time of seroconversion, and are detectable before

measurable antibody production. (For this reason, neopterin levels are used in blood and organ donation screening.) In the early asymptomatic phases the levels are low, but remain above normal in about 80% of HIV positive individuals, and rise gradually with the progression of the disease. Levels decrease with HAART (Wirleitner *et al.* 2005). (See figure 2.5).

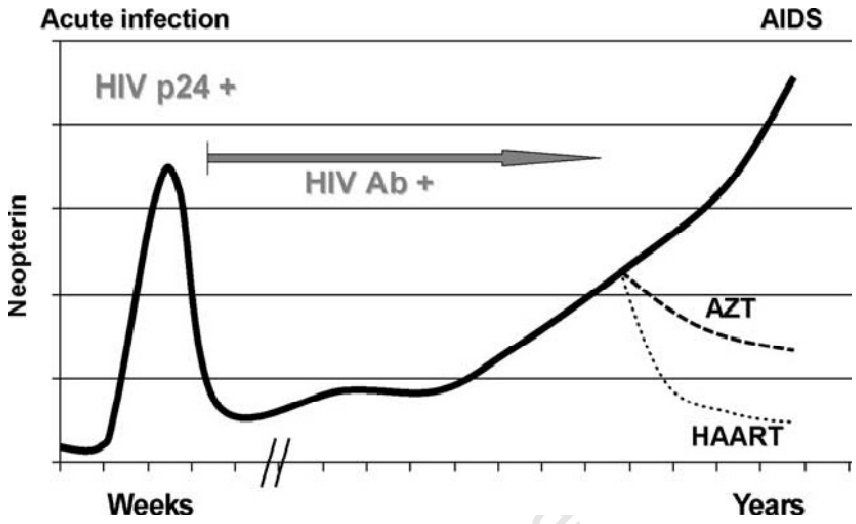

Figure 2.5: *Schematic diagram of the course of neopterin development over time in HIV positive patients.*

From: Wirleitner B, Schroeksnadel K, Winkler C, Fuchs D. Neopterin in HIV-1 Infection. *Molecular Immunology* 2005; **42**: 183-194.

Neopterin has been shown to have a significantly inverse correlation with CD4+/CD8+ T cell ratios and absolute T cell numbers (Fuchs *et al.* 1988). Neopterin is able to discriminate the different clinical stages of HIV infection as well as CD4 count (Hutterer *et al.* 1987). Neopterin levels in the serum and urine have therefore been shown to be useful markers in the course and progression of HIV disease in general (Hagberg *et al.* 2010; Kitchen *et al.* 2008; Mildvan *et al.* 2005). Neopterin is able to pass though the BBB and serum levels usually have a linear relationship to CSF levels (Andersson *et al.* 2001).

Higher neopterin levels have been demonstrated in the CSF of patients with HIV-associated cognitive impairment, as compared to neurologically asymptomatic patients (Fuchs *et al.* 1989; Brew *et al.* 1990; Hagberg *et al.* 2010). These levels were also shown to have a correlation with cognitive function, in that higher levels were found in the more impaired patients when compared to the minimally impaired patients (Hagberg *et al.* 2010). Neopterin levels decreased in response to ART, yet remained elevated above normal level (Hagberg *et al.* 2010; Hagberg *et al.* 2004). Blood levels of neopterin were also found to be higher in the cognitively impaired group than in the neurologically asymptomatic group (in those with CD4 counts

higher than 200 cells/ul), but to a lesser extent than in the CSF, indicating intrathecal immune activation as a primary source of neopterin in HAND (Hagberg *et al.* 2010).

2.4.3.2 Osteopontin

Osteopontin is a soluble protein found in most body fluids. It is produced by a variety of cells, including macrophages. As its name suggests, it was originally identified as a substance found in bone. It forms part of the bone matrix and assists with the regulation of mineralization. It was subsequently also discovered to be a pro-inflammatory cytokine with immune modulating effects (O'Regan *et al.* 2000; Wang *et al.* 2008). It can therefore be seen as an inflammatory marker.

Osteopontin was shown to have a potentially important role in the pathogenesis of HAND, through its effects on monocytes, in a study by Burdo *et al.* (2007). These researchers used *in vitro* models to examine the effects of osteopontin on human monocytes. One model consisted of a simulated BBB made of endothelial cells grown on a collagen gel. Although it was not found to have classic chemotactic effects for monocytes, it was shown that osteopontin has retention effects on monocytes (especially the CD14+/CD16+ variety). When applied to the human context, this means that osteopontin can prevent reverse transmigration over the BBB and out of the CNS (Burdo *et al.* 2007). Osteopontin was also shown to protect these monocytes from apoptosis (Burdo *et al.* 2007). Given that the monocyte/macrophages are the principal cell types for HIV replication in the CNS, and are important in mediating the resultant neurotoxic inflammatory cascade, these effects which ultimately increase the monocyte/macrophage population within the CNS must have an important role in the development of HAND (Burdo *et al.* 2007).

Burdo *et al.* also showed that plasma osteopontin levels were raised in monkeys with Simian immunodeficiency virus encephalitis, as compared to control Simian immunodeficiency virus monkeys without encephalitis (Burdo *et al.* 2007).

Osteopontin was recently also shown to be raised in the plasma of HIV positive human individuals, with minimal decrease with HAART (Chagan-Yasutan *et al.* 2009). Furthermore, plasma levels of osteopontin were shown to be significantly increased in patients with HIV dementia, and a significant correlation was shown between plasma osteopontin levels and HIV CNS dysfunction (Burdo *et al.* 2008). This study examined both humans and rhesus monkeys.

The monkey component of the study also had a longitudinal arm looking at plasma osteopontin levels in relation to the development of simian AIDS and encephalitis. Here it was found that the plasma osteopontin levels increased before the onset of neurological abnormalities (Burdo *et al.* 2008), suggesting a possible predictive role for the substance.

Another study showed that osteopontin can stimulate HIV-1 replication (through activation of the nuclear factor kappa B (NFκB) pathway), and further confirmed that high levels of osteopontin, this time in cerebrospinal fluid, remained even with HAART (Brown *et al.* 2011).

2.4.3.3 C-Reactive Protein (highly sensitive)

The SMART study, which looked primarily at the effects of episodic versus continuous antiretroviral therapy, also included a sub-study on biomarkers. One of the studied substances was C-reactive protein (CRP), a systemic pro-inflammatory marker, which was shown to be associated with mortality risk in HIV positive patients (Kuller *et al.* 2008). Two recent studies have shown a correlation between elevated serum CRP and cognitive decline in elderly HIV negative patients (Hoth *et al.* 2008; Komulainen *et al.* 2007). The relationship of serum CRP to HAND has not been investigated (Wright *et al.* 2009).

2.4.4 Neurodegenerative biomarkers

Markers of neurodegeneration include neurofilament protein, tau and S100 beta (Morris *et al.* 2010).

2.4.4.1 Neurofilament protein

Neurofilament (NF) protein is a major structural element of neurons, especially the large myelinated variety, where it is involved in the maintenance of axonal integrity. It is released not only from dying, but also dysfunctioning neurons (Petzold 2005). It has three subunits – light (L), intermediate (I) and heavy (H).

The light subunit has been well studied in the CSF, where it has been shown to be a sensitive biomarker of axonal damage, both for acute conditions as well as for various neurodegenerative diseases (Mellgren *et al.* 2007; Rosen *et al.* 2004; Rosengren *et al.* 1999) CSF NFL has been shown to be raised significantly but non-specifically in HAD, to decrease with

HAART, as well as to rise with HAART interruption (Abdulle *et al.* 2007; Gisslen *et al.* 2005; Mellgren *et al.* 2007). Another study found CSF NFL to have predictive qualities for the development of HAD (Gisslen *et al.* 2007).

Neurofilament heavy chain (NFH) is highly phosphorylated and is therefore more stable than NFL, and much more resistant to proteases. This means it can be measured in the peripheral blood (Petzold 2005). Plasma NFH has been shown to be a marker of neuronal injury in multiple sclerosis (Gresle *et al.* 2011) as well as other neurodegenerative conditions, like Alzheimer's disease and Vascular Dementia (Petzold 2005).

NFH has also been studied in HIV infection. Anderson *et al.* examined NFH levels in the CSF of HIV positive individuals, as well as the CSF of individuals with multiple sclerosis and other neurological disorders (Anderson *et al.* 2011). NFH levels were shown to be significantly higher in HIV disease than in other neurological diseases. However the levels within HIV disease did not relate specifically to the level of cognitive function, with raised CSF levels found even in cognitively normal patients (Anderson *et al.* 2011). This is important as it shows that detectable CNS neurodegeneration is occurring before the onset of neurological symptoms. Another interesting finding in this study pointing to the possible utility of NFH as a biomarker for HAND, was that on serial CSF determination, those who deteriorated cognitively also had an associated increase in their CSF NFH levels (Anderson *et al.* 2011). No correlation was found between the CSF NFH levels and the CPE of the HAART regimens used (Anderson *et al.* 2011).

2.4.4.2 Tau

Tau is a microtubule-associated protein, found in the axons of CNS neurons, where it has important structural functions, as well as being involved in axoplasmic flow. It is released extracellularly after CNS injury. It can therefore be seen as a marker of axonal CNS damage (Kavalci *et al.* 2007; Ramlawi *et al.* 2006). Two main types can be measured: total tau and phosphorylated tau. Both reflect neuronal damage non-specifically. Both forms have been shown to be elevated in the CSF of neurologically symptomatic HIV patients (Brew *et al.* 2005).

Tau has also been successfully assayed in serum, and correlated to CNS injury following head injury and stroke (Shaw *et al.* 2002; Bitsch *et al.* 2002). Another study correlated increased serum tau with neurocognitive decline after cardiopulmonary bypass surgery (Ramlawi *et al.*

2006). Interestingly, this study also showed increased CRP and inflammatory cytokines to be associated with neurocognitive decline (Ramlawi *et al.* 2006).

2.4.4.3 S-100 beta

S-100 is an acidic calcium-binding protein which is found in dimer forms with alpha and beta subunits (Brew *et al.* 2008). S-100 beta (S-100β) is found almost exclusively in astrocytes (Pemberton *et al.* 2001). It has been shown to be a marker of astrocytosis (Pemberton *et al.* 2001) and raised levels are found in a number of inflammatory, degenerative and traumatic CNS conditions (Lamers *et al.* 2003), making it a useful biomarker for brain injury (Chaves *et al.* 2010). More specifically, raised CSF S-100β has been shown to be associated with increased HAND severity and a quicker decline to HAD (Pemberton *et al.* 2001). S-100β can be measured in the CSF as well as the blood (Lamers *et al.* 2003). A recent study examined serum S-100β levels in Alzheimer's disease, and found a positive correlation between these levels and the severity of dementia (Chaves *et al.* 2010). S-100β has a short half-life, therefore constantly elevated levels indicate continuous release from damaged tissue, making it a worthwhile marker of ongoing neurodegeneration (Stroick *et al.* 2006).

Most HAND biomarker research has focussed on single markers, either in the CSF or blood, but no one marker has yet been shown to fulfil one or more of the desired functions with sufficient sensitivity and specificity. It has been suggested that investigating the different types of markers together in a combined approach may produce better results (Price *et al.* 2007).

Chapter 3 – Aims and hypotheses

This study had two main aims:

Firstly, to determine whether HAART improved cognitive function in HIV positive patients in South Africa, and more specifically whether this effect differed according to the CNS penetration-effectiveness of the antiretroviral regimen used.

Secondly, to determine whether the proposed inflammatory and neurodegenerative biomarkers related to the severity of cognitive impairment at baseline in HAART-naïve patients, and whether initial levels of these biomarkers could be correlated with the change in cognitive function a year later.

In order to address these aims, specific hypotheses were drafted.

Hypothesis one: HAART of at least ten months will improve cognitive function in HIV positive ARV-naïve patients with a CD4 count of less than 350 cells/ul and cognitive dysfunction at baseline. That is, participants with impaired cognitive function at baseline, as assessed using a battery of neuropsychological tests to derive a global deficit score, will show improvement one year later, after at least ten months of HAART therapy; while participants with impaired cognitive function at baseline, and a CD4 count less than 350 cells/ul, who have not been on HAART therapy will not show improvement one year later.

It was stipulated that all study participants analysed to test this hypothesis, should have a CD4 count of less than 350 cells/ul. This was necessary for an accurate comparison to determine the effects of HAART, as otherwise differing levels of immunosuppression (as reflected by the CD4 count) would have been a confounding factor in this analysis.

Of note, HAART was not purposefully withheld from any study participants. Reasons for the participants with a CD4 count of less than 350 cells/ul not to have received HAART included the change in the national HAART initiation threshold during the study period, and in some instances, failure of HAART initiation, despite eligibility, at the ART clinics. The reasons for the latter were uncertain. As this work forms part of a greater study, which was initiated prior to

this study, it was apparent at the time of the hypothesis drafting that such a group existed for further analysis.

At least ten months of HAART therapy was chosen as the cut-off point for the HAART analysis, as the follow-up assessment of cognitive function was done after twelve months. This was to ensure that the baseline assessment was a true pre-HAART assessment of the participants, when they were theoretically at their worst in terms of immunosuppression and the effects of untreated HIV disease. As the follow-up period was not adjustable, if a shorter treatment duration cut-off time had been chosen, for example six months, there might have been a longer period between the baseline cognitive assessment and initiation of HAART. This would have allowed for a further period of decline in cognitive function and CD4 count, meaning that the baseline would not have been a true baseline.

Hypothesis two: HAART is protective of cognitive function; that is, HIV positive patients with normal cognitive function at baseline who then use HAART for at least 10 months, will still have normal cognitive function at the one year follow-up visit, as opposed to HIV positive patients with normal cognitive function at baseline who do not receive HAART.

Hypothesis three: HAART regimens with greater CNS penetration-effectiveness (regimen CPE more than seven) will be more effective in improving cognitive outcomes in HIV positive patients, compared with HAART regimens with a lower CNS penetration-effectiveness (regimen CPE seven or less); such that patients on HAART regimens with a CPE of more than seven will have greater cognitive improvement at the one year follow-up visit, than those on regimens with a CPE of less than or equal to seven.

The categorisation of the CPE regimen scores into “low” and “high” was done according to the scheme devised by Letendre *et al.* (2010; 2011) (see section 2.2.3.3, p22). A regimen score is calculated by the sum of the individual agent scores. A regimen with a total score of seven or less is considered to have poor effect in the CNS, whilst a score of more than seven is considered optimal in terms of achieving HIV viral suppression within the CNS (Letendre *et al.* 2010; 2011).

Examination of the cognitive effects of high versus low CPE regimens had become clinically relevant, given the change in the South African National Antiretroviral Therapy guidelines in early 2010 (see p27).

Hypothesis four: Serum levels of the inflammatory markers neopterin, osteopontin and the neurodegenerative marker neurofilament H protein, will be significantly different in the HIV positive group as compared to the HIV negative control groups.

Three different control groups needed to be included in this study: Firstly, HIV negative participants, drawn from the same demographic areas as the HIV positive study subjects, and according to the same inclusion and exclusion criteria (except for HIV status). This was required to compare the effect of HIV on the biomarker levels.

Secondly, a group of HIV negative participants with another neurodegenerative disorder causing cognitive impairment, to compare the biomarker profile of HAND to this other disorder. As there was an Alzheimer's disease (AD) study being conducted by other members of my research group and for which I was involved with patient assessments, this was an obvious choice. However, given the very different demographic settings of the HAND and the AD studies, a third group of HIV negative participants was required, as demographically similar controls to the AD participants.

With the above groups it was possible to observe the effect of both HIV infection and cognitive dysfunction on the biomarker levels.

Unfortunately only three potential biomarkers could be tested due to practical constraints (limited stored serum volumes) and financial restrictions.

Ethical permission was not granted for CSF sampling, and therefore the chemicals had to be examined within the serum.

Hypothesis five: Serum levels of the inflammatory markers neopterin, osteopontin and the neurodegenerative marker neurofilament H protein, will relate to cognitive function in HIV positive individuals. That is, highest levels will be measured in HIV positive patients with severe cognitive impairment, lower levels in mild to moderate impairment, and even lower levels in HIV positive patients with normal cognition.

This was an important hypothesis for examining the ability of the selected chemicals to function as diagnostic biomarkers.

Given the current understanding of HAND pathogenesis, as an entity caused largely by neurodegeneration secondary to the inflammatory cascade initiated by HIV in the CNS, it seemed likely that markers of inflammation and neurodegeneration would correlate with clinical disease severity.

Hypothesis six: Higher initial serum levels of the proposed inflammatory and neurodegenerative markers will be associated with a change in cognitive function (as measured by the change in global deficit score) after approximately one year; such that higher initial marker levels will be associated with greater cognitive improvement one year later, after at least ten months on HAART, but in those patients not on HAART, higher initial serum levels of the markers will be associated with a decline in cognitive function at the one year follow-up visit.

Once again, given the currently understood pathogenesis of HAND, it seemed likely that higher initial levels of the markers would be associated with a decline in cognitive function without HAART; that is, as the natural disease progression would occur. However, with HAART, there would be viral control, and therefore potential suppression of the inflammatory response. In theory, this would lead to clinical improvement. It was hypothesised that the potential for improvement would be greatest in those with higher levels of inflammation and neuronal damage at baseline.

Hypothesis seven: Higher initial serum levels of the proposed inflammatory and neurodegenerative markers will be associated with the change in cognitive function according to the CNS penetration-effectiveness of the HAART regimen used; that is, participants with higher initial serum levels of the proposed inflammatory and neurodegenerative markers will have greater improvement in cognition if they are on HAART regimens with a CPE of more than seven, as opposed to regimens with a CPE of less than or equal to seven.

Similar logic was followed here as in hypothesis six, except that in hypothesis seven, the effect of the high and low CPE categories was being examined. It was hypothesised that with increased blood-brain barrier penetration, those HAART regimens of high CPE ranking would be more effective in dampening the inflammatory response and reducing the resulting neuronal damage. Once again, it was assumed that this effect would be greatest in those with higher levels of inflammation and neuronal damage at baseline, due to the greater potential for improvement.

Chapter 4 – Methods

4.1 Introduction and overview of methods

This project formed part of a collaborative research programme into HIV-Associated Neurocognitive Disorders in the Western Cape, by the Departments of Psychiatry and Neurology, at the University of Cape Town. A large database of patient information had already been, and continued to be, collected. Additionally, frozen serum samples had been stored at -80°C for further investigations.

Participants for this study were recruited randomly from three primary level ARV clinics in the Cape Town area from the beginning of 2008 until mid 2010. These participants were all at the stage of counselling and preparation for beginning HAART therapy. Demographically similar HIV negative control patients were recruited from HIV testing centres at the same clinics.

At baseline, the participants completed various questionnaires, a neuropsychological test battery (specifically designed to identify cognitive deficits often found in HAND), a full neurological examination and had blood taken for investigations. The nadir CD4 count was recorded for each patient. Magnetic Resonance Imaging scans were also performed.

After one year, the patients were followed up with repeat testing and examination, as well as documentation of their ARV therapy details.

This component of the larger study involved a retrospective review of previously collected patient clinical information, as well as the arrangement for the reassessment of some of the participants, and the performance of new laboratory investigations.

Data from two different cohorts of patients were used. The first cohort was recruited from the beginning of 2008 and completed follow-up by mid 2010. The second cohort was recruited from the same clinics from August 2009, and assessed at baseline (pre-HAART) in a very similar way to the first cohort. However, these participants form part of a different study by the GSH HIV Mental Health Group, which is cross-sectional in nature and therefore does not include follow-up assessment a year later. The follow-up of a subgroup of participants was therefore

arranged, with selection according to when the participants were initially seen, to allow for the one year reassessment to fall within the second half of 2011.

The first part of this study was cross-sectional. It looked at levels of a few selected serum biomarkers in HIV positive participants, as well as in HIV negative control participants. Both groups include cognitively normal and cognitively impaired participants. The biomarkers which were identified for further research in this study are the inflammatory markers (neopterin and osteopontin) and the neurodegenerative marker neurofilament heavy chain protein (NFH). Unfortunately only a few markers could be investigated due to practical constraints (limited stored serum sample volumes) and financial limitations.

The second part of the study was longitudinal. Biomarker levels were measured on the stored baseline, pre-HAART blood samples, using commercially available assays. These levels were then related to the cognitive status of the participants at baseline, and to the cognitive status one year later. In some participants this meant after a year of HAART therapy, whilst others remained HAART-naïve.

Additionally, correlates between change in cognitive function and the CNS penetration-effectiveness of the specific ARV regimen used were investigated. This was the main reason for including the second cohort of participants who were recruited later, as they would have been initiated on the new South African first-line HAART regimen, as opposed to the first cohort which started the old first-line regimen, allowing for a review of the effect of the CPE differences.

4.2 Study Design

The design had two main components:

Firstly, a cross-sectional analysis of biomarker levels and their correlation with HIV status, and cognitive dysfunction.

Secondly, a longitudinal investigation into the change in cognitive function of HIV positive participants over a period of approximately one year, and the relationship of this change to HAART in general, CPE ranking of HAART regimens, and initial biomarker levels.

4.3 Sample Size

This project made use of available stored serum samples and clinical data. Whilst new clinical information was collected from the second cohort of HIV positive participants, there was not much scope to increase the sample size, as there was the limitation of the number of participants who had been assessed at baseline, within the required time period. Overall, 125 HIV positive participants were included in the study.

The numbers included in the two main sections of the study were equivalent to similar studies conducted recently. A systematic review, which examined fifteen studies looking at the effect of HAART on cognitive function, reported that sample sizes within these studies ranged from 14 to 126 participants (Joska *et al*. 2010a). Sample sizes in recent biomarker studies that yielded significant results were: for NFH, n = 76 (Anderson *et al.* 2011), for osteopontin, n = 95 (Burdo *et al.* 2008), and for serum neopterin, n = 110 (Andersson *et al.* 2001) and n = 152 (Mildvan *et al.* 2005).

A post hoc power analysis was done in consultation with a statistician, for the primary outcome of neuropsychological response to HAART after one year. The power to detect a 0.25 difference in GDS between the two group means, with a sample size of 125, alpha 0.05, for a preHAART mean GDS of 0.8 and a standard deviation of 0.6, was 90.89%.

4.4 Recruitment of participants

HIV positive participants for this study were recruited from three primary level ARV clinics in the Cape Town area, namely Nolungile Clinic in Site C Khayelitsha, Woodstock Community Health Centre and Mitchells Plain Community Health Centre. Staff at the clinics assisted in the recruitment of participants. Patient folders were drawn at random on a particular day, to be approached and screened for possible inclusion in the study. If these patients signed informed consent and were found to meet the criteria, study visits were scheduled at Groote Schuur Hospital.

Demographically similar HIV negative control patients were recruited from HIV testing centres at the same clinics. The counsellors at these centres assisted with the recruitment. After the voluntary counselling and testing process was complete, if the patient had a negative result, they were approached for possible inclusion in the study.

Another small group of HIV negative participants with cognitive dysfunction due to probable Alzheimer's disease (AD) was included in the study as positive controls for the biomarker analysis. These participants were recruited as part of another study by the Division of Neurology, Groote Schuur Hospital, which is investigating Alzheimer's disease. These patients were all older than 60 years and met NINCDS/ADRDA criteria for probable Alzheimer's disease (McKhann *et al.* 1984; Dubois *et al.* 2007). They were recruited from Groote Schuur Hospital outpatient clinics (primarily the Division of Geriatric Medicine's Memory Clinic), private medical practices and old age homes in the Cape Town area. These participants underwent a full neurological examination, cognitive assessment and routine blood tests. Some participants underwent computerised tomography (CT) scanning for diagnostic clarification. In addition, the participants had given consent for their blood samples to be stored and used for further research investigations.

4.4.1 Inclusion criteria for the HAND study

The following criteria needed to be met for inclusion in the study:

- Participants had to sign informed consent.
- Participants needed to be between 18-35 years (to exclude age-related confounding factors).
- Participants needed to have completed formal schooling up to Grade Seven.

The HIV positive participants

- Had to have attended the clinic at least once prior to the visit on which they were approached.
- Had to have been diagnosed with HIV infection within the last six months.
- Had to be at the stage of counselling and preparation for beginning HAART therapy.

4.4.2 Exclusion criteria for the HAND study

Participants were excluded if they were found to have one or more of the following:

- Uncontrolled medical conditions
- Significant psychiatric or CNS diseases
- Substance or alcohol abuse (within the last three months)
- Previous moderate-severe head injury (loss of consciousness for more than 30 minutes after the injury, or injury severe enough to necessitate an overnight hospital admission)

Screening questionnaires were used to assist with identifying inclusion and exclusion criteria, as well as for collecting demographic information. (Beck Depression Inventory, The Alcohol Use Disorders Inventory, Substance Abuse and Mental illness Screener, Life Events Questionnaire of Brugha, The Childhood Trauma Questionnaire, Patient's Assessment of Own Functioning, Activities of Daily Living)

4.5 Ethics

The larger HAND research project had been approved by the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (REC 263/2007) (amendment 19 May 2008) and (REC 203/2008). An additional amendment for the specific new analyses included in this study was also approved (amendment 28 July 2011). The greater Alzheimer's disease research project, from which some of the control patients were drawn, also had full ethics approval (REC 270/2007).

4.6 Assessment of participants

Clinical assessments were conducted at Groote Schuur Hospital in the Psychiatry Outpatients Department. MRI scans were performed at the Cape Universities Brain Imaging Centre (CUBIC) at Tygerberg Hospital.

At baseline, all HIV positive participants completed a medical assessment and a neuropsychological test battery, had an MRI scan and had blood taken for investigations. At the follow-up visit approximately one year later, the neuropsychological assessments were repeated, and some participants had repeat medical assessments. Additionally information regarding the participants' HAART therapy was collected. This included the duration of therapy as well as the particular agents used.

The demographically similar HIV negative control participants only attended the baseline visit, and only completed the neuropsychological test battery. A small sub-group (10) was selected randomly for MRI scanning and blood investigations (blood for storage for later analysis).

The Alzheimer's disease control patients had blood samples taken at their baseline visits as part of their Alzheimer's disease research assessments. Some of these blood samples were stored for further research investigations at a later point.

4.6.1 Baseline

4.6.1.1 General evaluation

All participants completed some general evaluations as well as the focussed medical and psychological assessments. The following tests were performed: socio-demographic questionnaire, Miniature International Neuropsychiatric Interview plus, International HIV Dementia Rating Scale, The Neuropsychiatric Inventory, Cape Town HIV Consortium risk behaviour scale, Karnofsky Performance Scale, Camberwell Assessment of Need and the Sheehan Disability Scale.

4.6.1.2 Neurological evaluation

HIV positive participants underwent a medical assessment, according to a scheme devised by the study team. This assessment had a focussed history to detect neurological difficulties, and then an examination to detect signs of peripheral neuropathy as well as abnormalities of the motor system (tone and power changes), co-ordination, involuntary movements, primitive reflexes and a timed gait test.

4.6.1.3 Neuropsychological evaluation

The neuropsychological test battery was chosen by the Groote Schuur Hospital Department of Psychiatry, to test domains known to be commonly affected by HIV infection. It was based on the battery used by the HIV Neurobehavioural Research Center, at the University of California. The aim was to use similar tests to that used internationally, to allow for comparison of results. However the tests also needed to be locally suitable and to this end three local neuropsychologists were consulted. Small changes were made, such as substituting words in the word lists, and idioms, to make the tests more locally suitable. All the material for the tests was also translated into isiXhosa, which is the first language of the majority of the participants. Testing was done in the participants' first language where possible.

Neuropsychological Tests according to Domain	***What the test entails***
Motor skills	
Grooved Pegboard Test (Klove 1963)	Timed test in which pegs (with both a round and a flat side) must be inserted into slots within a wooden board, making it a test of manual dexterity, fine motor coordination and motor speed; performed with the dominant and non-dominant hand.
Finger Tapping Test	Test in which the tip of the thumb must be tapped against the tip of each other finger on the hand in sequence. This task is timed and must be performed five times, with the dominant and non-dominant hand in turn.
Memory (learning, recall)	
The Hopkins Verbal Learning Test (HVLT) (Brandt 1991)	A list of words is read to the participant. These words must be immediately repeated (three trials are allowed, with a rereading of the list in between). This creates the learning score. The words must then be recalled after 15 minutes, and also be recognised from a longer list of words with other similar distracter words. These tasks create the recall score.
The Brief Visuospatial Memory Test (BVMT) (Benedict 1997)	Participants view 6 geometric figures laid out in a 2x3 array on a page for 10 seconds. They must then reproduce this as closely as possible from memory. Three learning attempts are required. 25 minutes later the test is repeated. At this stage the participants are also requested to identify the 6 figures from a list of 12 similar figures.
The Rey Osterrieth Complex Figure (RCF) (Corwin *et al.* 1993)	Participants are required to reproduce a complicated line drawing, first by copying from the given image, and then later from memory at 3 and 30 minutes after the initial copying task.

Psychomotor processing speed	
Trail Making Test A (TMTA) (Reitan 1958)	Timed test in which circled numbers must be connected in ascending sequence.
Color Trail Making I (D'Elia *et al.* 1996)	Timed test in which circled numbers must be connected in ascending sequence. (The colour of the circled numbers alternates as the numbers ascend, but there is only one of each number.)
Wechsler Adult Intelligence Scale, 3rd Edition - WAIS III (Digit symbol coding and symbol search) (Wechsler 1997a)	In digit symbol coding, the participant is presented with digit-symbol pairs. They are then subsequently required to write the appropriate symbol next to a list of digits, as quickly as possible. Symbol search requires correctly identifying given symbols from a list of similar symbols, under time pressure.
Attention (and working memory)	
Wechsler Memory Scale III (Mental Control) (Wechsler 1997b)	The participants are required to recite known sequences, such as numbers from 1-20, the alphabet, days of the week and months of the year. With the exception of the alphabet, these tasks are then repeated in reverse (*i.e*. counting 20-1, days' order backwards etc). Finally the participant needs to say the days of the week sequentially, alternating each day with an increasing multiple of 6 (*i.e.* 0 Sunday, 6 Monday, 12 TueSDay etc). All these tasks are done under time pressure.
Mental Alternation Test (MAT) (Jones *et al.* 1993)	Participants initially count to twenty, then recite the alphabet, then do both at the same time, alternating numbers and letters in ascending sequence. This is done under time pressure.
Executive Function	
Stroop Colour Word Test (SCWT) (Stroop 1935)	In this task, the participant is asked to recognise colours, then read the names of colours in black and white print, before being shown a sheet with the names of colours, printed in another colour (e.g. RED written in blue ink). Here, they are required to say the colour that they see and not read the word printed. This test is also timed.

Color Trail Making II (D'Elia *et al.* 1996)	Timed test in which circled numbers must be connected in ascending sequence, alternating between two different colours of circle. (The correct colour must be chosen each time – each number appears in both colours.)
Wisconsin Card Sorting Test (WCST) (Heaton 1993)	Stimulus cards are presented to the participant with differing shapes of different colours and quantities printed on them. The participant is then given another set of cards to "match" to the stimulus cards. The tester decides how the cards are to be matched, *i.e.* according to shape, colour or quantity. The participant is not told how to match the cards, but is instructed when they have made a correct or incorrect match. The criteria for matching are altered throughout the test, meaning the participant needs to keep finding the new pattern.
Wechsler Adult Intelligence Scale, 3rd Edition - WAIS III (Block design and Matrix reasoning) (Wechsler 1997a)	The block design and matrix reasoning subtests were performed. In block design the participant is given four 3-dimensional blocks with different prints on each face. They are then shown a geometric pattern which must be created by correct placement of the blocks next to each other (by choosing the appropriate face to display). This response is timed. In matrix reasoning, the participants are shown a pattern of 3 blocks, they must then choose the best fit block to complete the design from a selection of 4 possibilities.
Verbal Fluency (and executive functioning)	
Category Fluency Test (Animals & Fruit/Vegetables) (Spreen *et al.* 1998)	Participants must name as many animals (or fruits and vegetables) as possible within one minute.

Table 4.1: *Neuropsychological test battery*

4.6.1.4 Special investigations

4.6.1.4.1 MRI

MRI scans were performed at Tygerberg Hospital's CUBIC Unit. These scans were useful in excluding other CNS pathologies, as well as being important for other parts of the greater HAND research study. Other than for their exclusionary function, they do not form a part of this research project.

4.6.1.4.2 Blood investigations

HIV positive participants had blood drawn for tests, including a CD4 count, haemoglobin, serum iron, total protein, albumin, vitamin B12 and serum folate, as well as for storage for further research investigations such as apolipoprotein E genotyping, and inflammatory and neurodegenerative markers.

HIV negative control participants had their HIV status confirmed with ELISA testing. A small subgroup (10) had blood taken for storage for future investigations, such as inflammatory and degenerative markers.

The blood samples that were not immediately sent to the diagnostic laboratory were spun down via centrifuge, to separate the serum, and the serum samples were then archived in a freezer in the Groote Schuur Hospital Old Main Building K47, at -80° C.

Unfortunately a serological test for syphilis was not included in the baseline blood investigations. As it was felt that this was an important omission,the patients' clinic records and the NHLS database were searched to find results of such tests which were performed by chance around the time of the study assessment. Three patients were excluded from further analysis within this study due to positive Rapid Plasma Reagin (RPR) results, confirmed with fluorescent treponemal antibody-absorption test (FTA-Abs). Of the other 125 who were included, a chronologically suitable negative result was able to be found for approximately eighty percent. For the rest, no syphilis testing had been performed. All 125 participants were included in the results analysis, yet did some further calculations to examine the differences between the group with a known negative RPR result, and the group with an unknown result.

4.7 Assessment of raw data

4.7.1 Neuropsychological data: Global Deficit Scores and classification of neuropsychological impairment

HIV negative demographically similar control patients (n = 103) underwent testing with a full neuropsychological battery, encompassing several different domains of cognitive function. This created a set of "normal" raw scores for each test, which allowed computation of the "normal" mean and standard deviation for each test, in the specific study population. This was important to be able to accurately define impairment in this setting, as there were no published normal values for the South African population. From these values, standard scores (Z and T scores) (see Figure 4.1) could be calculated for each participant who completed the neuropsychological battery.

Z score = raw score of participant – mean score of population

standard deviation of population

The T score was calculated from the Z score to avoid the use of negative numbers and decimals: T = 50+10Z (rounded to the nearest whole number).

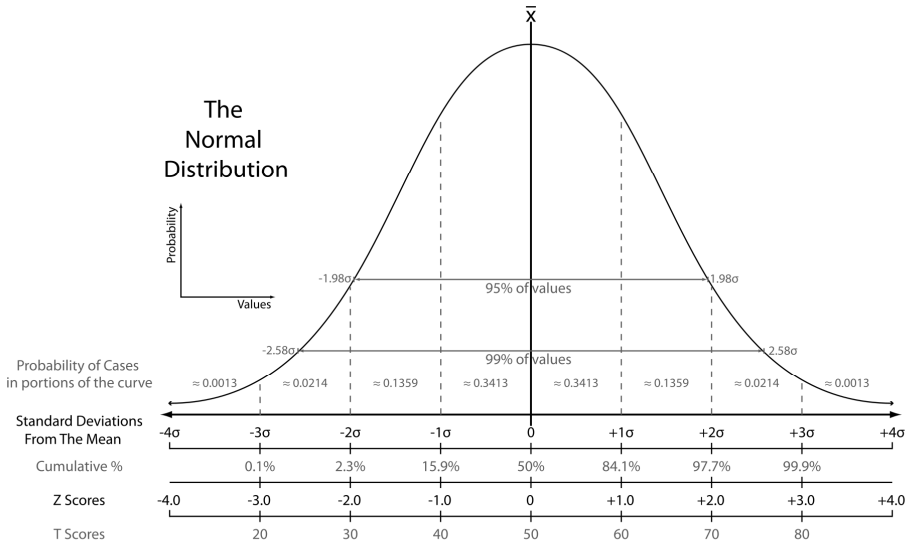

Figure 4.1: *The Normal Distribution*

From: Wikipedia.org, "Standard Score" [http://en.wikipedia.org/wiki/Standard_score - accessed 21/11/2011]

Summary scores for each ability domain were calculated by adding the individual Z scores for each test covering that domain, and dividing by the number of such tests. The specific scores used for each domain were: non-dominant hand scores for the finger tapping test and grooved pegboard test (motor), HVLT recall and BVMT recall scores (learning), MAT and WMS III mental control scores (attention), Digit Symbol Coding, TMTA and Colour Trails I (processing speed), Colour Trails II, Stroop Colour Word, WCST perseverative errors and RCF copy scores (executive function) and animal and fruit/vegetable fluency scores (verbal). This "summary Z score" for each domain was then converted to a T score.

T scores were then converted to Deficit Scores, according to the following schema (Heaton *et al.* 1995):

T scores	Deficit Score
≥ 40	0
39-35	1
34-30	2
29-25	3
24-20	4
≤ 19	5

The Global Deficit Score was then calculated by adding the deficit scores from each domain, and dividing by the total number of added scores. As T scores of 40 or more (which indicate performance within normal limits) were assigned a deficit score of 0, whilst T scores in the impaired range were assigned progressively higher scores, this method of summarising the results from a neuropsychological test battery emphasised poorer performance, which allowed for the detection of more subtle impairment that may otherwise be lost in simple mean calculations across test results.

A cut point (for determination of global impairment) of ≥0.50 has been shown to have very good diagnostic power: sensitivity 0.77, specificity 0.92, positive predictive value 0.88, negative predictive value of 0.83 and overall diagnostic accuracy rate (as compared to clinical ratings by experienced neuropsychologists) of 0.85. The likelihood ratio of detecting neuropsychological impairment is 9.38 (Carey *et al.* 2004).

In the same study by Carey *et al.* (2004), it was shown that 93% of the participants who had a global deficit score of ≥0.50, also had impairment in at least two ability domains as determined by clinical ratings. This is important as the diagnostic criteria for HAND require diagnosis of impairment in at least two ability domains (Antinori *et al.* 2007).

Although the cut point of ≥0.50 has been shown to have the best diagnostic accuracy in terms of impairment, different cut points could be used. By definition, a global deficit score of 0 implies normality, whilst larger numbers imply worse neuropsychological impairment.

For the purpose of this study, the participants were grouped into three categories of neuropsychological impairment: normal (GDS <0.25), mild-moderate impairment (0.25 ≤ GDS <0.75) and severe impairment (GDS ≥0.75). This approach was devised by Joska *et al.* (2011c), and the three groups were shown to correlate with the HAND diagnoses of normal function, ANI/MND (as these essentially have the same degree of neuropsychological impairment, but differ on functional status) and HAD. The kappa value for the correlation was 0.491, indicating a moderate correlation.

Using this approach instead of the defined HAND categories avoided the need to undertake a functional assessment of the participants which has been found to be difficult to do objectively (Valcour *et al.* 2011a).

4.7.2 HAART regimens: CPE ranking

At the time of the follow-up visit, information regarding the participants' antiretroviral therapy was collected. Firstly, whether HAART had been initiated or not. If HAART had been initiated, further information was collected, regarding the duration of therapy, as well as the clinical and laboratory response to HAART. Details regarding which particular ARV agents had been prescribed and taken were also recorded.

This information was then used to classify participants on the basis of their HAART therapy. CNS penetration-effectiveness scores were assigned to each antiretroviral agent, according to the revised 2010 schema by Letendre *et al.* (See Table 4.2) (Letendre *et al.* 2008 and 2010).

	Central Nervous System Penetration-Effectiveness Ranking			
Antiretroviral Drug Class	**4**	**3**	**2**	**1**
Nucleoside analogue reverse transcriptase inhibitors (NRTIs)	Zidovudine	Abacavir Emtricitabine	Didanosine Lamivudine Stavudine	Tenofovir Zalcitabine
Non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs)	Nevirapine	Delavirdine Efavirenz	Etravirine	
Protease inhibitors (PIs)	Indinavir/ritonavir	Darunavir/ritonavir Fosamprenavir/ritonavir Indinavir Lopinavir/ritonavir	Atazanavir Atazanavir/ritonavir Fosemprenavir	Nelfinavir Ritonavir Saquinavir Saquinavir/ritonavir Tipranavir/ritonavir
Entry/fusion inhibitors		Maraviroc		Enfuvirtide
Integrase strand transfer inhibitors		Raltegravir		

Table 4.2: *Revised Central Nervous System Penetration-Effectiveness Ranking*

Letendre S *et al.* Revised Central Nervous System Penetration-Effectiveness Ranking. *17th Conference on Retroviruses and Opportunistic Infections* 2010, Abstract 172.

The total regimen score was then calculated by adding the individual scores for each agent in the regimen. A total score of seven or less means that the regimen is considered to have poor CNS penetration-effectiveness, whilst a total score of more than seven implies good CNS penetration-effectiveness (Letendre *et al.* 2011).

4.8 Laboratory analysis

4.8.1 Preparation of samples

The blood samples that were not immediately sent to the laboratory were prepared for long term storage. The blood collection tubes were centrifuged at 4000rpm for 10minutes, to separate the serum, which was then pipetted into suitable cryo-tubes. The serum samples were placed in a freezer at -80° C. Some of the samples were stored in the Groote Schuur Hospital Old Main Building K47, and some at the Institute of Infectious Diseases and Molecular Medicine.

At the time of analysis, the samples were thawed and aliquotted for use in the three different tests. All samples were tested for each analyte in duplicate.

The neopterin and neurofilament heavy chain levels were determined by commercially available Enzyme-linked Immunosorbant Assay (ELISA) kits from DRG® and Innovation Beyond Limits (IBL) respectively. The assays were performed at Groote Schuur Hospital Old Main Building, according to the manufacturers' instructions.

The osteopontin levels were measured with Luminex technology, at the Centre for Proteomic & Genomic Research (CPGR).

4.8.2 Neopterin measurements

The neopterin ELISA kit used was a competitive ELISA from DRG (EIA-1476). As with all ELISAs it utilised the ability of antibodies to bind to their corresponding antigens.

In this assay each well of the plate is coated with a polyclonal antibody with a high affinity for neopterin. Twenty five microlitres of the standards, controls and samples, which contained the unlabelled form of neopterin (the antigen) were added to the wells, together with an enzyme conjugate which contained labelled antigen. The labelled and unlabelled antigen then competed for the available binding sites on the antibodies present in the well. After a 2 hour incubation period, the plate was washed to remove all unbound antigen, before a substrate (3,3',5,5'-Tetramthylbenzidine) was added to allow colour development. After 30 minutes a stopping solution (dilute sulphuric acid) was added, to halt the enzyme reaction, and the

absorbance was read at 450nm using an ELISA plate reader (Organon Teknika Microwell System Reader 530).

The absorbance values were inversely proportional to the amount of neopterin in the standards and samples, as a higher concentration of unlabelled antigen would mean that less labelled antigen would have been able to bind to the antibodies, which would have resulted in a weaker colour signal and therefore a lower absorbance reading at the end of the test.

The computer programme, Microsoft Excel, was used to plot the standard curve, by following the calculation instructions in the kit manual. Firstly, the average absorbance for each standard, control and sample was calculated. Then the percentage of bound antigen was calculated.

The net absorbance was determined by subtracting the non-specific binding (blank) absorbance from the sample absorbance value. This net absorbance is then divided by the net zero standard absorbance (the absorbance in these wells should be 100%) to obtain the percentage bound.

$$\%B/Bo = \frac{\text{Abs (sample)} - \text{Abs (NSB)}}{\text{Abs (zero std)} - \text{Abs (NSB)}} \times 100$$

A graph was then created for the standard values, with the percentage bound on the Y axis and the concentration of neopterin on the X axis. This standard curve was then used to determine the neopterin concentration for each sample, using the calculated percent bound.

4.8.3 Neurofilament heavy chain measurements

The Human Phosphorylated Neurofilament H ELISA kit was obtained from Biovendor: Research and Diagnostic Products (RD191138300R). It was a sandwich ELISA, used to detect sample antigen. The plate wells were pre-coated with chicken polyclonal anti-pNF-H antibody. One hundred microlitres each of sample (diluted three fold as required in the instruction manual), standards and quality controls (containing the antigen) were then added and incubated for an hour. After washing, a detection antibody (rabbit polyclonal anti-pNF-H) was added and the plate incubated again for one hour. After washing, HRP conjugated antibody against the rabbit antibody was added, to bind with the already bound rabbit antibody. After further incubation

(one hour), and a final wash, the substrate solution (3,3',5,5'-Tetramthylbenzidine) was added to bind to the conjugated antibody. After 15 minutes, a stop solution was added, and the absorbance was then read with a microplate reader (Organon Teknika Microwell System Reader 530) set at 450nm, with the reference wavelength set to 620nm. The absorbance was proportional to the concentration of pNF-H. (See figure 4.2 for an illustration of the sandwich ELISA principle).

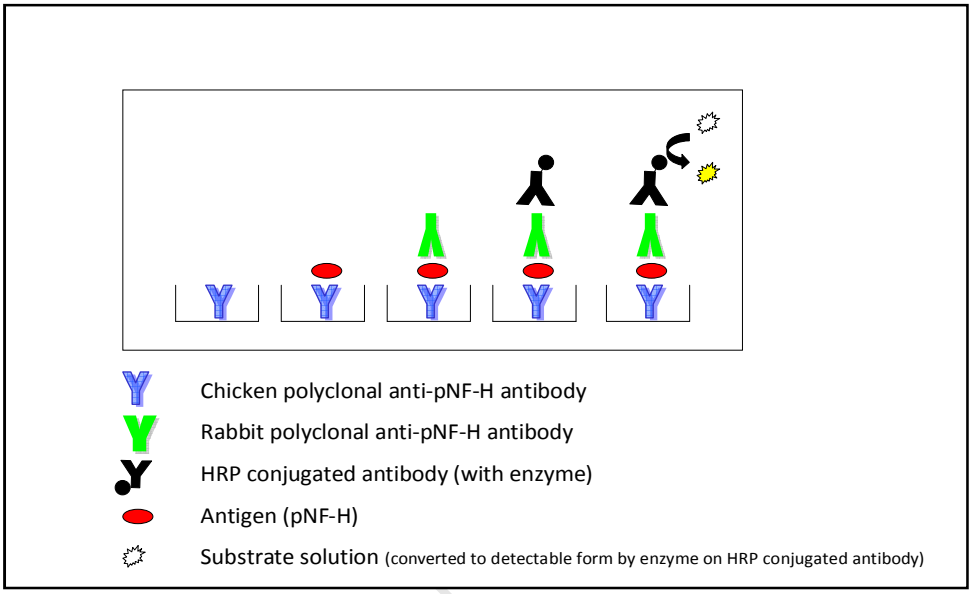

Figure 4.2: *Sandwich ELISA principle*

Modified from: Vinocur JM. ELISA.Wikipedia.org. 2006
[http://en.wikipedia.org/wiki/ELISA – accessed 18/11/2011]

The computer programme Microsoft Excel was then used to generate the standard curve, by plotting the absorbance values of the standards, against their known concentrations. The equation defining this standard curve was then calculated, and used to determine the concentration of pNF-H in each sample by using the absorbance values.

4.8.4 Osteopontin measurements

Osteopontin was measured in the samples using Luminex® xMAP® technology, and a Milliplex® MAP kit (cat. # HBN1A-51K). The procedures were performed at the Centre for Proteomic and Genomic Research, by the laboratory technicians, according to the manufacturers' instructions included in the kit.

Luminex® xMAP® technology utilises fluorescent-coded beads (minute polystyrene microspheres internally dyed with red and infrared flourophores) and immunoassay and flow

cytometry principles to enable detection of multiple analytes on a single sample volume (Luminex Corporation; Milliplex MAP HBN1A-51K kit book).

Each microsphere set was colour-coded with a specific concentration of the two dyes to allow a unique signal. Each bead was then coated with a specific capture antibody to bind its designated analyte once the sample (12.5uL) was added to the microplate wells. Thereafter a biotinylated detection antibody was introduced, and then incubated with Streptavidin-Phycoerythrin conjugate, which was the reporter molecule (Luminex Corporation; Milliplex MAP HBN1A-51K kit book) (See Figure 4.3).

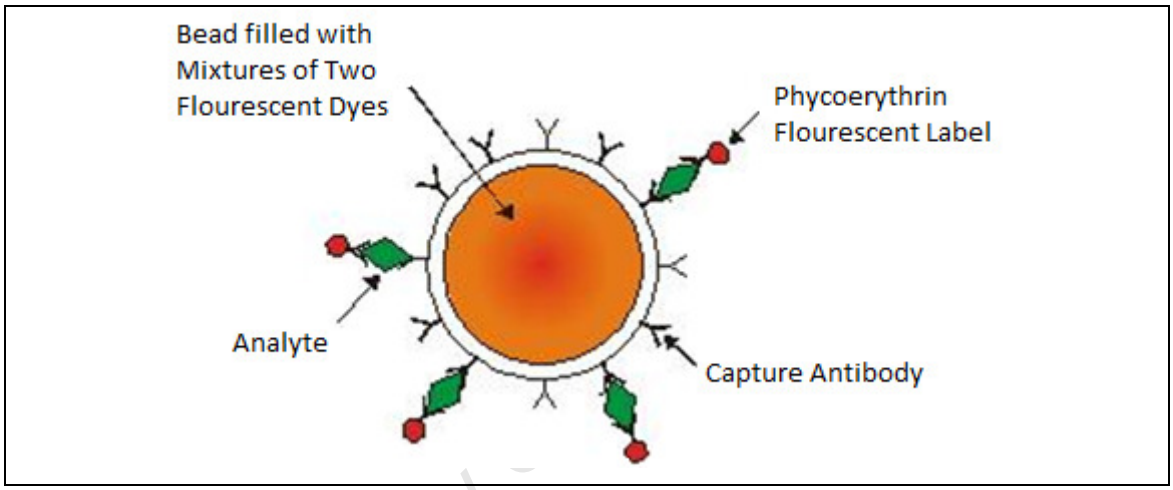

Figure 4.3: *xMAP® technology bead*

From: http://www.biotekinstruments.ru/ru/resources/articles/wash-luminex-xmap-assays.html [Accessed 04/01/2012]

The microspheres were then passed through the Luminex analyser, in a similar way to that used with flow cytometry. A red laser excited the dyes within the microspheres which allowed for classification of the type of microsphere (that is, what the bead had been labelled to measure). A second laser then excited the phycoerythrin fluorescent label, which, as the reporter molecule, showed binding of the analyte (See Figure 4.4). Multiple readings were performed for each bead set to validate the result. Digital signal processers within the Luminex analyser then quantified the results of the bioassay according to the fluorescent signals read, and sent the results (including the standard curve) directly to the attached computer system (Luminex Corporation; Milliplex MAP HBN1A-51K kit book).

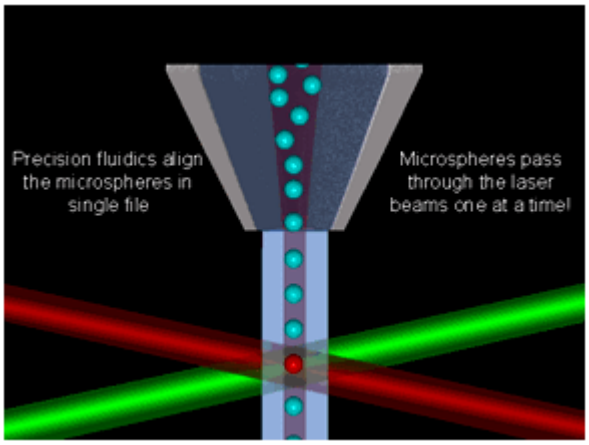

Figure 4.4: *Reading within the Luminex® analyser*
From: http://www.origene.com/xmap/ [Accessed 04/01/2012]

The kit used in this project was a Human Bone Panel Kit (1A), which allowed for the measurement of seven different analytes for each sample. However, as only osteopontin was of interest in this project, the other beads were not utilised.

4.9 Collation of data

All data from the larger studies were compiled onto a Microsoft Excel spreadsheet, by the data capturers. The data collected for this study (demographic data for the controls, follow-up data from the second cohort of HIV positive participants, the HAART data with revised CPE scores, the RPR data and all the biomarker data) were added.

The data were then prepared for analysis. Missing data were followed up in the source files, and added where possible.

4.10 Analysis of data

The data were then transferred into the Statistics/Data Analysis (STATA®) Software programme (version 11) for statistical analysis. The level of statistical significance was set at $\alpha = 0.05$. Distributions of the variables were determined using the Shapiro-Wilk test, and then parametric or nonparametric methods used as appropriate. Further details on specific analyses performed can be found in the results section.

4.11 Summary of methods: flow diagram of the study process

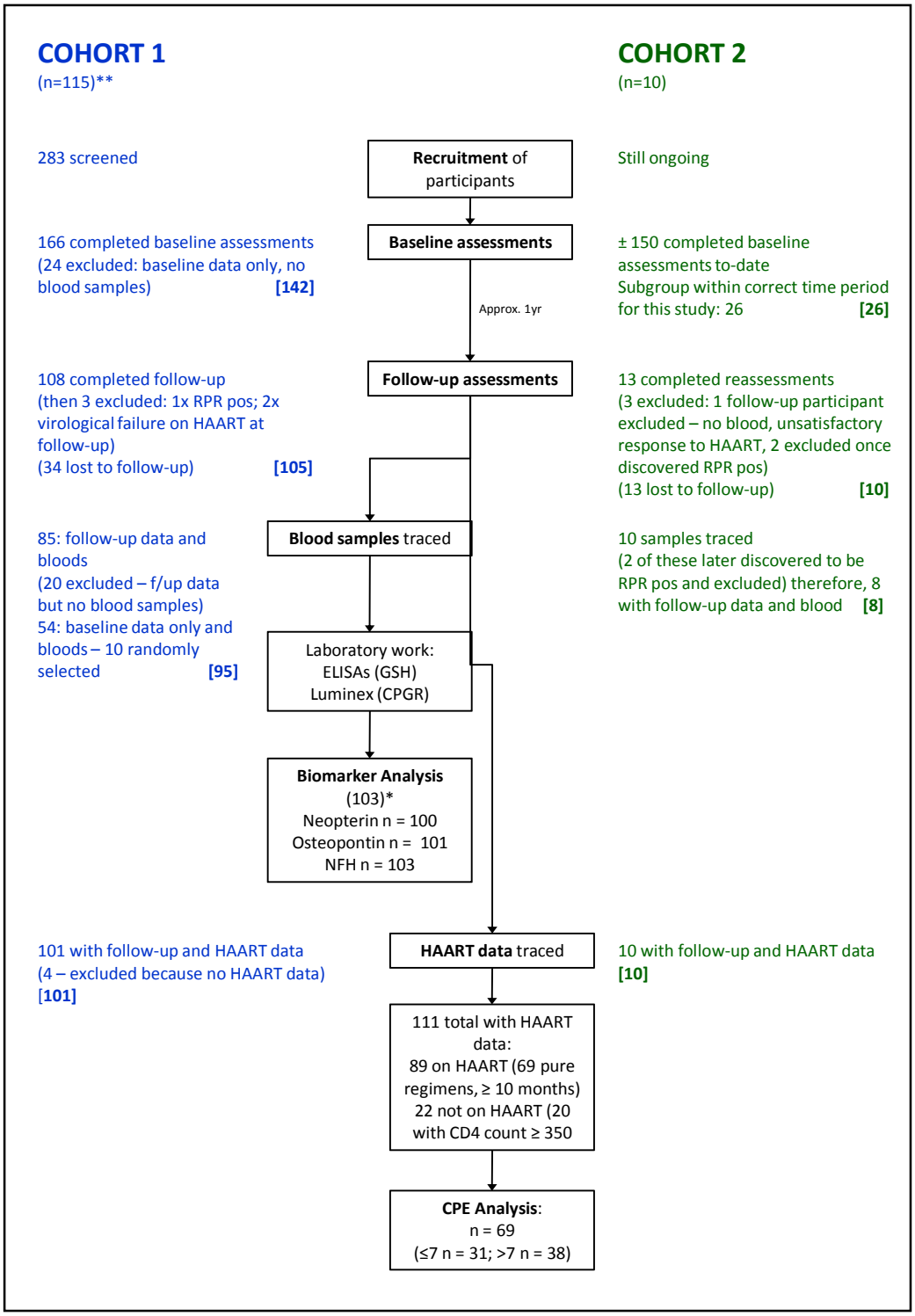

Figure 4.5: *Flow diagram of study process*

*The available neopterin kits only allowed for the measurement of 101 samples. NFH and OPN kits allowed for the measurement of 104 samples. 3 participants had to be excluded once found to be RPR positive. CPGR samples had already gone for processing once this was discovered, therefore only 101 useable sample results were yielded for OPN. For neopterin and NFH, 1 of the RPR positive participants' blood had already been analysed, however, in order not to waste the kits, I randomly selected 2 further participants from cohort 1 with baseline scores only and blood samples available (the rest of the data from these 2 participants were also included in the study). Therefore 100 neopterin, and 103 NFH measurements were yielded.

** The 115 included from cohort 1 is made up of the 105 who satisfactorily completed follow-up, as well as the 10 whose blood samples and baseline assessment data only were used for the biomarker analysis.

Chapter 5 – Results of the study

5.1 Subject characteristics

In total 248 participants were included in this study. The breakdown is indicated in Table 5.1 below.

HIV positive	
Total	125*
with follow-up data	[115]
with HAART data	[111]
With blood samples for biomarker analysis	[103]
With follow-up data and blood samples	[93]

HIV negative controls	
Total	123
Young, demographically similar to HIV+ for NP testing (and blood samples) (cognitively normal)	[103 (10)]
Old, probable Alzheimer's disease patients (cognitively impaired)	[10]
Old, demographically similar to AD patients (cognitively normal)	[10]

Total	**248**

Table 5.1: *Breakdown of study participant numbers*

*125 is the total of the 115 participants who completed follow-up together with the 10 participants whose baseline data and blood samples were utilised for the biomarker analysis.

Demographic characteristics, including sex, age, years of education and home language were collected where possible for all participants. CD4 count data were collected for the HIV positive participants. A Shapiro-Wilk Test was performed for the continuous variables. Years of education was found to have a normal distribution (SW, $p = 0.070$), as were the baseline CD4 counts (SW, p-value 0.400) and follow-up CD4 counts (SW, $p = 0.190$). Age was analysed separately for the two distinct age ranges in the study, the younger groups (SW, $p = 0.090$) and the older groups (SW, $p = 0.250$). Categorical data were analysed with percentage distributions (See Table 5.2).

	Mean age ± SD (in years)	Sex	Mean level of education ± SD (in years)	Home language	Mean baseline CD4 count ± SD (in cells/ul)*	Mean follow-up CD4 count ± SD (in cells/ul)
HIV+ participants						
All n = 125	29.7 ± 3.43	77% Female 23% Male	10 ± 1.8	91% Xhosa 3% English 1% Afrikaans 5% Other (Test Language: 64% Xhosa; 35% English; 1% Afrikaans)	165.8 ± 69.6	353.85 ± 140.92
HAART n = 69*	29.4 ± 3.2	75% Female 25% Male	10.2 ± 1.7	94% Xhosa 2% English 4% Other	162.32 ± 62.2	355.62 ± 128.26
No HAART n = 20*	30.2 ± 3.6	70% Female 30% Male	10.4 ± 1.8	90% Xhosa 10% Other	181.2 ± 83.0	319.93 ± 154.57
HIV- controls						
Young, demographically similar to HIV + n = 103**	25.3 ± 5.0	63% Female 37% Male	10.8 ± 1.4	98% Xhosa 1% Afrikaans 1% Other (Test Language: 84% Xhosa, 13% English; 3% Afrikaans)	N/A	N/A
Probable AD n = 10	74.2 ± 7.7	50% Female 50%% Male	10.11 ± 4.4	80% English 20% Afrikaans (Test Language: 100% English)	N/A	N/A
Demographically similar to AD n = 10	75.1 ± 4.6	80% Female 20% Male	14.6 ± 3.9	89% English 11% Afrikaans (Test Language: 100% English)	N/A	N/A

Table 5.2*: Demographic characteristics of study participants*

(SD = standard deviation; n = count; N/A = not applicable)

*****Only those with baseline CD4 count <350 cells/ul were included in these calculations, as this was the group used for all further calculations where CD4 count was important. (n = 120 for total HIV+ with CD4 count <350 cells/ul). **The HIV negative demographically similar control group comprised 103 participants, who underwent neuropsychological testing to determine the specific population norms. Additionally, 10 of these participants had blood drawn for investigations. The demographic details of this subgroup of 10 participants were also characterised. All of the comparisons detailed in the text and tables over the next few pages (between this control group (103) and the other study groups) were also conducted for the subgroup of 10. The findings that were significant in the comparisons with the larger group (103), remained significant when the analyses were repeated with the smaller group (10). Similarly, those findings that were non-significant, remained non-significant. The details of these analyses are therefore not included.

Comparisons of the demographic characteristics were done across the groups using one-way analysis of variance (ANOVA) tests. The groups were found to be comparable in terms of gender [F (3, 239)* = 2.46, *p* = 0.063] but significantly different in terms of age [F (3, 238) = 670.40*, p* = **<0.001**] and level of education [F (3, 238) = 17.82, *p* = **<0.001**]. (*= degrees of freedom between groups, within groups).

The age difference was to be expected given the obviously different age ranges for the HIV and AD studies respectively. In order to take the above differences into account, a comparison was done between the demographically similar groups. The HIV positive group was compared to its demographically similar group (see Table 5.3), and the Alzheimer's disease group was compared to its demographically similar group (see Table 5.5), using Student's t-tests for age and education, and a Chi-squared test for sex.

	HIV+	*Young Controls*	*df*	*t*	*p*
Age in years	29.7 (3.4)	25.3 (5.1)	221	7.76	**< 0.001**
Education in years	10.1 (1.8)	10.8 (1.4)	221	-3.08	**0.002**
Sex	77% Female 23% Male	63% Female 37% Male	χ^2 = 4.87 df = 1 *p* = **0.027**		

Table 5.3: *Demographic comparison of HIV positive participants and cognitively healthy young controls*
Means are presented with standard deviations in parentheses.
df = degrees of freedom; t = statistic t; *p* = p value

The young control group was recruited from the same areas, with the same criteria (except for HIV status) as the HIV positive group (see Table 5.4). When subjected to statistical testing however, these two groups are in fact not significantly demographically similar (see Table 5.3). The HIV positive group was slightly older, with a lower level of education. There were also proportionally fewer males in the HIV positive group. Although statistically significantly different, these groups are still clinically comparable.

Recruitment clinic	*% of HIV positive group recruited from each clinic*	*% of HIV negative control group recruited from each clinic*
Nolungile Clinic, Khayelitsha	79%	81%
Woodstock Clinic	17%	19%
Mitchells Plain CHC	4%	0%

Table 5.4: *Percentage distribution from recruitment clinics*

As detailed in Table 5.5, the Alzheimer's disease group was a similar age to its control group, however had, on average, fewer years of education. The sex distribution was equal in the AD group, but there were more females than males in the control group.

	AD	*Old Controls*	*df*	*t*	*P*
Age	74.2 (7.7)	75.1 (4.6)	17	-0.03	0.761
Education	10.1 (4.4)	14.6 (3.9)	17	-2.36	**0.031**
Sex	50% Female 50% Male	80% Female 20% Male	χ^2 = 42.65 df = 1 *p* = **< 0.001**		

Table 5.5: *Demographic comparison of Alzheimer's disease participants and cognitively healthy older controls*
Means are presented with standard deviations in parentheses.
df = degrees of freedom; t = statistic t; *p* = p value

The HIV positive group was the main focus of this study and therefore further baseline analysis was done for this group. Two areas needed to be explored further to allow for the later analyses to test the stated hypotheses.

Firstly, the group needed to be analysed and compared in terms of cognitive functional ability. Three groups were created according to baseline global deficit score, as described in the methods section (see 4.7.1, p49). A comparison of baseline characteristics was done using one-way ANOVA tests. The breakdown of numbers and the results of the ANOVAs are shown in Table 5.6. The only significant difference between the groups was found in terms of the level of education: the most severely impaired group had less education on average than the other two groups.

Baseline cognitive function groups (HIV+ group)					
	Normal GDS < 0.25	Mild-Moderate Impairment 0.25≤GDS<0.75	Severe Impairment GDS ≥0.75	One-way ANOVA results	
	n = 52 (median GDS = 0)	n = 37 (median GDS = 0.53)	n = 36 (median GDS = 1.14)	F (df)	*P*
Mean baseline CD4 ± SD	188.2 ± 106.7	182.2 ± 111.8	170.3 ± 92.0	0.31 (2, 121)	0.730
Mean level of education ± SD	10.9 ± 1.2	10.1 ± 1.85	8.9 ± 1.8	16.53 (2, 122)	**<0.001**
Sex	83% Female 17% Male	70% Female 30% Male	75% Female 25% Male	0.97 (2, 122)	0.381
Mean age ± SD	28.9 ± 3.1	30.3 ± 3.9	30.3 ± 3.2	1.01 (14, 110)	0.450

Table 5.6: *Comparison of HIV positive baseline cognitive function groups (all HIV positive participants)*
n = count; SD = standard deviation; F = statistic F; (df) = degrees of freedom between groups and within groups); p = p value

Table 5.7 below shows the numbers once the groups have been adjusted to include only those with CD4 count <350 cells/ul and HAART data (either no HAART or one HAART regimen for at least 10 months). These smaller groups were required to meet the conditions stated in some of the hypotheses. Once again, the only significant difference between the three groups was in terms of the level of education. The most severely impaired group had the lowest level of education, the mild to moderately impaired group the second lowest, and the normal group, the highest level of education.

	Normal GDS < 0.25	*Mild-Moderate Impairment* 0.25≤GDS<0.75	*Severe Impairment GDS ≥0.75*	*One-way ANOVA results*	
	n = 41	n = 30	n = 18	F (df)	*P*
Mean baseline CD4 count (in cells /ul) ± SD	173.05 ± 68.72	156.48 ± 67.05	168.22 ± 67.02	0.86 (70, 17)	0.683
Mean level of education (in years) ± SD	11.02 ± 1.15	10.2 ± 1.79	8.44 ± 1.46	5.96 (8, 80)	<0.001
Sex	83% Female 17% Male	63% Female 37% Male	72% Female 28% Male	1.52 (1, 87)	0.221
Mean age (in years) ± SD	28.83 ± 2.89	30.17 ± 3.9	30.67 ± 2.91	0.73 (12, 76)	0.719

Table 5.7: *Comparison of HIV positive baseline cognitive function groups (participants with CD4 count less than 350 cells/ul and HAART information)*
n = count; SD = standard deviation; F = statistic F; (df) = degrees of freedom between groups and within groups); p = p value

In order to analyse the effect of HAART on cognitive function, baseline analysis was conducted to determine the characteristics of the different groups. HAART information was only obtainable from those participants who returned for follow-up assessment. The median follow-up period was 12 months, interquartile range was 3 months. HAART data were obtained for 111 of the 115 participants who completed follow-up. (Reasons for not having HAART data were patient uncertainty on details and unclear recording of details on data capture forms.)

Of these 111 participants, 89 had been initiated on HAART since the baseline assessment, whilst 22 had not. Once those whose CD4 counts were greater than 350 cells/ul, and those who had not been on only one HAART regimen for at least 10 months, were excluded, the numbers for the HAART analysis became 69 on HAART and 20 not on HAART.

	HAART n = 69	*No HAART* n = 20	*Statistical comparisons*
Mean baseline CD4 count in cells/ul (SD)	162.32 (62.24)	181.15 (82.97)	t-test: df 86, t = -1.10, *p* = 0.275
Median baseline GDS (IQR)	0.27 (0.73)	0.33 (0.47)	Wilcoxon rank-sum test: z = -0.154 , *p* = 0.878

Table 5.8: *Comparison of the groups who did and did not receive HAART*
n = count; SD = standard deviation; IQR = interquartile range; df = degrees of freedom; t = statistic t; p= p value; z = statistic z

The HAART and no HAART groups were not significantly different in terms of baseline CD4 count or baseline GDS.

For the CPE analysis, the group of participants on one consistent regimen of HAART for more than 10 months were examined (n = 69). Table 5.9 shows the distribution of participants across the CPE categories. As the numbers in each smaller group were so small, no further demographic comparisons were done.

CPE of HAART Regimen	*n =*	*CPE category*	*Total n for category*
6	10	Low	31
7	21		
8	13	High	38
9	5		
10	20		

Table 5.9: *Breakdown of CNS penetration-effectiveness (CPE) categories*
n = count

A final baseline characteristic that was examined was the effect of knowing the RPR status of the participants. As stated in the methods section, syphilis testing was not part of the larger study protocol and was therefore not performed at the baseline assessment. It was felt that this information was important given the high prevalence of sexually transmitted infections in the South African population and the potential neurological effects of syphilis infection. Results of syphilis testing done for the participants at local clinics, at approximately the time of the baseline assessment, were therefore traced. Participants discovered to be RPR positive were immediately excluded from the study (n = 3). For the other 125 participants, it was possible to trace negative results for 99. The other 26 participants appeared not to have been tested. It was therefore decided to examine the other baseline characteristics with respect to this grouping (known negative result versus no result).

	RPR negative	*RPR unknown*	*Statistical comparisons*
Mean age (in years) ± SD	29.59 ± 3.53	30.19 ± 3.04	*t-test*: 123 df, t = -0.80 , *p* = 0.425
Mean level of education (in years) ± SD	10.22 ± 1.74	9.58 ± 1.98	*t-test*: 123 df, t = 1.64 , *p* = 0.104
Sex	75% Female 25% Male	81% Female 19% Male	*t-test*: 123 df, t = 0.54 , *p* = 0.594
Mean baseline CD4 count (in cells/ul) ± SD	181.13 ± 99.52	181.42 ± 120.01	*t-test*: 122 df, t = 0.01 , *p* = 0.992
Median baseline GDS (IQR)	0.33 (0.73)	0.73 (1.0)	*Wilcoxon rank-sum test*: z = -2.41, *p* = **0.016**
GDS change (IQR)	0 (0.27)	-0.2 (0.47)	*Wilcoxon rank-sum test*: z = 1.99, *p* = **0.046**

Table 5.10: *Comparison of the groups in which RPR was negative and unknown*
SD = standard deviation; IQR = interquartile range; df = degrees of freedom; t = statistic t; p= p value; z = statistic z

Table 5.10 shows the two groups to be equal in terms of age, level of education, sex distribution and baseline CD4 count. The groups were however different in terms of baseline GDS: the RPR unknown group had a higher median score, indicating poorer cognitive function. The GDS change was also greater for the RPR unknown group, which means they had greater improvement in cognitive function at follow-up.

A Kruskal-Wallis test was used to examine possible differences in the baseline cognitive function groups, according to the distribution of RPR negative to RPR unknown. A significant difference was detected. χ^2 = 4.14, 1 df, *p* = 0.042. Table 5.11 shows that there were proportionately more RPR unknowns in the severely impaired group than in the other two baseline cognitive function groups.

	RPR negative	*RPR unknown*
Normal GDS < 0.25	n = 45	n = 7
Mild-Moderate Impairment 0.25 ≤ GDS <0.75	n = 30	n = 7
Severe Impairment GDS ≥0.75	n = 24	n = 12

Table 5.11: *Breakdown of numbers in the baseline cognitive function groups according to RPR data* (n = count)

5.2 Results by Hypothesis

5.2.1 Hypothesis one and two

Hypothesis one stated that HAART of at least ten months would improve cognitive function in HIV positive ARV-naïve patients with a CD4 count of less than 350 cells/ul and cognitive dysfunction at baseline; that is, participants with impaired cognitive function at baseline, would show improvement one year later, after at least ten months of HAART therapy, while participants with impaired cognitive function at baseline, and a CD4 count less than 350 cells/ul, who had not been on HAART therapy, would not show improvement one year later.

Hypothesis two stated that HAART would be protective of cognitive function; that is, HIV positive patients with normal cognitive function at baseline who then used HAART for at least ten months, would still have normal cognitive function at the one year follow-up visit, as opposed to HIV positive patients with normal cognitive function at baseline who did not have exposure to HAART.

The entire group of HIV positive participants with follow-up data (n = 115) was analysed. How many of these participants maintained or improved their cognitive function, and how many worsened in terms of cognitive function was assessed. This was done by looking at the follow-up GDS with respect to the baseline GDS. As less than 0.25 is considered normal, and progressively higher numbers indicate worsening cognitive function, a positive change in GDS score indicates worsening, whilst a negative change indicates improvement. No change in GDS implies maintenance of cognitive function at the baseline level. Table 5.12 shows that the majority of the HIV positive study participants maintained or improved their cognitive function after one year.

All HIV+ with follow-up data (n = 115)	
Maintained or improved cognitive function	86 (75%)
Worsened cognitive function	29 (25%)

Table 5.12: *Distribution of cognitive responses at follow-up*
n = count

The change in cognitive function with respect to HAART status was then analysed. Only those with CD4 count less than 350 cells/ul, and those either not on HAART, or on HAART for at least ten months were included, as stipulated in the hypotheses (n = 89) (See Table 5.13).

	GDS change category breakdown	*Chi – squared test*	*Median GDS change*	*Wilcoxon rank-sum test to compare GDS change*
HAART n = 69	Maintained or improved cognitive function = 56 (81%)	$\chi^2 = 5.70$ df = 1 *p* = **0.017**	-0.07 (0.27)	z = -2.09 *p* = **0.036**
	Worsened cognitive function = 13 (19%)			
No HAART n = 20	Maintained or improved cognitive function = 11 (55%)		0 (0.36)	
	Worsened cognitive function = 9 (45%)			

Table 5.13: *Comparison of cognitive responses at follow-up between the HAART and no HAART groups*
n = count; χ^2 = chi-squared; df = degrees of freedom; p = p value; t = statistic t; z = statistic z

A Pearson's Chi-squared test was performed to compare the change in cognitive function categories, and this indicated a significant difference in cognitive response between the two groups (HAART and no HAART). χ^2 (1, n = 89) = 5.70, *p* = **0.017**. More of those who received HAART maintained or improved cognitive function than those who had not received HAART. A Wilcoxon rank-sum test was also performed to compare the actual GDS change as a continuous variable between the two groups. Again, a significant difference was detected, with the HAART group having a greater GDS improvement. z = -2.09, *p* = **0.036**. This indicates that HAART is beneficial for cognitive function in those with CD4 count less than 350 cells/ul. This is in keeping with hypothesis one.

A Kruskal-Wallis test was done to compare GDS change across the baseline cognitive function groups. (This test was chosen due to the non-parametric distribution of the GDS change variable. SW, *p* = **< 0.001**. Of note, baseline GDS was also found to have a non-parametric distribution. SW, *p* = **< 0.001**.)

There were significant differences in GDS change between the three baseline cognitive function groups, for the HIV positive group with follow-up data as a whole (Table 5.14), as well as those who had received HAART for at least ten months (Table 5.15). In both instances, the most severely impaired group had the greatest improvement in GDS at one year. This indicated that GDS change was also associated with the baseline level of cognitive function; that is, those that were more cognitively impaired to begin with, had a greater improvement in GDS change after one year. Figure 5.1 and 5.2 reflect this graphically.

For all HIV+ participants with follow up data (n = 115)			
	Median baseline GDS (IQR)	*Median GDS change (IQR)*	*Kruskal-Wallis Test*
Normal GDS < 0.25 n = 51	0 (0.13)	0 (0.13)	χ^2= 33.26 df = 2 *p* = **< 0.001**
Mild-Moderate Impairment 0.25 ≤ GDS <0.75 n = 37	0.53 (0.34)	-0.13 (0.33)	
Severe Impairment GDS ≥0.75 n = 27	1.07 (0.60)	-0.67 (0.6)	

Table 5.14: *Comparison of median GDS change across the baseline cognitive function groups*
n = count; χ^2 = chi-squared; df = degrees of freedom; p = p value; IQR = interquartile range

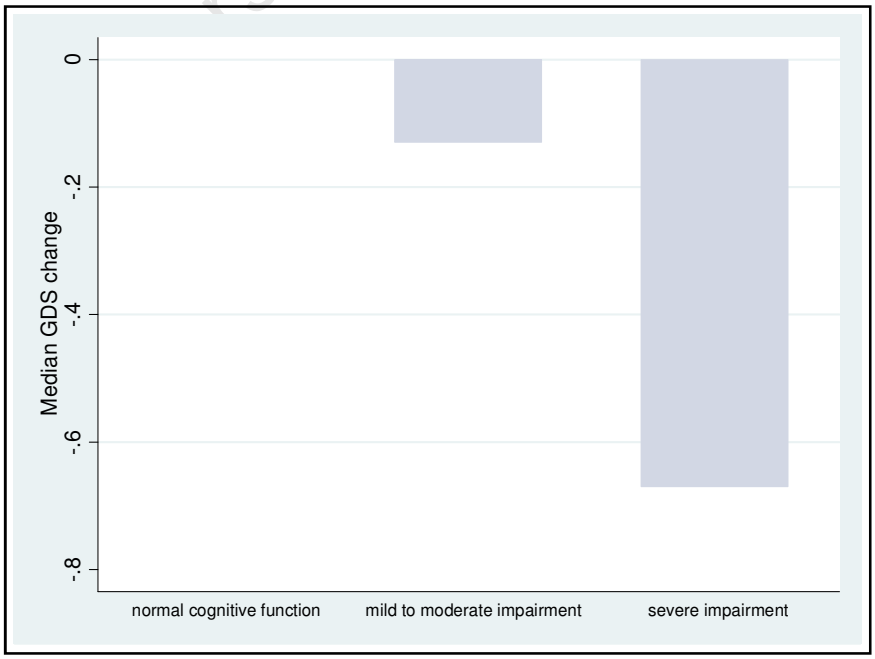

Figure 5.1: *Comparison of median GDS change across the baseline cognitive function groups*
(NB: GDS change of "0" implies maintenance of cognitive function; negative GDS change implies improvement; positive GDS change implies worsening.)

For HIV+ participants with follow up data, and on HAART ≥ 10 months (n = 69)			
	Median baseline GDS (IQR)	*Median GDS change (IQR)*	*Kruskal-Wallis Test*
Normal GDS < 0.25 n = 33	0 (0.13)	0 (0)	$\chi^2 = 19.74$ df = 2 *p* = **< 0.001**
Mild-Moderate Impairment 0.25 ≤ GDS <0.75 n = 21	0.60 (0.27)	-0.2 (0.46)	
Severe Impairment GDS ≥0.75 n = 15	1.20 (0.66)	-0.47 (0.67)	

Table 5.15: *Comparison of median GDS change across the baseline cognitive function groups (in the subgroup who received HAART)*
n = count; χ^2 = chi-squared; df = degrees of freedom; p = p value; IQR = interquartile range

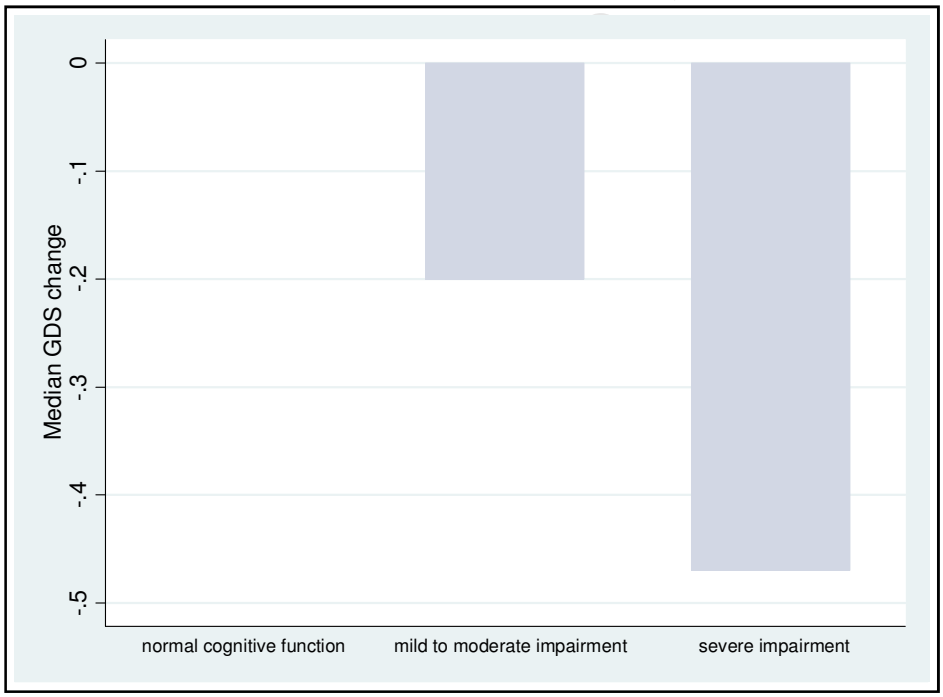

Figure 5.2: *Comparison of median GDS change across the baseline cognitive function groups (in the subgroup who received HAART)* (NB: GDS change of "0" implies maintenance of cognitive function; negative GDS change implies improvement; positive GDS change implies worsening.)

Further analysis was done to examine the effect of HAART on cognitive change at one year, within the baseline cognitive function groups (see Table 5.16). A significant difference in GDS change attributable to HAART, was found only for those with normal cognitive function: those who received HAART maintained their GDS at one year, whilst those without HAART deteriorated. This result indicates that HAART is protective of cognitive function, which

supports hypothesis two. The result for those with mild-moderate impairment bordered on significance, with those receiving HAART having a much greater improvement in GDS than those who did not.

Baseline cognitive function group	*HAART status*	*GDS change category breakdown*	*Fisher's Exact test*	*Median GDS change (IQR)*	*Wilcoxon rank-sum test to compare GDS change*
Normal cognitive function GDS < 0.25	HAART (n = 33)	Maintained or improved cognitive function = 26 (79%)	*p* = **0.034**	0 (0)	z = -2.33 *p* = **0 .020**
		Worsened cognitive function = 7 (21%)			
	No HAART (n = 8)	Maintained or improved cognitive function = 3 (38%)		0.1 (0.27)	
		Worsened cognitive function = 5 (62%)			
Mild-Moderate Impairment 0.25≤GDS <0.75	HAART (n = 21)	Maintained or improved cognitive function = 18 (85%)	*p* = 0.237	-0.24 (0.46)	z = -1.95 *p* = **0.051**
		Worsened cognitive function = 3 (15%)			
	No HAART (n = 9)	Maintained or improved cognitive function = 6 (67%)		-0.07 (0.2)	
		Worsened cognitive function = 3 (33%)			
Severe Impairment GDS ≥0.75	HAART (n = 15)	Maintained or improved cognitive function = 12 (80%)	*p* = 0.554	-0.47 (0.67)	z = -0.594 *p* = 0.553
		Worsened cognitive function = 3 (20%)			
	No HAART (n = 3)	Maintained or improved cognitive function = 2 (67%)		-0.27 (1.27)	
		Worsened cognitive function = 1 (33%)			

Table 5.16: *Comparison of cognitive responses at follow-up between the HAART and no HAART groups, across the baseline cognitive function groups*
n = count; IQR = interquartile range; p = p value; z = statistic z

5.2.2 Hypothesis three

Hypothesis three stated that HAART regimens with greater CNS penetration-effectiveness (regimen CPE more than seven) would be more effective in improving cognitive outcomes in HIV positive patients, compared with HAART regimens with a lower CNS penetration-effectiveness (regimen CPE less than or equal to seven); such that patients on HAART regimens with a CPE of more than seven would have a greater improvement in cognitive function at the one year follow-up visit, than those on regimens with a CPE of less than or equal to seven.

Table 5.17 reiterates the breakdown of participants according to the CPE categories. A total of 69 participants were included in this analysis.

Breakdown of CPE categories (pure HAART regimens, ≥10 months, only. Total n = 69)			
CPE of HAART Regimen	n =	CPE category	Total n for category
6	10	low	31
7	21		
8	13	high	38
9	5		
10	20		

Table 5.17: *Breakdown of CNS penetration-effectiveness (CPE) categories*
n = count

A two-by-two table (Table 5.18) was constructed and a Pearson's Chi-squared test performed to examine the effect of CPE category on cognitive response at the one year follow-up visit. No significant difference in cognitive response between the two groups (high and low CPE) was found. χ^2 (1, n = 69) = 0.51, p = 0.473.

	High CPE (>7)	*Low CPE (≤7)*	Total
Maintained or improved cognitive function	32 (84%)	24 (77%)	58
Worsened cognitive function	6 (16%)	7 (23%)	13
Total	38	31	

Table 5.18: *Comparison of cognitive responses at follow-up between the high and low CPE groups*

A comparison of median GDS change between the two groups (low CPE and high CPE) was also done, using a Wilcoxon rank-sum test (see Table 5.19). No significant difference was found.

	Median GDS change (IQR)	*Wilcoxon rank-sum test to compare GDS change*
Low CPE	0 (0.2)	z = 0.99
High CPE	-0.13 (0.4)	*p* = 0.320

Table 5.19: *Comparison of median GDS change at follow-up between the high and low CPE groups*
IQR = interquartile range; z = statistic z, p = p value

Table 5.20 shows the effect of CPE category on cognitive response at one year (within the baseline cognitive function groups), using a categorical variable (cognitive response category) as well as a continuous variable (median GDS change). Fisher's Exact tests were done for the categorical variable and Wilcoxon rank-sum tests for the continuous variable. No significant results were detected.

Baseline cognitive function group	*CPE category*	*GDS change category breakdown*	*Fisher's Exact test*	*Median GDS change (IQR)*	*Wilcoxon rank-sum test to compare GDS change*
Normal cognitive function GDS < 0.25	Low CPE (n = 15)	Maintained or improved cognitive function = 13 (87%)	*p* = 0.283	0 (0)	z = -0.54 *p* = 0.587
		Worsened cognitive function = 2 (13%)			
	High CPE (n = 18)	Maintained or improved cognitive function = 13 (72%)		0 (0.07)	
		Worsened cognitive function = 5 (28%)			
Mild-Moderate Impairment 0.25 ≤ GDS <0.75	Low CPE (n = 10)	Maintained or improved cognitive function = 7 (70%)	*p* = 0.090	-0.2 (0.67)	z = 0.53 *p* = 0.596
		Worsened cognitive function = 3 (30%)			
	High CPE (n = 11)	Maintained or improved cognitive function = 11 (100%)		-0.27 (0.46)	
		Worsened cognitive function =0 (0%)			
Severe Impairment GDS ≥ 0.75	Low CPE (n = 6)	Maintained or improved cognitive function = 4 (67%)	*p* = 0.341	-0.34 (1.80)	z = 1.18 *p* = 0.237
		Worsened cognitive function = 2 (33%)			
	High CPE (n = 9)	Maintained or improved cognitive function = 8 (89%)		-0.73 (0.60)	
		Worsened cognitive function = 1 (11%)			

Table 5.20: *Comparison of cognitive responses at follow-up between the high and low CPE groups across the baseline cognitive function categories.*
n = count; IQR = interquartile range; p = p value; z = statistic z

The CPE groups were then examined directly; that is, without categorisation into high and low. Below is a breakdown of the numbers found in each baseline cognitive function group (Table 5.21).

Baseline cognitive function group	*Revised CPE*					Totals
	6	**7**	**8**	**9**	**10**	
Normal cognitive function GDS < 0.25	4	11	6	2	10	33
Mild-Moderate Impairment 0.25 ≤ GDS <0.75	4	6	5	2	4	21
Severe Impairment GDS ≥0.75	2	4	2	1	6	15
Totals	10	21	13	5	20	69

Table 5.21: *Breakdown of number of participants according to regimen CPE score and baseline cognitive function group*

A Kruskal-Wallis test was done to compare the median GDS change across the CPE divisions. No statistically significant difference was found (see Table 5.22).

CPE	*Median GDS change (IQR)*	*Kruskal-Wallis Test*
6	0 (0.33)	$\chi^2 = 3.45$ df = 4 *p* = 0.485
7	-0.13 (0.27)	
8	-0.07 (0.2)	
9	-0.07 (0.2)	
10	-0.165 (0.465)	

Table 5.22: *Comparison of median GDS change across the regimen CPE groups*
CPE = CNS penetration-effectiveness score; IQR = interquartile range; χ^2= Chi-squared; df = degrees of freedom; p = p value

Finally just the lowest CPE regimen was compared with the highest, namely CPE = 6 and CPE = 10, to look for differences in terms of effect on cognitive function. No significant result was found (Table 5.23).

	GDS change category breakdown	*Fisher's Exact test*	*Median GDS change (IQR)*	*Wilcoxon rank-sum test to compare GDS change*
CPE = 6 (n = 10)	Maintained or improved cognitive function = 6 (60%)	*p* = 0.143	0 (0.33)	z = 1.67 *p* = 0.095
	Worsened cognitive function = 4 (40%)			
CPE = 10 (n = 20)	Maintained or improved cognitive function = 17 (85%)		-0.165 (0.47)	
	Worsened cognitive function = 3 (15%)			

Table 5.23: *Comparison of highest and lowest regimen CPE according to cognitive response at follow-up*
CPE = CNS penetration-effectiveness score; n = count; IQR = interquartile range; p = p value; z =statistic z

Overall, no difference was found in terms of cognitive outcomes at one year, in participants using high versus low CPE regimens. Therefore no evidence in support of hypothesis three was found.

5.2.3 Hypothesis four

Hypothesis four stated that serum levels of the inflammatory markers neopterin, osteopontin and the neurodegenerative marker neurofilament heavy chain would be significantly different in the HIV positive group as compared to the HIV negative control groups.

Neopterin

The distribution of the neopterin values for the samples was found to be not normal when a Shapiro-Wilk test was applied (SW, p = **< 0.001**). Non-parametric analysis methods were thus used.

The median neopterin levels in each group were compared using a Kruskal-Wallis test, and a significant difference was detected (see Table 5.24): the HIV positive group had much higher median neopterin levels than any of the HIV negative groups. Figure 5.3 shows this graphically.

Study participant group	*Median neopterin level in ng/mL (IQR)*	*Kruskal-Wallis Test*
HIV+ (n = 100)	4.00 (3.73)	χ^2 = 38.65 df = 3 p = **< 0.001**
HIV- , young, demographically similar to HIV+ (n = 10)	1.81 (0.34)	
HIV- , probable AD (n = 10)	1.98 (0.93)	
HIV- , old, demographically similar to AD (n = 10)	2.22 (1.47)	

Table 5.24: *Comparison of median serum neopterin levels across the study participant groups*
n = count; IQR = interquartile range; + = positive; - = negative; χ^2= Chi-squared; df = degrees of freedom; p = p value

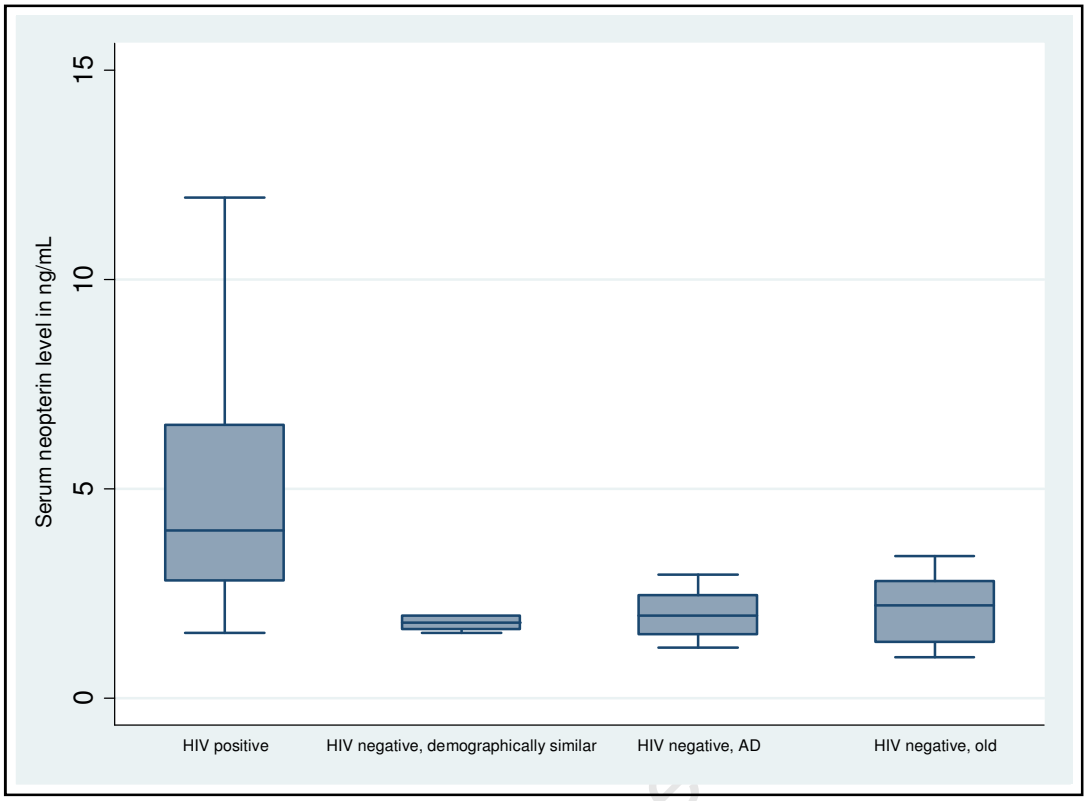

Figure 5.3: *Comparison of serum neopterin levels across the study participant groups*

The median neopterin levels in the control groups only, were then compared using Kruskal-Wallis tests. No significant difference was found (Table 5.25).

	Kruskal-Wallis Test
Comparing all three control groups	χ^2 = 0.11 df = 2 *p* = 0.946
AD versus old controls	χ^2 = 0.02 df = 1 *p* = 0.880
Cognitively normal old versus young controls	χ^2 = 0.006 df = 1 *p* = 0.940

Table 5.25: *Comparisons of median serum neopterin levels across the control groups*
χ^2= Chi-squared; df = degrees of freedom; p = p value

The HIV positive, cognitively normal participants were then compared to the young, demographically similar HIV negative participants, in terms of median neopterin level, by using a Wilcoxon rank-sum test. A significant difference was found. z = 3.87, *p* = **< 0.001** (See Table 5.26).

	Median neopterin level in ng/mL (IQR)	*Wilcoxon rank-sum test*
HIV positive, cognitively normal only n = 37	3.86 (2.80)	z = 3.87 *p* = **< 0.001**
HIV negative, demographically similar controls n = 10	1.81 (0.34)	

Table 5.26: *Comparison of median neopterin levels between cognitively normal demographically similar participants, differing only in terms of HIV status.*
n = count; IQR = interquartile range; z = statistic z; p = p value

The HIV positive, cognitively impaired participants were then compared to the probable AD participants, in terms of median neopterin level, by using a Wilcoxon rank-sum test. A significant difference was found: z = 3.738, *p* = **< 0.001** (See Table 5.27).

	Median neopterin level in ng/mL (IQR)	*Wilcoxon rank-sum test*
HIV positive, cognitively impaired only n = 63	4.28 (3.82)	z = 3.74 *p* = **< 0.001**
HIV negative, probable AD controls n = 10	1.98 (0.93)	

Table 5.27: *Comparison of median neopterin levels between cognitively impaired HIV positive participants and cognitively impaired HIV negative participants (AD).*
n = count; IQR = interquartile range; z = statistic z; p = p value

Finally, the HIV positive, cognitively impaired participants together with the AD participants, were compared (as one group) to the cognitively normal controls (as one group) in terms of median neopterin level, by using a Wilcoxon rank-sum test. A significant difference was found. z = 4.46, *p* = **< 0.001** (See Table 5.28).

	Median neopterin level in ng/mL (IQR)	*Wilcoxon rank-sum test*
All cognitively impaired participants n = 73	3.63 (4.00)	z = 4.46 *p* = **< 0.001**
All cognitively normal participants n = 20	1.82 (1.06)	

Table 5.28: *Comparison of median neopterin levels between cognitively impaired and cognitively normal participants, regardless of HIV status*
n = count; IQR = interquartile range; z = statistic z; p = p value

These results, taken in conjunction with the above results, support hypothesis four, in terms of neopterin. That is, that neopterin levels in the HIV positive group were significantly different to those in the HIV negative control groups.

Osteopontin

The distribution of the osteopontin values for the samples was found to be not normal when a Shapiro-Wilk test was applied (SW, p = **< 0.001**). Non-parametric analysis methods were thus used.

The median osteopontin levels in each group were compared. No significant difference was detected (see Table 5.29). Figure 5.4 shows this graphically.

Study participant group	*Median osteopontin level in pg/mL (IQR)*	*Kruskal-Wallis Test*
HIV+ (n = 101)	271 (579.2)	χ^2 = 3.15 df = 3 p = 0.370
HIV-, young, demographically similar to HIV+ (n = 10)	509.05 (293.3)	
HIV-, probable AD (n = 10)	266.15 (422.0)	
HIV-, old, demographically similar to AD (n = 10)	456.4 (254.2)	

Table 5.29: *Comparison of median serum osteopontin levels across the study participant groups*
n = count; IQR = interquartile range; + = positive; - = negative; χ^2= Chi-squared; df = degrees of freedom; p = p value

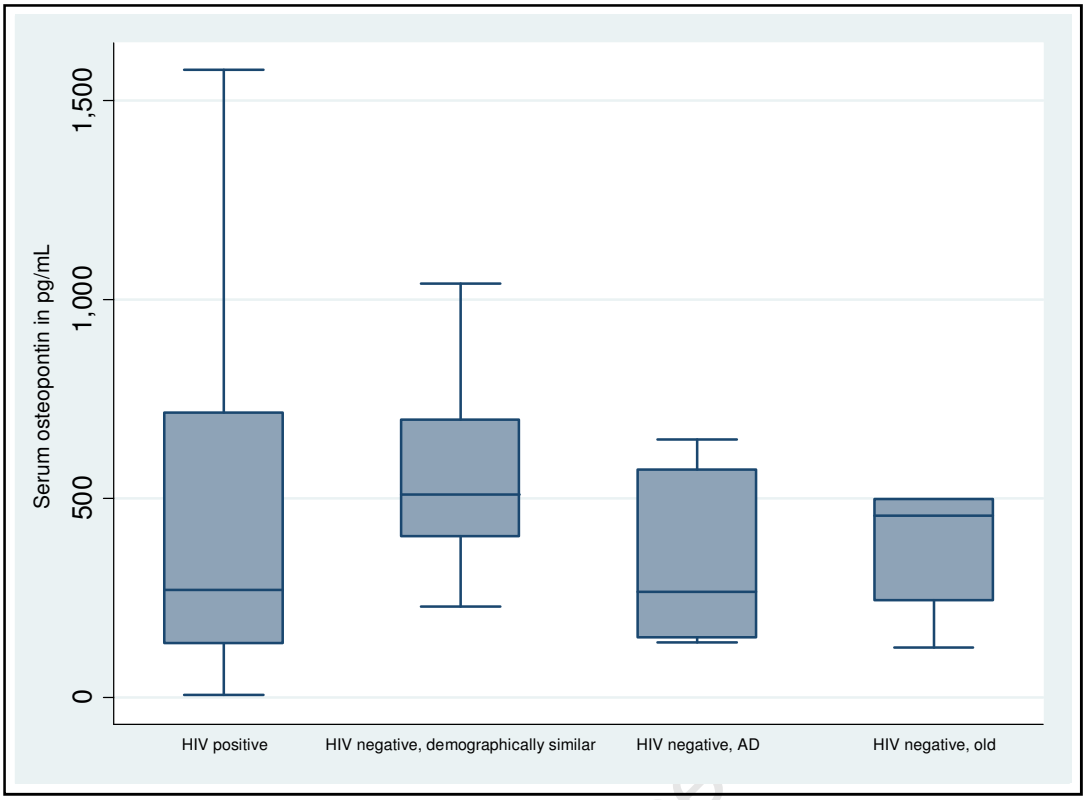

Figure 5.4: *Comparison of serum osteopontin levels across the study participant groups*

The median osteopontin levels in the control groups only were then compared using Kruskal-Wallis tests. No significant difference was found (Table 5.30).

	Kruskal-Wallis Test
Comparing all three control groups	$\chi^2 = 2.91$ df = 2 $p = 0.233$
AD versus old controls	$\chi^2 = 0.69$ df = 1 $p = 0.406$
Cognitively normal old versus young controls	$\chi^2 = 0.37$ df = 1 $p = 0.545$

Table 5.30: *Comparisons of median serum osteopontin levels across the control groups*
χ^2= Chi-squared; df = degrees of freedom; p = p value

The HIV positive, cognitively normal participants were then compared to the young, demographically similar HIV negative participants, in terms of median osteopontin level, by using a Wilcoxon rank-sum test. No significant difference was found. z = -1.61, $p = 0.107$ (see Table 5.31).

	Median osteopontin level in pg/mL (IQR)	*Wilcoxon rank-sum test*
HIV positive, cognitively normal only n = 37	232 (526.8)	z = -1.61 *p* = 0.107
HIV negative, demographically similar controls n = 10	509.05 (293.3)	

Table 5.31: *Comparison of median osteopontin levels between cognitively normal demographically similar participants, differing only in terms of HIV status.*
n = count; IQR = interquartile range; z = statistic z; p = p value

The HIV positive, cognitively impaired participants were then compared to the probable AD participants, in terms of median osteopontin level, by using a Wilcoxon rank-sum test. No significant difference was found. z = 0.142, *p* = 0.887 (Table 5.32).

	Median osteopontin level in pg/mL (IQR)	*Wilcoxon rank-sum test*
HIV positive, cognitively impaired only n = 64	291.15 (615)	z = 0.142 *p* = 0.887
HIV negative, probable AD controls n = 10	266.15 (422)	

Table 5.32: *Comparison of median serum osteopontin levels between cognitively impaired HIV positive participants and cognitively impaired HIV negative participants (AD).*
n = count; IQR = interquartile range; z = statistic z; p = p value

Finally, the HIV positive, cognitively impaired participants together with the AD participants, were compared (as one group) to the cognitively normal controls (as one group) in terms of median osteopontin level, by using a Wilcoxon rank-sum test. No significant difference was found. z = -1.45, *p* = 0.147 (see Table 5.33).

	Median neopterin level in ng/mL (IQR)	*Wilcoxon rank-sum test*
All cognitively impaired participants n = 74	291.15 (569.7)	z = -1.45 *p* = 0.147
All cognitively normal participants n = 20	456.9 (256)	

Table 5.33: *Comparison of median osteopontin levels between cognitively impaired and cognitively normal participants, regardless of HIV status*
n = count; IQR = interquartile range; z = statistic z; p = p value

As no significant results were found in the osteopontin analysis, hypothesis four has not been proven for osteopontin.

Neurofilament H protein

Unfortunately the neurofilament heavy chain assays only yielded results for 2 of the samples out of 103. For the rest of the samples, the concentration of NFH was below the limit of the assays' detection. The data were thus not able to be used for analysis in terms of this hypothesis.

5.2.4 Hypothesis five

Hypothesis five stated that serum levels of the inflammatory markers neopterin, osteopontin and the neurodegenerative marker neurofilament H protein, would relate to cognitive function in HIV positive individuals; that is, highest levels would be measured in HIV positive patients with severe cognitive impairment, lower levels in mild to moderate impairment, and even lower levels in HIV positive patients with normal cognition.

Neopterin

To examine this hypothesis, the HIV positive participants were divided into their baseline cognitive function groups, and the median neopterin levels were then compared using a Kruskal-Wallis test. No significant difference was found (Table 5.34). Figure 5.5 shows the levels of neopterin across the groups in graphical form.

Baseline cognitive function group	*Median serum neopterin level in ng/mL (IQR)*	*Kruskal-Wallis Test*
Normal cognitive function GDS < 0.25 (n = 37)	3.86 (2.80)	$\chi^2 = 1.29$ df = 2 *p* = 0.524
Mild-Moderate Impairment 0.25 ≤ GDS <0.75 (n =33)	3.63 (3.83)	
Severe Impairment GDS ≥0.75 (n =30)	4.75 (4.38)	

Table 5.34: *Comparison of median serum neopterin levels across the baseline cognitive function groups*
n = count; IQR = interquartile range; χ^2= Chi-squared; df = degrees of freedom; p = p value

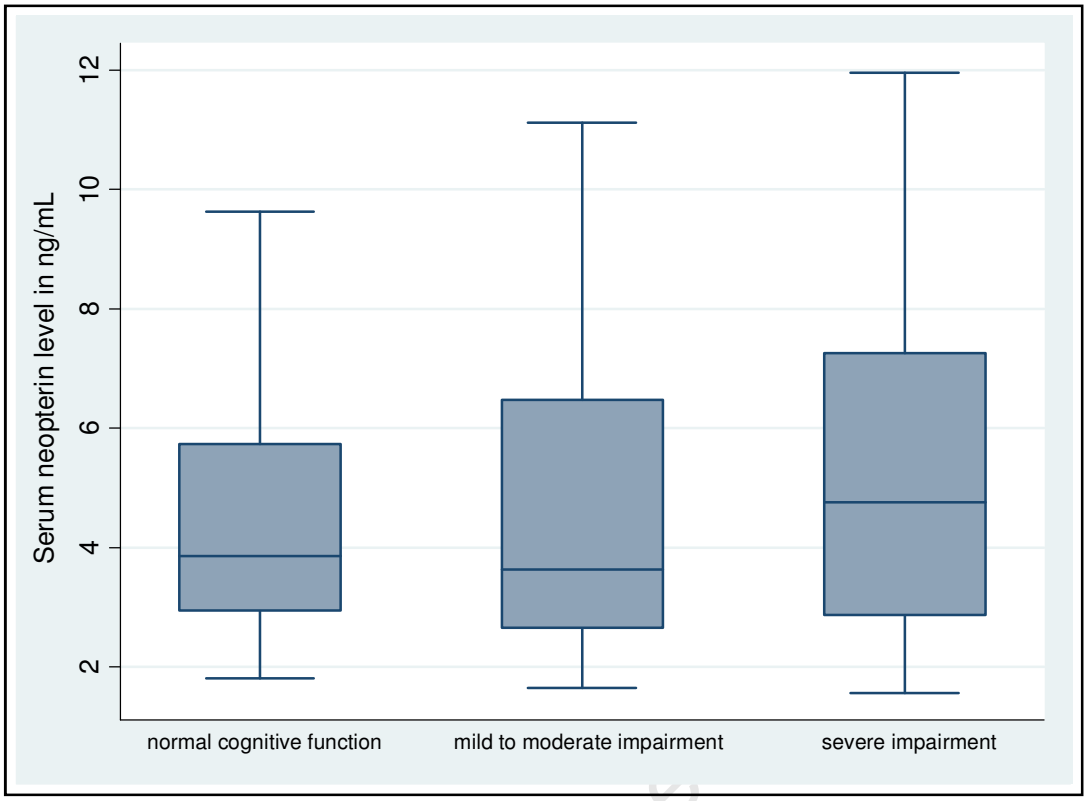

Figure 5.5: *Comparison of serum neopterin levels across the baseline cognitive function groups*

The median neopterin level of the cognitively normal group was compared to that in the severely impaired group, using a Wilcoxon rank-sum test. Again no significant difference was found. z = -1.11, p = 0.267.

A different approach was attempted, which involved a median split of the neopterin values, which created "low" and "high" neopterin level categories for the HIV positive participants. These categories were then compared across the baseline cognitive function groups (see Table 5.35), and a Pearson's Chi-squared test performed to examine the effect. No significant difference was found. χ^2 (2 df) = 1.91, p = 0.386.

Baseline cognitive function group	*Neopterin category (according to median split)*	
	Low	High
Normal cognitive function GDS < 0.25	n = 21	n = 16
Mild-Moderate Impairment 0.25 ≤ GDS <0.75	n = 17	n = 16
Severe Impairment GDS ≥0.75	n = 12	n = 18

Table 5.35: *Breakdown of numbers within high and low neopterin categories according to baseline cognitive function groups*
n = count

Serum levels of neopterin did not relate to cognitive function in HIV positive patients in this study.

Osteopontin

Once again, the HIV positive participants were divided into their baseline cognitive function groups. The median osteopontin levels were then compared using a Kruskal-Wallis test. A significant difference was found (see Table 5.36). The group with mild to moderate cognitive impairment had slightly lower levels than the normal group, however the severe impairment group had the highest levels of osteopontin. Figure 5.6 shows this result in graph format.

Baseline cognitive function group	*Median osteopontin level in pg/mL (IQR)*	*Kruskal-Wallis Test*
Normal cognitive function GDS < 0.25 (n = 37)	232.00 (526.8)	$\chi^2 = 7.32$ df = 2 *p* = **0.026**
Mild-Moderate Impairment 0.25 ≤ GDS <0.75 (n = 33)	226.3 (318.7)	
Severe Impairment GDS ≥0.75 (n = 31)	478.80 (1286.8)	

Table 5.36: *Comparison of median serum osteopontin levels across the baseline cognitive function groups*
n = count; χ^2= Chi-squared; df = degrees of freedom; p = p value

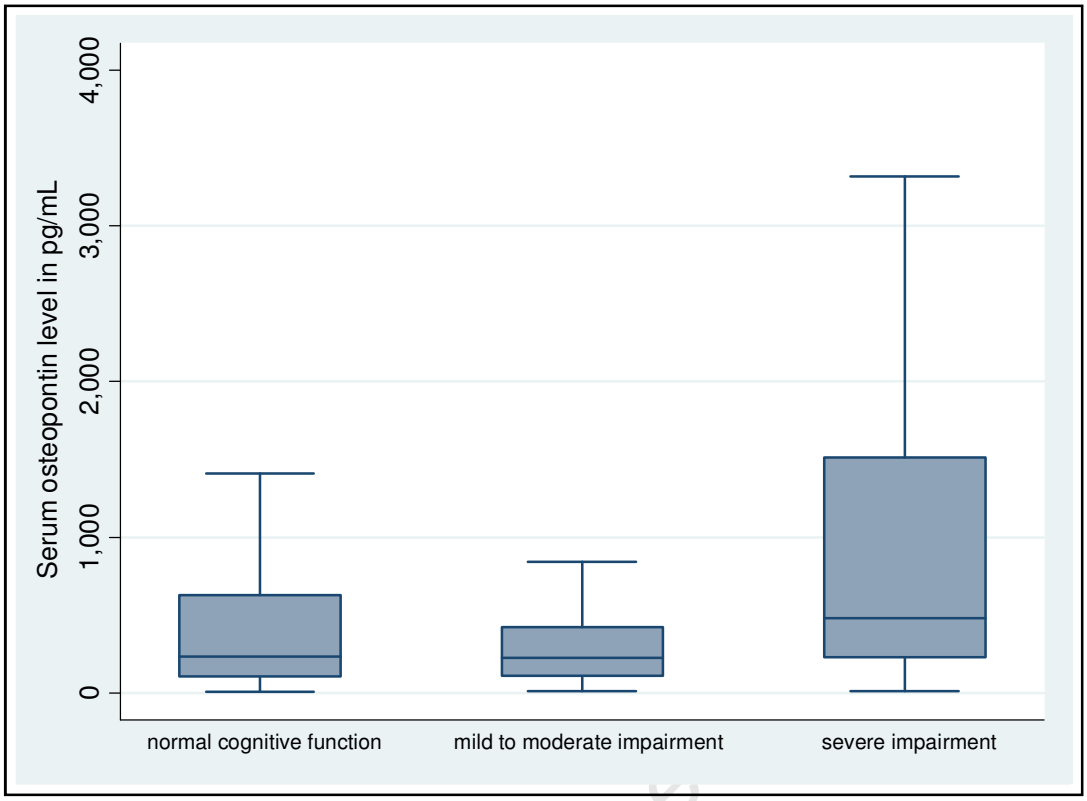

Figure 5.6: *Comparison of serum osteopontin levels across the baseline cognitive function groups*

The median osteopontin level of the cognitively normal group was compared to the severely impaired group, using a Wilcoxon rank-sum test. Again a significant difference was found.
z = -2.11, *p* = **0.035**.

A median split of the osteopontin values was then performed, which created "low" and "high" osteopontin level categories for the HIV positive participants. These categories were then compared across the baseline cognitive function groups (see Table 5.37), and a Pearson's Chi-squared test performed to examine the effect. No significant difference was found. χ^2 (2 df) = 4.037, *p* = 0.133.

Baseline cognitive function group	Osteopontin category (according to median split)	
	Low	*High*
Normal cognitive function GDS < 0.25	n = 21	n = 16
Mild-Moderate Impairment 0.25 ≤ GDS <0.75	n = 19	n = 14
Severe Impairment GDS ≥0.75	n = 11	n = 20

Table 5.37: *Breakdown of numbers within high and low osteopontin categories according to baseline cognitive function groups*
n = count

Serum levels of osteopontin did show some relation to cognitive function in HIV positive patients in this study. There was a significant difference between the normal cognitive function group and the severely impaired group, with the severely impaired group having markedly elevated levels. Interestingly, the mild to moderately impaired group had slightly lower levels than the normal cognitive function group.

Neurofilament H protein

Unfortunately the neurofilament heavy chain assays only yielded results for 2 of the samples out of 103. For the rest of the samples, the concentration of NFH was below the limit of the assays' detection. The data were thus not able to be used for analysis in terms of this hypothesis.

5.2.5 Hypothesis six

Hypothesis six stated that higher initial serum levels of the proposed inflammatory and neurodegenerative markers would be associated with a change in cognitive function (as measured by the change in global deficit score) after approximately one year; such that higher initial marker levels would be associated with greater cognitive improvement one year later, after at least ten months on HAART, but in those patients not on HAART, higher initial serum levels of the markers would be associated with a decline in cognitive function at the one year follow-up visit.

Neopterin

For this analysis, all participants with both neopterin levels and follow-up data were included. n = 93.

In examining the effect of baseline serum neopterin level on the change of cognitive function after one year, a difference in median neopterin level between the cognitive response groups was first looked for, using a Wilcoxon rank-sum test. A significant difference was found. Those who improved in cognitive function or maintained their cognitive function, had higher baseline serum neopterin level than those who worsened (see Table 5.38). Figure 5.7 shows this result in graph format.

GDS change category	*Median baseline serum neopterin in ng/mL (IQR)*	*Wilcoxon rank-sum test*
Maintained or improved cognitive function (n = 71) (76%)	4.18 (4.09)	z = 2.28 *p* = **0.023**
Worsened cognitive function (n = 22) (24%)	3.14 (2.02)	

Table 5.38: *Comparison of median baseline serum neopterin level between the cognitive response groups*
n = count

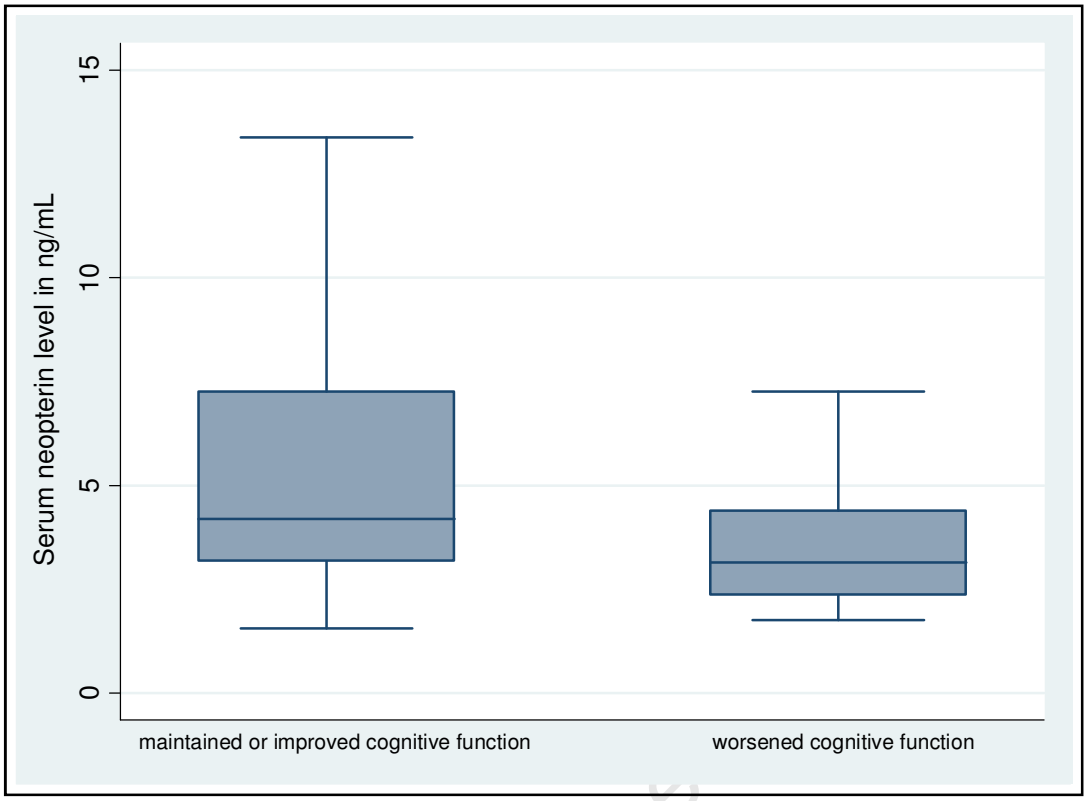

Figure 5.7: *Comparison of baseline serum neopterin level between the cognitive response groups*

A median split was once again applied. The difference in cognitive response was then examined for the two neopterin categories, and a Chi-squared test performed to assess the relationship. The actual median GDS change was also compared between the two groups using a Wilcoxon rank-sum test. A significant difference was found: more of those participants in the high neopterin level category maintained or improved their cognitive function than those with low neopterin levels (see Figure 5.8). The high neopterin group also had a greater improvement in GDS at one year (see Table 5.39).

Neopterin category (according to median split)	*GDS change group*		*Chi-squared test*	*Median GDS change (IQR)*	*Wilcoxon rank-sum test*
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 32	n = 15	χ^2 = 3.59	0 (0.33)	z = 2.39
High	n = 39	n = 7	df = 1 *p* = **0.058**	-0.165 (0.47)	*p* = **0.017**

Table 5.39: *Comparison of cognitive response and median GDS change between the high and low baseline serum neopterin categories (according to median split)*
n = count; χ^2= Chi-squared; df = degrees of freedom; p = p value; z = statistic z

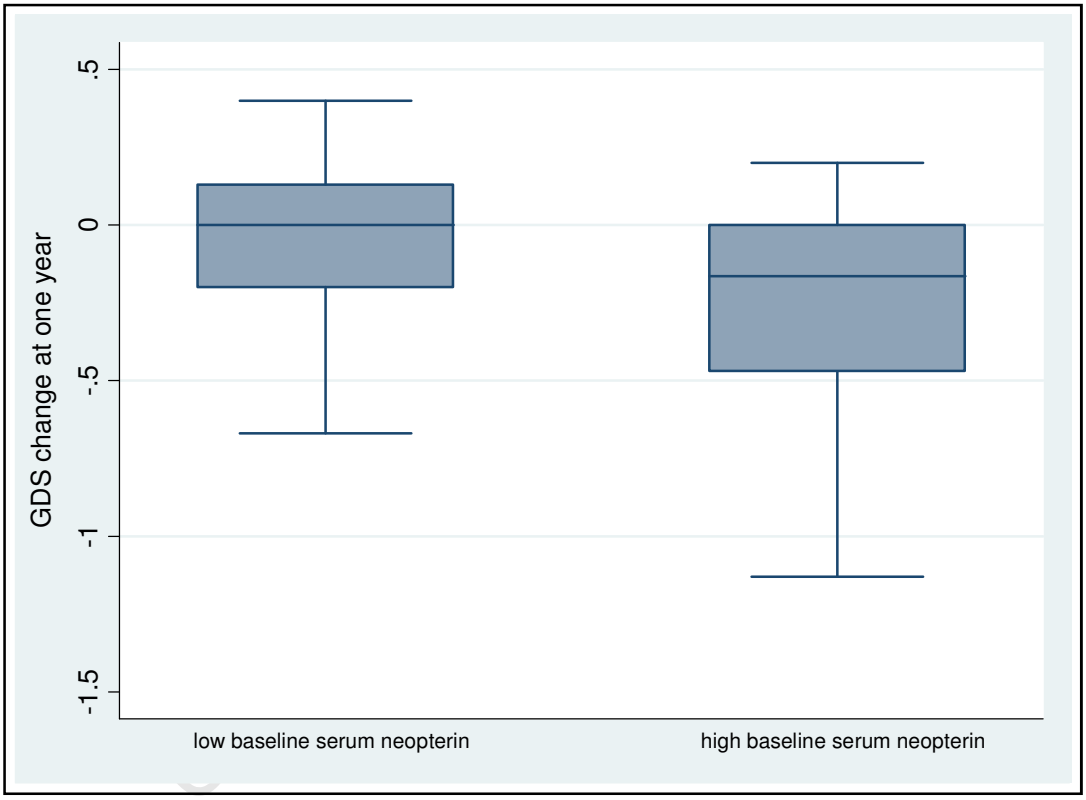

Figure 5.8: *Comparison of GDS change between the high and low baseline serum neopterin categories*

NB: GDS change of "0" implies maintenance of cognitive function; negative GDS change implies improvement; positive GDS change implies worsening

A tertile split was done and the above analysis repeated (see Table 5.40). The group with high baseline neopterin levels still had significantly more participants who maintained or improved cognitive function at one year, than the lower neopterin level group.

Neopterin category (according to tertile split)	*GDS change group*		*Fisher's Exact test*	*Median GDS change (IQR)*	*Wilcoxon rank-sum test*
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 14	n = 10	*p* = **0.018**	-0.065 (0.5)	z = 1.625
High	n = 20	n = 2		-0.165 (0.47)	*p* = 0.104

Table 5.40: *Comparison of cognitive response and median GDS change between the high and low baseline serum neopterin categories (according to tertile split)*
n = count; p = p value; z = statistic z; IQR = interquartile range

The effect of HAART on the above was then analysed. n = 68 for these analyses as only those participants with neopterin results, and HAART data (either no HAART, or HAART for more than or equal to ten months) and CD4 count more than 350 cells/ul, could be included. No significant results were found (see Tables 5.41 and 5.42).

Received HAART (n = 54)					
Neopterin category (according to median split)	GDS change group		Fisher's Exact test	Median GDS change (IQR)	Wilcoxon rank-sum test
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 20	n = 5	*p* = 1.00	-0.07 (0.2)	z = 0.709
High	n = 24	n = 5		-0.13 (0.4)	*p* = 0.478
No HAART (n = 14)					
Neopterin category (according to median split)	GDS change group		Fisher's Exact test	Median GDS change (IQR)	Wilcoxon rank-sum test
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 4	n = 4	*p* = 0.301	0.035 (0.63)	z = 0.91
High	n = 5	n = 1		-0.065 (0.27)	*p* = 0.362

Table 5.41: *Comparison of cognitive response and median GDS change between the high and low baseline serum neopterin categories, for the HAART and no HAART groups*
n = count; p = p value; z = statistic z

Within the HAART and no HAART groups, no significant difference in terms of cognitive response was found, between the high and low baseline neopterin groups.

Table 5.42 shows that there was no significant difference between the different combinations of HAART, no HAART, and high and low CPE groups in terms of GDS change.

	Median GDS change (IQR)	*Kruskal-Wallis Test*
HAART, low neopterin (n = 25)	-0.07 (0.2)	$\chi^2 = 2.27$ df = 3 *p* = 0.518
HAART, high neopterin (n = 29)	-0.13 (0.4)	
No HAART, low neopterin (n = 8)	0.04 (0.63)	
No HAART, high neopterin (n = 6)	-0.07 (0.27)	

Table 5.42: *Comparison of median GDS change across the different groups of HAART and neopterin level category*
n = count; IQR = interquartile range; χ^2= Chi-squared; df = degrees of freedom; p = p value

A Wilcoxon rank-sum test was also done to compare the HAART, high baseline serum neopterin group to the no HAART, high neopterin group, as specified in hypothesis six. There was no significant difference between the two groups. z = -0.42, *p* = 0.670. Table 5.43 shows the distribution of study participants across the HAART-neopterin level combination categories.

	Maintained or improved cognitive function	*Worsened cognitive function*
HAART, low neopterin	n = 20	n = 5
HAART, high neopterin	n = 24	n = 5
No HAART, low neopterin	n = 4	n = 4
No HAART, high neopterin	n = 5	n = 1

Table 5.43: *Breakdown of numbers according to cognitive response across the different groups of HAART and neopterin level category*
n = count

The findings in this study are partly in support of hypothesis six. That is, higher initial serum levels of neopterin were found to be associated with a change in cognitive function. Those with higher baseline neopterin levels were more likely to maintain or improve their cognitive function at one year. However, no difference with respect to HAART status was detected.

Osteopontin

For this analysis, all participants with both osteopontin levels and follow-up data were included. n = 93.

In examining the effect of baseline serum osteopontin level on the change of cognitive function after one year, a difference in median osteopontin level between the cognitive response groups was first looked for. No significant difference was found (see Table 5.44 and Figure 5.9).

GDS change category	*Median baseline serum osteopontin in pg/mL (IQR)*	*Wilcoxon rank-sum Test*
Maintained or improved cognitive function (n = 71)	269.8 (599.5)	z = -0.44 *p* = 0.658
Worsened cognitive function (n = 22)	322.15 (888.5)	

Table 5.44: *Comparison of median baseline serum osteopontin level between the cognitive response groups*
n = count; IQR = interquartile range; z = statistic z; p = p value

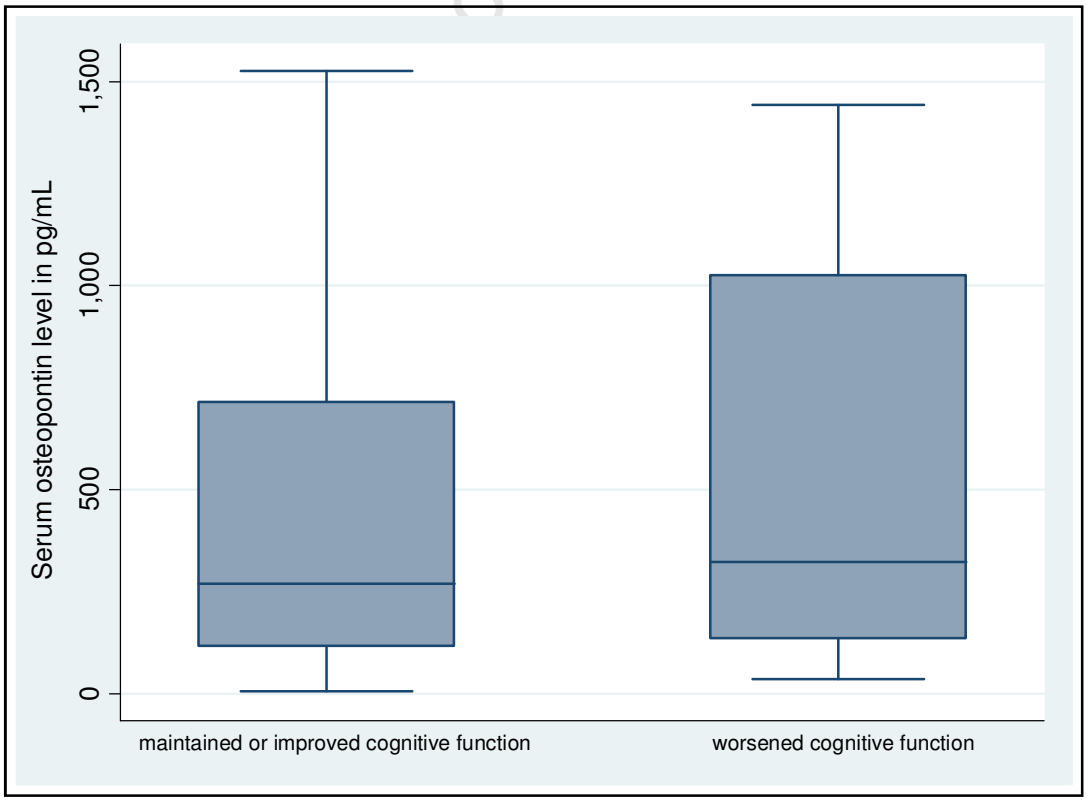

Figure 5.9: *Comparison of baseline serum osteopontin level between the cognitive response groups*

A median split was once again applied. The difference in cognitive response was then examined for the two osteopontin categories, and a Chi-squared test performed to assess the relationship. The actual median GDS change was also compared between the two groups using a Wilcoxon rank-sum test. No significant differences were found. (See Table 5.45 and Figure 5.10).

Osteopontin category (according to median split)	*GDS change group*		*Chi-squared test*	*Median GDS change (IQR)*	*Wilcoxon rank-sum test*
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 38	n = 9	χ^2 =1.07 df = 1 *p* = 0.301	-0.13 (0.2)	z = 0.31 *p* = 0.757
High	n = 33	n = 13		-0.13 (0.54)	

Table 5.45: Comparison of cognitive response and median GDS change between the high and low baseline serum osteopontin categories (according to median split)
n = count; χ^2 = Chi-squared; df = degrees of freedom; p = p value; z = statistic z

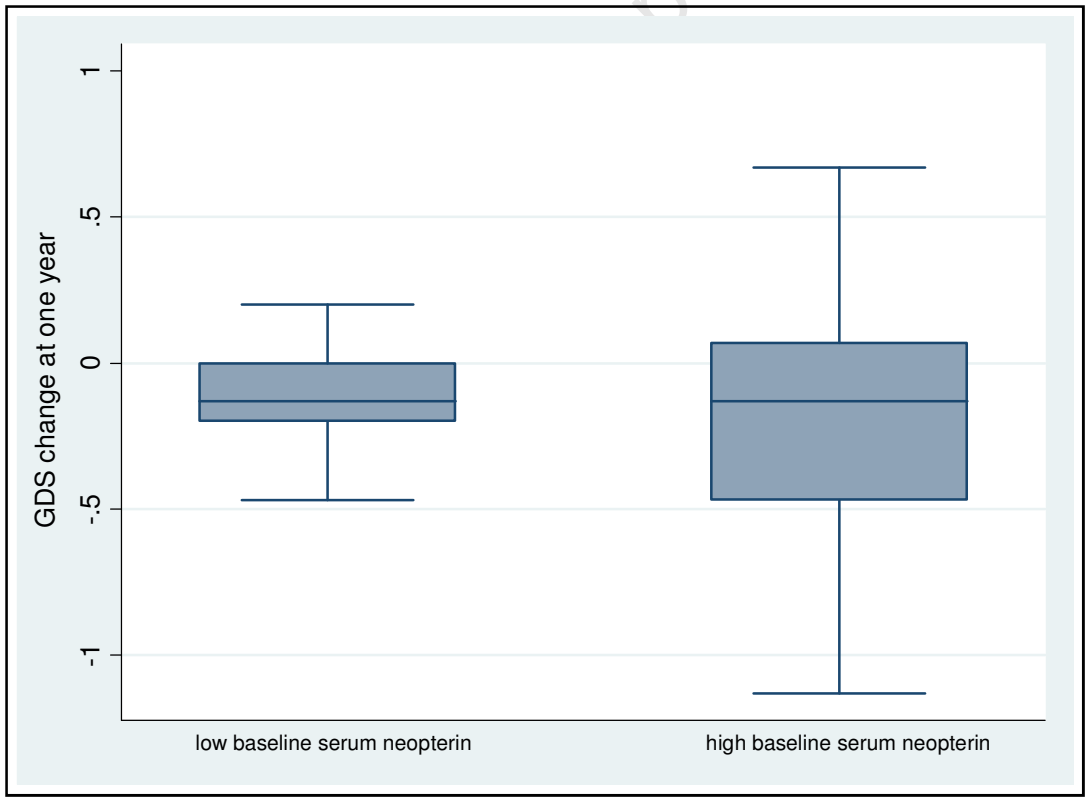

Figure 5.10: *Comparison of GDS change between the high and low serum osteopontin categories*

NB: GDS change of "0" implies maintenance of cognitive function; negative GDS change implies improvement; positive GDS change implies worsening

A tertile split was done (to be able to compare the extremes of the osteopontin levels) and the above analysis repeated. Again, no significant difference was found between the groups in terms of cognitive function (see Table 5.46).

Osteopontin category (according to tertile split)	*GDS change group*		*Chi-squared test*	*Median GDS change (IQR)*	*Wilcoxon rank-sum test*
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 20	n = 5	$\chi^2 = 0.18$ df = 1 $p = 0.675$	-0.07 (0.2)	z = 0.67 $p = 0.506$
High	n = 18	n = 6		-0.13 (0.61)	

Table 5.46: *Comparison of cognitive response and median GDS change between the high and low baseline serum osteopontin categories (according to tertile split)*
n = count; χ^2= Chi-squared; df = degrees of freedom; p = p value; z = statistic z

The effect of HAART on the above was then analysed. n = 67 for these analyses as only those participants with neopterin results, and HAART data (either no HAART, or HAART ≥10 months) and CD4 count >350, could be included. Within the HAART group, one significant difference was detected. More of those with a low osteopontin level maintained or improved cognitive function at one year, than those with high baseline osteopontin. No other significant results were found (see Table 5.47).

Received HAART (n = 53)					
Osteopontin category (according to median split)	GDS change group		Fisher's Exact test	Median GDS change (IQR)	Wilcoxon rank-sum test
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 26	n = 2	$p =$ **0.034**	-0.13 (0.235)	z= -0.86
High	n = 17	n = 8		-0.07 (0.53)	$p = 0.388$
No HAART (n = 14)					
Osteopontin category (according to median split)	GDS change group		Fisher's Exact test	Median GDS change (IQR)	Wilcoxon rank-sum test
	Maintained or improved cognitive function	Worsened cognitive function			
Low	n = 4	n = 2	$p = 0.657$	0 (0.2)	z = 0.13
High	n = 5	n = 3		-0.07 (0.57)	$p = 0.897$

Table 5.47: *Comparison of cognitive response and median GDS change between the high and low baseline serum osteopontin categories, for the HAART and no HAART groups*
n = count; p = p value; z = statistic z

	Median GDS change (IQR)	*Kruskal-Wallis Test*
HAART, low osteopontin (n =28)	-0.13 (0.24)	$\chi^2 = 2.13$ df = 3 *p* = 0.539
HAART, high osteopontin (n =25)	-0.07 (0.53)	
No HAART, low osteopontin (n =6)	0 (0.2)	
No HAART, high osteopontin (n =8)	-0.07 (0.57)	

Table 5.48: *Comparison of median GDS change across the different groups of HAART and osteopontin level category*
n = count; χ^2= Chi-squared; df = degrees of freedom; p = p value

For the different combinations of HAART, no HAART, and high and low osteopontin, no significant difference was found in terms of median GDS change at one year (see Table 5.48).

A Wilcoxon rank-sum test was also done to compare median GDS change in the HAART, high baseline serum osteopontin group to the no HAART, high serum osteopontin group. There was no significant difference between the two groups. z = -0.17, *p* = 0.866. Table 5.49 shows the distribution of participants across the HAART and osteopontin level categories.

	Maintained or improved cognitive function	*Worsened cognitive function*
HAART, low osteopontin	n = 26	n = 2
HAART, high osteopontin	n = 17	n = 8
No HAART, low osteopontin	n = 4	n = 2
No HAART, high osteopontin	n = 5	n = 3

Table 5.49: *Breakdown of numbers according to cognitive response across the different groups of HAART and osteopontin level category*
n = count

Overall, baseline serum osteopontin levels were not found to be related to the change in cognitive function at one year.

Neurofilament H protein

The neurofilament H data were not able to be used for analysis in terms of this hypothesis.

5.2.6 Hypothesis seven

This hypothesis stated that higher initial serum levels of the proposed inflammatory and neurodegenerative markers would be associated with the change in cognitive function according to the CNS penetration-effectiveness of the HAART regimen used; that is, participants with higher initial serum levels of the proposed inflammatory and neurodegenerative markers, would have greater improvement in cognition if they were on HAART regimens with a CPE of more than seven, as opposed to regimens with a CPE of less than or equal to seven.

The sample used to examine this hypothesis is small as only participants on HAART for at least ten months, and for whom blood samples were available, could be included. n = 54.

Neopterin

Median GDS change was compared across the categories of high and low CPE versus high and low baseline serum neopterin level (according to a median split). A Kruskal-Wallis test was used for the comparison. Table 5.50 shows the result, which borders on significance, indicating that there could be a difference in cognitive response between the different categories of high and low neopterin and high and low CPE.

	Median GDS change (IQR)	*Kruskal-Wallis Test*
Low CPE, low neopterin (n = 13)	-0.13 (0.2)	χ^2 = 7.43 df = 3 *p* = **0.056**
Low CPE, high neopterin (n = 12)	0 (0.24)	
High CPE, low neopterin (n = 12)	-0.04 (0.43)	
High CPE, high neopterin (n = 17)	-0.2 (0.34)	

Table 5.50: *Comparison of median GDS change across the different groups of CPE and neopterin level categories*
n = count; χ^2= Chi-squared; df = degrees of freedom; p = p value

Two further comparisons were done. The high CPE, high neopterin was compared to the low CPE, low neopterin group, as these groups represented the extreme situations. A Wilcoxon

rank-sum test was used to compare the medians of the two groups. No significant difference was found. z = 1.55, *p* = 0.122.

The high CPE, high neopterin group was then compared to the low CPE, high neopterin group. A Wilcoxon rank-sum test was used to compare the medians of the two groups. A significant difference was found. z = 2.32, *p* = **0.020**. This finding is in support of hypothesis seven for neopterin, that is, higher baseline serum neopterin levels were associated with greater improvement in GDS in those who received HAART of a high CPE regimen, as opposed to those who received low CPE regimens.

Osteopontin

Median GDS change was compared across the categories of high and low CPE, versus high and low baseline serum osteopontin level (according to a median split). A Kruskal-Wallis test was used for the comparison. Table 5.51 shows the result. No significant difference was detected.

	Median GDS change (IQR)	*Kruskal-Wallis Test*
Low CPE, low osteopontin (n = 11)	-0.13 (0.20)	χ^2 = 3.56 df = 3 *p* = 0.313
Low CPE, high osteopontin (n = 13)	0 (0.40)	
High CPE, low osteopontin (n = 17)	-0.13 (0.53)	
High CPE, high osteopontin (n = 12)	-0.17 (0.47)	

Table 5.51: *Comparison of median GDS change across the different groups of CPE and osteopontin level categories*
n = count; χ^2= Chi-squared; df = degrees of freedom; p = p value

Two further comparisons were done. The high CPE, high osteopontin was compared to the low CPE, low osteopontin group, as these groups represented the extreme situations. A Wilcoxon rank-sum test was used to compare the medians of the two groups. No significant difference was found. z = 0.78, *p* = 0.437.

The high CPE, high osteopontin group was then compared to the low CPE, high osteopontin group. A Wilcoxon rank-sum test was used to compare the medians of the two groups. No significant difference was found. $z = 1.12$, $p = 0.260$.

No evidence in support of hypothesis seven was found for osteopontin; that is, the baseline serum levels of osteopontin did not relate to the change in cognitive function at one year, according to the CPE of the HAART regimen used.

Neurofilament H protein

The neurofilament H data were not able to be used for analysis in terms of this hypothesis.

5.3 Summary of results

Subject characteristics

The HIV positive group was found to be comparable to the HIV negative control groups. The only significant difference was in terms of level of education (attributable to the much higher level in the control group for the AD participants).

Within the HIV positive group, the baseline cognitive function groups were found to be comparable in terms of CD4 count, sex and age. A significant difference was found only for level of education, in that those who were more impaired had had a lower level of education. These findings were also true for the smaller sub-group of participants with CD4 count less than 350 cells/ul and HAART data.

The group who received HAART was comparable to that which did not receive HAART in terms of CD4 count and baseline GDS.

Hypothesis results

Hypothesis one

The group who received HAART had significantly more participants with maintained or improved cognitive function, and a significantly greater improvement in GDS at the one year follow-up visit, than the group who had not received HAART.

Those with higher GDS at baseline (that is poorer cognitive function) had a significantly greater improvement in GDS at the one year follow-up visit. The GDS change was greatest for those who were most severely impaired, and then received HAART.

Hypothesis two

HAART was found to be protective of cognitive function. Amongst those participants with normal cognitive function at baseline, significantly more of those who received HAART remained cognitively normal at the one year follow-up visit than those who did not receive HAART.

Hypothesis three

No significant difference in cognitive response was found between the groups receiving HAART of higher and lower CPE regimens.

Hypothesis four

Serum neopterin levels were found to be significantly higher in the HIV positive group as compared to the HIV negative control groups.

No significant difference was found for the osteopontin levels between the HIV positive group and the HIV negative control groups.

Due to the assays yielding only very few detectable levels, the neurofilament H data were not able to be used for analysis.

Hypothesis five

Serum neopterin level was found not to relate to the level of cognitive function in the HIV positive group.

Serum osteopontin levels showed some relation to cognitive function in the HIV positive group. That is, significantly higher levels were found in the severely impaired group as compared to the normal cognitive function group.

Hypothesis six

Baseline serum neopterin levels were found to be associated with the change in cognitive function at the one year follow-up visit. That is, significantly more of those with higher baseline serum neopterin levels maintained or improved their cognitive function at the one year follow-up visit than those with lower baseline serum neopterin levels. HAART status had no effect on this relationship.

Baseline serum osteopontin levels were not associated with the change in cognitive function one year later.

Hypothesis seven

Higher baseline serum levels of neopterin were found to be significantly associated with the change in cognitive function after one year, according to the CPE of the HAART regimen used. The group with high baseline levels of neopterin together with a high CPE HAART regimen, had significantly greater improvement in cognitive function than the group with high baseline levels of neopterin together with a low CPE HAART regimen.

No association was found for baseline serum osteopontin levels and change in cognitive function with respect to the CPE of the regimen used.

Chapter 6 – Discussion

This study investigated the effect of antiretroviral therapy on cognitive function in HIV positive individuals in Cape Town, South Africa. Furthermore, it sought to investigate a few potential serum biomarkers for HAND, to assist with diagnosis and potentially, prognostication in our setting.

Overall, HAART was found to be beneficial in protecting, and in some cases, improving cognitive function. The CPE of the regimen used was not found to be an important factor in terms of cognitive outcomes, which is of local public health importance, given our recent ART protocol change.

A suitable serum biomarker for use in HAND in South Africa was not identified, although some potentially clinically useful information was detected. Neopterin levels were higher in the HIV positive group than in the HIV negative controls. Neopterin did not relate to baseline cognitive function in the HIV positive group. However, higher baseline neopterin levels were associated with maintenance of, or improvement in, cognitive function after one year. Osteopontin levels did not differ significantly between the HIV positive and negative groups, but did relate to cognitive function in the HIV positive group, with highest levels found in the most severely impaired. Baseline osteopontin was not associated with change in cognitive function. In this study, neurofilament heavy chain was not present in the peripheral blood in a concentration that allowed detection.

The following discussion first focuses on potential confounding factors arising from the demographic characteristics of the study participants, before commenting on the results of the hypotheses. Limitations of the study are then addressed before providing recommendations for future research.

Study participant demographic characteristics

The HIV positive group was found largely to be comparable to the HIV negative control groups. Besides age, which would clearly differ significantly given the vastly different target age ranges of the HAND study population and the AD study population, the only significant difference was in terms of level of education. This was attributable mainly to the much higher level in the

older HIV negative, cognitively normal group. This group was however included only as a comparison for the AD participants' findings, and was therefore not relevant as a control group for the HIV positive group. The younger, HIV negative control participants were recruited from the same demographic areas as the HIV positive participants, and according to the same criteria (besides HIV status). The fact that statistically significant differences in demographic features were found, is probably not clinically relevant given that these two groups merely represented different random samples from the same population.

Within the HIV positive baseline cognitive function groups, a significant difference was also found in terms of education, in that those who were most severely impaired, had on average, the lowest level of education.

The differing levels of education need further explanation.

Firstly, the two study cohorts (HIV and AD) are from very different socio-economic backgrounds. Higher socio-economic status is often associated with increased level of education, which is reflected in the higher number of years amongst the older, cognitively normal control group.

Secondly, lower level of education is a recognised risk factor for dementia (Stern 2009). This relates to the concept of cognitive reserve, which is basically the brain's ability to sustain insult before the appearance of a functional deficit. In a systematic review conducted by Valenzuela and Sachdev, increased cognitive reserve was found to be associated with a significantly decreased risk for incident dementia (0.54 OR, CI 95% 0.49-0.59) (Valenzuela and Sachdev 2006). Factors found to increase reserve were higher educational and occupational attainment, higher intelligence quotient, and mentally stimulating leisure activities (Valenzuela and Sachdev 2006; Stern 2009). Lower level of education has also been linked to increased risk of cognitive impairment in HIV in a number of studies (Joska *et al.* 2010b; Balestra *et al.* 2011).

Thus, it is not surprising that the attained level of education was significantly lower in the severely cognitively impaired groups (for both HIV and AD) with respect to their cognitively normal, otherwise demographically similar control groups.

It is also possible that level of education is in fact a confounding variable. That is, those with less education scored lower in neuropsychological testing as a result of having had less

education, and were therefore less experienced with a test procedure, and not necessarily because they were more cognitively impaired. While this is concerning, the neuropsychological test battery remains the current gold standard for cognitive assessment.

The effect of HAART on cognitive function (hypothesis one and two)

The study of the effect of HAART on cognitive function in the immunosuppressed HIV positive population was opportunistic. Clearly, it would not have been ethical to purposefully withold HAART from this population (given HAART's well known positive systemic effects on viral control and resultant immune reconstitution) merely for the sake of studying cognitive responses. However this study opportunity arose as a result of local circumstances.

The very recent change in the South African National ART Guidelines, from a HAART initiation threshold of 200 cells/ul to 350cells/ul, meant that given the timing of the study, there was a sample of participants with a CD4 count less than 350cells/ul, who were not eligible for HAART in South Africa at that time.

Secondly, there is unfortunately currently always a group of eligible people in South Africa who do not receive HAART as they should do. This is due to a number of factors, including, the vast number of HIV positive people in South Africa, the resultant high burden on the healthcare system, with a lack of adequate resources at many of the HIV clinics, as well as poor health education and residual HIV-associated social stigma.

Given this population of CD4 count-matched HAART-naïve participants, it was possible to show that HAART is in fact beneficial for cognitive function. The groups needed to have comparable CD4 counts, as advanced immunosuppression and more specifically, lower nadir CD4 count, have been shown to be risk factors for HAND (Childs *et al.* 1999; Balestra *et al.* 2011; Ellis *et al.* 2011). As the CD4 counts of the two compared groups (HAART and no HAART) were not significantly different, CD4 count (and therefore stage of disease) did not represent a confounding factor in this analysis.

The finding in this study of HAART being beneficial for maintaining or improving cognitive function is consistent with current literature on the subject. HAART's positive effect on cognition has previously been demonstrated in sub-Saharan Africa (Sacktor *et al.* 2006), and, as stated in chapter two, a recent systematic review revealed that studies largely agree:

HAART induces a significant improvement in cognitive status, typically within 6 months of treatment initiation (Joska *et al.* 2010a). Other recent literature has, however, questioned HAART's positive effect on cognitive function, given the continually high incidence rate of HAND in the HAART era. Many reasons have been proposed (see 2.2.3.3, p22), including potential toxicity of the ARVs themselves. These hypotheses require further investigation. However, the results of this study support the use of HAART for the prevention and treatment of HAND in South Africa, amongst the immunocompromised HIV positive population, as more participants who received HAART maintained or improved cognitive function than those who did not receive HAART.

HIV positive participants with higher global deficit score at baseline (that is, poorer cognitive function) had a greater improvement in GDS at the one year follow-up visit. The GDS change was greatest for those who were most severely impaired and received HAART. This is in agreement with the finding by Cysique *et al.* (2009) that severe baseline cognitive impairment was a predictor of neuropsychological improvement at follow-up. It is also consistent with previous observations which led to the clinical characterisation of HAD as a potentially reversible dementia (Nath *et al.* 2008). The reason that the most severely impaired participants had the greatest improvement in GDS could however merely reflect the fact that these participants had greater scope for improvement. One cannot become more "normal" than "normal", therefore those who were mildly or moderately impaired, could only improve their GDS to a smaller degree than those who were more severely impaired at baseline.

The overall analysis of cognitive response at the one year follow-up visit for all participants who completed follow-up, showed a high rate (75%) of a positive cognitive response; that is, maintenance or improvement in cognitive function. This most likely also reflects the positive effect of HAART on cognition, as 77% of the participants used in this analysis had been initiated on HAART since the baseline assessment. (HAART data were available for 111 of the 115 follow-up participants. Of the 111, 89 had been initiated on HAART since the baseline assessment. Only 69 were used in the formal HAART response analysis as they needed to have been on one regimen only, for at least 10 months, which were requirements for the HAART hypotheses.)

Neuropsychological improvement due to HAART therapy has been shown to be greatest for HAART-naïve individuals (Letendre *et al.* 2004), typically within six months of initiation of treatment, which is possibly why there was such a high positive cognitive response rate in this

study. It has been hypothesised that the high level of inflammation found in HIV has a detrimental effect on cognition, relating to dysfunctional neurons, before actual cell death occurs. It is therefore possible that HAART is able to dampen this inflammatory response to some extent, by reducing the viral load, which allows for neuronal functional recovery, and therefore improvement in cognitive ability.

Whilst practice effects must always be kept in mind with repetition of neuropsychological test batteries, the interval between the testing was long in this study, and the positive effect of HAART on cognition is biologically plausible.

The effect of HAART regimen CNS penetration-effectiveness (CPE) on cognitive outcomes

One of the proposed reasons for the continually high incidence rate of HAND in the HAART era relates to the difficulty posed by the blood-brain barrier for the passage of drugs into the CNS. The CPE ranking system was devised by Letendre *et al* (2008; revised 2010), according to the ability of different ARV agents to cross the BBB and achieve control of HIV replication within the CNS. However, studies looking at the correlation between cognitive outcomes and higher CPE regimens have shown mixed results (Tozzi *et al.* 2009; Rourke *et al.* 2011; Ciccarelli *et al.* 2011; Letendre *et al.* 2004; Marra *et al.* 2009). Tozzi *et al.* and Letendre *et al.* both found an association between higher CPE regimens and improved neuropsychological function, Rourke *et al.*, and Ciccarelli *et al.* found no association between CPE scores and cognitive outcomes, and Marra *et al.* found an association between higher CPE scores and poorer cognitive performance.

This study found no association between the CPE of the HAART regimen used and the cognitive outcome at a one year follow-up assessment. Whilst this is in agreement with other international study findings, it is important to consider that the numbers for the CPE analysis in this study were small, and the length of follow-up relatively short. Given these factors, and the ongoing mixed results in the literature, it is still uncertain whether the CPE of the HAART regimen used is an important factor in treating HAND or not. The result of this study is however beneficial in terms of South African public health, given the recent ART protocol change, to a new first-line HAART regimen with a low CPE ranking of only six. Concerns have been raised about the potential impact on cognitive function, due to the widespread use of this regimen, however the findings in this study indicate that those concerns may have been unnecessary, at least in the first year of therapy.

HAND biomarker investigation

This study sought to investigate HAND biomarkers further, especially for use in the South African setting. Blood was used instead of CSF for several reasons.

Most importantly, at the time of the study's initiation, the ethics committee had not granted permission for the collection of CSF for investigation as part of the study, as the participants were well out-patients, and lumbar puncture was deemed not otherwise clinically necessary, invasive, and potentially dangerous. Blood collection was given ethical approval, as it is considered minimally invasive and safe for the participants.

The safety concerns associated with lumbar puncture will always be present for consideration in patient care. A blood biomarker would therefore be far better overall, especially for use in primary health care. Other considerations are the short time and minimal expertise required to take blood rather than CSF, which are important for a clinically feasible biomarker for the South African setting, where HIV care is often provided by nursing sisters at a primary level.

CSF does naturally provide a closer reflection of what is occurring within the CNS, yet given the altered permeability of the BBB in HIV infection, it was hypothesised that the substances of interest would be detectable in the peripheral blood in sufficient levels for potential use as biomarkers.

Serum neopterin in HAND (hypothesis four, five, six and seven)

In this study serum neopterin levels were found to be significantly higher in the HIV positive group as compared to the HIV negative control groups. This was expected as it has been reported in the literature that neopterin levels are raised in the body fluids of HIV positive individuals throughout the course of their illness, rising with disease progression (Wirleitner *et al.* 2005). This reflects neopterin's status as a marker for cellular immune activation, being produced by stimulated macrophages. The vast majority (120 out of 125) of the HIV positive participants in this study had a CD4 count of less than 350 cells/ul, indicating immunosuppression and an advanced stage of HIV disease. Therefore raised serum neopterin levels were to be expected in this group.

Serum neopterin level did not relate to the level of cognitive function in the HIV positive group in the current study. This contrasts with the findings of Hagberg *et al.* (2010) where blood neopterin levels were shown to have a correlation to cognitive function, in that higher levels were found in the more impaired patients when compared to the minimally impaired patients. Whilst this correlation was strong in the Hagberg study for CSF levels, the blood neopterin level-cognitive function correlation only held for participants with a CD4 count of more than 200 cells/ul. Considering that the mean CD4 count in my study was 166 cells/ul, this could explain why no correlation with cognitive function was detected.

Baseline serum neopterin levels were found to be associated with the change in cognitive function at the one year follow-up visit. That is, significantly more of those with higher baseline serum neopterin levels maintained or improved their cognitive function at the one year follow-up visit than those with lower baseline serum neopterin levels. HAART status had no effect on this relationship. This is an interesting and paradoxical finding. Higher serum neopterin levels have been found in several studies to be predictive of HIV disease progression (Hagberg *et al.* 2010; Kitchen *et al.* 2008; Mildvan *et al.* 2005). Given the importance of inflammation in HIV disease pathogenesis, this makes sense. The findings in this study are therefore unexpected, as one would think that worsening inflammation would not predict cognitive improvement. Perhaps this is due to the fact that neopterin was measured in the blood rather than CSF, and was therefore not giving an accurate reflection of the inflammation occurring within the CNS. It would have been interesting to know what the serum neopterin levels were in the participants at the follow-up assessment. However, unfortunately no blood was collected at this time. That may have given a clearer indication of the true inflammatory trend.

Should it have been that higher baseline neopterin levels correlated with greater cognitive improvement in those who received HAART, only, this would have been more logical. It could be that in those with greater levels of inflammation at baseline, there is greater scope for improvement with HAART, due to suppression of the inflammatory response. However, HAART status did not have an effect on the relationship between baseline serum neopterin and cognitive response at one year. It is, however, possible that this is merely a spurious finding, due to small numbers in this analysis (n = 68: 54 on HAART and 14 not on HAART).

Higher baseline serum levels of neopterin were also found to be significantly associated with the change in cognitive function after one year, according to the CPE of the HAART regimen used. The group with high baseline levels of neopterin together with a high CPE HAART

regimen, had significantly greater improvement in cognitive function than the group with high baseline levels of neopterin together with a low CPE HAART regimen. This is also an interesting finding, which suggests that perhaps this study does support the use of higher CPE regimens after all. The fact that the participants with higher baseline neopterin levels did better cognitively if they received a higher penetrating regimen, suggests that perhaps the increased HAART penetration was able to target the increased inflammation to a greater degree (by allowing for better CNS viral control which would reduce the inflammatory stimulus), allowing for greater improvement in cognitive function. However, problems with this argument include that no clear benefit for the higher CPE regimen was seen when analysed alone, and considering the finding discussed in the previous paragraph it is unlikely that the serum neopterin levels are giving a clear reflection of the degree of CNS inflammation. It must be remembered, however, that this was a small study and perhaps the numbers are not reflecting the situation accurately, in which case any of the above may or may not be true findings.

Serum osteopontin in HAND (hypothesis four, five, six and seven)

In this study, no significant difference was found for the osteopontin levels between the HIV positive group and the HIV negative control groups. Chagan-Yasutan *et al.* found osteopontin to be raised in the plasma of HIV positive human individuals, with minimal decrease with HAART. Yet the numbers were very small in their study (n = 8) (Chagan-Yasutan *et al.* 2009).

On simple inspection of the median values found in the current study, within the different study groups, no clear distinction was seen between the HIV positive and negative groups, but what was evident was that the HIV positive group and the AD group in fact both had lower levels overall than the two completely cognitively normal groups. This finding was not significant (p = 0.159), but is still perhaps an interesting observation that could be explored further in another study with larger numbers. Perhaps osteopontin may still have a role as a marker of cognitive dysfunction in neurodegenerative disorders.

Serum osteopontin levels showed some relation to cognitive function in the HIV positive group. That is, significantly higher levels were found in the severely impaired group as compared to the normal cognitive function group. This is in partial agreement with the findings of Burdo *et al.* (2008) who found a significant correlation between plasma osteopontin levels and HIV CNS dysfunction. However in my study, the osteopontin levels did not increase sequentially as the level of cognitive function declined, as was found in the Burdo study. In the

current study the mild to moderately impaired group actually had slightly lower levels of osteopontin than the cognitively normal group (see table and graph on page 88 and 89). This could be an effect due to the divisions of the cognitive function groups; that is, the mild to moderately impaired group perhaps was too heterogeneous to group together. The normal and severely impaired groups clearly represent extremes, whilst the middle group in fact represents a spectrum of impairment in its own right. This could explain why a clear trend in terms of osteopontin level was not detected.

The same study by Burdo *et al.* (2008) mentioned above, examined both humans and rhesus monkeys. The monkey component of the study also had a longitudinal arm looking at plasma osteopontin levels in relation to the development of simian AIDS and encephalitis. Here it was found that the plasma osteopontin levels increased before the onset of neurological abnormalities (Burdo *et al.* 2008), suggesting a predictive role for the substance. In my study, baseline serum osteopontin levels were not associated with the change in cognitive function one year later and therefore a predictive role in humans was not detected.

Serum neurofilament heavy chain in HAND (hypothesis four, five, six and seven)

In this study, neurofilament heavy chain was not present in the peripheral blood in a concentration that allowed detection. This is unfortunate as it would have been interesting to compare serum findings with those of Anderson *et al.* for the CSF (see page 33).

Study limitations

This study had limitations.

Being partly opportunistic in utilising pre-existing information and serum samples as an adjunct to a larger study, this work was cost-effective. However the larger study had been initiated well before the time of this project. This meant that a lot of the work for this study had to be done retrospectively, and therefore the way that the participants were assessed, could not be altered.

Ideally more blood could have been collected for storage for later assays; both an increased amount at the baseline assessment and a second sample at the follow-up assessment. This would have allowed for more biomarkers to be investigated (although there were also

financial limitations in this regard), as well as allowing for a second measurement to give an indication of the trend of the analytes for each participant.

The number of investigations done at baseline could also have increased, to attempt to exclude further possible asymptomatic opportunistic infections, especially given that serum inflammatory markers were being measured. There is a high prevalence of sub-clinical opportunistic infections within the HIV positive population in South Africa, reflected by the high rate of immune reconstitution inflammatory syndrome (IRIS) on HAART initiation (10% in one study, by Murdoch *et al.* (2008).

This is linked to the issue of known negative, versus unknown, RPR status. The fact that the study participants were not tested as part of the formal study assessments was unfortunate, given the high rate of sexually transmitted infections in the South African population as a whole, and the even higher rate amongst the HIV positive population. Syphilis infection may also have neurological effects, although generally only with very advanced disease. Although it was attempted to remedy this omission by tracing the results of testing done at the local clinics or hospitals, it was not possible to find test results for all the participants. In the comparison between the two groups (RPR negative versus RPR unknown, see results table on page 66), they were found to be similar in terms of most factors except for cognitive function. Overall, the median baseline GDS was higher in the RPR unknown group (indicating worse cognitive function). Furthermore, the proportion of RPR unknown to RPR negative was greater in the cognitively impaired group, than in the other two baseline cognitive function groups (table on page 66). Whilst it is possible that there were a few positive RPR participants in the RPR unknown group, which could have been advanced syphilis with resulting cognitive dysfunction, it is more likely that the increased number of unknown RPRs in this severely impaired group, is due to decreased health seeking behaviour secondary to the already existing HIV-related cognitive impairment.

All of the above noted, it is, however, important to remember that HIV positive individuals will probably always have sub-clinical infections contributing to a systemic inflammatory response, which has been hypothesised to be an important driver of CNS inflammation and neurodegeneration (Perry *et al.* 2007), so therefore possible undetected opportunistic infections in this study were most likely not confounding factors.

There was a relatively short time-scale of follow-up. One year could be considered not quite long enough for assessing cognitive outcomes. Perhaps with a longer period of follow-up, a different response rate might have been detected, and potentially a difference in the high and low CPE regimens might also have become apparent. Other studies to determine the cognitive response to HAART have however utilised similar time frames for follow-up assessment, for example Cysique *et al.* (2009) did repeated neuropsychological testing at 12, 24, 36 and 48 weeks after initiation of HAART. These researchers found a mean improvement in cognitive function with HAART, especially for those with higher CPE regimens.

Perhaps the HIV negative demographically similar controls, who underwent neuropsychological testing to create the set of normal ranges for the study population, could have had repeat testing at one year. This would have allowed for a second set of normal ranges, which could have been used to control for improvement in GDS due to practice effects.

Although there were sound reasons for using blood instead of CSF in the biomarker analysis, the most important being the lack of ethical approval for CSF collection at the time, this could be viewed as a limitation of the study. For the investigation of biological conditions within the CNS, cerebrospinal fluid is unrivalled due to its proximity to the brain. However, systemic inflammation does play a role in influencing the state of the CNS, and therefore the measurement of blood analytes still has an important role. Practical issues around CSF collection should also not be ignored when considering potential biomarkers.

There were relatively small numbers in the study, although the numbers were adequate to yield some significant results. The relatively small study numbers are due to the poor follow-up rate, and limited time available to complete the study.

The follow-up rate was considered as a possible source of bias: those who did not return for the follow-up assessment could have deteriorated significantly more than those who did complete follow-up. Reasons for this could include poor memory or severe apathy (both features of HAND). In this way, important trends may have been lost. However, it is also possible that some participants who had become significantly better in terms of cognition also did not return for follow-up. Education around this study being purely for research purposes and not for curative purposes, was given in the informed consent process. It is, however, always possible that participants felt that their condition might have improved through

involvement in this research. Thus those whose cognition improved markedly, may have no longer felt the need to return for follow-up.

A brief analysis was therefore done on the greater study population to determine the baseline cognitive status of those who did not return for follow-up: 19% had normal cognitive function, 38% had mild to moderate impairment and 43% had severe impairment. In contrast, the distribution of baseline cognitive status, amongst the participants who did return for follow-up was: 44% normal, 32% mild to moderately impaired and 23% severely impaired. These data reflect a fair distribution across the cognitive function groups. However, an inverse relation was seen with more initially cognitively normal participants returning for follow-up, and more initially severely impaired participants failing to return. Although unavoidable, this may indicate a selection bias in terms of follow-up, in favour of the participants with better cognition.

However, the most likely reason for the overall poor follow-up rate is purely practical. These participants are from a low socio-economic background, meaning a stable dwelling and fixed contact telephone number are not standard. Poverty and the inability to lose a day of work could also be important factors in not returning to the study. It should be remembered too, that these participants all had HIV disease, mostly with advanced immunosuppression, and therefore death due to opportunistic infection was always a possibility. This may be true even more so for those with greater cognitive impairment, who could well have had more advanced HIV disease.

Despite these limitations, this study has been worthwhile in producing further information on HAND as a clinical entity in South Africa. South Africa represents a high HIV prevalence area, but limited research is done here compared to the developed world. It is a region where clade C HIV-1 is found, which is relatively understudied compared to clade B. There is also a distinctly demographically different population to that found in the US and Europe, where the majority of HAND research is conducted, as well as a healthcare system which faces different challenges. South African research findings are therefore important as they have more local relevance.

Chapter 7 – Conclusions and recommendations

Overall, this study has added to the pool of evidence which shows that HAART is beneficial for cognitive function. HAART regimen CPE was not found to be an important factor in cognitive outcomes after one year. This is a promising finding in the protocol-bound South African context, where the current first-line HAART regimen has a low CPE ranking. This study has however highlighted the need to focus on improving HAART initiation programmes to ensure that all those who are currently eligible are in fact receiving HAART.

As the greater evidence base is still divided on the issue of whether higher CPE regimens are more beneficial for cognitive outcomes, further prospective CPE studies with larger samples and longer follow-up times need to be conducted, ideally, also within South Africa.

Serum neopterin and osteopontin levels may have clinical use; however, further research is required to identify biomarkers for HAND in South Africa. Further studies looking at these and other potential markers (alone or in combination), in larger populations, and with serial levels, are required.

References

Abdulle S, Mellgren A, Brew BJ, Cinque P, Hagberg L, Price RW *et al*. CSF neurofilament protein (NFL) – a marker of active HIV related neurodegeneration. *Journal of Neurology* 2007; **254** (8): 1026-1032.

Ancuta P, Kamat A, Kunstman KJ, Kim EY, Autissier P, Wurcel A *et al*. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS One* 2008; **3**: e2516.

Anderson C, Sacktor N, McArthur JC, Nath A. Significant Neuronal Injury in Asymptomatic HIV Infection. Presented at *18th Conference on Retroviruses and Opportunistic Infections* 2011, Abstract 407.

Andersson LM, Hagberg L, Fuchs D, Svennerholm B, Gisslen M. Increased blood-brain barrier permeability in neuro-asymptomatic HIV-1-infected individuals – correlation with cerebrospinal fluid HIV-1 RNA and neopterin levels. *Journal of NeuroVirology* 2001; **7**: 542-547.

Anthony I, Bell J. The Neuropathology of HIV/AIDS. *International Review of Psychiatry* 2008; **20** (1): 15-24.

Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M *et al*. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* 2007; **69**: 1789-1799.

Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a public health approach. 2010 Edition. World Health Organisation. Available at: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf (accessed 23/06/2011)

Balestra P, Tozzi V, Zaccarelli M, Libertone R, Cataldo G, Liuzzi G *et al*. Prevalence and risk factors for HIV associated neurocognitive disorders (HAND), 1996-2010: results from an observational cohort. *Sixth IAS Conference on HIV Pathogenesis, Treatment and Prevention* July 2011; Abstract MOAB0103.

Banks WA, Freed EO, Wolf KM, Robinson SM, Franko M, Kumar VB. Transport of human immunodeficiency virus type 1 pseudoviruses across the blood-brain barrier: role of envelope proteins and adsorptive endocytosis. *Journal of Virology* 2001; **75**: 4681-4691.

Bell JE. An update on the neuropathology of HIV in the HAART era. *Histopathology* 2004; **45**: 549-559.

Benedict RHB. Brief Visuospatial Memory Test – Revised. Odessa: Psychological Assessment Resources 1997.

Bitsch A, Horn C, Kemmling Y, Seipelt M, Hellenbrand U, Stiefel M *et al*. Serum Tau protein level as a marker of axonal damage in acute ischaemic stroke. *European Neurology* 2002; **47**: 45-51.

Bobardt MD, Salmon P, Wang L, Esko JD, Gabudza D, Fiala M *et al*. Contribution of proteoglycans to human immunodeficiency virus type 1 brain invasion. *Journal of Virology* 2004; **78** (12): 6567-6584.

Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. *Nature Medicine* 1997; **3** (1): 42 – 47.

Bottiggi KA, Chang JJ, Schmitt FA, Avison MJ, Mootoor Y, Nath A *et al*. The HIV Dementia Scale: Predictive power in mild dementia and HAART. *Journal of the Neurological Sciences* 2007; **260;** 11-15.

Bouwman FH, Skolasky RL, Hes D, Selnes OA, Glass JD, Nance-Sproson TE *et al*. Variable progression of HIV-associated dementia. *Neurology* 1998; **50**: 1814-1820.

Brandt J. The hopkins verbal learning test: Development of a new memory test with six equivalent forms. *Clinical Neuropsychologist* 1991; **5** (2): 125-142.

Brew BJ, Perdices M, Darveniza P, Edwards P, Whyte B, Burke WJ *et al*. The neurological features of early and "latent" human immunodeficiency virus infection. *Australian and New Zealand Journal of Medicine* 1989; **19** (6): 700-705.

Brew BJ, Bhalla RG, Paul M, Gallardo H, McArthur JC, Schwartz MK *et al*. Cerebrospinal fluid neopterin in human immunodeficiency virus type 1 infection. *Annals of Neurology* 1990; **28**: 556-560.

Brew BJ, Pemberton L, Blennow K, Wallin A, Hagberg L. CSF amyloid beta42 and tau levels correlate with AIDS dementia complex. *Neurology* 2005; **65**: 1490-1492.

Brew BJ, Letendre SL. Biomarkers of HIV related central nervous system disease. *International Review of Psychiatry* 2008; **20** (1): 73-88.

Brew BJ, Letendre S. Biomarkers of HIV-Related Central Nervous System Disease. In: Paul RH, Sacktor NC, Valcour V, Tashima KT, editors. HIV and the Brain: New challenges in the modern era. New York: Humana Press, 2009: 49-75.

Brown A, Islam T, Adams R, Nerle S, Kamara M, Eger C *et al*. Osteopontin enhances HIV replication and is increased in the brain and cerebrospinal fluid of HIV-infected individuals. *Journal of Neurovirology* 2011; DOI 10.1007/s13365-011-0035-4.

Burdo TH, Wood MR, Fox HS. Osteopontin prevents monocyte recirculation and apoptosis. *Journal of Leukocyte Biology* 2007; **81**: 1504-1511.

Burdo T, Ellis R, Fox H. Osteopontin Is Increased in HIV-Associated Dementia. *The Journal of Infectious Diseases* 2008; **198**: 715-22.

Burt TD, Agan BK, Marconi VC, Weijing H, Kulkarni H, Mold JE *et al*. Apolipoprotein (apo) E4 enhances HIV-1 cell entry in vitro, and the APOE ε4/ε4 genotype accelerates HIV disease progression. *Proceedings of the National Academy of Sciences* 2008; **105** (25): 8718-8723.

Butters N, Grant I, Haxby J, Judd LL, Martin A, McClelland J *et al.* Assessment of AIDS-related cognitive changes: recommendations of the NIMH workshop on neuropsychological assessment approaches. *Journal of Clinical and Experimental Neuropsychology* 1990; **12** (6): 963-978.

Carey CL, Woods SP, Gonzalez R, Conover E, Marcotte TD, Grant I *et al*. Predictive Value of Global Deficit Scores in Detecting Neuropsychological Impairment in HIV Infection. *Journal of Clinical and Experimental Neuropsychology* 2004; **26** (3): 307 – 319.

Chagan-Yasutan H, Saitoh H, Ashino Y, Arikawa T, Hirashima M, Li S *et al*. Persistent Elevation of Plasma Osteopontin Levels in HIV Patients Despite Highly Active Antiretroviral Therapy. *The Tohoku Journal of Experimental Medicine* 2009; **218**: 285-292.

Chaves ML, Camozzato AL, Ferreira ED, Piazenski I, Kochhann R, Dall'Igna O *et al*. Serum levels of S100B and NSE proteins in Alzheimer's disease patients. *Journal of Neuroinflammation* 2010, **7**: 6 (http://www.jneuroinflammation.com/content/7/1/6).

Cherner M, Ellis RJ, Lazzaretto D *et al*. Effects of HIV-1 infection and aging on neurobehavioural functioning: preliminary findings. *AIDS* 2004; **18** (Suppl 1): S27-S34.

Cherner M, Cysique L, Heaton RK, Marcotte TD, Ellis RJ, Masliah E *et al*. Neuropathologic confirmation of definitional criteria for human immunodeficiency virus-associated neurocognitive disorders. *Journal of Neurovirology* 2007; **13**: 23-28.

Childs EA, Lyles RH, Selnes OA, Chen B, Miller EN, Cohen BA *et al*. Plasma viral load and CD4 lymphocytes predict HIV-associated dementia and sensory neuropathy. *Neurology* 1999; **52** (3): 607-613.

Ciccarelli N, Fabbiani N, Di Giambenedetto S, Fanti I, Baldonero E, Bracciale L *et al*. Efavirenz associated with cognitive disorders in otherwise asymptomatic HIV-infected patients. *Neurology* 2011; **76** (16): 1403-1409.

Clifford DB, McArthur JC, Schifitto G, Kieburtz K, McDermott MP, Letendre S *et al*. A randomized clinical trial of CPI-1189 for HIV-associated cognitive-motor impairment. *Neurology* 2002; **59** (10): 1568-1573.

Clifford DB, Smurzynski M, Park LS, Yeh TM, Zhao Y, Blair L *et al*. Effects of active HCV replication on neurologic status in HIV RNA virally suppressed patients. *Neurology* 2009; **73** (4): 309-314.

Clinical Guidelines for the Management of HIV & AIDS in Adults and Adolescents. National Department of Health, South Africa 2010. Available from: http://www.doh.gov.za/docs/index.html (accessed 23/06/2011)

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW *et al*. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 1993; **261**: 921- 923.

Corder EH, Robertson K, Lannfelt L, Bogdanovic N, Eggertsen G, Wilkins J *et al*. HIV-infected subjects with E4 allele for APOE have excess dementia and peripheral neuropathy. *Nature Medicine* 1998; **4** (10): 1182-1184.

Corwin J, Bylsma FW. Translations of excerpts from Andre Rey's Psychological examination of traumatic encephalopathy and PA Osterrieth's The Complex Figure Copy Test. *Clinical Neuropsychologist* 1993; **7**: 3-15.

Cysique LA, Vaida F, Letendre S, Gibson S, Cherner M, Woods SP *et al.* Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. *Neurology* 2009; **73** (5): 342-348.

Cysique LA, Murray JM, Dunbar M, Jeyakumar V, Brew B. A screening algorithm for HIV-associated neurocognitive disorders. *HIV Medicine* 2010; **11**: 642-649.

D'Elia LF, Satz P, Uchiyama CL, White T. Color Trails Test: Professional Manual. Odessa: Psychological Assessment Resources 1996.

De Ronchi RD, Faranca I, Berardi D, Scudellari P, Borderi M, Manfredi R *et al*. Risk factors for cognitive impairment in HIV-1-infected persons with different risk behaviours. *Archives of Neurology* 2002; **59** (2): 812-818.

De Vries HE, Kuiper J, de Boer AG, Van Berkel TJC, Breimer D D. The blood-brain barrier in neuroinflammatory disease. *Pharmacological Reviews* 1997; **49** (2): 142-155.

Dore BJ, McDonald A, Li Y, Kaldar JM, Brew BJ. Marked improvement following AIDS dementia complex in the era of highly active antiretroviral therapy. *AIDS* 2003; **17**: 1539-1545.

Dubois B, Feldman HH, Jacova C, DeKosky ST, Barberger-Gateau P, Cumming J *et al*. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurology* 2007; **6:** 734-746.

Dunlop O, Goplen AK, Liestål K, Myrvang B, Rootwelt H, Christopherson B *et al*. HIV dementia and apolipoprotein E. *Acta Neurologica Scandanavia* 1997; **95**: 315-318.

Edinger AL, Mankowski JL, Doranz BJ, Margulies BJ, Lee B, Rucker J *et al*. CD4-independent, CCR5-dependent infection of brain capillary endothelial cells by a neurovirulent simian immunodeficiency virus strain. *Proceedings of the National Academy of Sciences* 1997; **94** (26): 14742 – 14747.

Ellis R, Langford D, Masliah E. HIV and antiretroviral therapy in the brain: neuronal injury and repair. *Nature Reviews Neuroscience* 2007; **8**: 33-44.

Ellis R J, Badiee J, Vaida F, Letendre S, Heaton RK, Clifford D *et al*. Nadir CD4 Count is a Predictor of HIV Neurocognitive Impairment in the Era of Combination Antiretroviral Therapy. *AIDS* 2011; **25**: doi: 10.1097/QAD.0b013e32834a40cd.

Ernst T, Chang L. Effect of aging on brain metabolism in anti-retroviral naïve HIV patients. *AIDS* 2004; **18** (Suppl 1): S61-S67.

Evans SR, Yeh TM, Sacktor N, Clifford DB, Simpson D, Miller EN *et al*. Selegiline transdermal system (STS) for HIV-associated cognitive impairment: open-label report of ACTG 5090. *HIV Clinical Trials* 2007; **8** (6): 437-446.

Everall IP, Heaton RK, Marcotte TD, Ellis RJ, McCutchan JA, Atkinson JH *et al*. Cortical Synaptic Density is Reduced in Mild to Moderate Human Immunodeficiency Virus Neurocognitive Disorder. *Brain Pathology* 1999; **9**: 209-217.

Firnhaber C, Reyneke A, Schulze D, Malope B, Maskew M, MacPhail P *et al*. The prevalence of hepatitis B co-infection in a South African urban government HIV clinic. *South African Medical Journal* 2008; **98** (7): 541-544.

Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Wachter H. Neopterin as a Marker in HIV Infection. *Clinical Chemistry* 1988; **34** (2): 466-467.

Fuchs D, Chiodi F, Albert J, Asjo B, Hagberg L, Hausen A *et al*. Neopterin concentrations in cerebrospinal fluid and serum of individuals infected with HIV-1. *AIDS* 1989; **3** (5): 285-288.

Gabuzda DH, Hirsch MS. Neurologic Manifestations of Infection with Human Immunodeficiency Virus: Clinical Features and Pathogenesis. *Annals of Internal Medicine* 1987; **107** (3): 383-391.

Gannon P, Khan MZ, Kolson DL. Current understanding of HIV-associated neurocognitive disorders pathogenesis. *Current opinion in Neurology* 2011; **24**: 275 - 283.

Gartner S. HIV Infection and Dementia. *Science* 2000; **287**: 602-604.

Ghafouri M, Amini S, Khalili K, Sawaya B. HIV-1 associated dementia: symptoms and causes. *Retrovirology* 2006; **3**: 28. doi: 10.1186/1742-4690-3-28.

Gisslen M, Rosengen L, Hagberg L, Deeks SG, Price RW. Cerebrospinal fluid signs of neuronal damage after antiretroviral treatment interruption in HIV-1 infection. *AIDS Research Therapy* 2005; **2**: 6. doi: 10.1186/1742-6405-2-6.

Gisslen M, Hagberg L, Brew BJ, Cinque P, Price RW, Rosengren L. Elevated Cerebrospinal Fluid Neurofilament Light Protein Concentrations Predict the Development of AIDS Dementia Complex. *Journal of Infectious Diseases* 2007; **195:** 1774-8.

Glass JD, Fedor H, Wesselingh SL, McArthur JC. Immunocytochemical quantitation of human immunodeficiency virus in the brain: Correlations with dementia. *Annals of Neurology* 1995; **38** (5): 755-762.

Gonzalez E, Rovin BH, Sen L, Cooke G, Dhanda R, Mummidi S *et al*. HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration of tissues and MCP-1 levels. *Proceedings of the National Academy of Sciences* 2002; **99** (21): 13795-13800.

Gonzalez-Scarano F, Martin-Garcia J. The Neuropathogenesis of AIDS. *Nature Reviews Immunology* 2005; **5**: 69-78.

Grant I, Atkinson H, Hesselink JR, Kennedy CJ, Richman DD, Spector SA *et al*. Evidence for Early Central Nervous System Involvement in the Acquired Immunodeficiency Syndrome (AIDS) and Other Human Immunodeficiency Virus (HIV) Infections: Studies with Neuropsychologic Testing and Magnetic Resonance Imaging. *Annals of Internal of Medicine* 1987; **107**: 828- 836.

Grant I. Neurocognitive disturbances in HIV. *International Review of Psychiatry* 2008; **20** (1): 33-47.

Gresle MM, Butzkueven H, Shaw G. Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis. *Multiple Sclerosis International* 2011; Article ID 315406. doi: 10.1155/2011/315406.

Gupta JD, Satishchandra P, Gopukumar K, Wilkie F, Waldrop-Valverde D, Ellis R *et al*. Neuropsychological deficits in human immunodeficiency virus type 1 clade C-seropositive adults from South India. *Journal of Neurovirology* 2007; **13** (3): 195-202.

Haase A. Pathogenesis of lentivirus infections. *Nature* 1986; **322:** 130-136.

Hagberg L, Andersson LM, Abdulle S, Gisslen M. Clinical Application of Cerebrospinal Fluid Neopterin Concentrations in HIV Infection. *Pteridines* 2004; **15** (3): 102-106.

Hagberg L, Cinque P, Gisslen M, Brew B, Spudich S, Bestetti B *et al*. Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection. *AIDS Research and Therapy* 2010; **7**: 15. (http://www.aidsrestherapy.com/content/7/1/15)

Heaton RK, Chelune GJ, Talley JL, Kay GG, Curtiss G. Wisconsin Card Sorting Test Manual: Revised and expanded. Odessa: Psychological Assessment Resources; 1993.

Heaton RK, Grant I, Butters N, White D, Kirson D, Hampton Atkinson J *et al*. The HNRC 500 – Neuropsychology of HIV infection at different disease stages. *Journal of the International Neuropsychological Society* 1995; **1**: 231-251.

Heaton K, Marcotte TD, Mindt MR, Sadek J, Moore DJ, Bentley H *et al*. The impact of HIV-associated neuropsychological impairment on everyday functioning. *Journal of the International Neuropsychological Society* 2004; **10**: 317-331.

Heaton RK, Clifford DB, Franklin JR, Woods SP, Ake C, Vaida F *et al*. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: the CHARTER study. *Neurology* 2010; **75**: 2087-2096.

Heaton RK, Franklin DR, Ellis RJ, McCutchan A, Letendre SL, Le Blanc S *et al*. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *Journal of Neurovirology* 2011; **17**: 3-16.

Heseltine PN, Goodkin K, Atkinson JH, Vitiello B, Rochon J, Heaton RK *et al*. Randomized double-blind placebo-controlled trial of peptide T for HIV-associated cognitive impairment. *Archives of Neurology* 1998; **55** (1): 41-51.

Ho DD, Sarngadharan MG, Resnick L, Dimarzo-Veronese F, Rota TR, Hirsch MS. Primary Human T-Lymphotrophic Virus Type III Infection. *Annals of Internal Medicine* 1985; **103** (6 pt1): 880 – 883.

Hoth K, Haley A, Gunstad J, Paul R, Poppas A, Jefferson A *et al*. Elevated C-Reactive protein is related to cognitive decline in older adults with cardiovascular disease. *Journal of the American Geriatric Society* 2008; **56**: 1898-1903.

Huber C, Batchelor JR, Fuchs D, Hausen A, Lang A, Niederwieser D *et al*. Immune Response-associated production of neopterin. *Journal of Experimental Medicine* 1984; **160**: 310-316.

Hult B, Chana G, Masliah E, Everall I. Neurobiology of HIV. *International Review of Psychiatry* 2008; **20** (1): 3-13.

Human Phosphorylated Neurofilament H ELISA kit handbook. Biovendor Research and Diagnostic Products. Cat. No. : RD191138300R. Version 98 090911 20.

Hutterer J, Fuchs D, Eder G *et al*. Neopterin as a discriminating and prognostic parameter in healthy homosexual, ARC and AIDS patients. *Wiener Klinische Wochenschrift* 1987; **99** (15): 531-535.

Jones BN, Teng EL, Folstein MF, Harrison KS. A New Bedside Test of Cognition for Patients with HIV Infection. *Annals of Internal Medicine* 1993; **119** (10): 1001-1004.

Joska JA, Gouse H, Paul RH, Stein D, Flisher AJ. Does highly active antiretroviral therapy improve neurocognitive function? A systematic review. *Journal of Neurovirology* 2010a; **16**: 101-114.

Joska JA, Westgarth-Taylor J, Myer L, Hoare J, Thomas KGF, Combrinck M *et al*. Characterization of HIV-Associated Neurocognitive Disorders Among Individuals Starting Antiretroviral Therapy in South Africa. *AIDS Behaviour* 2010b; doi 10.1007/s10461-010-9744-6.

Joska JA, Fincham DS, Stein DJ, Paul RH, Seedat S. Clinical Correlates of HIV-Associated Neurocognitive Disorders in South Africa. *AIDS Behaviour* 2010c; **14**: 371-378.

Joska JA, Combrinck MI, Valcour VG, Hoare J, Leisegang F, Mahne AC *et al*. Association between apolipoprotein E4 genotype and human immunodeficiency virus-associated dementia in younger adults starting antiretroviral therapy in South Africa*. Journal of Neurovirology* 2010d; doi 10.3109/13550284.2010.513365.

Joska JA, Hoare J, Stein DJ, Flisher AJ. The neurobiology of HIV dementia: implications for practice in South Africa. African Journal of Psychiatry 2011a; **14**: 17-22.

Joska JA, Westgarth-Taylor J, Hoare J, Thomas KGF, Paul R, Myer L *et al*. Validity of the International HIV Dementia Scale in South Africa. *AIDS Patient Care and STDs* 2011b; **25** (2): doi: 10.1089/apc.2010.0292.

Joska JA, Westgarth-Taylor J, Hoare J, Thomas KGF, Paul R, Myer L *et al*. Neuropsychological Outcomes in Adults Commencing Antiretroviral Therapy in South Africa. *Fourth International Meeting on HIV Infection and the Central Nervous System* July 2011c; Abstract OC2, also presentation by Joska JA on 15 July 2011.

Kanmogne GD, Kennedy RC, Grammas P. HIV-1 gp120 proteins and gp160 peptides are toxic to brain endothelial cells and neurons: Possible pathway for HIV entry into the brain and HIV-associated dementia. *Journal of Neuropathology and Experimental Neurology* 2002; **61** (11): 992-1000.

Kavalci C, Pekdemir M, Durukan P, Ilhan N, Yildiz M, Serhatlioglu S *et al*. The value of serum tau protein for the diagnosis of intracranial injury in minor head trauma. *American Journal of Emergency Medicine* 2007; **25**: 391-395.

Ketzler S, Weis S, Haug H, Budka H. Loss of neurons in the frontal cortex in AIDS brains. *Acta Neuropathologica* 1990; **80**: 92-94.

Kitchen M, Quigley MA, Mwinga AM, Fuchs D, Lisse IM, Porter JDH *et al.*HIV Progression and Predictors of Mortality in a Community-Based Cohort of Zambian Adults*. Journal of the International Association of Physicians in AIDS Care* 2008; **7** (1): 17-26.

Klove H. Clinical neuropsychology. In: The Medical Clinics of North America. Forster FM (Ed). New York: W B Saunders, 1963.

Komulainen P, Lakka T, Kivipelto M, Hassinen M, Penttila I, Helkala E *et al*. Serum high sensitivity C-reactive protein and cognitive function in elderly women. *Age and Ageing* 2007; **36**:443-448.

Kramer L, Bauer E, Funk G, Hofer H, Jessner W, Steindl-Munda P *et al*. Subclinical impairment of brain function in chronic hepatitis C infection. *Journal of Hepatology* 2002; **37** (3): 349-354.

Kuller L, Tracy R, Belloso W, De Wit S, Drummond F, Lane C H *et al*. Inflammatory and Coagulation Biomarkers and Mortality in Patients with HIV Infection. *PLoS Med* 2008; **5** (10) e203: 1496-1508.

Kumar A, Borodowsky I, Fernandez B, Gonzalez L, Kumar M. Human immunodeficiency virus type 1 RNA levels in different regions of human brain: Quantification using real-time reverse transcriptase-polymerase chain reaction*. Journal of Neurovirology* 2007; **13**: 210-224.

Laeyendecker O, Li X, Arroyo M, McCutchan F, Gray R, Wawer M *et al*. The Effect of HIV Subtype on Rapid Disease Progression in Rakai, Uganda. Abstract 44LB, *13th Conference on Retroviruses and Opportunistic Infections* 2006.

Lamers K, Vos P, Verbeek M, Rosmalen F, van Geel WJA, van Engelen BGM. Protein S-100B, neuron-specific enolase (NSE), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) in cerebrospinal fluid (CSF) and blood of neurological patients. *Brain Research Bulletin* 2003; **61**: 261-264.

Langford TD, Letendre SL, Larrea GJ, Masliah E. Changing patterns in the Neuropathogenesis of HIV During the HAART Era. *Brain Pathology* 2003; **13**: 195-210.

Lawler K, Mosepele M, Ratcliffe S, Seloilwe E, Steele K, Nthobatsang R *et al*. Neurocognitive impairment among HIV-positive individuals in Botswana: a pilot study. *Journal of the International AIDS Society* 2010; **13**:15. doi: 10.1186/1758-2652-13-15.

Letendre SC, Ellis R, Marcotte T, Heaton R, McCutchan JA, Grant I. Individuals co-infected with hepatitis C (HCV) and HIV are more cognitively impaired than those infected with either virus alone. Presentation at *4th International Symposium on Neurovirology and 10th Conference on Neuroscience of HIV Infection* 2002.

Letendre SL, McCutchan JA, Childers ME, Woods SP, Lazzaretto D, Heaton RK. Enhancing Antiretroviral Therapy for Human Immunodeficiency Virus Cognitive Disorders. *Annals of Neurology* 2004; **56**: 416-423.

Letendre SL, Woods SP, Steven P, Ellis RJ, Atkinson JH, Eliezer M *et al*. Lithium improves HIV-associated neurocognitive impairment. *AIDS* 2006; **20** (14): 1885-1888.

Letendre S, Marquie-Beck J, Capparelli E, Best B, Clifford D, Collier A *et al*. Validation of the CNS Penetration-Effectiveness Rank for Quantifying Antiretroviral Penetration Into the Central Nervous System. *Archives of Neurology* 2008; **65** (1): 65-70.

Letendre S *et al*. Revised Central Nervous System Penetration-Effectiveness Ranking. *17th Conference on Retroviruses and Opportunistic Infections* 2010, Abstract 172.

Letendre S. The CPE Score. Lecture given at *Fourth International Meeting on HIV Infection and the Central Nervous System*. Rome, Italy. 16 July 2011.

Levine A J, Singer EJ, Shapshak P. The Role of Host Genetics in the Susceptibility for HIV-associated Neurocognitive Disorders. *AIDS Behaviour* 2009; **13**: 118 – 132.

Liner J, Robertson K, Meeker RB. Antiretroviral neurotoxicity. Poster presentation at *39th Annual Neuroscience Meeting*, Chicago; 2009.

Liu NQ, Lossinsky AS, Popik W, Li X, Gujuluva C, Kriederman B *et al*. Human immunodeficiency virus type 1 enters brain microvascular endothelia by micropinocytosis dependent on lipid rafts and the mitogen-associated protein kinase signalling pathway. *Journal of Virology* 2002; **76** (13): 6689-6700.

Luminex Corporation website:
http://www.luminexcorp.com/TechnologiesScience/xMAPTechnology/
[Accessed 04/01/2012]

Marra CM, Zhao Y, Clifford DB, Letendre S, Evans S, Henry K *et al*. Impact of Combination Antiretroviral Therapy on Cerebrospinal Fluid HIV RNA and Neurocognitive Performance. *AIDS* 2009; **23** (11): 1359 - 1366.

Masliah E, Achim CL, Ge N, Deteresa R, Terry RD, Wiley CA. Spectrum of human immunodeficiency virus-associated neocortical damage. *Annals of Neurology* 1992a; **32**: 321-329.

Masliah E, Ge N, Achim CL, Hansen LA, Wiley CA. Selective neuronal vulnerability in HIV encephalitis. *Journal of Neuropathology and Experimental Neurology* 1992b; **51**: 585-593.

McArthur J, Hoover D, Bacellar H, Miller EN, Cohen BA, Becker JT *et al*. Dementia in AIDS patients: Incidence and risk factors. *Neurology* 1993; **43**: 2245-2252.

McArthur J C. HIV Dementia: an evolving disease. *Journal of Neuroimmunology* 2004; **157:** 3-10.

McArthur JC, Brew B, Nath A. Neurological complications of HIV infection. *Lancet Neurology* 2005; **4**: 543-555.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan E. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 1984; **34:** 939-942.

McPhail ME, Robertson KR. Neurocognitive Impact of Antiretroviral Treatment: Thinking Long-Term. *Current HIV/AIDS Reports* 2011. doi: 10.1007/s11904-011-0091-7.

Mellgren A, Price RW, Hagberg L, Rosengren L, Brew BJ, Gisslen M. Antiretroviral treatment reduces increased CSF neurofilament protein (NFL) in HIV-1 infection. *Neurology* 2007; **69**: 1536-41.

Mildvan D, Spritzler J, Grossberg SE, Fahey JL, Johnston DM, Schock BR *et al*. Serum Neopterin, an Immune Activation Marker, Independently Predicts Disease Progression in Advanced HIV-1 infection. *Clinical Infectious Diseases* 2005; **40**: 853-8.

Milliplex® MAP kit handbook. Human bone Panel 1A, 96 well Plate Assay, Cat. # HBN1A-51K.

Mishra M, Vetrivel S, Siddappa NB, Ranga U, Seth P. Clade-specific differences in neurotoxicity of human immunodeficiency virus-1 B and C tat of human neurons: significance of dicysteine C30C31 motif. *Annals of Neurology* 2008; **63** (3): 366-376.

Moore DJ, Masliah E, Rippeth JD, Gonzalez R, Carey CL, Cherner M *et al*. Cortical and subcortical neurodegeneration is associated with HIV neurocognitive impairment. *AIDS* 2006; **20**: 879-887.

Morris KA, Davies NWS, Brew BJ. A guide to the interpretation of neuroimmunological biomarkers in the combined antiretroviral therapy-era of HIV central nervous system disease. *Neurobehavioral HIV Medicine* 2010; **2**: 59-72.

Murdoch DM, Venter WDF, Feldman C, Van Rie A. Incidence and risk factors for the immune reconstition inflammatory syndrome in HIV patients in South Africa: a prospective study. *AIDS* 2008; **22** (5): 601-610.

Nath A, Conant K, Chen P, Scott C, Major EO. Transient exposure to HIV-1 tat protein results in cytokine production in macrophages and astrocytes: a hit and run phenomenon. *The Journal of Biological Chemistry* 1999; **274** (24): 17098-17102.

Nath A, Maragos W, Avison M, Schmitt F, Berger J. Accelerated HIV dementia with methamphetamine and cocaine use. *Journal of Neurovirology* 2001; **7**: 66-71.

Nath A, Schiess N, Venkatesan A, Rumbaugh J, Sacktor N, McArthur J. Evolution of HIV dementia with HIV infection. *International Review of Psychiatry* 2008; **20** (1): 25-31.

Navia B, Jordan B, Price R. The AIDS dementia complex: I. Clinical features. *Annals of Neurology* 1986; **19** (6): 517-524.

Navia BA, Dafni U, Simpson D, Tucker T, Singer E, McArthur JC *et al*. A phase I/II trail of nimodipine for HIV-related neurologic complications. *Neurology* 1998; **51** (1): 221-228.

Neopterin ELISA kit User's Manual. DRG International. Ref EIA-1476.

Nowacek A, Gendelman HE. NanoART, neuroAIDS and CNS drug delivery. *Nanomedicine (London)* 2009; **4** (5): 557-574.

O'Regan AW, Nau GJ, Chupp GL, Berman JS. Osteopontin (Eta-1) in cell-mediated immunity: teaching an old dog new tricks. *Immunology Today* 2000; **21** (10): 475 - 478.

Orrell C, Walensky RP, Losina E, Pitt J, Freedberg KA, Wood R. HIV type-1 clade C resistance genotypes in treatment-naïve patients after first virological failure in a large community antiretroviral treatment programme. *Antiviral Therapy* 2009; **14**: 523-531.

Pemberton L, Brew BJ. Cerebrospinal fluid S100beta and its relationship with AIDS dementia complex. *Journal of Clinical Virology* 2001; **22**: 249-253.

Perry VH, Cunningham C, Holmes C. Systemic infections and inflammation affect chronic neurodegeneration. *Nature Reviews Immunology* 2007; **7**: 161-167.

Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. *Journal of the Neurological Sciences* 2005; **233**: 183-198.

Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet* 1993; **342**: 697-699.

Power C, Selnes OA, Grim JA, McArthur JC. HIV Dementia Scale: a rapid screening test. *Journal of Acquired Immune Deficiency Syndrome Human Retrovirology* 1995; **8** (3): 273 – 8.

Price RW, Epstein LG, Becker JT, Cinque P, Gisslen M, Pulliam L *et al*. Biomarkers of HIV-1 CNS infection and injury. *Neurology* 2007; **69**: 1781-1788.

Quasney MW, Zhang Q, Sargent S, Mynatt M, Glass J, McArthur J. Increased Frequency of the Tumour Necrosis Factor-alpha-308 A Allele in Adults With Human Immunodeficiency Virus Dementia. *Annals of Neurology* 2001; **50**: 157-162.

Rackstraw S. HIV-related neurocognitive impairment – A review. *Psychology, Health & Medicine* 2011; **16** (5): 548-563.

Ragin A, Wu Y, Du H, Ochs R, Epstein L. Injury to the brain is evident early in HIV infection. *18th Conference on Retroviruses and Opportunistic Infections* 2011; Abstract 55LB.

Ramlawi B, Rudolph J, Mieno S, Feng J, Boodhwani M, Khabbaz K *et al*. C-Reactive Protein and inflammatory response associated to neurocognitive decline following cardiac surgery. *Surgery* 2006; **140**: 221-226.

Rao VR, Sas AR, Eugenin EA, Siddappa NB, Bimonte-Nelson H, Berman JW *et al*. HIV-1 clade specific differences in the induction of neuropathogenesis. *The Journal of Neuroscience* 2008; **28** (40): 10010-10016.

Reading within the Luminex® analyser (image). Taken from: http://www.origene.com/xmap/ [Accessed 04/01/2012]

Reger M, Welsh R, Razani J, Martin DJ, Boone KB. A meta-analysis of the neuropsychological sequelae of HIV infection. *Journal of the International Neuropsychological Society* 2002; **8**: 410-424.

Reitan RM. Validity of the Trail making Test as an indicator of organic brain damage. *Perceptual and Motor Skills* 1958; **8**: 271-276.

Robertson KR, Smurzynski M, Parsons TD, Wu K, Bosch RJ, Wu J *et al*. The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS* 2007; **21**: 1915 – 1921.

Robertson KR, Su Z, Margolis DM, Krambrinck A, Havlir DV, Evans S *et al*. Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology* 2010; **74**: 1260-1266.

Rosen H, Karlsson J, Rosengren L. CSF levels of neurofilament is a valuable predictor of long-term outcome after cardiac arrest. *Journal of the Neurological Sciences* 2004; **221**: 19-24.

Rosengren L , Karlsson J, Sjogren M, Blennow K, Wallin A. Neurofilament protein levels in CSF are increased in dementia. *Neurology* 1999; **52**: 1090-1093.

Rourke SB, Carvalhal A, Zipursky AR, Bekele T, McCombe J, Rachlis A *et al*. Examining the impact of CNS penetration-effectiveness of combination antiretroviral treatment (cART) on neuropsychological outcomes in persons living with HIV: findings from the Ontario HIV Treatment Network (OHTN) cohort study. *6th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention*. 17-20 July 2011. Abstract MOAB0104.

Ryan EL, Morgello S, Isaacs K, Naseer M, Gerits P, Manhattan HIV Brain Bank. Neuropsychiatric impact of hepatitis C on advanced HIV. *Neurology* 2004; **62** (6): 957-962.

Sacktor N, Schiffito G, McDermott MP, Marder K, McArthur JC, Kieburtz K. Transdermal Selegiline in HIV-associated cognitive impairment: pilot, placebo-controlled study. *Neurology* 2000; **54** (1): 233-235.

Sacktor NC, Wong M, Nakasujja N, Skolasky RL, Selnes OA, Musisi S *et al*. The International HIV Dementia Scale: a new rapid screening test for HIV dementia. *AIDS* 2005; **19** (13): 1367 – 1374.

Sacktor NC, Nakasujja N, Skolasky RL, Robertson K, Wong M, Musisi S *et al.* Antiretroviral Therapy improves cognitive impairment in HIV+ individuals in Sub-Saharan Africa. *Neurology* 2006; **67** (2): 311-314.

Sacktor N, Nakasujja N, Robertson K, Clifford DB. HIV-associated cognitive impairment in sub-Saharan Africa – the potential effect of clade diversity. *Nature Clinical Practice Neurology* 2007; **3** (8): doi 10.1038/ncpneuro0559.

Sacktor N, Nakasujja N, Skolasky RL, Rezapour M, Robertson K, Musisi S *et al*. HIV subtype D is associated with dementia, compared to subtype A, in immunosuppressed individuals at risk of cognitive impairment in Kampala, Uganda. *Clinical Infectious Diseases* 2009; **49** (5): 780-786.

Safety and tolerability of the antioxidant OPC-14117 in HIV-associated cognitive impairment. The Dana Consortium on the Therapy of HIV Dementia and Related Cognitive Disorders. *Neurology* 1997; **49** (1): 142-146.

Saunders AM, Strittmatter WJ, Schmechel DE, George-Hyslop PH, Pericak-Vance MA, Joo SH *et al*. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993; **43**: 1467-1472.

Schifitto G, Sacktor N, Marder K, McDermott P, McArthur JC, Kieburtz K *et al*. Randomized trial of the platelet-activating factor antagonist lexipafant in HIV-associated cognitive impairment. Neurological AIDS Research Consortium. *Neurology* 2002; **59** (10): 391-396.

Schifitto G, Yiannoutsos CT, Ernst T, Navia BA, Nath A, Sacktor N *et al*. Selegiline and oxidative stress in HIV-associated cognitive impairment. *Neurology* 2009a; **73** (23): 1975-1981.

Schifitto G, Zhong J, Gill D, Peterson DR, Gaugh MD, Zhu T *et al*. Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. *Journal of Neurovirology* 2009b; **15** (2): 176-186.

Schifitto G, Zhang J, Evans SR, Sacktor N, Simpson D, Millar LL *et al*. A multicenter trial of selegiline transdermal system for HIV-associated cognitive impairment. *Neurology* 2007; **69** (13): 1314-1321.

Schweinsburg BC, Taylor MJ, Alhassoon OM, Gonzalez R, Brown GG, Ellis RJ *et al*. Brain mitochondrial injury in human immunodeficiency virus-seropositive (HIV+) individuals taking nucleoside reverse transcriptase inhibitors. *Journal of Neurovirology* 2005; **11**: 356-364.

Shaw GJ, Jauch EC, Zemlan FP. Serum cleaved tau protein levels and clinical outcomes in adult patients with closed head injury. *Annals of Emergency Medicine* 2002; **39**: 254-7.

Shiramizu B, Gartner S, Williams A *et al*. Circulating proviral HIV DNA and HIV-associated dementia. *AIDS* 2005; **19**: 43-52.

Shiramizu B, Ratto-Kim S, Sithinamsuwan P, Nidhinandana S, Thitivichianlert S, Watt G *et al*. HIV DNA and dementia in treatment-naïve HIV 1 infected individuals in Bangkok, Thailand. *International Journal of Medical Sciences* 2007; **4** :13-18

Simioni S, Cavassini M, Annoni J, Rimbault Abraham A, Bourquin I *et al*. Cognitive Dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS* 2010; **24** (9): 1243- 1250.

Simioni S, Cavassini M, Annoni J, Hirschel B, Du Pasquier R. HIV-associated neurocognitive disorders: a changing pattern. *Future Neurology* 2011; **6** (1): 81-95.

Singh KK, Ellis RJ, Marquie-Beck J, Letendre S, Heaton RK, Grant I *et al*. CCR2 polymorphisms affect neuropsychological impairment in HIV-1-infected adults. *Journal of Neuroimmunology* 2004; **157**: 185-192.

Smith CA, van Gorp W, Ryan ER, Ferrando SJ, Rabkin J. Screening Subtle HIV-Related Cognitive Dysfunction: The Clinical Utility of the HIV Dementia Scale*. Journal of Acquired Immune Deficiency Syndromes* 2003; **33** (1): 116-118.

Snider WD, Simpson DM, Nielson S, Gold WMJ, Metroka CE, Posner JB. Neurological complications of acquired immune deficiency syndrome: Analysis of 50 patients. *Annals of Neurology* 1983; **14**: 403-418.

South African Department of Health. Circular on new criteria for initiating of adults on ART at CD4 count of 350 cells/ul and below. August 2011.

Spector SA, Singh KK, Gupta S, Cysique LA, Jin H, Letendre S *et al*. APOE epsilon4 and MBL-2 O/O genotypes are associated with neurocognitive impairment in HIV-infected plasma donors. *AIDS* 2010; **24** (10): 1471-1479.

Spreen O, Strauss E. A compendium of neuropsychological tests: Administration, norms and commentary, 2nd Edition. New York: Oxford University Press; 1998: 447-464.

Spudich SS, Ances BM. Central Nervous System Complications of HIV Infection, in : Highlights of the 18th Conference on Retroviruses and Opportunistic Infections. *Topics in Antiviral Medicine* 2011; **19** (2): 48-57.

Stern Y. Cognitive Reserve. *Neuropsychologica* 2009; **47** (10): 2015-2028.

Stroick M, Fatar M, Ragoschke-Schumm A, Fassbender K, Bertsch T, Hennerici MG. Protein S-100B – A Prognostic Marker for Cerebral Damage. *Current Medicinal Chemistry* 2006; **13**: 3053-3060.

Stroop JR. Studies of interference in serial verbal reaction. *Journal of Experimental Psychology* 1935; **18**: 643-662.

The Normal Distribution (image). Taken from Wikipedia.org, "Standard Score". [http://en.wikipedia.org/wiki/Standard_score - accessed 21/11/2011]

Tozzi V, Balestra P, Salvatori M, Vlassi C, Liuzzi G, Giancola M *et al*. Higher CPE Score Improved Cognition, Changes in Cognition During Antiretrovial Therapy: Comparison of 2 Different Ranking Systems to Measure Antiretroviral Drug Efficacy on HIV-Associated Neurocognitive Disorders. *Journal of Acquired Immune Deficiency Syndromes* 2009; **52** (1): 56-63.

UNAIDS Report on the Global AIDS Epidemic 2010. Available at: http://www.unaids.org/globalreport/Global_report.htm (accessed 23/06/2011)

Valcour V, Shikuma C, Shiramizu B, Watters M, Poff P, Selnes O *et al*. Higher frequency of dementia in older HIV-1 individuals: The Hawaii aging with HIV-1 Cohort. *Neurology* 2004; **63**: 822-827.

Valcour VG, Shiramizu B, Sithinamsuwan P, Nidhinandana S, Ratto-Kim S, Ananwaranich J *et al*. HIV DNA and cognition in a Thai longitudinal HAART initiation cohort: the SEARCH 001 cohort study. *Neurology* 2009; **72**: 992-998.

Valcour V, Sithinamsuwan P, Letendre S, Ances B. Pathogenesis of HIV in the Central Nervous System. *Current HIV/AIDS Reports* 2011a; **8** : 54-61.

Valcour V, Sailasuta N, Chalermchai T, Marovich M, Lerdlum S, Suttichom D *et al*. HIV Brain Viral and Inflammatory Signature during Acute Infection. *18th Conference on Retroviruses and Opportunistic Infections* 2011b; Abstract 54

Valenzuela MJ, Sachdev P. Brain reserve and dementia: a systematic review. *Psychological Medicine* 2006; **36**: 441-454.

Vally Z. HIV-associated neurocognitive disorders. *South African Journal of Psychiatry* 2011; **17** (4): 98-102.

Van Harmelen JH, Van der Ryst E, Loubser AS, York D, Madurai S, Lyons S *et al.* A predominantly HIV Type 1 Subtype C-Restricted Epidemic in South African Urban Populations. *AIDS Research and Human Retroviruses* 1999; **15** (4): 395-398.

Vasan A, Renjifo B, Hertzmark E, Chaplin B, Msamanga G, Essex M *et al*. Different rates of disease progression of HIV type 1 infection in Tanzania based on infecting subtype. *Clinical Infectious Diseases* 2006; **42**: 843-852.

Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurology* 2011; **10**: 241-252.

Vinocur JM. ELISA. Wikipedia.org. 2006.[http://en.wikipedia.org/wiki/ELISA – accessed 18/11/2011]

Wang K, Denhardt D. Osteopontin: Role in immune regulation and stress responses. *Cytokine and Growth Factor Reviews* 2008; **19**: 333-345.

Wechsler D. Wechsler Adult Intelligence Scale, 3rd Edition. San Antonio: The Psychological Corporation; 1997a.

Wechsler D. Wechsler Memory Scale, 3rd Edition. San Antonio: The Psychological Corporation; 1997b.

Wirleitner B, Schroeksnadel K, Winkler C, Fuchs D. Neopterin in HIV-1 Infection. *Molecular Immunology* 2005; **42**: 183-194.

Wong MH, Robertson K, Nakasujja N, Skolasky R, Musisi S, Katabira E *et al*. Frequency of and risk factors for HIV dementia in an HIV clinic in sub-Saharan Africa. *Neurology* 2007; **68** (5): 350-355.

Woods SP, Moore DJ, Weber E, Grant I. Cognitive Neuropsychology of HIV-Associated Neurocognitive Disorders. *Neuropsychology Review* 2009; **19**: 152-168.

Wright E. Neurological disease: the effects of HIV and antiretroviral therapy and the implications for early antiretroviral therapy initiation. *Current Opinion in HIV and AIDS* 2009; **4** (5): 447-452.

xMAP® technology bead (image). Taken from http://www.biotekinstruments.ru/ru/resources/articles/wash-luminex-xmap- assays.html - accessed 04/01/2012]

Yepthomi T, Paul R, Vallabhaneni S, Kumarasamy N, Tate D, Solomon S *et al*. Neurocognitive consequences of HIV in Southern India: a preliminary study of clade C virus. *Journal of the International Neuropsychological Society* 2006; **12** (3): 424-430.