A Follow-up Study Evaluating the Effects of HIV Related Cognitive Impairment

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Declaration

I declare that this thesis has not been submitted for examination at this or any other university and it is entirely my own work.

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Dedication

This thesis is dedicated to my Mother, a wonderful woman, without whose support and encouragement I would never have been able to complete this work. She is unconditionally loving, kind and tireless in her devotion to her children and grandchildren. She is my rock and any enlightenment that emanates from my beacon is entirely due to her energy and inspiration.

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Summary

This thesis reports the results of a follow-up study involving an Irish cohort of people living with HIV (PLWH) who were screened between 2010 and 2013 for HIV related cognitive impairment (CI), known as HIV Associated Neurocognitive Disorder (HAND). Four studies were undertaken between 2014 and 2018, all of which addressed different aspects of HAND. The first project followed a sub-cohort of PLWH with neuropsychology testing to evaluate the trajectory of HAND in the context of sustained viral suppression. In the second project, a subset of PLWH underwent follow-up brain imaging to evaluate for progressive grey matter degeneration with the use of Voxel Based Morphometry (VBM) and for white matter microstructural changes using Diffusion Tensor Imaging (DTI). The third project compared the hospital services and resource use in PLWH screening positive for CI with PLWH screening negative. Finally, the burden and management of epilepsy and seizures in HAND was evaluated.

The primary aim of this study was to evaluate the natural course of HAND in the era of highly active antiretroviral therapy (HAART). On neuropsychological follow-up, this cohort showed an overall stability, with only a minority of PLWH progressing to severe cognitive impairment. MRI showed marked widespread inflammatory / microstructural changes in the white matter at baseline, with relative stability at follow-up. Indolent, however, insignificant cortical grey matter loss was also observed over time. PLWH who screened positive for CI were shown to have poorer clinical outcomes and used hospital resources more intensively than those screening negative. Lastly, PLWH were found to have a

higher burden of seizures than the general population and often failed to achieve seizure control and disengaged from specialist services.

Table of Contents

List of Figures

List of Tables

1. Introduction

1.1 Background

Human immunodeficiency virus (HIV) is a lentivirus, a genus of retroviruses. Its hallmark is the reverse transcription of viral RNA into the host DNA. It is acquired via sexual contact, infected blood products, use of contaminated needles in intravenous drug users, and by vertical transmission from mother to child (1). HIV primarily infects immune system cells, CD4+T cells, macrophages, and dendritic cells. It impairs the affected immune cells function and gradually destroys them, thereby suppressing the infected individual's immune system and predisposing them to opportunistic infections (*Pneumocystis jirovecii* formerly *Pneumocystis carinii, Cytomegalovirus, Toxoplasma gondii, Candida albicans, Cryptococcus neoformans, Mycobacterium tuberculosis, Mycobacterium avium complex* and others are amongst them), and some malignancies (most commonly Kaposi's sarcoma and non-Hodgkin's lymphoma) (2).

According to 2001 report of the Joint United Nations Programme on HIV-AIDS (UNAIDS), HIV infection was the fourth leading cause of death worldwide at the beginning of the 21st century. Although HIV/AIDS related deaths are no longer among the top 10 causes of death, HIV and its complications continue to be one of the most significant public health challenges and remain one of the leading causes of disability-adjusted life years (DALY) worldwide (3). It is estimated that a total of 36.7 million people lived with HIV in 2016 and this figure reached 36.9 million at the end of 2017 (4, 5). The vast majority of people living with HIV

(PLWH) in 2016 and 2017 were working age adults. This is impacting negatively on the global economy and individual families. This study project is focused on one of the disabling HIV infection complications – HIV Associated Neurocognitive Disorder or so called HAND.

1.2 HIV History

HIV infection was unknown until the 1980's. Sporadic cases of acquired immune deficiency syndrome were reported prior to the 1970's and it is believed that the current HIV epidemic started in the mid or late 1970's (6). However, it was not until 1981, when five cases of a rare lung infection called *Pneumocystis carinii pneumonia* (PCP) were reported in previously healthy homosexual men (MSM - men having sex with men) in Los Angeles, and an unusually aggressive cancer termed Kaposi's sarcoma was reported in a group of men in New York and California (7), that concern emerged regarding a common pathogenesis. By the end of 1981, there were a total 270 reported cases of severe immune deficiency associated with opportunistic infections and cancers among MSM, which suggested that the cause of the immune deficiency was sexually transmitted (8). In June 1982, the new syndrome was initially called gay-related immune deficiency (GRID) by the Centers for Disease Control and Prevention (CDC) (9). However, at the same time, there were emerging reports of PCP in people who injected drugs, in haemophiliacs, and in Haitians. Subsequently, in September 1982, for the first time, the CDC used the term Acquired Immune Deficiency Syndrome (AIDS) (9-11).

In May 1983, Luc Antoine Montagnier with his colleagues at the Pasteur Institute in France reported the discovery of a new retrovirus that could be the cause of

AIDS and called it Lymphadenopathy-Associated Virus (LAV) (12). In April 1984, a team led by Robert Gallo in the US National Cancer Institute isolated the retrovirus Human T Lymphotropic virus Type III (HTLV-III) and announced that it was the likely cause of AIDS (13, 14). In January 1985, in a joint conference with the Pasteur Institute, it had been concluded that LAV and HTLV-III were an identical organism (15). In May 1986, the International Committee on Taxonomy of Viruses renamed it to Human Immunodeficiency Virus (HIV) (16).

The first commercial enzyme linked immuno-sorbent assay test (ELISA), to detect antibodies to the virus in the serum was licensed by the US Food and Drug Administration (FDA) in March 1985 (17). Two years later, in March 1987, the FDA approved the first antiretroviral drug, zidovudine (AZT), as a treatment for HIV (18). In June 1995, the FDA approved the first protease inhibitor (PI), which marked the beginning of a new era of highly active antiretroviral treatment (HAART) (19). Once HAART was introduced, an immediate decline of between 60% and 80% in rates of AIDS-related deaths and hospitalisations was noted, thus improving survival of HIV infected individuals and transforming HIV infection into a chronic illness (20). In September 2015, the World Health Organisation (WHO) issued the new treatment guidelines recommending that all HIV positive individuals should be placed on antiretroviral treatment as soon as possible after their diagnosis, regardless of their WHO clinical stage and CD4 count (21).

1.3 HIV Infection

1.3.1 HIV Virus Structure

HIV is a lentivirus that belongs to Retroviridae family, as it contains *reverse transcriptase,* which irreversibly transcribes viral RNA into the host cell DNA. The HIV *virion*, or the virus particle consists of *genome*, *capsid* and *envelope*. The HIV *genome* encodes the virus genetic information within two identical unpaired positive-sense single RNA strands enclosed inside the *capsid*. The *capsid* is a cone shaped "coat" formed by viral protein *Gag p24*, which, together with other nucleocapsid protein *Gag p7*, protects the *genome* from nucleases during the invasion into the prospective host cell (22). The HIV *genome* codes for nine *genes*: three *structural genes – pol, gag, env,* and six *regulatory genes – tat, rev, nef, vif, vpu,* and *vpr*. Of them, *tat* and *rev* are essential *regulatory genes* (1).

The HIV virus has a bilayer phospholipid *envelope* derived from the host cell. Virus derived envelope proteins, *gp41* and *gp120*, are encoded by the *env* gene and form envelope trimmer *spike complexes.* These mediate virus entry into the host cell (22). Glycoprotein *gp120* determines the HIV *viral tropism. Gp120* has a high affinity for CD4 receptors that are present on the surface of T4 lymphocytes, macrophages, and monocytes and attaches the virus to these cells. In this way, *gp120* plays a crucial role in the host cell invasion and initiation of HIV replication (1).

1.3.2 HIV Virus Types and Strains

There are two major HIV virus types, HIV-1 and HIV-2. HIV-1 is the most common one and accounts for 95% of worldwide infections. HIV-2 is less

common and is considered to be less virulent (23). The two types are distinguished by the different envelope glycoprotein antibody reactivity. The retroviruses have high mutation, recombination, and replication rates. These contribute to high genetic diversification and survival in the hostile environment. There are many genetically distinct groups and variants within the main HIV types. The genetic diversity has implications in HIV diagnosis, viral load monitoring, anti-retroviral therapy response, and resistance development. HIV-1 has four main groups, M, N, P, and O. Group M, “major”, is the most common of them and is divided into nine subtypes, or *clades,* A, B, C, D, F, G, H, J, and K (24). Clade B is the dominant subtype in Europe, Australia, and in the Americas. Although it is only responsible for about 11% of the worldwide HIV infections, it is the most studied one. Clade C causes about 48% of global HIV infections. It is more common in India and South Africa (25).

1.3.3 Mechanism of Infection

Once the HIV virus enters the host organism either through direct inoculation via contaminated needles, blood products transfusion, across mucosal membranes or via the placenta, it first encounters the dendritic cells. Dendritic cells express a HIV specific receptor – DC-SIGN. Viral glycoprotein *gp120* binds to dendritic DC-SIGN and the virus is transported to lymphoid tissue. Here, dendritic cells act as antigen presenting cells and prime the naïve T cells (26).

To infect the host cell, the virus is required to bind at two sites: CD4+ receptor and one of the chemokine co-receptors. Chemokine co-receptor CXCR4 is required for the T cell tropic strains (X4 virus) and CCR5 chemokine co-receptor

is required for the macrophage tropic strains (R5 virus) (27). Rapid viral replication and widespread dissemination ensues after the successful transmission and, ultimately, results in progressive CD4+ T cell depletion (28). An actively replicating virus can destroy immune cells through direct cytopathic mechanisms, by interfering with cell metabolism as a result of high multiplication rate, via membrane destruction during “budding”, or through infection and killing of lymphoid progenitor cells. Anti-HIV immune responses may be involved in indirect killing of T cells, as the host is trying to control the infection (1, 2, 27-29).

1.3.4 HIV Infection Staging

Once acquired, the HIV virus persists and causes lifelong infection. The WHO uses the presence or absence of immunological and clinical symptoms and their severity to classify the course of HIV infection into four clinical stages (30). The most recently updated WHO clinical staging is presented in Table 1.1.

Table 1.1. WHO Clinical Staging of HIV/AIDS for Adults and Adolescents with Confirmed HIV Infection

Clinical stage 1 - Asymptomatic
Asymptomatic Persistent generalized lymphadenopathy
Clinical stage 2 - Mild symptoms
Moderate unexplained weight loss (<10% of presumed or measured body weight) Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media and pharyngitis) Herpes zoster Angular cheilitis

Recurrent oral ulceration
Papular pruritic eruptions
Seborrhoeic dermatitis
Fungal nail infections
Clinical stage 3 - Advanced symptoms
Unexplained severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for longer than one month
Unexplained persistent fever (above 37.6°C intermittent or constant, for longer than one month)
Persistent oral candidiasis
Oral hairy leucoplakia
Pulmonary tuberculosis (current)
Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 × 109 per litre) or chronic thrombocytopaenia (<50 × 109 per litre)
Clinical stage 4 - Severe symptoms
HIV wasting syndrome
Pneumocystis pneumonia
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site)
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extra-pulmonary tuberculosis
Kaposi's sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extra-pulmonary cryptococcosis including meningitis
Disseminated non-tuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis (with diarrhoea)
Chronic isosporiasis
Disseminated mycosis (coccidiomycosis or histoplasmosis)
Recurrent non-typhoidal Salmonella bacteraemia
Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours
Invasive cervical carcinoma

Atypical disseminated leishmaniasis Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

WHO clinical staging of HIV adapted from WHO Report on HIV/ADS Programme: Case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children, 2007 (30).

Last revised in 2008, the CDC classification of HIV infection, which is intended for surveillance purposes, is based on the presence or absence of an AIDS defining illness, combined with laboratory data on CD4+ T lymphocytes count and/or percentages, and requires laboratory evidence of HIV infection (Table 1.2) (31).

Table 1.2. CDC Staging of HIV Infection

HIV infection stage	Laboratory evidence	Clinical evidence
Stage 1	Laboratory confirmation of HIV infection* **and** CD4+ T-lymphocyte count of ≥500 cells/μL **or** CD4+ T-lymphocyte percentage of ≥29%	None required but no AIDS-defining condition
Stage 2	Laboratory confirmation of HIV infection* **and** CD4+ T-lymphocyte count of 200-499 cells/μL **or** CD4+ T-lymphocyte percentage of 14-28%	None required but no AIDS-defining condition
Stage 3 (AIDS)	Laboratory confirmation of HIV infection* **and** CD4+ T-lymphocyte count of <200 cells/μL **or** CD4+ T-lymphocyte percentage of <14%	**or** documentation of an AIDS-defining condition (with laboratory confirmation* of HIV infection)
Stage Unknown	Laboratory confirmation of HIV infection* **and** no information on CD4+ T-lymphocyte count **or** CD4+ T-lymphocyte percentage	**and** no information on presence of AIDS-defining conditions

*Criteria for laboratory confirmation of HIV infection
Positive result from an HIV antibody screening test (e.g., reactive enzyme immunoassay [EIA]) confirmed by a positive result from a supplemental HIV antibody test (e.g., Western blot or indirect immunofluorescence assay test).
or
Positive result or report of a detectable quantity (i.e., within the established limits of the laboratory test) from any of the following HIV virologic (i.e., non-antibody) tests: - HIV nucleic acid (DNA or RNA) detection test (e.g., polymerase chain reaction [PCR]) - HIV p24 antigen test, including neutralisation assay - HIV isolation (viral culture)

CDC staging of HIV infection and criteria for HIV infection confirmation adapted from "Revised Surveillance Case Definitions for HIV Infection Among Adults, Adolescents, and Children Aged <18 Months and for HIV Infection and AIDS Among Children Aged 18 Months to < 13 Years --- United States, 2008" (31).

1.3.5 HIV Treatment

1.3.5.1 HIV treatment initiation

The main treatment goal is to achieve consistent viral suppression and, in this way, reduce the HIV associated morbidity and mortality (21, 32). Since the guidelines were first issued in 2002, there is progressively more evidence to support the benefit of early and immediate treatment (32). The current WHO guidelines on when to start antiretroviral therapy (ART) were last revised and updated in 2015 (21). The WHO recommends initiating ART therapy in all individuals living with HIV, independent of their disease clinical stage and at any CD4+ count. However, in the countries with limited resources, treatment initiation is prioritised for those with advanced clinical stage or severe disease, and for adults whose CD4+ count is at/below 350 cell/mm^3 and children whose CD4+ count is at/below 750 cell/mm^3 (21, 33).

1.3.5.2 HIV treatment agents

Currently, there are more than twenty five antiretroviral drugs available. These are classified according to their mode of action (Table 1.3) (34). Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs) inhibit reverse transcriptase (RT), thus blocking the virus replication. Non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs) interfere with virus replication by preventing RT from completing reverse transcription. Protease inhibitors (PIs) block viral protease and preclude viral maturation, or the budding. Co-receptor inhibitors (CIs/CCR5 antagonists) block chemokine receptor CCR5, preventing R5 virus strains from infecting host cells. Integrase inhibitors (IIs) block viral enzyme integrase, preventing the viral genome from integrating into the host cell DNA. Fusion inhibitors (FIs) stop the virus envelope merging with the host cell membrane and thus prevent virus entry into the cell. Highly Active Antiretroviral Therapy (HAART), a term coined in the late 1990's, describes the viral suppression effectiveness of a combination of antiretroviral therapy drugs, with different mechanisms of action. A number of fixed dose combinations are available to facilitate adherence to complex treatment regimens (32).

Table 1.3. List of Available Antiretroviral Drugs

Drug class	Drug name
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Abacavir, Didanosine, Emtricitabine, Lamivudine, Stavudine, Zidovudine, Zalcitabine
Nucleotide analog Reverse Transcriptase Inhibitors (NtRTIs)	Tenofovir

Drug class	Drug name
Non-Nucleoside analog Reverse Transcriptase Inhibitors (NNRTIs)	Delavirdine, Efavirenz, Etravirine, Nevirapine, Rilpivirine
Protease Inhibitors (PIs)	Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir, Amprenavir
Integrase Inhibitors (IIs)	Dolutegravir, Raltegravir
Fusion Inhibitors (FIs)	Enfuvirtide (Ibalizumab - reviewed for FDA approval)
Co-receptor Inhibitor (CIs) / CCR5 Antagonist	Maraviroc
Approved Fixed-Dose Combinations	Abacavir + dolutegravir + lamivudine, or ABC/DTG/3TC (Triumeq) Abacavir + lamivudine, or ABC/3TC (Epzicom) Abacavir + lamivudine + zidovudine, or ABC/3TC/ZDV (Trizivir) Efavirenz + emtricitabine + tenofovir, or EFV/FTC/TDF (Atripla, Tribuss) Elvitegravir + cobicistat + emtricitabine + tenofovir, or EVG/COBI/FTC/TAF or ECF/TAF (Genvoya) Elvitegravir + cobicistat + emtricitabine + tenofovir, or EVG/COBI/FTC/TDF or ECF/TDF (Stribild) Emtricitabine + rilpivirine + tenofovir, or FTC/RPV/TAF (Odefsey) Emtricitabine + rilpivirine + tenofovir, or FTC/RPV/TDF (Complera) Emtricitabine + tenofovir, or TAF/FTC (Descovy) Emtricitabine + tenofovir, or TDF/FTC (Truvada) Lamivudine + zidovudine, or 3TC/ZDV (Combivir)

1.4 Neurologic Complications of HIV

Despite the introduction of effective HAART treatment, neurologic complications of HIV infection remain common and may occur at any infectious stage (35). The rate of HIV associated neurologic complications in various case series has been quoted to be as high as 40-70% in the AIDS, or in the symptomatic HIV infected population (36-38). HIV can affect the nervous system indirectly by leaving the infected individual vulnerable to opportunistic infections and primary central nervous system lymphoma (PCNSL). The most commonly described central nervous system (CNS) opportunistic infections in the context of advanced HIV stage (CD4+ count <200 cells/ml) are cryptococcal meningitis, CNS toxoplasmosis, and progressive multifocal leukoencephalopathy (PML). Other opportunistic infections include TB meningitis, Cytomegalovirus (CMV) encephalitis, polyradiculopathy, mononeuritis multiplex, and less frequent Herpes Zoster Virus (HZV) neuropathies, and encephalitis caused by Herpes Simplex Viruses (HSV-1 and HSV-2 encephalitis) (Table 1.4) (39).

HIV may also directly affect both, the peripheral and central nervous systems. It produces distinct clinical syndromes such as HIV associated dementia (HAD) and milder forms of HIV related cognitive impairment, HIV encephalopathy (HIVE), seizures, vacuolar myelopathy, and distal predominantly sensory axonal polyneuropathy (40-42).

Table 1.4. Classification of the Neurological Complications of HIV Infection Based on Underlying Disease Pathophysiology

Immunological dysregulation
Autoimmune disease (early and middle phases of HIV infection)
Acute-phase encephalitis, neuropathies
Subacute and chronic idiopathic demyelinating polyneuropathies
Multiple-sclerosis-like disease
Immunosuppression: opportunistic infections/neoplasm (late phase of HIV infection)
Cerebral toxoplasmosis
Progressive multifocal leukoencephalopathy (PML)
Primary CNS lymphoma (PCNSL)
CMV encephalitis, polyradiculopathy, mononeuritis multiplex
HIV-driven
AIDS dementia complex
Distal predominantly sensory polyneuropathy
Secondary conditions
Metabolic/toxic
Hypoxic encephalopathies
Narcotic overdose
Nucleoside neuropathies
Zidovudine myopathy
Psychiatric disorders
Reactive anxiety, depression

Classification of the neurological complications of HIV infection based on underlying disease pathophysiology adapted from Richard W Price, Neurological complications of HIV infection, Lancet, 1996 (39).

1.5 Cognitive Impairment in HIV

HIV related cognitive impairment (CI) is an important neurologic complication of HIV. At least half of HIV infected individuals complain of or exhibit varying degrees of cognitive dysfunction, impaired memory and concentration,

psychomotor slowing, difficulties processing and learning new information, lack of motivation and apathy (43). In a case series by Navia et al., cognitive impairment was reported to be the presenting or the only sign of AIDS in 25% of patients (43).

1.5.1 HAND Terminology and Nomenclature History

Soon after the HIV epidemic emerged, there was a growing body of evidence that its causative organism not only affected the immune system of its host, but could possibly affect the brain "directly", causing acquired persistent cognitive decline. An unusual "mental impairment" in those who suffered from "stubbornly resistant to treatment infections and cancers" was first noted in the early 1980's (44). At the time, HIV related progressive dementia was described by Snider et al. as a triad of cognitive decline, behaviour changes, and impaired motor performance, with preserved alertness and was called *subacute encephalitis* or *subacute encephalopathy* (44). Prior to the introduction of HAART, dementia associated with HIV was a common feature of the late stages of AIDS and almost inevitably led to person's demise. It was seen in up to 50% of HIV infected patients prior to death (45).

In 1986, Navia et al. described in detail this unique constellation of neurobehavioral features and its correlation with the autopsy findings in 46 patients who suffered from unexplained cognitive impairment often associated with behaviour or motor dysfunction during the course of AIDS, and coined the term *AIDS Dementia Complex* (ADC) for it (43). According to the severity of impairment, Price et al. classified the ADC into stages and categories and

thoroughly described each of them (46). Patients with *severe dementia* were usually bedridden, with severe intellectual disability, markedly limited intellectual and social capacity (often mute), and required full time assistance. Patients with *moderate dementia* were impaired in two or more cognitive domains, and were not able to function independently, but were able to independently perform simple activities of daily living. Those with *mild dementia* were capable of independent functioning, despite experiencing cognitive symptoms and objective deficits in one cognitive area (Table 1.5).

Table 1.5. AIDS Dementia Complex Clinical Staging

Stage 0 (Normal)
- Normal mental and motor function
Stage 0.5 (equivocal and subclinical)
- Absent, minimal or equivocal symptoms without impairment of work or capacity to perform activities of daily living. - Mild signs (snout response, slower ocular or extremity movements) may be present. - Gait and strength are normal.
Stage 1 (mild)
- Able to perform all but the more demanding aspects of work or activities of daily living, but with unequivocal evidence (signs and symptoms that may include performance on neuropsychological testing) of functional intellectual or motor impairment. - Can walk without assistance.
Stage 2 (moderate)
- Able to perform basic activities of self-care, but cannot walk or maintain the more demanding aspects of daily life.

- Ambulatory, but may require a single prop.
Stage 3 (severe)
- Major intellectual incapacity (cannot follow news or personal events, cannot sustain complex conversation, considerable slowing of all outputs) and motor disability (cannot walk unassisted, requires walker or personal support, usually with slowing and clumsiness of arms as well).
Stage 4 (end stage)
- Nearly vegetative. - Intellectual and social comprehension and output are at rudimentary level. - Nearly or absolutely mute. - Paraparetic or paraplegic, with urinary and faecal incontinence.

Clinical staging of ADC, adapted from Richard W Price, The AIDS dementia complex, Journal of Infectious Diseases, 158, 1079-1083, 1988 (46).

In 1991, another group, AIDS Task Force of American Academy of Neurology (AAN) led by Robert S Janssen, renamed ADC to *HIV-1 Associated Dementia* (HAD) and outlined the major differences between the clinical features of *Minor Cognitive / Motor Disorder* (MCMD) associated with HIV-1 and the three subcategories of HAD (*HAD with motor symptoms*, *HAD with behavioural or psychosocial symptoms*, and *HAD with both motor and behavioural/psychosocial symptoms*) (47).

Later, in 2007, Antinori et al. reviewed the 1991 AAN terminology and diagnostic criteria for cognitive disorders associated with HIV and proposed the new term, *HIV Associated Neurocognitive Disorders* (HAND), to include *HIV associated Dementia* (HAD) and HIV associated *Mild Neurocognitive Disorder* (MND). They also described a group of HIV positive individuals with subclinical cognitive

impairment and suggested the inclusion of a new nosology termed *Asymptomatic Neurocognitive Impairment* (ANI) (48).

1.5.2 Research Diagnostic Criteria for HAND

Along with the revised definitions and terminology, Antinori et al. proposed the new research criteria for HAD, MND, and ANI, as outlined in Table 1.6. The purpose of the new criteria was to refine a few issues with the previous classification and criteria. In order to address the overlap between the previously existing criteria for HAD with mild functional decline and MCMD, the number of domains that should be examined, along with the number of cognitive domains and the degree of impairment in those domains that would permit classification of severity, was defined. The revised criteria also reflect the possible reversibility of HIV related cognitive dysfunction. Lastly, the new criteria permitted inclusion of milder forms of cognitive impairments identified on formal neuropsychological testing that had not progressed enough to interfere substantially with everyday functioning, such as in ANI (48).

Table 1.6. Revised Research Criteria for HIV-Associated Neurocognitive Disorders (HAND)

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.**

HIV-1-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 standard deviation below the mean for age-education-appropriate norms on standardised neuropsychological tests. The neuropsychological tests 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 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.**

HIV-1-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 standard deviations, or greater than demographically corrected means.
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.
4. There is no existing evidence of another, pre-existing cause for the dementia.**

*If there is a prior diagnosis of ANI, MND, or HAD, but currently the individual does not meet the criteria, the diagnosis of ANI, MND, or HAD in remission can be made.

** If the individual with suspected ANI, MND, or HAD also satisfies criteria for a major depressive episode or substance dependence, the diagnosis of ANI, MND, or HAD, respectively, should be deferred to a subsequent examination conducted at a time when the major depression has remitted or at least 1 month after the cessation of substance use.

Note 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 treatment of depression.

Revised research criteria for HAND adapted from Antinori et al., Updated research nosology for HIV-associated neurocognitive disorders, Neurology, Vol 69, Issue 18, 2007 (48).

The Antinori et al. revised research criteria have been used in the former cross-sectional study “Neuropsychological Assessment of Patients with a Positive Screen for Cognitive Impairment” conducted at St. James’s Hospital, Dublin, by P. McNamara et al. (49) , and in the current follow-up study.

1.6 Change with HAART

1.6.1 HIV Incidence and Prevalence Change

The introduction of effective Highly Active Antiretroviral Therapy (HAART) in 1995 has substantially transformed the course of the HIV epidemic. The HIV epidemic trajectory can be now divided into three phases: (i) from 1980's to 1997 when the incidence of new HIV infections reached its peak (3.3 million/year), (ii) from 1997 to 2005, when HIV incidence had a steep fall after the introduction of HAART, and (iii) from 2005 to 2016, when it plateaued at about 2.6 million/year (4).

Once introduced into clinical practice, HAART also reduced the AIDS-related deaths and transformed HIV infection into a chronic disease (19, 20). HIV infected individuals started to live longer. Brodt et al. observed a more than 70% decline of AIDS defining conditions in a cohort of 1,003 HIV seropositive MSM over the course of four years, from 1992 to 1996 (50). However, despite the decreasing incidence of the new cases over the past decade, the prevalence has been on the rise, with the number of people living with HIV reaching 38.8 million in 2015 (4, 51).

1.6.2 HAND Incidence and Prevalence Change

After the introduction of HAART, not only did the incidence of new HIV infections and AIDS drop dramatically, but also the incidence of AIDS related dementia halved, as described in the Multicentre EuroSIDA study. This study included 7,300 HIV seropositive individuals from over 15 countries (52). In the MACS Study (Multicenter AIDS Cohort Study), HIV related dementia incidence also plummeted by 50%, from 21.1% in 1990 to 10.5% in 1998 (35). McArthur in his

review, “HIV dementia an evolving disease”, reported that incidence of severe to moderate HIV related dementia fell from 6.6% in 1989 to 1% in 2000 (53). In another retrospective analysis of HIV outpatient clinic visits between 1995 and 1998 by Maschke et al., the incidence of ADC fell from 17.1% in 1995-96 to 11.2% in 1997-98 (38). However, it was noted by Sacktor et al. that the more subtle forms of impairment still developed in as many as 20% of treated HIV infected individuals (54).

More recent studies show that, despite the decreased incidence of HAD, the prevalence of the milder forms of HAND is on the rise. In the CHARTER study (CNS HIV Antiretroviral Therapy Effects Research), 52% of the 1,555 HIV seropositive individuals recruited from 2003 to 2007 had some degree of neuropsychological impairment. The specific diagnoses were estimated to be 33% for ANI, 12% for MND, and only 2% for HAD (55). In the MACS study, HAND affected 33% of the 364 HIV positive patients seen between 2007 and 2008, and of them 14% had ANI, 14% had MND, and 5% had HAD (56).

1.6.3 Change of Temporal Course and Pattern of HAND

The pattern and course of HIV related cognitive dysfunction has also evolved since it was first described in the 1980’s. Prior to the availability of HAART, HIV related dementia had a distinct pattern of neurocognitive impairment with its distinct course. It usually developed insidiously in the setting of the AIDS diagnosis, with many individuals experiencing an abrupt acceleration of cognitive dysfunction, while some of them had sudden development of mental decline over the course of a few days. The total duration of the dementia phase was one to

nine months. Once severely demented, the median survival was one to six months, with opportunistic infections or aspiration pneumonia being the usual cause of death (43). Introduction of the effective treatment, however, has changed the time course of the disease by improving survival. In a retrospective analysis of the Australian AIDS Notification Data by Dore et al., a greater survival was observed in those diagnosed with ADC, from 11.9 months in 1993–1995 to 48.2 months in 1996–2000. Furthermore, in those with ADC and a CD4 count of less than 100 cell/ml at the time of diagnosis, survival improved from a mean of five months in the 1993-1995 to 38.5 months in 1996-2000 (57).

In the pre-HAART era, HIV dementia had a prototypic “subcortical” pattern (43). Severe cognitive impairment ensued after the initial milder symptoms of inattention, mental slowness, difficulty in performing serial 7’s, and impaired judgement, which then progressed to little or no meaningful response to surroundings, rendering the patient fully dependant. These symptoms were accompanied with behavioural changes (gradual social withdrawal, or agitation and socially inappropriate behaviour, frank psychosis) and motor dysfunction (more frequently worsening ataxia, hypertonia, paraplegia, tremor, frontal release signs, and myoclonus and, less frequently, seizures) (43, 53). With the introduction of HAART, the severity of cognitive impairment evolved to a milder phenotype, with prevailing MND and ANI presentations (55, 56). The clinical features of dementia have also changed, from a predominantly “subcortical” frontostriatal pattern to a mixed “cortical and subcortical” pattern, with prominent cortical involvement with severe memory impairments (53). Cortical involvement in the post-HAART era was also demonstrated on PET CT by Brew et al. (58).

1.7 Risk Factors for HAND Development

Navia et al. in an autopsy case series of 70 patients with AIDS described 46 cases who had otherwise unexplained severe mental retardation and nearly two thirds of them had no focal or other metabolic confounding disorder (43). It was also noted that demented patients had a higher incidence of systemic CMV *(Cytomegalovirus)* or MAI *(Mycobacterium avium intracellulare)* infection than non-demented patients, which suggested that development of dementia required the establishment of severe immune deficiency. Cognitive impairment was regarded in 1999 by McArthur et al. as a common complication of AIDS, with up to 15% of patients with AIDS experiencing dementia and 15% having milder forms of cognitive dysfunction (59). Low nadir CD4+ count (the lowest recorded count) was found to be an important risk factor for the development of cognitive impairment with an incidence of HIV related dementia being as high as 7% in those with CD4+ counts of less than 100 cell/ml in the MACS study (39). Of note, it was observed in the same study that cognitive impairment developed at any CD4+ count, varying from 0.5% in those with CD4+ counts of more than 500 cell/ml, to 1.3-1.7% in those with CD4+ counts of 201-500 cell/ml, and up to 3.0% in those with CD4+ counts between 101 and 200 cell/ml.

Becker et al. in a study on a HIV seropositive ageing cohort found a 23% dementia prevalence in those older than 50 years of age compared to 9% in those who were younger, whilst a milder cognitive impairment prevailed in the youngest group (22% vs 14% in over 50's) (60). The prevalence of HIV dementia was higher in the HIV seropositive population group over the age of 50 in a Hawaiian cohort, compared to those from the younger group (61). Cherner et al.

in their study compared 67 HIV infected patients aged 50 and older with 52 HIV infected patients aged less than 35 and noted age associated decline in most of the cognitive domains. Also, in the older group, cognitive impairment prevalence was twice higher in those with a detectable virus in the cerebrospinal fluid (CSF). Whereas in the younger group, detectable virus in the CSF did not confer a higher risk for the development of cognitive impairment (62).

In a cross-sectional CSF study by Ellis et al. involving 97 HIV seropositive subjects, cognitive impairment was associated with higher viral RNA levels in the CSF but only in those diagnosed with AIDS (3.1 vs 1.8 log 10/ml) (63). Notably, CSF levels of HIV RNA higher than 200 copies/ml were predictive of subsequent development of neurocognitive decline at follow-up in a cohort of 139 HIV positive individuals, with 76 of those initially cognitively intact and 58% of them on ART regimens (64).

Some studies suggested that the HIV virus clade type might be a determinant for the development of neuropsychological decline and that individuals infected with clade D have a greater chance of developing HAD, followed by those infected with clades B, C, and A (65). The clade type was determined in 60 subjects by sequence analysis of *gag* and *gp41* regions in a follow-up study in Uganda, where clade C, D, and A infections are predominant. All of them were immunosuppressed and ART naïve at baseline. Eight of the nine (89%) subjects who were clade D infected met diagnostic criteria for HAD in comparison with seven of the 33 (24%) clade A infected patients with HAD (66). However, this was a small number study. Moreover, in another Ugandan study, 54 HIV infected children had the HIV subtype determined and those infected with clade A

performed worse on memory and learning domains than those infected with clade D virus (67). In a study performed in South India, on 119 clade C infected individuals who were not on ART regimens, Gupta et al. described mild to moderate cognitive impairment in 60.5% of the cohort and none of them met diagnostic criteria for HAD (68). However, in a brain volumetric study, which included 17 clade B and 17 clade C infected ART naïve individuals, Ortega et al. found that both groups had significant volume decline, without specific clade effect (69). Data from currently available studies are somewhat conflicting with respect to clade association with HAND, thus larger scale studies are needed to clarify whether one or the other clade is more neurovirulent. Furthermore, a Guinea-Bissau study assessed 22 HIV-2 infected subjects and 45 seronegative controls with the use of international HIV dementia scale (IHDS) and showed no significant difference in performance amongst the groups, suggesting that HIV-2 is less neurovirulent (70).

McCombe et al. examined predictive factors for the development of symptomatic HAND in a cohort of 1,320 seropositive subjects receiving ART and found that low nadir CD4+ count, age, increased longevity with HIV infection, and high baseline viral load were associated with a diagnosis of symptomatic HAND on both, the univariate and multivariate analyses. While African origin, was predictive of symptomatic HAND on the univariate analysis only (71).

1.8 Underlying Mechanisms of HAND

1.8.1 Neuropathology

First reports on identification of AIDS-related virus HTLV-III/LAV in the CSF and brains of adults and children suffering from AIDS encephalopathy or other neurological complications of AIDS came to light in 1985, suggesting that the virus causing AIDS (HIV) was capable of entering the CNS (72, 73) and like other lentiviruses it was a neuroinvasive virus (74). It was then postulated that AIDS related neurocognitive symptoms were possibly a direct effect of the virus entering the CNS, infecting the brain tissue (neurotropic virus), and leading to neurologic symptoms of ADC (neurovirulent virus). The first attempt to describe the morphopathological picture of HAD was made in 1986. In their autopsy series on 70 patients suffering from AIDS, of whom 46 had clinical manifestation of dementia, Navia et al. found distinct histological abnormalities predominantly in the white matter, subcortical structures, such as centrum semiovale, basal ganglia (putamen, caudate, claustrum, and to a lesser degree globus pallidus), and brainstem, mostly pons (75). White matter changes were mainly described as white matter pallor, focal white matter loss and, in a half of the cases, vacuolation involving centrum semiovale, internal capsule, brainstem, and cerebellum was seen. These findings were associated with foamy macrophages and multinucleated, sometimes giant cell infiltrates and reactive astrocytosis in the most severe cases. However, these findings were noted in only two thirds of the demented patients, although similar milder changes were present in a half of non-demented cases, and were referred to as subclinical changes.

Everall et al. also found a 38% frontal cortex neuronal loss in HIV patients. The authors quantitatively assessed the number of neurons in the frontal cortex in eleven patients with HIV and compared them with the neuronal counts in eight controls who died of systemic illnesses (76). The neural density was 306 x 102/mm^3 in the HIV group, which was comparable to 499 x 102/mm^3 in the control group. There was no significant difference amongst the HIV disease clinical stage sub-groups. In their stereological study, Oster and colleagues found about 37% of global neuronal loss in all neocortical areas in patients with AIDS. However, this finding did not correlate well with the severity of neurocognitive symptoms (77). Another morphopathological finding is gliosis, ranging from 8% in the neurocognitively intact patients, to 11% in those with asymptomatic impairment, and 25% in those with HAD (78).

1.8.2 HIVE and its Association with HAND

The HIV virus particles were demonstrated by electron microscopy within the multinucleated cells in the brains of those suffering from AIDS associated encephalopathy (79, 80). The AIDS associated encephalopathy, or later termed HIV encephalopathy (HIVE), is histologically defined by the pathognomonic presence of multinucleated giant cells associated with the evidence of productive viral infection and the absence of opportunistic infection (81). Multinucleated cells represent fused infected and non-infected macrophages and microglia. The productive viral infection is demonstrated by microglia and perivascular macrophages staining positive for HIV antigens on immunohistochemical study, by a high copy number of pro-viral DNA on Southern blot analysis, or by the presence of viral particles in the multinucleated cells on electron microscopy.

HIVE was a common autopsy finding in the first fifteen years of the HIV epidemic. Martinez et al. described it in 33.5% of his 200 autopsy case series, ranging from 28% in MSM and heterosexual men populations, to 59.5% in intravenous drug users (82). HIVE was found in 56% of drug users and 17% of MSM men with AIDS in an autopsy study by Bell et al. (83). In this study, productive infection was also present in the grey matter in about 50% of HIVE cases, despite its known predilection for subcortical structures and central white matter. Severe dementia in this autopsy series correlated better with HIVE in those who had grey matter involvement, than in those who only had white matter involvement, in whom milder forms of impairment prevailed.

1.8.3 Neuroinvasion and the Blood Brain Barrier

HIVE is frequently found in the advanced stage of HIV infection and presumes the presence of productive infection of CNS. However, when does the virus enter the CNS during the course of the HIV infection?

1.8.3.1 Evidence of early neuroinvasion

Ho et al. isolated the virus from the CSF during the acute HTLV-III (HIV) seroconversion associated with acute aseptic meningitis (84). The authors also cultured the virus from the spinal cord of a patient with myelopathy and from the sural nerve of a patient with peripheral neuropathy. Both of these clinical entities are features of more advanced stages of HIV infection. Examination of brains of HIV infected drug users who died accidentally before developing any clinical signs showed evidence of inflammation with T cell reaction, perivascular cuffing, and low grade leptomeningitis, with an increased number of microglial cells. This

also suggested that HIV virus invasion into the CNS occurs at the time of primary infection (85). The inflammatory infiltrates in the pre-clinical stage were dominated by CD8+ and CD20+ lymphocytes. All of the above findings, together with former evidence of productive infection in the brains of those with AIDS associated HIVE or HAD, support the theory that HIV virus can enter the CNS early after the transmission and either persists in the brain or re-infects it during the course of the HIV disease.

1.8.3.2 Role of the Blood Brain Barrier

How does HIV enter the CNS? Microvascular endothelial cells in the brain vessels and the tight junctions between these cells control traffic of systemically circulating substances and cells creating the blood brain barrier (BBB). BBB “isolates” and protects the brain parenchyma from infectious agents and circulating toxins (86). However, the BBB is selectively permeable and trafficking of immune cells, monocytes and some T lymphocytes through the BBB is part of the physiological immune surveillance, which in theory may “open the door” for intracellular infectious agents (87). Moreover, the brain parenchyma consists of two groups of cells, first – neurons, astrocytes and oligodendrocytes all of which derive from ectodermal plate, and second – parenchymal microglia and perivascular microglia or perivascular macrophages that derive from mesodermal plate. Perivascular macrophages are bone marrow derived (BMD) cells from monocyte lineage that settle in the brain and are replenished by the circulating monocytes throughout an individual’s life course (88). Perivascular macrophages, together with parenchymal microglia, represent the “resident” immunocompetent cells of the brain. Parenchymal microglia expresses the same cell-surface antigen

phenotype as the mononuclear cells of the phagocytic system (88). Notably, animal models showed that about 20% of microglia are re-populated throughout the lifespan (89).

In addition, perivascular macrophages and microglia express the HIV receptors, CD4 receptor together with CCR5 and CXCR4 co-receptors, rendering them susceptible to HIV invasion. All of the above create a “gap” in the BBB and open the “gate” to the HIV virus, which enters the CNS through the so called “Trojan Horse” mechanism as a passenger inside the infected circulating immune cells (lymphocytes or monocytes), and settles then in the microglia (90, 91).

1.8.4 Immune Response and HAND

Although HIVE implies productive virus infection and correlates with HAD to a certain extent, this is not always the case. Bell and colleagues described cases of HAD with no evidence of productive infection on autopsy, as well as cases that histopathologically demonstrated HIVE that had no clinical correlate (83).

1.8.4.1 Role of monocytes and cytokines

In their autopsy study, which included 51 cases, Glass and colleagues found that HAD correlated better with monocyte infiltration (macrophages and microglia) than with the evidence of productive infection, suggesting that HAD might be the product of indirect effect of HIV through actions of activated macrophages and microglia (92). Moreover, Fisher and colleagues found an increased number of CD14+ CD16+ immune cells in the microglial nodules and perivascular infiltrates in the brains of individuals with AIDS and dementia (93). A surge in CD14+

CD16+ monocytes generation in the bone marrow is stimulated by an abnormal production of cytokines such as macrophage colony stimulating factor (M-CSF) in the later stages of AIDS. These cells are thought to be the virus reservoir in the CNS and to be implicated in the brain tissue injury through induction of neuroinflammation. They are phenotypically more phagocytic and express higher levels of pro-inflammatory cytokines such as tumour necrosis factor - α (TNF-α), interleukin (IL-1) and other histocompatibility antigens (94, 95). High levels on TNF-α, IL-1 and interferon-γ (IFN-γ) were found in the brains of HIV infected individuals at all stages of HIV infection by Tyor and colleagues. These findings also support the role of immune activation and neuroinflammation in the HIV neuropathogenesis (96). High plasma monocyte chemoattractant protein-1 (MCP-1) released by activated monocytes was also correlated with subcortical tissue damage demonstrated on MRI (97). High plasma and CSF levels of MCP-1 were associated with HAD and time to death by Sevigny and colleagues in their case series (98).

1.8.4.2 Role of astrocytes

Messam et al., as well as Sabri et al. and other researchers, also demonstrated restricted HIV infection of astrocytes (99, 100). The mechanism of HIV infecting the astrocytes thus far is not clearly understood, as astrocytes do not express CD4 receptors but only express chemokine co-receptors CCR5 and CXCR4. Astrocytes are normally involved in maintaining homeostasis in the CNS and in supporting neurons by buffering neurotoxic compounds such as glutamate and also by production of neurotrophic cytokines. Disruption of astrocytes main

functions is believed to be implicated in the HIV associated neuronal loss. These and other postulated pathogenic mechanisms are represented in the Figure 1.1.

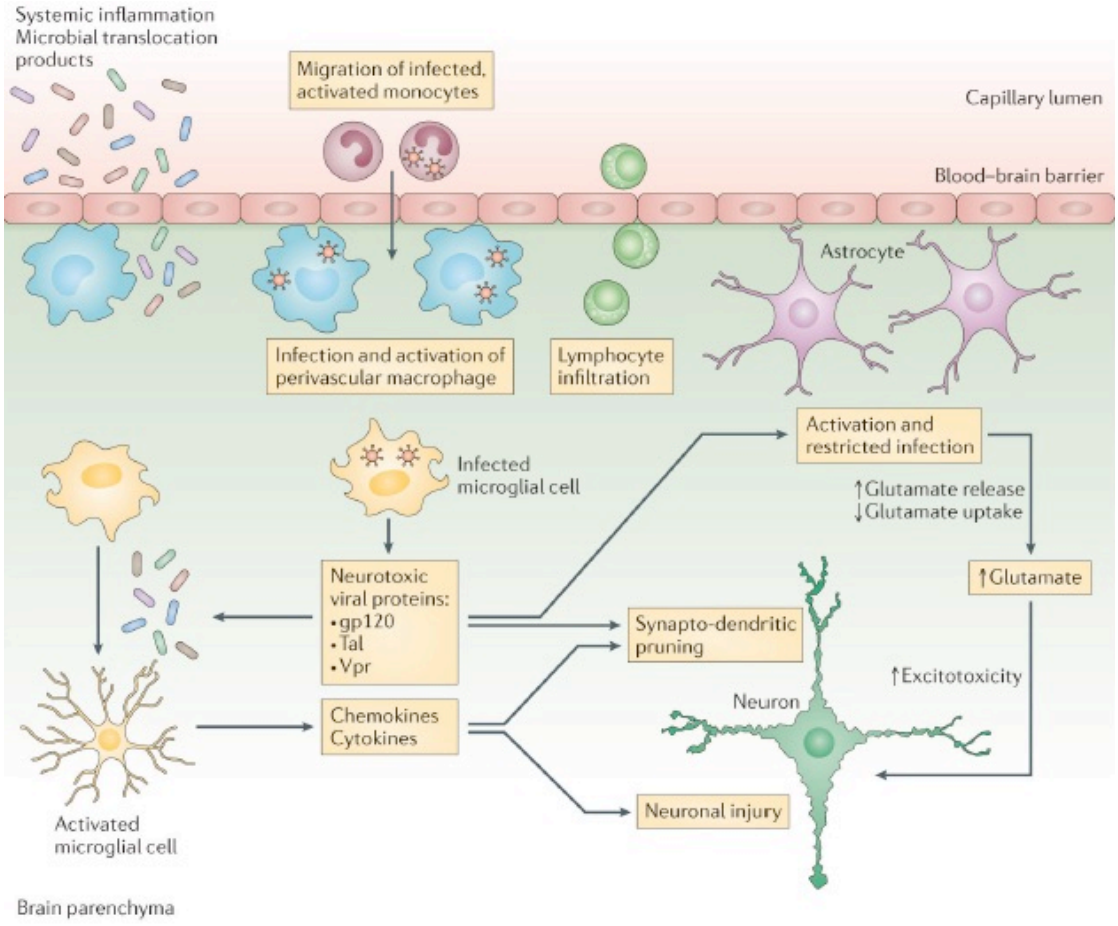


Figure 1.1. Neuropathogenic mechanisms that contribute to HIV associated neurocognitive disorders.

Copy adapted from Saylor et al., HIV-associated neurocognitive disorder--pathogenesis and prospects for treatment, Nature reviews, Neurology, 12, 234-248, 2016 (101).

1.8.4.3 CD8+ encephalitis

After the introduction of HAART, an innate immune response to low level HIV antigen, associated with widespread perivascular CD8+ lymphocytic infiltrates

was also found to underlie progressive neurocognitive impairment in some cases. CD8+ encephalitis usually presents with severe neurocognitive decline in the context of suppressed HIV viral loads (102). Productive infection was not found in the brains of these patients and they responded dramatically to immune suppressive therapy with corticosteroids. Perivascular post-contrast enhancements on the MRI T1 sequence, which resolved on follow-up in those who survived, were seen by Lescure and colleagues in their case series of 14 patients (103). In their study, which involved CSF analysis of 31 participants, Schrier and colleagues found that CSF CD8+ cells that express IFN-γ correlated with HAND severity in ART treated patients, but not the cytolytic CD8+ that express CD107a (104). The CD8+ cytolytic activity was dependent on CD4+ levels being higher than 400/ml and low CSF HIV RNA levels (<103 copies/ml), which supports the theory of aberrant immune response involvement in CD8+ encephalitis.

1.8.4.4 IRIS

After the introduction of HAART, a rapidly deteriorating neurocognitive decline upon ART initiation was reported in several studies and was attributed to the immune reconstitution inflammatory syndrome (IRIS) (105, 106). Brain biopsy showed diffuse perivascular infiltration with macrophages and predominantly CD8+ T lymphocytes with toxic granulations in the leptomeninges, grey and white matter. IRIS is associated with paradoxical neurological deterioration, despite the improvements in CD4+ counts and achieved viral suppression after the therapy initiation. It results from the rapid recovery of the immune system, which targets infectious agents and can develop within the first few months of therapy.

Increased baseline CD8+ and low CD4+, with active or subclinical opportunistic infection at the time of ART commencement, can heighten the risk of IRIS development. It is involved in the pathogenesis of other AIDS-related CNS conditions such as CMV, tuberculosis, cryptococcal infection, and PML (107).

1.8.4.5 CSF escape

Symptomatic CSF escape in the context of a preserved immune system is another rapidly progressive neurocognitive syndrome. It presents in patients on HAART with detectable HIV RNA in the CSF while plasma viral load is stably suppressed (108). It is caused by aberrant immune responses, mainly CD8+ activation, to low level of antigens and demonstrates diffuse inflammatory changes in the brain on neuroimaging. It has a dramatic course that can be reversed with treatment adjustment, to include ART therapy that suppresses CSF viral load more effectively (109).

1.8.4.6 HAART CSF penetration effectiveness

Typically, initiation of HAART substantially reduces the plasma viral load (110). However, this is not the case in the CSF. It may take more than two months for HAART to effectively suppress the virus in the CSF. In their study, Polis and colleagues measured HIV RNA load in the CSF before initiating HAART, at two months and at six months post HAART initiation in 25 subjects who had a baseline plasma VL >8,000 copies/ml. Patients were ART naïve prior to entering the study and were commenced on a four drug therapy. At baseline, the majority of participants (23/25) had a HIV RNA load in the CSF of >50 copies/ml. A plasma VL below 50 copies/ml was achieved within 28 weeks of treatment. At two

months follow-up, 36% of them still had a CSF HIV RNA load of >50 copies/ml, but by six months all had <50 copies/ml (64). Such a delay might be explained by ART ability to enter CNS/CSF. Detection of ART in the CSF can be considered a surrogate marker of ART penetration into CNS.

The blood brain barrier regulates antiretroviral drugs trafficking into the CNS. Moreover, ART penetration into CNS/SCF is further restricted by the ART drugs plasma-protein binding ability. Therefore, different ART agents penetrate into CNS/CSF at different levels and some of them may not even achieve detectable levels. The ART effectiveness in a specific tissue is determined by its penetration and concentration in that tissue. However, ART concentrations and viral load in the CNS cannot be measured directly. Therefore, drug concentrations in the CSF and CSF VL are used as surrogate markers of the above (34).

Poor CSF/CNS penetration of ART agents might have implications in the CNS viral suppression, which, if not controlled can promote HAND (64). Antinori and colleagues measured ART drugs concentration in plasma and CSF in 63 subjects, 78% of whom had neurological complications. Didanosine, efavirenz, nelfinavir, and concomitantly administered ritonavir and saquinavir had undetectable CSF levels in this study. The authors demonstrated higher CSF concentrations for the newer ART agents, nevirapine, lamivudine, stavudine, and indinavir, with a median CSF-to-plasma concentration ratio of 0.63, 0.23, 0.20, and 0.11, respectively (110). Nevirapine and indinavir have the lowest plasma-protein binding ability in their class. The mean CSF viral load was significantly lower in those who were taking at least one therapeutic agent that was detectable in the CSF but did not correlate with the number of detectable drugs in the CSF,

or their CSF concentration (110). Letendre and colleagues assessed the possible benefits of the CSF penetrating ART agents and the CSF viral load suppression on the neuropsychological performance. The global deficit score (GDS) was measured on neuropsychological testing at baseline and at a median of 15 weeks follow-up in 31 cognitively impaired individuals who began new ART therapy. In this study, those who were on a greater number of CSF detectable drugs showed a greater CSF viral load reduction (111). The CSF viral load suppression independently predicted improvement of the GDS, suggesting that cognitively impaired HIV infected individuals might benefit from ART regimens that include CSF penetrating drugs.

Later, in another work, Letendre and colleagues developed and validated a ranking system for antiretroviral drugs, which quantified an individual's ART regimen CNS/CSF penetration effectiveness (CPE), defined as a greater propensity to suppress CSF viral loads. In this study, 467 subjects who took 166 different ART combinations had HIV RNA levels measured in paired plasma and CSF samples. Low CPE ranks (=<1.5) were associated with increased chances of having detectable CSF VL. Those subjects on ART regimens with low CPE ranks were observed to be more likely to develop a severe CNS condition (34). This system was re-evaluated and modified by the same group of researchers in 2010 (Table 1.7). The new ranking system was more strongly associated with CSF VL and was thought to better reflect CNS ART distribution (112).

Cysique et al. assessed performance on neuropsychology tests in 31 participants at four time intervals over a 48 weeks follow-up after initiating ART. An ART drug CPE score equal to or above 2 was predictive of better performance at follow-up

in this study (113). Robertson and colleagues, however, found neuropsychological improvement at 6 months follow-up in 48 subjects, despite the ART regimens with poor CPE used (114). Marra and colleagues, who assessed neurocognitive status in 79 participants at baseline, 24 and 52 weeks after initiating ART, found that ART drugs with greater CNS penetrance were controlling the CSF VL more effectively but were associated with worse neuropsychological outcomes at follow-up (115).

Table 1.7. CNS Penetration Effectiveness Ranking

Drug class	CPE Score			
	4	3	2	1
Nucleoside Reverse Transcriptase Inhibitors	Zidovudine	Abacavir Emtricitabine	Didanosine Lamivudine Stavudine	Tenofovir Zalcitabine
Non-nucleoside Reverse Transcriptase Inhibitors	Nevirapine	Delavirdine Efavirenz	Etravirine	
Protease Inhibitors	Indinavir/r	Darunavir/r Fosamprenavir/r Indinavir Lopinavir/r	Atazanavir Atazanavir/r Fosamprenavir	Nelfinavir Ritonavir Saquinavir/r Tipranavir/r
Entry/Fusion Inhibitors		Maraviroc		Enfuvirtide
Integrase Strand Transfer Inhibitors		Raltegravir		

CPE - central nervous system penetration effectiveness; /r - ritonavir-boosted.

Higher CPE scores reflect estimates of better penetration or effectiveness in the central nervous system (i.e. a ranking of 4 indicates the best penetration or effectiveness).

Adapted from Letendre et al., Neurologic complications of HIV disease and their treatment, Topics in HIV medicine, publication of the International AIDS Society, 18, 45-55, 2010, (112).

Garvey et al., in their retrospective review of nearly 20,000 individuals who commenced ART between 1996 and 2007, also showed that very low CPE ranks (=/<4) were associated with higher mortality but not severe CNS disorders (HIVE, PML, toxoplasmosis and cryptococcal meningitis) (116). In a study which included 5,882 participants, McManus et al. did not find any statistically significant survival benefit of ART with high CPE (117). In their work, Shikuma and colleagues found that ART monocyte efficacy score was associated with better cognitive function rather than high CPE (118). Thus far, the evidence from different studies is inconclusive to support high CPE ART regimens in an attempt to reduce the incidence of HAND. However, this can be helpful when dealing with the issue of CNS escape or CNS compartmentalisation. It is important to note that higher CPE might confer a theoretically possible neurotoxic effect (119).

1.8.4.7 CNS compartmentalisation and quasispecies

Distinct genetic variants of HIV-1 populations in peripheral blood and CSF have been demonstrated on sequence analysis in one subject in an 18 months follow-up study that included eleven patients with primary HIV infection (120). In another two year longitudinal study by Sturdevant and colleagues on paired CSF and blood samples from 72 ART naïve individuals, 30% of participants showed CNS viral replication associated with CSF inflammatory response at some time points. In 16% of them, there was evidence of CNS compartmentalisation of one of the

two transmitted virus lineages. This supports the theory that HIV can adapt to CNS conditions and use it as a tissue reservoir (121). CNS compartmentalisation is thought to be facilitated by T-lymphocytes rather than macrophages or microglia during the early infection phase, as CSF sequestered virus populations were associated with T-lymphocyte (mainly CD8+) CSF pleocytosis. Furthermore, IFN-γ expressing CD8+ in the CSF has been associated with HAND before (121).

Vazquez-Santiago and colleagues explored the presumed genetic heterogeneity in the different body compartments on paired plasma and CSF samples collected at 12 months interval from a patient with ANI. They too demonstrated CCR5 (R5) genetic variants in the CSF on *env* sequencing, as opposed to X4 or dual-tropic variants in the plasma. *Env* determines the cell tropism or the type of cell the virus is going to invade. CNS compartmentalisation of distinct *env* strains persisted throughout the course of this study, despite the long term ART therapy. However, this was only a one case study (122). Antinori et al. postulated that CNS virus replication might be independent from that of other compartments, as they found drug resistant virus strains (quasispecies) in the CSF, which carried drug mutations distinct from plasma strains in 18 of the 40 examined subjects (110). In the CNS, the virus either stays latent or multiplies at low rates and triggers a chronic inflammatory process. Thus, we can presume that CNS compartmentalisation may play its role in the HAND pathogenesis by maintaining immune activation and chronic inflammation.

1.8.5 Possible Neurotoxic Effects

1.8.5.1 Viral toxins

Mechanisms other than direct destruction of neurons by the HIV virus might be implicated in the pathogenesis of HAND. This is supported by finding little or no presence of productive infection in neurons, oligodendrocytes, and astrocytes, which is explained by the absence of CD4 receptors that are necessary for virus penetration. Also, there is no finding of direct neuronal lysis on autopsy in HIV positive subjects with HAD (123). Furthermore, infiltrates of infected macrophages, microglia, and MNGC are frequently found on morphopathology. These infiltrates are surrounded by focal oedema and demyelination, supporting the theory of indirect effect of HIV and its products on neuronal tissue. Several viral proteins (*env pg120, pg41, Tat, Vpr, Nef*) together with cytokines released by activated immune cells can dysregulate normal physiology of neuronal cells, trigger apoptotic cascades, and lead to neurodegeneration (Figure 1.1) (90, 123).

In vitro experiments showed that envelope protein *gp120* shed by the virus, binds to oligodendrocyte and type-2 astrocyte galactosylceramides and other proteoglycans, leading to an increase in intracellular Ca2+ ions and thus, induce excitotoxicity (124). Moreover, in a more recent study, Zhou and colleagues showed that *gp120* induces intracellular Ca2+ influx through activation of postsynaptic N-Methyl-D-Aspartate (NMDA) receptors in the hippocampus of *gp120* transgenic rats that displayed neurological similarity to HAND (125).

In their work, Zhu and colleagues found that *gp120* induced hippocampal neuron apoptosis through enhanced activity of outward delayed rectifier currents

channels (Ik) (a subtype of voltage gated K+ channels) (126). Bachis et al. reported evidence of apoptosis in the neurons that "internalised" *gp120*, which was injected into rat striatum and hippocampus. Not only did the neurons internalise the injected *gp120*, a process mediated by CXCR4 chemokine receptor, but they were also capable of retrograde transport of *gp120* to distal neurons that project to the injected areas (127). Authors suggested that CXCR4 mediated the ability of the neurons to sequester and transport *gp120* distally. This together with the earlier described *gp120* neurotoxic effects, can contribute to a widespread neuronal apoptosis. Furthermore, Peters et al. demonstrated that certain brain-derived *env gp120* have a higher ability to infect cells with low levels of CD4 and / or CCR5 such as macrophages and microglia. This highly fusigenic and macrophage-tropic *env gp120* subtypes were found in the brains of patients with neurologic complications. Their higher fusigenicity may explain their higher neuropathogenicity (128).

It was also reported that circulating *gp120* alters BBB permeability. Exposure of human brain microvascular endothelial cells (HBMEC) to gp120 derived from both CCR5 macrophage-tropic and CXCR4 lymphocyte-tropic viruses reduced the endothelial junctions' tightness by decreasing the production of tight junction protein - *occludin*, with a subsequent increase in monocyte trafficking through the *in vitro* BBB model. *Gp120* was shown to increase production of matrix metalloproteases (MMP-2 and MMP-9). These, in turn, can contribute to neuronal tissue injury by damaging the vascular basement membrane of the BBB, induction of myelin degradation, induction of oxidative stress, and apoptosis of endothelial and neuronal cells (129). *Gp120* can also induce neuronal apoptosis

by activating caspase-mediated neuronal cell death through increased production of TNF-α by non-neuronal cells, which then interacts with neuronal cell TNF receptors (90).

TNF-α and other proinflammatory cytokine induced activation of nitric oxide synthase (iNOS) can induce Nitric Oxide (NO) synthesis, which is another potential neuronal injury mediator. In their study, Adamson et al. showed that a high expression of *env* protein *gp41* correlated with high levels of an immunologic isoform (type II) of nitric oxide synthase (iNOS) in those with severe HAD. They showed *gp41* induced synthesis of iNOS in mixed neuronal and glial cultures associated with NO induced neuronal death (130).

Other HIV proteins, *Tat* and *Vpr* can potentially be involved in the neurotoxic virus effects. *Tat* is a HIV transactivator protein that is released by infected cells and, if present in high quantities, can be neurotoxic through mechanisms that involve multiple intracellular-signalling pathways. *In vitro*, *Tat* was shown to decrease expression of claudin-1, claudin-5, and zonula occludens (ZO)-2, which are important tight junction proteins. Thus, it can have a detrimental effect on BBB and contribute to inflammatory cell trafficking into the CNS (131). Experimental intraventricular injection of *Tat* into male rats resulted in perivascular infiltration of mononuclear cells, gliosis, and apoptosis. These changes are thought to be mediated through induction of TNF-α in the infected immune cells, upregulation of CXCR4 on the surface of resting CD4+ cells, and other chemokines upregulation which were described in the *in vitro* studies (132). However, *Tat* has not been found as a free protein in CSF or serum. Therefore, its role in the pathogenesis of HAND, although possible, is not entirely elucidated.

Viral protein R (*Vpr*), has been detected in CSF, and *in vitro*, was shown to cause neurons and human neuron-precursor cells apoptosis via upregulating the apoptosis genes involved in the mitochondrial apoptotic pathways (133). Furthermore, Rom et al. showed that *Vpr* can be released by infected immune cells and taken up by non-infected neurons. Once inside the neurons, *Vpr* increased the level of intracellular Ca2+ through inhibiting its release from the cells, despite the fact that it increased cell membrane permeability. Excess of intracellular Ca2+ induces the reactive oxygen species (ROS) signalling pathway, which leads to a reduction in ATP production and inhibits mitochondria transport to axonal and dendritic synaptic connections. *Vpr* induced mitochondrial transport and function decline may lead to neuronal cell death (134).

Another viral protein, *Nef* (HIV negative factor) was also shown to induce neuronal cell apoptosis. Trillo-Pazos and colleagues showed a neuronal population reduction by 32% in human neuronal cultures *in vitro* after the *Nef* exposure for six days (135). *Nef* was found in the brains of HIV individuals with neuronal damage, and an over expression of a certain subtype of *Nef* (subtype D) was associated with HAD (136-138). Khan et al. showed that *Nef* is capable of modulating beta amyloid (Aβ) expression in the neuronal cells and induce Aβ peptides production. In this way it could possibly contribute to HIV induced neurodegeneration and HAND (139).

1.8.5.2 Neurotoxic effect of HAART

Neurotoxic effect of certain ART agents as a potential causative mechanism of HAND persistence in the era of HAART has also been proposed (140).

Zidovudine induced mitochondrial dysfunction in the muscle was first described in 1987 (141). Subsequent studies on NRTIs, which are very well known to cause peripheral neuropathy (PN), showed that these drugs cause mitochondrial toxicity in multiple tissues, including axons and Schwann cells, by inhibiting the mitochondrial DNA polymerase γ (142). Chen et al. found that PIs conferred additional risk for development of PN (143). Some PIs, such as, lopinavir and ritonavir can exert neurotoxic effects by inducing oxidative stress and dysregulation of Ca2+ homeostasis (144). Akay et al. showed PI induced hippocampal synaptophysin loss, indicative of synaptic injury and neuronal loss, in two animal models, despite the effective control of simian immunodeficiency virus (SIV) replication in the periphery and in the CNS (145). Robertson and colleagues evaluated the direct effects of 15 antiretroviral drugs on the rat cortical neuron colonies, which were challenged for 1 week with one of the drugs or a combination of drugs. Authors noted dendritic beading, simplification of the dendritic processes, and neuronal shrinkage. Abacavir, efavirenz, etravirine, nevirapine, and atazanavir had higher toxicities, while darunavir, emtricitabine, tenofovir, and maraviroc showed less toxic effects. No additive toxic effects were seen with combinations that are used in clinical practice (146). To complement the *in vitro* and animal model studies that support neurotoxic effect of some ART agents, in a recent study, Robertson and colleagues enrolled 167 individuals from AIDS Clinical Trials Group (ACTG) who had good viral suppression on ART and chose to interrupt treatment, which was re-initiated later. On average, cognitive status improved at follow-up, as shown by neuropsychological test scores after the ART regimens discontinuation (147). However, there is also evidence that

some ART drugs, such as the CCR5 blocker, maraviroc, might have neuroprotective effects (148).

1.8.6 HIV and HAART Related Cardiovascular Risk Factors and Risk of Developing Cognitive Impairment

Mechanisms other than pure ART neurotoxicity that might contribute to persistence of milder forms of HAND in the HAART era have also been explored. Strategies for Management of Anti-Retroviral Therapy (SMART) trial in HIV-infected individuals, which included 292 participants, showed that continuous ART therapy was associated with a surge in the cardiovascular risk factors (149). Furthermore, participants who had prior cardiovascular disease (CVD), hypertension, and hypercholesterolemia were more likely to perform poorer on neuropsychological testing. While other risk factors, such as CD4+ counts and ART CPE, did not correlate with neurocognitive impairment in this study.

1.8.6.1 HIV and HAART related DM

People living with HIV were found to be more likely to suffer from Diabetes Mellitus (DM) and Metabolic Syndrome (MetS), as well as CVD. A recent Canadian study involving a cohort of 1,065 HIV positive individuals found that HIV infected people were 1.39 more likely to have DM than people without HIV of similar age (150). The incidence of DM was found to be higher in those who were treated with ART in the earlier phase of the epidemic between 1997 and 1999 and had been exposed to the older ART agents. Cognitive dysfunction ranging from mild CI through to dementia is one of the complications of longstanding DM,

even in the absence of HIV. The long-term risk of dementia increases in patients with type II DM by a factor of two (151).

1.8.6.2 HIV and HAART related HTN

Prolonged HAART use was significantly associated with a higher prevalence of Systolic Hypertension (SH) (152). In 5,578 participants in the Multicenter AIDS Cohort Study (MACS), blood pressure measurements recorded between 1984 and 2003 were analysed. The prevalence of SH among men taking HAART for less than 2 years was similar to that among HIV negative men, but was significantly higher thereafter (153).

1.8.6.3 HIV and HAART related metabolic syndrome, hypertriglyceridemia and hypercholesterolemia

With respect to metabolic syndrome (MetS) in HIV seropositive patients, the prevalence rate in the Western countries was estimated to be between 7% and 45% (154). Following the introduction of combination ART (cART) in the late 1990's, patients were observed to have drug related body fat distribution changes that were associated with metabolic abnormalities such as hypertriglyceridemia, insulin resistance, and high serum cholesterol (155, 156). In the SYMONE (SIndrome Metabolica ONE) study on a large Italian HIV cohort of 1,243 subjects, with a mean age of 43.2, MetS prevalence was shown to be as high as 23.3% in men and 17.4% in women (156). High triglycerides levels were found in 50% of this cohort and were attributed to the widespread use of PI class ART. Hypertriglyceridemia was shown to be a common finding in AIDS patients even without exposure to ART (157). All of the above are important risk factors for

cardiovascular disease and can be implicated in the development of cognitive dysfunction and the persisting high prevalence of HAND in the era of HAART.

1.8.7 Increasing HIV Population Age and Other Confounding Factors

With the introduction of highly active antiretroviral therapies, mortality from HIV/AIDS related causes has profoundly dropped and the life expectancy has increased, with HIV infected individuals living well into their 60's and 70's (4). By 2015, 50% of people living with HIV in the developed countries were predicted to be older than 50 years of age (158). Hence, there is growing concern that the prevalence of HAND will continue to rise with the ageing of this population.

1.8.7.1 Increasing risk of HAND with age

Advanced age was associated with an increased risk of HAND in HIV positive population in cross-sectional studies (60-62, 159, 160). Moreover, in a longitudinal study, Seider and colleagues tested verbal and visuospatial learning and memory at baseline and at one year follow-up in 54 HIV positive and 30 HIV negative participants aged 40 to 74. Authors found that age and HIV status predicted a greater rate of cognitive decline at follow-up (161). They suggested that HIV is associated with an accelerated cognitive ageing in this population. These trends persisted even after the advent of HAART (162).

Furthermore, Cassol and colleagues, in a CSF study of 100 participants (46 HIV positive and 54 HIV negative), in the HIV group found metabolites that were also present in the older HIV negative controls. This finding was suggestive of an accelerated ageing, or so called "inflammageing", in the HIV positive individuals.

Particularly, researchers identified neurotransmitters (glutamate, N-acetyl aspartate), markers of glial activation (myo-inositol), and ketone bodies (beta-hydroxybutyric acid and 1,2-propanediol) (163).

Some individuals may be genetically more at risk of developing cognitive impairment, conferred by apolipoprotein E4 isoform (APOE4). This was found to be an independent risk factor for HAD in older participants in a Hawaiian HIV positive ageing cohort (164).

There are also concerns that, with the increasing age, this population, not only undergoes accelerated normal physiologic brain ageing, but also may be more susceptible to other age-related neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and Fronto-Temporal Dementia, which may confound the HAND diagnosis. These conditions are associated with deposition of abnormal proteins in the brain, such as amyloid-β (Aβ), ubiquitin, tau or α-synuclein. Abnormal deposition of these compounds in the HIV positive individuals was assessed for in various studies.

Accumulation of Aβ, which is a characteristic feature of Alzheimer's disease, was described in ADC. However, in HIV, Aβ plaques are diffuse and tend to deposit in the neuronal soma and along the axonal tracts, as opposed to Alzheimer's disease, where they are predominantly found in neurites (165). There is evidence to suggest that long-term ART might be associated with Aβ deposition (165, 166). In the study by Green and colleagues, 162 AIDS autopsies undertaken between 1983 and 2001 were assessed for Aβ deposition. Interestingly, in the post-HAART patients, predominantly frontal cortex (in 50%) and slightly less

hippocampal and basal ganglia Aβ deposition was a more common finding, compared to the pre-HAART patients (165). Elevation of phosphorylated Tau (pTau) is predominantly seen in older AD individuals. Notably, it is also seen at early age in the brains of HIV infected people, and higher levels of pTau are seen in those exposed to ART (167). However, studies assessing pTau are somewhat controversial. Smyth et al. did not find any correlation between pTau and the burden of HIV RNA in the brain (168). Gisslen and colleagues assessed amyloid and tau metabolism biomarkers in the CSF of HIV positive patients with and without cognitive impairment and in AD patients. They found that, although amyloid precursor protein levels were low and total Tau levels were elevated in some patients with ADC and those with opportunistic CNS infections, the pTau levels remained low, indicating neuronal injury without pTau accumulation, in contrast to that of AD. Clifford and colleagues also measured Aβ42, Aβ40, total Tau, and pTau in cognitively unimpaired HIV individuals, HIV individuals with HAND, and seronegative patients with mild AD. They too found that Aβ42 level in HAND patients was similar to that in AD patients, but pTau was only elevated in the AD group. This suggests that mechanisms of brain injury in HAND differ from those in AD (169).

This is further supported by the work of Ances and colleagues, in which amyloid-binding agent (11) C-Pittsburgh Compound B ((11)C-PiB) was assessed in 10 cognitively intact HIV infected individuals and in 20 seronegative controls. It was found that HIV positive participants did not have the (11)C-PiB parameters, that would be suggestive of fibrillary amyloid deposition in the brain, despite the low Aβ42 levels in the CSF (170). Furthermore, Ances et al., in their more recent

study, which included 16 HIV positive participants (of them eleven cognitively intact and five with HAND) and 10 Alzheimer's disease participants, showed that even when diagnosed with HAND, the HIV patients do not exhibit the (11)C-PiB uptake levels characteristic for Alzheimer's disease participants (170). Khanlow and colleagues found α-synuclein deposits in the substantia nigra in 16% of the 73 clinically well-characterized HIV infected individuals aged 50 and older from the National NeuroAIDS Tissue Consortium series. This is a higher rate than that found in the older healthy persons, but there was no clinical correlate to this finding (171). However, Tisch et al. described a case series of three patients with relatively young onset of parkinsonism in the context of HIV and HAART therapy, and hypothesised that HAART might interfere with the ubiquitin proteasome pathway and lead to loss of nigrostriatal neuronal reserve (172).

1.8.7.2 Social determinants of health and cognitive function

Attempts have been made to better understand the socioeconomic determinants of cognitive function across longitudinal studies of ageing cohorts with HIV (173). Low socio-economic status and persistent poverty which results in poor access to good quality education and healthcare, good neighbourhoods and better jobs have been demonstrated to have an effect on behaviour and cognition (or at least on the performance on available neuropsychological tests) in immigrant populations (174, 175). McCombe and colleagues observed trends of increasing risk of symptomatic HAND in those with lower educational attainment (odds ratio of 0.65 for completing secondary school as opposed to non-completing secondary school; and odds ratio of 0.60, 0.49, and 0.38 for completing some post-secondary education, for graduating from post-secondary education, and

completing a professional degree, Master's or PhD degree, respectively) (71). The effect of education has been recognised along with the effect of age in the neuropsychological testing not only in healthy population but also in HIV individuals (58, 176, 177). Most well-known and largely used neuropsychology batteries require corrections for age and education for meaningful interpretation of individual raw test results. Therefore, researchers and clinicians are encouraged to use validated tests with normative data based upon large cross-cultural samples.

Other studies found that an increased number of active life style factors (engagement in social, mental and physical activities) was associated with better cognitive performance among HIV infected individuals (178). However, authors caution that the opposite explanation can also be true, i.e. those with preserved cognitive function are more inclined to engage in all those activities. Housing status is a significant predictor of health outcomes and medication adherence in the HIV population (179). Homeless people with HIV/AIDS are at an increased risk of negative health outcomes including alcohol and drug misuse.

Drug abuse and alcohol misuse are frequent confounders in people living with HIV and should be considered when assessing the effect of HIV on the brain and cognition (180). In an earlier work of Chiesi et al., intravenous drug use was demonstrated to confer a higher risk for developing HIV associated dementia (181). Drug use alone was shown to cause activation of microglia (182), hypoxic-ischemic changes with cerebral oedema, ischemic neuronal damage and neuronal loss in the non-HIV infected population (183). Immunohistochemical analysis of hippocampus, brainstem and basal ganglia of opioid users

demonstrated excess of neurodegenerative proteins (β-amyloid precursor protein, neurofibrillary tangles, and hyperphosphorylated tau) in the drug abusers when compared to controls (184, 185). However, no correlated cognitive dysfunction was demonstrated in these studies.

Other studies found that alcohol consumption can exacerbate HIV pathogenesis, including cognitive performance (186). A dose-related and state-dependent impairment in the acquisition of new information have long been recognized in benzodiazepines users (187, 188). Assessing the neuropsychological functioning of persons with HIV and substance misuse is beset with challenges (189). The accurate assessment of alcohol and drug use is complicated by poor recall, deliberate misreporting, inconsistent patterns of use over time, and polysubstance abuse concurrently and over time, and so self-reports of current and past use can be misleading. Individual responses to alcohol and drug use are also heterogeneous so the extent and duration of abuse can affect individuals differently.

1.8.7.3 Role of the cognitive reserve

The impact of cognitive reserve in maintaining intact cognitive function among older HIV positive individuals was assessed in their work by Folley and colleagues. Cognitive reserve was measured by an average score that included the number of years of formal education and the reading ability assessed using the Reading subtest of the third edition of Wide Range Achievement Test. This study included 129 individuals classified by HIV serostatus, 102 HIV positive and 27 HIV negative, by age, and neuropsychological impairment. Authors showed

that those who demonstrated the highest levels of cognitive reserve capacity in the subgroups with high risk for CI (older and HIV positive) remained cognitively intact, suggesting that higher cognitive reserve has a neuroprotective effect in this at risk population (190). In another study, Sheppard et al. assessed the cognitive status of 146 HIV positive individuals at baseline and at 14 months follow-up. Cognitive reserve was measured using the number of years of formal education, estimated verbal intelligence quotient (IQ) measured by the Wechsler Test of Adult Reading (WTAR), and the Hollingshead score of highest occupation level. They too found that lower baseline cognitive reserve was independently predictive of incident neurocognitive decline in the older HIV positive participants (191). Although there is no unanimously agreed method of evaluating cognitive reserve, all studies are in agreement that it does confer protection against cognitive decline, even in at risk HIV positive individuals.

1.9 Summary

HIV is a neuroinvasive, neurotropic, and neurovirulent organism that affects the nervous system and frequently causes neurological disorders, including HIV associated neurocognitive disorder (HAND). HAART transformed HIV infection into a chronic condition with the life expectancy of HIV infected individuals nearly approaching that of HIV negative population. Although introduction of HAART has substantially reduced the incidence of the most severe form of HIV related cognitive impairment – HIV associated dementia, the prevalence of subtler forms of HAND is increasing even in those with undetectable viral load. Based on earlier prevalence studies, it is estimated that up to 24 Million people worldwide could have been affected by HAND in 2015.

Cognitive impairment has major implications in everyday functioning, as it may cause disengagement with healthcare services and treatment noncompliance due to forgetfulness or poor judgement. Poor compliance and engagement with services can result in poor disease control, reduced quality of life, poor functioning at work, increased mortality, and subsequently higher healthcare costs. The increasing prevalence of HAND leads to new therapeutic and management challenges and possibly an increased financial burden on society. To better assist in prevention and management of HAND, it is important that screening tools and biomarkers of disease progression are evaluated and implemented in routine clinical care.

Despite the extensive research in the field of HIV and its related cognitive impairment, it is still little known about the evolution and natural course of HAND. In the pre-HAART era, HAD was typically described as a subcortical dementia with a triad of progressive cognitive decline, behavioural abnormalities, and motor dysfunction. Since the introduction of HAART, this classical pattern is evolving and other patterns are being recognised. HIV has a predilection for the white matter, but the former SJH cross-sectional study by McNamara et al., 2015, and other international studies show that there is also grey matter involvement, demonstrated by neuropsychological tests and MRI data (49).

HAND pathophysiology is also poorly understood. There is a growing body of evidence that, although the invasion of the brain microglia and macrophages by the virus is an important first step in initiating the brain injury, it is actually the indirect mechanism that might be more responsible for the HAND pathophysiology. Virus invasion causes chronic immune cell activation that

produces inflammatory cytokines and, together with viral proteins, triggers apoptotic pathways, which ultimately lead to neuron death and neurodegeneration.

The increased deposition of α-synuclein and β-amyloid in the brains of older HIV infected individuals also suggest an increased risk of developing neurodegenerative disease, especially with the ageing of this population. With the increasing age of PLWH, and likewise in the seronegative population, there is a higher risk of developing cerebrovascular disease secondary to hypertension, diabetes, cardiac disease, hypercholesterolemia, and potentially, metabolic conditions, such as vitamin B12 deficiency or hypothyroidism, all of which can further exacerbate the risk of cognitive impairment.

There is also growing concern that HAART might exert a neurotoxic effect by interfering with neuronal and glial cell metabolism that can further contribute to the development of cognitive dysfunction.

The pathophysiology of HAND is multifactorial and all of these factors may play their role. However, it is not clear yet how they interact with each other and why some HIV infected individuals develop HAND and some do not. Alternatively, they are all going to ultimately develop HAND but the neurodegenerative process is halted by the effective ART treatment and other individual protective mechanisms, such as cognitive reserve. Thus, to answer all these questions, it is imperative that the trajectory of HAND is investigated across the adult lifespan. Such longitudinal research is important because it can more directly identify clinical and demographic factors that place an individual at a greater risk for

pathological neurocognitive decline. This may also allow for the development of early intervention strategies to prevent further decline.

1.10 Hypothesis, Aims and Objectives

1.10.1 Hypothesis

This work is centred on the hypothesis that HIV virus enters the CNS early in the course of the disease, causing early inflammation when neurons are primarily damaged indirectly as infected macrophages, lymphocytes, and microglia release cytokines and other neurotoxic substances, and evolves later into a neurodegenerative process from apoptosis and other programmed cell death mechanisms triggered by the inflammatory process. This model would predict a slow steady cognitive and functional decline in patients with HIV that would be demonstrated using neuropsychological and imaging tools, which may or may not be modulated by treatment in the post-HAART era.

1.10.2 Aims

The main aim of this work is to determine, using sophisticated neuropsychological and imaging tools, whether or not patients with HIV who have screened positive for cognitive impairment continue to deteriorate, as one might expect with a neurodegenerative process, or whether cognitive status can be stabilised and even improved with the use of HAART therapy.

Additional sub aims were to evaluate whether or not:

1. PLWH who also suffer from disease related cognitive dysfunction are more hospital resource intensive
2. PLWH have a higher burden of other neurological conditions (the rate and cause of seizures in PLWH were examined in more detail).

1.10.3 Objectives

The main objectives of this work were:

1. Neuropsychological follow-up assessment of a sub-cohort of 104 HIV positive patients attending HIV services at St James's Hospital and who screened positive for cognitive impairment and underwent detailed neuropsychological assessments at baseline
2. Follow-up MRI data acquisition in a subset of 50 patients from the 104 HIV positive sub-cohort of patients attending HIV services at St James's Hospital who screened positive for cognitive impairment and underwent detailed neuropsychological and neuroimaging assessments at baseline
3. Comparison of hospital service utilisation and costs incurred by HIV positive patients who screened positive for cognitive impairment (100 CI+ group) with those who screened negative (100 CI- group)
4. Evaluation of the rate and cause of seizures in a cohort of HIV positive patients (n=604) attending HIV services at St James's Hospital who underwent screening for cognitive impairment between 2010 and 2013.

1.11 Thesis Outline

The introductory chapter highlights that, despite the achievement of a good viral control and a considerable drop in HIV related mortality following the introduction

of HAART, it is still not known enough about how HAND progresses in the context of effective treatment. The main objectives of this thesis are to follow on a cross-sectional study undertaken at St James's Hospital between 2010 and 2014 by P. McNamara et al. (49), which described the neuropsychological profile, neurological features, and MRI and CSF findings of HAND in an Irish cohort of people living with HIV (PLWH), and to contribute further to the knowledge about the natural course of HAND in PLWH receiving the HAART treatment.

Chapter 2 examines and discusses the neurological and neuropsychological features of HAND at follow-up in a sample of 79 HIV positive patients attending services at St. James's Hospital. Chapter 3 examines and discusses the findings of the grey matter morphometry and white matter diffusivity measures on the follow-up brain MR imaging in a subset of 42 HIV positive patients. Chapter 4 compares and discusses hospital service utilisation over a six year follow-up period in a group of 200 HIV positive patients (100 of whom screened positive and 100 screened negative for cognitive impairment in the former cross-sectional study by McNamara et al. (192) concerned with the prevalence of positive screen for cognitive impairment in an Irish cohort of PLWH). Furthermore, in this chapter, hospital costs in the same 200 HIV positive group over a two year period will be compared and discussed. Chapter 5 is concerned with the discussion of burden of one of the other Neurological complications of HIV – seizures and epilepsy, and issues encountered with their management in PLWH. Finally, conclusions drawn from this research and further directions are presented in Chapter 6.

2. Follow-up Neuropsychological Assessment of HIV Positive Patients with a Positive Screen for Cognitive Impairment

2.1 Introduction

2.1.1 Outline of the Chapter

This chapter describes the follow-up assessments of the patients who previously participated in the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" by McNamara et al. (49). Subjects who participated in the previous cross-sectional study were re-enrolled and underwent detailed neuropsychological assessments to determine whether there was a progression of cognitive impairment since their earlier assessment. The underlying hypothesis of the study is that, despite the ART treatment, the neurocognitive impairments associated with HIV (i.e. HAND), will progress due to immune/inflammatory mediated neurodegeneration.

The early part of this chapter provides a review of the research conducted, to date that has attempted to evaluate and describe the natural history of HIV associated cognitive impairment. Research in this area is important because the presentation and course of HIV associated neurocognitive disorders have changed with the recent rapid advances in the treatments of HIV.

The aims and objectives of the study are then described, followed by a detailed description of the study methods and approach. Importantly, as well as collecting

comprehensive clinical history from the subjects, a range of neuropsychological assessment tools are described. Together, these are used to fully characterise the neurocognitive profile of the study subjects at follow-up. Because these same assessment tools were also used in the earlier study, useful comparisons over time can be made. Lastly, the results of detailed analysis are presented.

2.1.2 Background

Approximately 2,200 HIV+ individuals attend HIV services in St. James's Hospital. Previous work carried out at SJH showed that, out of 604 randomly selected patients who were tested using the Brief Neuro-Cognitive Screen (BNCS), 51.5% screened positive for CI (192). (A copy of BNCS is presented in Appendix 1). Of those who screened positive for CI, 104 participants were randomly selected and underwent detailed neurocognitive assessments in the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" (49). Out of 104 HIV+ patients who partook in the detailed study, 66 (63.5%) met diagnostic criteria for HAND (41.3% - ANI, 20.2% - MND, and 2% - HAD), with the most common deficit pattern being an amnestic and dysexecutive one. In the current study, follow-up assessments were carried out in 79 patients who underwent detailed testing at baseline and retained in the follow-up study.

2.1.3 HAND in the Context of HAART

Although it is important to know that HIV infected individuals can exhibit cognitive impairment at a given time during the course of their HIV disease, it is equally important to know the trajectory of this impairment and the natural course of

HAND. Long term neuropsychology follow-up is increasingly more relevant in the HAART era, because chronically HIV infected individuals are living longer, as the treatment is becoming more effective. It is important to know whether HAND will improve, remain stable, or progress in the context of effective viral suppression, and, when further cognitive decline is found, what the mechanisms of its progression are.

2.1.3.1 Follow-up studies in the pre-HAART era

The first attempts to evaluate the longitudinal course of HAND were made prior to the introduction of HAART (Table 2.1. A.). McKegney and colleagues (1990) followed up 46 HIV+ and 45 HIV- intravenous drug users (IVDU) at a mean interval of 7.4 months. They found selective deficits at baseline in some of the tests in the HIV+ group such as, dominant hand finger tapping, similarities and digit span forward, when compared to seronegative group. However, they observed an improvement on finger tapping test at follow-up and no further deterioration on the other tests (193). Bono et al. (1996) followed 42 HIV+ and 39 HIV- IVDU patients over twelve months. Although 31% of HIV- and 36% of HIV+ participants performed 2SD (standard deviations) below the mean of controls on at least two of the neuropsychology (NP) subtests at baseline, no progression was observed at follow-up. In this study, cognitive impairment detected at baseline assessments was attributed to substance misuse and its other related variables (194). In another study, Burges et al. (1994) assessed 61 MSM, 41 of them HIV- and 20 of them HIV+ at the time of their first HIV status test and at twelve months follow-up. There were no differences on neuropsychology tests performance between the HIV- and the asymptomatic HIV+ participants at

baseline and follow-up, and the mean scores on NP tests were within the normal range for both groups. However, the HIV+ subjects were more likely than HIV- participants to perform at a lower level at follow-up on one or more tests than predicted by multiple regression analysis based on their baseline performance (195). These studies showed that, although cognitive impairment was present at baseline in some of the subjects, it did not progress in the early asymptomatic stages of HIV, even if untreated. In addition, some of these studies observed improvements at follow-up. Some of these improvements were attributed to practice effects. However, these studies were limited by small participant numbers and short follow-up period.

Selnes et al. assessed a larger group of IVDU individuals (160 HIV- and 160 AIDS-free HIV+) at baseline, and at six months and twelve months follow-up. Authors found no difference in performance by HIV status at any visits, and improvements due to practice effects were observed in both groups. They too concluded that cognitive decline during the pre-symptomatic stages of HIV infection was rare, regardless of the route of acquisition of the virus. Performance on NP tests correlated with age and educational attainment in this study (196). In a small longitudinal study but with a longer follow-up interval, which assessed 21 HIV- and 21 HIV+ men who were not on antiretroviral therapy (thirteen of them with persistent generalised lymphadenopathy and eight with AIDS-related complex), Saykin et al. (1991) showed that, overall, neuropsychological profiles did not deteriorate significantly at 18 months follow-up. The AIDS-related complex group, however, showed continued impairment and abnormalities on measures of

activities of daily living. A mild decline in verbal memory was also noted in some patients (197).

Karlsen et al. (1993), in their study, followed 36 asymptomatic HIV infected individuals. During the two year follow-up period, these patients underwent six monthly neuropsychological tests. Interestingly, in this study, it was found that performance on NP testing significantly improved from time-1 to time-2 and from time-2 to time-3, and levelled off between time-3 and time-4, which could be explained by the practice effect phenomena observed on frequently repeated neuropsychology tests. They too concluded that cognitive impairment does not develop gradually in the asymptomatic stages of HIV-infection (198). However, all these studies were limited by relatively short follow-up periods, during which the practice effects are significant and can preclude identification of a meaningful change on neuropsychology testing.

Stern and colleagues (1995) followed up a larger cohort in the pre-HAART era (113 HIV+ and 55 HIV- men) and for a longer follow-up period of 4.5 years with six-monthly assessments. Both groups tended to perform better over time. However, this improvement was less marked or was eventually eliminated for tests of attention and language domains in the HIV+ individuals. In addition, HIV+ patients performed worse on the memory tests when compared with HIV- controls. Amongst the HIV+ group, practice effects were diminished or eliminated in the memory, executive function, language, and attention tests in those with lower CD4 counts and those with a more advanced disease (199). They also observed a more rapid decline in the executive, attentional and language tests performance in the 33 participants who died during the follow-up period. It was

concluded that HIV infection results in progressive cognitive change, which is related to the disease severity. In their study, Silberstein and colleagues (1993) assessed 40 HIV+ and 81 HIV- patients at baseline and at a mean of 47 months. They found statistically significant declines on finger tapping and Trail Making B tests in the HIV+ group and concluded that subtle cognitive deficits develop slowly over time and are correlated with the course of HIV infection (200). In their longitudinal study, which involved serial assessments during 36 months, Villa et al. (1996) also found that timed memory and psychomotor tasks, which require attention and learning, were the most sensitive in the earlier stages of the HIV related cognitive impairment, and the latter correlated with the virological/immunological status in the advanced stages of HIV infection (201). It seems that short duration follow-up studies in relatively stable, asymptomatic HIV+ individuals failed to identify gradual development of HIV related cognitive impairment, even in the pre-HART era. However, the longitudinal studies with a longer follow-up period demonstrated gradual development of deficits in memory, attention, speed of information processing, executive function, and language domains and these were associated with the progression of the HIV infection.

Thus, HIV virus was assumed to be the cause of the brain damage and AIDS dementia complex. Furthermore, HIV was identified in the brain tissue in patients with advanced disease in the early days of the HIV epidemic, supporting this assumption. After the widespread use of highly effective ART, this was further supported by the substantial fall in the incidence of the HIV associated dementia from 6.49 per 1,000 person-years (before 1997) to 0.66 per 1,000 person-years (2003−2006), as reported in the CASCADE cohort (Concerted Action on

SeroConversion to AIDS and Death in Europe) (202). In this cohort, patients having a current CD4+ count >350 cells/ml were less likely to develop HAD. However, the milder forms of HIV associated cognitive impairments are still prevalent and little is known about the underlying mechanisms and the natural course of the disease in the setting of HAART.

2.1.3.2 Post-HAART longitudinal studies

Soon after the introduction of a combination antiretroviral (c-ART) regimen that included protease inhibitors in 1996, it was found that this combination was very effective at suppressing the systemic HIV viral load. However, its impact on HIV associated cognitive impairment was not clear (Table 2.1. B.). Sacktor and colleagues (1999) followed a total of 411 HIV+ men from the Multicentre AIDS Cohort Study (MACS) and evaluated psychomotor speed as a marker of HAD. At baseline, 282 participants displayed psychomotor slowness. They were divided into four different treatment groups: antiretroviral naive (no antiretroviral medication treatment), monotherapy, combination antiretroviral therapy with and without protease inhibitors (combination/+PI and combination/-PI). Longitudinal performance on the following three tests: the Grooved Pegboard (GP) (non-dominant and dominant hands), Trail Making Test B and the Symbol Digit Modalities Test (SDMT) was compared to assess the psychomotor speed. Authors found that participants from combination/+PI group, who had an abnormal performance at baseline on non-dominant hand GP test, showed improvement at follow-up ($p=0.02$) when compared with ART naïve and monotherapy group. Also, relative to monotherapy and ART naïve groups, both

combination therapy groups, with PI and with no PI, showed improvements on SDMT (p=0.03 and p=0.01, respectively) (203).

Suarez and colleagues (2001) assessed a cohort of 91 HIV+ individuals, 80 of whom were on continuous HAART regimens at baseline and at a 12.3+/-8.3 months follow-up. At the baseline assessment, 53 were cognitively impaired (36 minor CI; 17 ADC) and 38 were not impaired. It was found that HAART improved subcortical function on tests of psychomotor speed, executive function, and verbal anterograde memory, although there was a high attrition rate (36%) in this study, as well as high mortality rate amongst those with CI (25%) (204). Authors concluded that memory impairments in the HIV+ immunosuppressed individuals were due to frontal subcortical dysfunctions.

In a one year follow-up study, Sacktor and colleagues (2013) assessed 77 HIV+ individuals from Sub-Saharan Africa, 11.7% of whom had normal neurocognitive function, 15.6% - ANI, 33.7% - MND, and 39.0% had HIV dementia at baseline. ART treatment was initiated after the baseline assessment in 31 of them. At follow-up, both groups showed significant improvements in the tests of verbal memory, executive function, motor, and psychomotor speed performance, as well as depression symptoms. Improvements on NP testing in the non-ART group did not differ from those in the ART group and the authors postulated that these were possibly due to practice effects, as well as due to mood and the overall quality of life improvements (205).

In yet another study, Sacktor and colleagues (2006) found high rates of dementia as assessed by International HIV Dementia Scale at baseline in 23 HIV+

individuals with advanced HIV infection (35% presented with Memorial Sloan Kettering (MSK) dementia stage 0.5 and 61% with MSK dementia stage 1). All participants initiated HAART after the baseline assessments and were re-assessed at three and six months follow-up. This study showed neurocognitive improvement on HAART, with the rate of dementia dropping to 26% at second visit and to 4% at third visit (206). Although these studies generally show improvements of cognitive status and a drop in prevalence of dementia with greater improvements of mainly subcortical function in those taking HAART, they have the caveat of a short term follow-up, which is subject to strong practice effects. Some of them also had a relatively small number of subjects and / or high attrition rates (Table 2.1. B.).

Cysique and colleagues (2010), in their one year follow-up study, showed that despite ongoing HAART, cognitive decline in HIV+ people is common. They assessed 192 HIV+ individuals, 56% of whom were on HAART at baseline and 60.9% were on HAART at follow-up, and 101 HIV- people. Neurocognitive decline was found in 27% of HIV+, compared to 5% in those who were seronegative. As in the pre-HAART era, cognitive decline was predicted by the AIDS status, lower nadir CD4+, and worse processing speed at baseline, and it was associated with lower CD4+ count and failure of viral suppression on HAART at follow-up (207). Immune recovery was slightly worse in the “decliners” group, despite the CD4+ count improvements in both groups at follow-up.

Not dissimilar results were reported by Hayman-Abello et al. (2007) in a follow-up study on 386 HIV+ individuals, 180 of whom completed follow-up assessments. HIV+ individuals on HAART outperformed those on a non-HAART regimens and

the ART naïve group on the GP Test in this study. While 26% of the HIV+ subjects deteriorated on follow-up testing, 65% had a stable cognitive profile and 10% exhibited improved cognitive functioning, which was predicted by the immune system response, initial cognitive impairment level, and estimated IQ. This study could not demonstrate a clear association between HAART and improvement in the cognitive status. HAART was associated with changes on cognitive domains such as attention, psychomotor speed, learning efficiency, and abstraction, in those with CD4+ cell count < 200 cell/ml (208).

Ciccarelly et al. (2015) also showed variable outcome at a two year interval in 245 HIV+ individuals, 150 of whom completed the follow-up assessments (37.5% attrition rate). The HIV disease was well controlled in the majority of the participants (88%). In this study, 119 (79.3%) patients remained stable, 15 (10%) subjects improved, and 16 patients (10.7%) got worse at follow-up. Higher CD4+ count was associated with less impairment, and dyslipidemia was associated with higher risk of impairment at follow-up (209). Dufouil and colleagues (2015) in their study in 400 HIV+ individuals (95% on ART), 283 of whom had follow-up assessments at a mean interval of 2 years, showed improved cognitive function in 70% of those who had dementia and in 40% of those who had MND at baseline. However, they also found evidence of cognitive decline, which was associated with diabetes and prediabetes, independently of HIV-related factors, age, and other cardiovascular risk factors (210).

Heaton and colleagues (2015) in 436 HIV+ individuals in the CHARTER study (CNS HIV Anti-Retroviral Therapy Effects Research), observed a further neurocognitive decline in 22.7% of participants at 16-72 months follow-up.

Neurocognitive status remained stable in 61% of participants and 16.5% experienced improvement. Being off antiretroviral therapy and low CD4+ counts predicted progressive neurocognitive dysfunction in this study. Those who declined were more likely to have detectable virus in plasma and CSF. Another predictor of cognitive decline was having a confounding co-morbidity, such as a lifetime psychiatric condition and a current depressive episode (211).

The relationship between HIV disease, symptoms of depression, HAART and neurocognitive performance was also evaluated by Gibbie and colleagues (2006). In their study, 129 HIV+ individuals were assessed at baseline, of whom 80 completed follow-up testing at two years. At baseline, 27% of participants met criteria for a current mood disorder. This study showed that neurocognitive performance at follow-up improved significantly in those who did not have depressive symptoms at baseline. Domains of psychomotor speed, spatial working memory and mental flexibility remained impaired in those with depression at baseline (212). However, Cysique and colleagues (2007) in a larger study in 227 HIV+ patients found no differences in the neuropsychological performance associated with lifetime or incident major depression at two year follow-up (213).

Later, Brouillette and colleagues (2016) reported stable cognitive function in 80% of the CHARTER study cohort (in 701 HIV+ participants) at a three year follow-up. Individuals who declined (15.8%) on at least one NP test were older, had a longer duration of HIV infection, and had worse baseline NP performance on every test (214). Furthermore, 226 HIV+ individuals with normal neurocognitive status and 121 with ANI from the CHARTER study were assessed six-monthly,

for a median of 45 months (28.7–63.7). It was found that those with ANI at baseline converted to symptomatic HAND faster than those with normal performance at baseline. Adjusted risk ratios for symptomatic HAND were 2.0 (confidence interval (CI) 1.1–3.6; $p = 0.02$) for self-reported (SR) functional impairment, 5.8 for practice-based (PB) functional impairment (CI 3.2–10.7; $p < 0.0001$), and 3.2 for either SR or PB (CI 2.0–5.0; $p < 0.0001$). It was concluded that ANI increases the risk for earlier development of symptomatic HAND by two to six times, supporting the possible prognostic value of ANI. Authors suggested that identifying those with asymptomatic impairment might provide a window of opportunity and that modifying treatment at this stage might delay further decline (215).

Table 2.1. Longitudinal Neuropsychological Studies in HIV Cohorts before and after the Introduction of HAART

Author / year	Population studied	Follow-up interval	Baseline	Follow-up findings
A. Pre-HAART longitudinal neuropsychology studies				
McKegney et al. 1990 (193)	45 HIV- IVDU 46 HIV+ IVDU (all from methadone clinic)	Mean 7.5 months	HIV+ had deficits in dominant hand finger tapping, similarities and digit span forwards.	HIV+ Improved finger tapping and no other deteriorations were observed at follow-up.
Saykin et al. 1991 (197)	21 HIV- men 21 HIV+ men (8 of them with AIDS related complex - ARC)	18 months	Mainly ARC patients showed mild impairments in language, memory, attention, and visual and auditory processing.	ARC group showed mild decline in verbal memory and measures of ADLs, although overall neuro-psychological profiles did not deteriorate.
Selnes et al. 1992 (196)	160 HIV- 160 AIDS-free HIV+	6 months and 12 months	No difference in performance by serostatus	Improvements in both groups and no difference in performance by serostatus was found.
Karlsen et al. 1993 (198)	36 HIV+	6 monthly testing for 24 months	All participants were asymptomatic at baseline.	Significant improvement in from time-1 to time-2 and time-2 to time-3, explained by practice effect with a level off between time-3 and time-4. No gradual development of NP impairment was found.
Silberstein et al. 1993 (200)	81 HIV- IVDU 40 HIV+ IVDU (all from methadone clinic)	Mean 47 months	No significant difference in performance between the groups was identified.	Declines on Finger Tapping and Trail Making B tests in the HIV+ group correlated with disease progression.
Burges et al. 1994 (195)	41 MSM HIV- 20 MSM HIV+	12 months	No difference between HIV+ and HIV- with mean test scores within normal range.	At follow-up, HIV+ group performed at a lower level than predicted on one or two tests but within normal range.
Stern et al. 1995 (199)	113 HIV+ men 55 HIV- men	6 monthly for 4.5 years	HIV+ patients performed worse on the memory tests.	Both groups performed better over time. In HIV+ improvements were less marked in language and attention. Less practice

Author / year	Population studied	Follow-up interval	Baseline	Follow-up findings
				effects were noted with lower CD4 counts, with rapid decline in the executive, attentional and language tests performance in the 33 participants who died during f/up.
Bono et al. 1996 (194)	42 HIV+ 39 HIV-	12 months	31% of HIV- and 36% of HIV+ participants performed 2 SD below mean on at least two tests	No progression in either group.
Villa et al. 1996 (201)	78 HIV+ (asymptomatic) 56 HIV+ had follow-up 32 HIV-	12 and 36 months	28.2% of asymptomatic HIV had abnormal cognitive testing results.	Worsening performance on timed memory and psychomotor tasks which require attention and learning, correlated with HIV disease status.
B. Post-HAART longitudinal neuropsychology studies				
Sacktor et al. 1999 MACS study (203)	129 HIV- MSM 282 HIV+ MSM divided in four treatment groups -ART naïve, -monotherapy, -combination/-PI, -combination/+PI	12 months	282 HIV+ participants displayed psychomotor slowness (the Grooved Pegboard non-dominant and dominant hands, Trail Making Test B, and the Symbol Digit Modalities Test).	Combination/+PI participants with baseline abnormal performance on non-dominant hand Grooved Pegboard test showed improvement at follow-up ($p=0.02$) when compared to ART naïve and monotherapy group. Relative to monotherapy and ART naïve groups, both combination therapy groups: with PI and with no PI, showed improvements on Symbol Digit Modalities Test.
Suarez et al. 2001 (204)	91 HIV+ (80 of them on continuous HAART)	12.3+/-8.3 months	53 were cognitively impaired (36 minor CI; 17 ADC) and 38 were cognitively intact.	Those on HAART showed improved subcortical function on tests of psychomotor speed, executive function and verbal anterograde memory.
Sacktor et al.	23 HIV+ with advanced HIV	3 and 6 months	35% - met criteria for MSK	Neurocognitive improvement was noted

Author / year	Population studied	Follow-up interval	Baseline	Follow-up findings
2006 (206)	disease (all initiated HAART after baseline)		dementia stage 0.5 and 61% - MSK dementia stage 1.	on HAART, with the rate of dementia dropping to 26% at second visit and to 4% at third visit.
Gibbie et al. 2006 (212)	129 HIV+ (80 completed follow-up assessments)	24 months	7% had HIV-associated cognitive changes, 34.8% had depressive symptoms and 27% of participants met criteria for a current mood disorder.	Neurocognitive performance at follow-up improved in all participants but more significantly in those who did not have depressive symptoms at baseline.
Cysique et al. 2007 (213)	227 HIV+ men	24 months	At baseline, participants did not meet criteria for a current major depressive episode (MDE).	98 participants had a lifetime history of MDE and 23 - met criteria for incident MDE at one of their follow-up evaluations. No differences in neuropsychological performance was associated with lifetime or incident major depression.
Hayman-Abello et al. 2007 (208)	386 HIV+ (180 completed the follow-up)	12-24 months	Those on HAART outperformed those on a non-HAART regimen and ART naïve on the GP Test.	26% deteriorated on follow-up testing, 65% had a stable cognitive profile and 10% improved cognitive functioning. Improvement was predicted by immune system response, initial cognitive impairment level, and estimated IQ. HAART was associated with improvements in attention, psychomotor speed, learning efficiency and abstraction in those with CD4 cell counts < 200 cell/ml.
Cysique et al. 2010 (207)	192 HIV+ (56% of whom were on HAART at baseline and 60.9% - on HAART at follow-up) 101 HIV- controls	12 months	At baseline 106 (55.2%) HIV+ participants met CDC-1993 criteria for AIDS.	27% of HIV+ showed neurocognitive decline. In HIV- group only 5% declined. Cognitive decline was predicted by AIDS status, lower nadir CD4, and was associated with lower CD4 count and

Author / year	Population studied	Follow-up interval	Baseline	Follow-up findings
				failure of viral suppression on HAART at follow-up.
Sacktor et al. 2013 (205)	77 HIV+ (treatment was initiated after the baseline assessment in 31 participants)	12 months	11.7 % of subjects displayed normal neurocognitive function; 15.6 % - ANI; 33.7 % - MND and 39.0 % had HAD.	Both groups (ART and no-ART) showed significant improvements in the tests of verbal memory, executive functioning, motor, and psychomotor speed performance.
Grant et al. 2014 (215)	347 HIV+ participants from the CHARTER study	6 monthly for a median of 45.2 months	At baseline, 226 were neurocognitively normal and 121 had ANI.	Those with ANI at baseline converted to symptomatic HAND faster than those with normal performance. ANI might have prognostic value.
Ciccarelly et al. 2015 (209)	245 HIV+ (88% had well controlled disease) (150 - completed f/up)	24 months	Higher CD4 count was associated with less impairment.	79.3% of subjects had stable cognitive function, 10% - improved, and 10.7% - worsened. Dyslipidaemia was associated with higher chances of progression at follow-up.
Dufouil et al. 2015 (210)	400 HIV+ (95% on ART) 283 completed follow-up	Mean 24 months	Those with diabetes (n=39) and prediabetes (n=33) performed worse at baseline.	70% of those who had dementia and in 40% of those who had MND at baseline showed improvements at follow-up. Authors found evidence of cognitive decline in those with diabetes and pre-diabetes.
Heaton et al. 2015 (211)	436 HIV+ from CTARTER study cohort	16-72 months (mean 35 months)	46% had neurocognitive impairment at baseline	61% of participants remained stable and 16.5% experienced improvement. Low CD4 counts, being off ART treatment, detectable plasma/CSF virus, life-time psychiatric condition and current depressive episode predicted progressive neurocognitive dysfunction.
Brouillette et al. 2016 (214)	701 HIV+ participants from the CHARTER study cohort	36 months	All participants completed a battery of 15NP tests covering 7	Stable cognitive function in 80% of participants. 15.8% of participants

Author / year	Population studied	Follow-up interval	Baseline	Follow-up findings
			cognitive domains all assessments.	showed decline in cognition. They were older, had a longer duration of HIV disease, and performed worse at baseline.

2.1.3.3 Summary

All of these longitudinal studies found that, in the context of HAART treatment, the majority of participants in the populations studied had stable cognitive function at relatively short interval follow-up. However, gradual progression of cognitive impairment was observed in a small proportion of HIV+ individuals. Although most authors found that, as in the pre-HAART era, further decline was associated with HIV related factors such as AIDS, nadir CD4+, immune response and detectable virus in the plasma or CSF, other factors such as age, dyslipidemia, diabetes, confounding psychiatric comorbidities, and performance on neuropsychology tests at baseline were also playing an important role. However, some of these studies were limited by small participant numbers, while other studies had high attritions rates, and thus, possibly more “decliners” were being missed at follow-up. The main reasons for attrition in most of the above studies were mentioned to be the refusal to take part in follow-up assessments. The other reasons for dropping out were moving to other city, hence accessing the services elsewhere. Some participants were excluded from the follow-up due to a drug misuse. A small proportion of participants died and some dropouts were lost to follow-up for unknown reasons. The follow-up intervals, number of assessments, and the tests used to evaluate cognitive status also varied in all of these studies.

In addition to different methodological approaches in terms of follow-up intervals and tests used, the researchers also applied different definitions of decline/improvement and different approaches to assess the trends of serial NP results, and meaningful change in cognitive function. For example, Suarez and

colleagues used the general estimating equations (204), whereas Gibbie et al. and Grund et al. used regression models of assessing change in global neuropsychological performance summary score (212, 216). Changes in the mean neuropsychological test performance for each of the neuropsychological tests were used to assess for decline or improvement by Sacktor et al. (206). Global deficit scores (GDS) and summary regression change score were used by Cysique and colleagues (213). While the CHARTER research group also used the change in HAND diagnosis, by applying Antinori et al. diagnostic criteria for HAND as a measure of cognitive change at follow-up (215). Brouillette et al. tested two definitions of change, a change equal to or greater than 0.5 standard deviation (SD) from the baseline score and a change that was greater than Standard Error of Measurement (SEM) estimate (214). While some researchers used self-reported functional impairment as a measure of functional decline (Becker et al.), others used validated scales to evaluate functional outcomes (Schifitto et al.) or a combination of both methods (Grant et al.) (60, 215, 217). Allowing for all of the differences in the populations studied and methodological approaches used, in general, available follow-up studies showed a decline of cognitive status in at least 4% - 27% of participants, stability in the majority of them (60% - 80%), and improvement in 10% - 24%.

2.2 Study Aims

This study has two aims:

- Aim 1: To determine whether or not there is progression of cognitive decline at follow-up in an established and well characterised Irish cohort of 104 HIV+ patients in the context of effective treatment.

- Aim 2: To establish the prevalence of various patterns of cognitive dysfunction at follow-up in an established and well characterised Irish cohort of 104 HIV+ patients.

2.3 Objectives

The objectives of this study are to:

- Recruit the 104 subjects who participated in the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" for the follow-up assessments
- Asses the neuropsychological status and profile at follow-up to evaluate the longitudinal course of the disease in context of effective treatment
- Asses the neurological examination status at follow-up
- Correlate neuropsychological status change with HIV indices (Viral Load and CD4+ count) and other demographic and clinical variables.

2.4 Ethical Approval

The follow-up study of cognitive impairment in a well characterised Irish cohort of HIV+ patients was conducted in accordance with the Good Clinical Practice guidelines and all experimental procedures adhered to the Declaration of Helsinki. The study was approved by the Combined Adelaide and Meath Hospital Incorporating the National Children's Hospital (AMNCH) and St. James's Hospital (SJH) Research Ethics Committee (REC Reference: 2014 Chairman's Action (7)). It was explained to patients that participation in the study was voluntary and that their refusal to participate or withdrawal from the study would not affect their ongoing medical care. A written informed consent was provided by all participants

before participation (Appendix 2). Before agreeing to participate in the follow-up study, all patients were given the patient information leaflet to read and they had the opportunity to ask any questions about the study. All patients were also given a phone number to contact the researcher if they had any further questions or if they wished to cancel or re-schedule their appointment.

2.5 Methodology

2.5.1 Study Design

This was a prospective, follow-up, observational study. Patients who attend HIV services in SJH, and who participated in the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" conducted at SJH between 4 August 2011 and 28 November 2013 (49), were asked to participate in the follow-up assessments.

2.5.2 Study Site

The current study and the preceding studies ("Prevalence of a Positive Screen for Cognitive Impairment on a Cohort of HIV Positive Patients Attending an Irish Clinic" and "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment") were conducted at St. James's Hospital, which is one of the largest hospitals in Ireland and the largest university teaching hospital in Dublin, with 950 beds. The Department of GenitoUrinary Medicine and Infectious Diseases (GUIDe) clinic provides the largest outpatient service for people living with HIV, which is accessed by circa 2,200 HIV+ individuals. Three HIV clinics are held weekly, including one clinic for patients co-

infected with hepatitis B / C viruses. SJH also has a dedicated inpatient service for HIV+ patients. The service is provided by a medical team led by Infectious Diseases and GenitoUrinary Medicine Consultants, clinical nurse specialists, clinical nutritionists, medical social workers, and a dedicated pharmacy team. SJH has the largest Clinical Research Facility (CRF) in Ireland in partnership with the Wellcome Trust and Trinity College Dublin (TCD). Recruitment for the follow-up study took place in the GUIDe clinic and an appointment for follow-up assessment was arranged for those who agreed to participate. The clinical examination and neuropsychological testing at follow-up (T_2) were conducted in one of the clinical rooms in CRF, which is adequately lit and well ventilated, quiet, and free from potential distractions and interruptions of a busy GUIDe clinic environment.

2.5.3 Study Population and Recruitment

Recruitment for the follow-up assessments (T_2) took place in the GUIDe clinic in SJH. Patients attending the HIV services in SJH, who met the inclusion and none of the exclusion criteria, were approached during their routine clinic appointment and were asked to participate in the follow-up study. The potential participant population included a subset of 104 HIV+ patients who screened positive for cognitive impairment in the "Prevalence of a Positive Screen for Cognitive Impairment on a Cohort of HIV Positive Patients Attending an Irish Clinic" and who underwent detailed neuropsychological testing in the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" at baseline (T_1). Recruitment and T_2 assessments started on 26th November 2014 and concluded on 20th December 2016.

2.5.4 Study Inclusion and Exclusion Criteria

Inclusion criteria:

To be able to participate in the study, patients had to be:

- HIV+
- Attending HIV services at St James's Hospital
- Over the age of 18
- Medically stable
- Competent to understand and give informed consent to participate in the study
- Former participants of the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" at T_1.

Exclusion criteria:

Patients could not partake in the study if they were:

- HIV negative
- Attending HIV services other than HIV clinic at St James's Hospital
- Under the age of 18
- Medically unstable, or acutely ill (patients could participate in the follow-up study at a later stage during T_2 when they were medically stable and met all of the inclusion criteria and none of the exclusion criteria)
- Unable to give informed consent to participate in the study
- Not participants of the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" at T_1.

2.5.5 Data Collection

Demographic data, including date of birth, gender, country of birth, handedness and English language spoken as first language, which were collected in the prevalence study, were included for the respective participants of the follow-up study. Other demographic data such as age at the time of follow-up (T_2), number of years spent in formal education, employment and current occupation history, smoking and alcohol history, and history of recreational/illicit substances use were updated and recorded into the study database.

Clinical and HIV disease related data collected during the prevalence study, including: year of diagnosis, mode of transmission, nadir CD4+ T cell count, CD4+ T cell count and HIV viral load at T_1 were included. Disease related data such as current CD4+ T cell count and HIV viral load at T_2, as well as documentation of sustained viral suppression during the follow-up period, were collected and entered into the study database.

Medical history for the presence of hepatitis B and C co-infection, history of infection with syphilis, history of hypertension, hypercholesterolemia and diabetes was updated. Psychiatric history of depression, anxiety, bipolar affective disorder (BPAD), schizophrenia and psychosis was also updated. History of neurological conditions and complications of HIV (CNS TB, CNS toxoplasmosis, encephalitis, cryptococcal meningitis, PML, IRIS, stroke, epilepsy, and neuropathy) and family history of neurodegenerative disorders recorded at the time of prevalence study were verified for updates and included.

History and current use of methadone, benzodiazepines, antiepileptic drugs, anti-psychotics, as well as anti-depressants, were updated. Current antiretroviral therapy history was also updated and recorded. It was documented if participants were currently on ART, or had only been taking ART to prevent mother to child transmission (MTCT). It was also documented if there were ART naïve patients or patients who were previously exposed to ART but not currently on ART amongst the T_2 participants. ART therapy history at T_1 was also included. Date of initiation of ART was updated for those participants who started ART therapy after the T_1 assessment. Time between HIV diagnosis and ART initiation was recorded.

Updated clinical and disease related data were obtained from the participants' paper-based healthcare records and from the electronic records used by GUIDe clinic staff. Blood test results were collected from St. James's Hospital electronic patient record (EPR) system. EPR and paper healthcare records were accessed with the participants' consent. Viral suppression was defined as a HIV viral load of less than 40 copies per ml.

Other clinical data collected at the time of follow-up assessment included presence of cognitive symptoms (word finding difficulties, difficulties remembering names or faces, forgetting recent conversations and repeating themselves, forgetfulness, which included difficulties maintaining personal schedules for hospital appointments and medication regimens, misplacing objects, difficulties with following directions or instructions, poor concentration, difficulties learning new information, making errors at work, being slower at completing tasks, safety concerns, behavioural changes, and personality changes) and neurological examination (cranial nerve examination, tone, power, reflexes, co-ordination,

sensation, apraxia, gait and rapid alternating movements). Neuropsychological data was recorded on the individual neuropsychological test sheets.

2.5.6 Missing Data

Nadir CD4+ count was not available for two patients who were diagnosed outside of Ireland. Missing data were excluded from the analysis involving this variable.

2.5.7 Data Protection

Data collected in this study were treated in accordance with Data Protection Act of 1988 & Data Protection Amendment Act of 2003 and was pseudo-anonymised. All collected data were transferred to a password protected computerised database using Excel 2013. The identifying information was treated in a strictly confidential manner. Each participant was assigned a unique study number at the time of the prevalence study. This unique study number was maintained and used to pseudo-anonymise the data at the T_1 and T_2 detailed assessments also. Hard copies of the clinical and neuropsychological assessments were stored in a locked office in the Department of Neurology in SJH. The consent forms did not contain any reference to the identifying study number and were also stored in this office. The candidate only had access to these documents at the time of T_2 assessment.

2.5.8 Neuropsychological Tests Administered

All patients underwent a full neurological history and examination at the T_2 assessment. Neurocognitive tests used in the “Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment”

at T_1 were also used during the follow-up assessments in the current study to accurately evaluate the neurocognitive status changes at T_2. These tests included the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), the Addenbrooke's Cognitive Examination revised (ACE-r), the Frontal Assessment Battery (FAB), and the Montreal Cognitive Assessment (MoCA). A copy of each of these tests is presented in Appendix 3. These tests were administered at T_2 in the same sequence as they were administered at T_1 to ensure consistency across the entire population studied, as well as consistency at the two time points. All tests were administered according to published instructions.

The RBANS test, which was originally developed for assessment of dementia was found to be useful in a number of clinical situations when a relatively rapid, easily administered, sensitive, and repeatable neurocognitive test is needed (218). It is useful for screening purposes for deficits in acute clinical settings (stroke, head injury, or other acute insult to the brain), as well as when assessing for progression of cognitive dysfunction in degenerative diseases, and when evaluating recovery during the rehabilitation. The RBANS consists of twelve subtests and assesses five cognitive domains: immediate memory, visuospatial/constructional, language, attention, and delayed memory. Quick and robust assessment in multiple domains is most useful when diagnostic criteria such as Antinori et al. criteria for HAND need to be applied. In RBANS, competency in each domain is assessed for by at least two subtests. The raw score for each of the subtests contributes to the index score of one of the five domains, which is adjusted for age. Immediate memory index score is obtained

from the conversion of list learning and story memory total raw scores. Figure copy and line orientation raw scores contribute to the index score for the visuospatial and constructional domain. Picture naming and semantic fluency raw scores are converted into an index score for the language domain. Digit span and coding raw scores are used to obtain an index score for the attention domain. List recall, list recognition, story recall, and figure recall raw scores are converted into an index score for the delayed memory domain. There are published RBANS standardised normative data for raw scores and index scores, as well as standard deviation scores and correlation coefficients for English-speaking populations of a wide age range, from 20 to 89 years. These are available in the RBANS stimulus booklet. There are also limited published data available on RBANS scores to account for practice and learning effects for general population (219). Therefore, it can be used to measure cognitive impairment in cross-sectional studies and for follow-up assessments. It can be easily administered and no special training is required. The wide age range normative data are useful in clinical cohorts such as SJH HIV cohort.

Modified scoring criteria for the figure copy subtest of the RBANS introduced by Duff et al. in 2007 were used for the figure copy and figure recall scoring in the follow-up study at T_2 (220). In their validation paper, Duff et al. felt that standard scoring criteria that came with the test tended to generate scores that show “circumscribed” deficit in this cognitive area. The modified criteria were shown to capture the functioning in the visuospatial/constructional domain more accurately when applied on a sample of almost 800 community dwelling population, as they

led to scores that were more consistent with the other RBANS subtests when compared to the scores generated by the standard criteria.

The ACE-r assesses five cognitive domains: orientation and attention, memory, verbal fluency, language, and visuospatial abilities. The total maximum possible score is 100 and consists of the sum of the scores in each of the assessed domains. No adjustment is made for the age or educational level of the participant. A score of less than 88 is considered to be abnormal. This cut off has 94% sensitivity and 89% specificity for detecting dementia (221). A domain was deemed to be abnormal if the patient's score was more than one standard deviation away from the mean normative data published by Mioshi et al. in their validation paper (221).

The FAB is a brief battery of six neuropsychological tasks designed to assess a single, executive function. More specifically FAB explores one's ability for abstract reasoning and conceptualisation, lexical verbal fluency and mental flexibility, motor programming and executive control of action, self-regulation and resistance to interference, inhibitory control, and environmental autonomy. The total score possible is 18. A standard cut-off score of 12 is suggested for use in clinical settings. Slachevsky et al. found that this cut-off score had the highest sensitivity and specificity (77% and 87%, respectively) to differentiate patients with Alzheimer's disease and Frontotemporal dementia. However, no adjustment was made for age or education and the populations studied were older than the HIV cohort assessed in the current study (222). Given the wide age range and educational level of the current study participants, adjustment for age and education is important. Therefore, normative data which was adjusted for age

(from 20 up to 95 years) and educational attainment (from 1-3 years up to >13 years) published by Appollonio et al. were deemed to be more appropriate and were applied at T_1 assessment and in the current study (223). An adjusted FAB score lower than 13.48 was considered to be abnormal. The following formula was used to calculate the adjusted FAB score:

Adjusted FAB score = raw FAB Score–1.43 x [log(100–age)–3.65]–0.98 x [square root (years of education)–3.15]

The MoCA assesses eight domains: visuospatial, executive, attention, concentration, working memory, language, delayed memory, and orientation (224). The total score possible is 30 and consists of the sum of the scores of each of the eight domains. One point was added for the participants who had spent 12 or less years in full time education. A total score of less than 26 was considered abnormal. The score has high sensitivity (90%) and specificity (87%) for detecting mild cognitive impairment. MoCA was shown to be a reasonable screening tool for CI detection in HIV+ individuals (225). There are twelve sub-tests within the MoCA to test the eight cognitive domains. The normative data for these sub-tests published by Nasreddine and colleagues were used to determine whether a domain was abnormal or not (224). If the patient's score in one of the domain's sub-tests was more than one standard deviation away from that of normal controls, the domain was deemed to be abnormal.

2.5.9 Functional Impairment Assessment

Functional impairment was interrogated during the neurological history taking and was based on the study participants' self-report of perception of difficulties with

cognitive function and activities of daily living (Appendix 4). Where available, self-reported history of functional impairment was complemented by the collateral history from reliable informants.

2.5.10 Determination of a Positive Screen for Anxiety or Depression at Follow-up

The Hospital Anxiety and Depression Scale (HADS) was used to screen the participants for clinically significant anxiety and depression at the time of follow-up. A score greater than 11 in the anxiety or depression subsection of this tool was considered to be indicative of a positive screen for anxiety or depression, respectively (226). The HADS scale has been validated for use in HIV+ individuals (227, 228). It was also used at the time of the initial cross-sectional study and is presented in Appendix 5. A review article evaluating performance of HADS in comparison with other more formal tools for assessment of anxiety and depression found it had a sensitivity and specificity of approximately 0.8 for both anxiety and depression (229). These properties were found to persist in general and psychiatric populations. HADS is widely used in hospital settings as a screening tool for symptoms of anxiety and depression in medically ill patients and can be quickly and easily administered. However, it is not a diagnostic tool for a clinical diagnosis of depression or anxiety.

2.5.11 Determination of a HIV Associated Neurocognitive Disorder (HAND) Diagnosis

Diagnostic criteria and definitions for HAND proposed by Antinori et al. were used to define the HIV associated neurocognitive disorders (48). Asymptomatic neurocognitive impairment (ANI) was defined as a deficit that was one standard deviation below the mean in at least two neurocognitive domains tested with no functional impairment. Mild neurocognitive disorder (MND) was defined as impairments in two domains that were at least one standard deviation below the mean with reported mild functional impairment. HIV associated dementia (HAD) was defined as a deficit in at least two domains that was two standard deviations below the mean with significant functional impairment. Full diagnostic criteria are presented in Table 1.6. Normative data of age-related index scores along with standard deviations are available for the RBANS. Therefore, RBANS results were used to determine the HAND diagnosis.

2.5.12 Calculation of Percent Retention Scores

Retention scores were calculated based on the RBANS list and story immediate and delayed memory subtests. Clark et al. in their percent retention scores validation study based on RBANS tests found that the healthy controls had mean percent retention scores of 77.11 (SD of 12.01) and 86.32 (SD of 12.51) on list memory and story memory, respectively (230). For the purposes of the “Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment” study, and the current follow-up study, an abnormal score was taken as the mean percent retention scores of healthy controls that were

published in the Clark et al. validation paper, minus two standard deviations. Subsequently, retention scores in the baseline and current study were deemed abnormal if patients recalled less than 53% of the acquired list items and less than 61% of the acquired story items. Using the mean score minus two standard deviations ensured that patients were scoring significantly lower than the healthy population. These percentages were slightly lower than the optimal cut-off scores for List Retention (LR) and Story Retention (SR) used to distinguish between healthy controls and patients diagnosed with Alzheimer's disease (< 60% and < 70%, respectively) in the validation paper. These cut off scores correctly classified 94.6% of normal controls (sensitivity = 0.959, specificity = 0.900) and 91.4% of subjects diagnosed with AD (sensitivity = 0.904, specificity = 0.950). Moreover, the normative data was based on a group of controls with a mean age of 72 years but the mean age of study participants in the "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment" cohort was 43 years. Since the majority of study subjects were significantly younger than the controls used in the validation paper, it was felt that study participants with normal cognitive function should score the same or higher than these normal controls. Therefore, for all of the above reasons, the scores below proposed cut off level should suggest abnormality. Retention scores using the RBANS list and story memory subtests were calculated as follows:

LR Score = List Recall Raw Score ÷ List Learning Raw Score Trial 4 × 100

SR Score = Story Recall Raw Score ÷ Story Memory Raw Score Trial 2 × 100

2.5.13 Determination of Abnormal List Recognition Scores

The RBANS supplement provides mean scores and standard deviations for list recognition subtest based on age (231). A list recognition score that was more than two standard deviations away from the mean score for that age group was judged to be abnormal.

2.5.14 Calculation of Cortical–Subcortical Deviation Score (CSDS)

Originally introduced by Randolph et al., and validated by Beatty et al., the cortical-subcortical deviation score is used to discriminate between “cortical” and “subcortical” dementias (232-234). The terms “cortical” and “subcortical” reflect certain patterns of neurocognitive impairment in different neurodegenerative conditions that are associated with different neuropathology. For example, impairments of memory and language are dominant features of Alzheimer’s disease, which is considered to be the prototypical “cortical” dementia. In contrast, attention and certain visuospatial functions are more prominently impaired in disorders such as Huntington’s disease, Parkinson’s disease, ischemic cerebrovascular disease, and Progressive Supranuclear Palsy, all of which are characterized by greater pathologic involvement of the subcortical white matter and/or subcortical nuclei.

Index scores obtained in the RBANS subtests, assessing delayed memory, language, attention, and visuospatial constructional domains were used for this purpose. The individual cortical–subcortical deviation score was calculated for each participant by subtracting the average of delayed memory and language indexes from the average of attention and visuospatial constructional indexes.

Values above zero are indicative of a cortical type of impairment and scores equal to or below zero are indicative of a subcortical type of impairment.

2.5.15 Test-Retest Stability

Since this was a repeated neuropsychological assessment, it was important to assess the test re-test coefficients, which are key elements when evaluating the change at follow-up. These show the degree to which the re-test score is systematic and free form error, or the degree to which each individual retains his/her relative position within the group from T_1 to T_2 irrespective of change (235). Test-retest reliability or stability was calculated using Pearson's correlation. Test reliability is usually presented as a correlation that ranges from +1.0 (strong correlation, as x increases, y increases) to 0 (no correlation, or no relationship) to -1.0 (as x increases, y decreases). Test re-test stability is affected by the time interval between the tests. Longer time intervals between the repeated tests lead to lower test reliability values. It is also affected by the tested person's age, i.e. younger adults have higher test-retest correlation values. In addition, not all cognitive domains generate the same test-retest correlation values. The normative stability or correlation coefficients for RBANS index scores (Form A to Form A re-test) were calculated in a cohort of 40 participants with normal cognitive status and with an average age of 70.7 years (SD=7.9). The mean interval of re-testing was 38.7 weeks (SD=2.8). These normative coefficients were available for comparison in the RBANS manual (218). In the current study, the correlation coefficients were expected to be lower, as the re-test time interval was longer than in the normative data presented in the RBANS manual.

However, the current cohort was much younger than the reference cohort, with an average age of 46.6 (SD 10.13) years at T_2.

2.5.16 Assessment of Reliable Change when Accounting for Practice Effects

With the re-administration of neurocognitive assessment tests, where there is prior exposure to the testing materials, there is a high likelihood of observing improved performance. This improved performance might be due to a clinical intervention or natural recovery in some cases. It might also occur in the absence of any intervention and it is due to prior experience or exposure to the test and loss of the test novelty at the time of repeated test administration, or so called practice effects (235). These practice effects might include remembering items from the test (declarative memory), remembering how to do the test, or becoming familiar with the test strategy (procedural memory) and can be magnified by the subject's baseline intelligence. Hence, from a clinical and research perspective, it is important to take into account practice effects as without doing so, there is a danger that a clinician/researcher might perceive improvement where the actual improvement of cognitive status might not exist. Notably, however, practice effects can lead to under detection of impairment at follow-up.

Many studies have looked at the impact of repeated testing on the re-test scores. The common finding of studies to date is that most improvements on retesting are observed between the first and second test with plateauing thereafter (236-239). In all of these studies, the interval between the first and second test is typically

measured in weeks rather than years, as in the current study. However, these effects have still been observed even two years after the baseline test (240).

It is also important to note that different cognitive domains are affected by the test repetition to differing extents. The normative data in the table 3.8 of the RBANS manual indicates that mean re-test index scores (retested at an average of 38.7 weeks) are generally higher in all domains except for the language, where the mean index scores were lower at re-test for the cohort studied (218). Similarly, Bartels and colleagues in their study on frequent repetitive cognitive testing (baseline, week 2-3, week 6, week 9, month 3, month 6, month 12) in a healthy adult population found that the largest improvement on performance occurred between the first and second test and the most important changes until month three were observed in executive functions (14.0 ± 10.7%), followed by learning/memory (13.3 ± 12.3%) and attention (11.9 ± 10.6%) (241).

Apart from aforementioned test items recall, procedural learning and familiarity with testing environment, tested person's intelligence and the domain tested, other factors such as age, mood, motivation and fatigue on the day of testing, availability of alternate test forms, interval between tests, regression to the mean, ceiling and floor effects can also influence the size of practice effects (235, 241). The ceiling effect, for example, means that if a subject gained the maximum score possible at baseline, it would be difficult to assess for improvement at follow-up. Similarly, the floor effect means that if a subject gained the lowest possible score at baseline it may not be possible to identify the progression of impairment at follow-up.

To estimate the possible impact of practice effects in the current follow-up study, the approach of Reliable Change Index that controls for practice effects (RCI + PE) was used for each of the RBANS subtest raw scores. This approach was described by Duff et al. in their review of methods for assessing reliable change in longitudinal neuropsychological studies (235):

$$(RCI + PE) = (T2 – T1) – (M2 - M1)/SED$$

where T1 is individual score at T_1 and T2 is individual score at T_2,

M1 is control group mean at T_1 and M2 is control group mean at T_2, and

SED is standard error of the difference which is calculated using the formula:

$$SED = \sqrt{2}\, S1^2(1 – r12)$$

where S1 is standard deviation at T_1 and

r12 is correlation coefficient between the scores at T_1 and T_2.

The current study, however, did not have access to a comparable reference group neither at baseline nor at follow-up to allow for the analysis that would account for possible practice effects at T_2. Therefore, a conservative approach was adopted, which relied on available published data that, at a minimum, included means and standard deviations for the test scores at baseline and follow-up and test re-test reliability coefficients. To estimate the impact of theoretical practice effect on the individual RBANS subtests at second re-test, first, the short interval re-test normative data published by Safaz et al. were used. The authors re-tested a group of 95 healthy individuals, with an age range of 20-

49 years at 4-6 weeks interval, using the Turkish version of the RBANS form A (242). Then, longer interval, second re-test norms published by Duff and colleagues were used (219). In the latter study, a cohort of 445 healthy community dwelling older adults, with a mean age of 72.89 (SD=5.52), completed RBANS Form A at baseline and at re-test at approximately one year interval.

2.5.17 Domain Based Analysis

The domain based analysis relied on the subtests of each of the neuropsychology tests in the battery. These subtests were grouped into the following seven neurocognitive domains:

1. Attention and Processing Speed
2. Working Memory
3. Delayed Memory
4. Executive Function
5. Language
6. Visuospatial skills
7. Orientation.

The allocation of the subtests across these domains is defined in Appendix 6. Each score was *z* transformed for each patient and then averaged to give an average *z* score for each domain. As there was no control group in this study, in order to perform the *z* transformation, the means and standard deviations were calculated for the 79 patients at T_1 and T_2. The following calculation was used for the z transformation:

$$z_i = (x_i - \mu) \div SD$$

z_i = *z* score for individual patient, x_i = individual score, μ = mean, SD = standard deviation.

2.5.18 Statistical Analysis

Categorical (qualitative) data were described using absolute numbers and percentages, i.e. relative frequencies. Means, standard deviations, and medians were obtained to present the quantitative (discrete and continuous) variables. Test-retest reliability was assessed by calculating Pearson correlation coefficient. Chi squared test was used for categorical data analysis (McNemar test and Stuart-Maxwell test, where appropriate). Paired samples T-test for parametric data and Wilcoxon Signed Rank test for non-parametric data were used to assess the quantitative variables differences at T_1 and T_2. Differences between T_1 and T_2 z-scores were assessed using analysis of variances (ANOVA). Alternatively, Kruskal Wallis test was used when the assumption of homogeneity of variances was violated. The results were considered to be statistically significant when p-values were less than or equal to 0.05 ($p \leq 0.05$). Bonferroni correction was applied to account for multiple testing, where appropriate. To carry out the statistical analysis, *IBM® SPSS® Statistics V24* was used. Data were presented in tables and, for the graphical representation, bar charts, box plots, and scatter plots were used.

2.5.19 Role of the PhD Candidate

The candidate recruited and re-enrolled all of the follow-up study participants and consented them before the follow-up assessments. The candidate created and maintained the follow-up study database. The candidate administered the

detailed neuropsychological testing for all of the study participants at follow-up and performed the statistical analysis. The candidate also applied the modified figure copy scoring criteria to the RBANS figure copy and figure recall subtest results obtained during the T_1 assessments to ensure consistency of scoring in these tests and to eliminate the inter-scorer agreement bias, as well as the possibility of improvement or progression by chance due to the use of different scoring criteria. Following this, for T_1 results, the index scores for Visuospatial/Constructional and Delayed memory domains were adjusted, and the HAND diagnosis was re-classified where applicable. The database was updated with the adjusted scores and HAND diagnoses.

2.6 Results

2.6.1 Description of Study Population

While 104 HIV+ individuals underwent the baseline assessments in the initial "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment", only 79 participants were retained in the follow-up study at T_2 and 25 were lost to follow-up. The mean follow-up interval was 36 months, ranging from 25 to 61 months. Characteristics of the original cohort of 104 participants, characteristics at T_1 and at T_2 of the 79 participants who were retained in the study and known characteristics of those who were lost to follow-up (LTFU) are described in Table 2.2.

Table 2.2. Patient Characteristics at T_1 and T_2

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
Demographics									
Age (in years)	Mean (SD)	43.2	(10.06)	42.0	(9.76)	43.6	(10.2)	46.6	(10.13)
	Median (range)	42.8	(22-71)	40.1	(22-64)	42.8	(23-71)	45.6	(25-74)
Gender	Male	84	(80.8%)	19	(76.0%)	65	(82.3%)	65	(82.3%)
	Female	20	(19.2%)	6	(24.0%)	14	(17.7%)	14	(17.7%)
Country of birth	Ireland	83	(79.8%)	21	(84.0%)	62	(78.5%)	62	(78.5%)
	Europe	6	(5.8%)	2	(8.0%)	4	(5.1%)	4	(5.1%)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
	Africa	14	(13.4%)	2	(8.0%)	12	(15.2%)	12	(15.2%)
	South America	1	(1.0%)	0	(0.0%)	1	(1.3%)	1	(1.3%)
English as a first language	Yes	90	(86.5%)	23	(92.0%)	67	(84.8%)	67	(84.8%)
Handedness	Right	92	(88.5%)	20	(80.0%)	72	(91.1)	72	(91.1)
	Left	12	(11.5%)	5	(20.0%)	7	(8.9%)	7	(8.9%)
Employment history	Employed	54	(51.9%)	9	(36.0%)	45	(56.9%)	47	(59.5%)
	Student	5	(4.8%)	0	(0.0%)	5	(6.3%)	7	(8.9%)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
	Unemployed	41	(39.5%)	15	(60.0%)	26	(32.9%)	18	(22.8%)
	Retired	4	(3.8%)	1	(4.0%)	3	(3.8%)	7	(8.9%)
Years of education	Mean (SD)	13.6	(4.1)	12.2	(4.3)	13.8	(3.9)	14.1	(4.0)
	Median (range)	14	(6-23)	11.0	(6-22)	14.0	(6-23)	14.0	(6-24)
HIV disease related characteristics									
Mode of transmission	Heterosexual	34	(32.7%)	5	(20.0%)	29	(36.7%)	29	(36.7%)
	MSM	48	(46.2%)	11	(44.0%)	37	(46.8%)	37	(46.8%)

Patient Characteristics		Subjects assessed at T_1 n = 104		Subjects lost to follow-up n = 25		Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79		Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79	
	IVDU	19	(18.3%)	8	(32.0%)	11	(13.9%)	11	(13.9%)
	Other	3	(2.9%)	1	(4.0%)	2	(2.5%)	2	(2.5%)
Time since diagnosis (years)	Mean (SD)	9.43	(7.29)	9.26	(7.47)	9.48	(7.28)	12.52	(7.28)
	Median (range)	7.45	(0.1-28.3)	7.48	(0.1-27.9)	7.41	(0.64-28.25)	10.57	(3.2 – 32.4)
Nadir CD4 count	Mean (SD)	246.64	(169.6)	263.76	(121.3)	241.08	(182.9)	241.08	(182.9)
	Median (range)	241.0	5-848	272.00	(5-460)	238.0	(9-848)	238.0	(9-848)
Current CD4 count	Mean (SD)	603.87	(289.91)	510.20	(289.26)	633.51	(285.55)	713.44	(257.70)
	Median (range)	585.5	(59-1485)	520	(59-1442)	618	(142-1485)	681.00	(164-1394)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
Virally suppressed	Yes	91	(87.5%)	20	(80.0%)	71	(89.9%)	76	(96.2%)
	No	4	(3.8%)	3	(12.0%)	1	(1.3%)	2	(2.5%)
	Not on ART at time of assessment	9	(8.7%)	2	(8.0%)	7	(8.9`%)	1	(1.3%)
Antiretroviral therapy	Naïve	7	(6.7%)	2	(8.0%)	5	(6.3%)	0	(0.0%)
	MTCT	2	(1.9%)	0	(0.0%)	2	(2.5%)	1	(1.3%)
	On ART	95	(91.3%)	23	(92.0%)	72	(91.1%)	78	(98.7%)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
Interval between T_1 and T_2 assessments (months)	Mean (SD)	n/a		n/a		n/a		36.45	(7.97)
	Median (range)	n/a		n/a		n/a		34.89	(25.13-60.98)
Continuous viral suppression between T_1 and T_2	Yes	n/a		n/a		n/a		58	(73.4%)
Exposure to HAART (total years)	Mean (SD)	6.51	(5.06)	5.88	(5.23)	6.82	(4.93)	9.28	(5.17)
	Median (range)	6.01	(0.01-16.5)	3.56	(0.0-16.5)	6.35	(0.05-16.44)	8.99	(1.3-20.8)
CNS Opportunistic Infections									

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
Hx of Cryptococcal Meningitis	Yes	1	(1.0%)	0	(0.0%)	1	(1.3%)	1	(1.3%)
Hx of CNS TB	Yes	2	(1.9%)	0	(0.0%)	2	(2.6%)	2	(2.6%)
Hx of Toxoplasmosis	Yes	2	(1.9%)	1	(4.0%)	1	(1.3%)	1	(1.3%)
Hx of Encephalitis	Yes	1	(1.0%)	0	(0.0%)	1	(1.3%)	1	(1.3%)
Hx of PML	Yes	1	(1.0%)	0	(0.0%)	1	(1.3%)	1	(1.3%)
Other Comorbidities									
Epilepsy	Yes	5	(4.8%)	1	(4.0%)	4	(5.1%)	4	(5.1%)

Patient Characteristics		Subjects assessed at T_1 n = 104		Subjects lost to follow-up n = 25		Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79		Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79	
Stroke	Yes	2	(1.8%)	1	(4.0%)	1	(1.3%)	1	(1.3%)
Hx of Depression	Yes	27	(26.0%)	3	(12.0%)	24	(30.4%)	34	(43.0%)
Hx of Bipolar Disorder	Yes	1	(1.0%)	0	(0.0%)	1	(1.3%)	1	(1.3%)
Hx of Anxiety	Yes	4	(2.9%)	3	(12.0%)	1	(1.3%)	1	(1.3%)
Hx of Schizophrenia	Yes	1	(1.0%)	0	(0.0%)	1	(1.3%)	1	(1.3%)
Hx of Psychosis	Yes	2	(1.8%)	0	(0.0%)	2	(2.5%)	2	(2.5%)
Neuropathy	Yes	2	(3.6%)	1	(4.0%)	1	(1.3%)	1	(1.3%)
Hypertension	Yes	11	(10.6%)	3	(12.0%)	8	(10.1%)	10	(12.7%)

Patient Characteristics		Subjects assessed at T_1 n = 104		Subjects lost to follow-up n = 25		Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79		Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79	
Hypercholesterolemia	Yes	14	(12.5%)	5	(20.0%)	9	(11.4%)	33	(41.8%)
Diabetes	Yes	4	(3.8%)	2	(8.0%)	2	(2.5%)	7	(8.9%)
Hx of Hepatitis B	Yes	8	(7.8%)	0	(0.0%)	8	(10.1%)	8	(10.1%)
Hx of Hepatitis C	Yes	26	(25.0%)	12	(48.0%)	14	(17.7%)	14	(17.7%)
Hx of Syphilis	Yes	15	(14.4%)	3	(12.0%)	12	(15.2%)	14	(17.7%)
Medications									
Benzodiazepines	Yes	22	(21.2%)	8	(32.0%)	14	(17.7%)	15	(19.0%)
Methadone Replacement	Yes	16	(15.4%)	8	(32.0%)	8	(10.1%)	11	(13.9%)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
Therapy									
Anti-depressants	Yes	14	(13.5%)	5	(20.0%)	9	(11.4%)	10	(10.1%)
Anti-seizure	Yes	6	(5.8%)	3	(12.0%)	3	(3.8%)	3	(3.8%)
Anti-psychotics	Yes	3	(2.9%)	0	(0.0%)	3	(3.8%)	3	(3.8%)
Lifestyle History									
Smoking history	Smoker	51	(49.0%)	15	(60.0%)	36	(45.6%)	38	(48.1%)
	Non-smoker	33	(31.7%)	7	(28.0%)	26	(32.9%)	26	(32.9%)
	Ex-smoker	20	(19.2%)	3	(12.0%)	17	(21.5%)	15	(19.0%)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
Alcohol history	Non drinker	19	(18.3%)	6	(24.0%)	13	(16.5%)	13	(16.5%)
Units per week	1-20 units	66	(63.5%)	12	(48.0%)	54	(68.4%)	53	(67.1%)
	>21 units	14	(25.0%)	5	(20.0%)	9	(11.4%)	10	(12.7%)
	Former drinker	5	(4.8%)	2	(8.0%)	3	(3.8%)	3	(3.8%)
Illicit Substance Use	Never	68	(65.4%)	11	(44.0%)	57	(72.2%)	57	(72.2%)
	Current non IV use	18	(17.3%)	5	(20.0%)	13	(16.5%)	12	(15.2%)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
	Current IV use	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
	Previous Hx	18	(17.3%)	9	(36.0%)	9	(11.4%)	10	(12.7%)
Family history of neurodegenerative disorders	Yes	16	(15.4%)	3	(12.0%)	13	(16.5%)	13	(16.5%)
Hospital Anxiety and Depression Scale									
Positive screen for anxiety	Yes	30	(28.8%)	7	(28.0%)	23	(29.1%)	22	(27.8%)
HADS Anxiety Score	Mean (SD)	7.93	(4.48)	7.96	(3.76)	7.92	(4.71)	7.73	(4.50)

Patient Characteristics		**Subjects assessed at T_1 n = 104**		**Subjects lost to follow-up n = 25**		**Subjects assessed at T_1 and T_2; characteristics at T_1 n = 79**		**Subjects assessed at T_1 and T_2; characteristics at T_2 n = 79**	
	Median (range)	7.00	(0-21)	7.00	(3-15)	8.00	(0-19)	7.00	(0-19)
Positive screen for depression	Yes	9	(8.7%)	2	(8.0%)	7	(8.9%)	9	(11.4%)
HADS Depression Score	Mean (SD)	5.41	(4.216)	4.44	(3.51)	5.37	(4.41)	5.13	(4.36)
	Median (range)	5.00	(0-20)	5.00	(0-13)	5.00	(0-20)	4.00	(0-18)

Similar to T_1, at T_2 the majority of the participants were male (82.3%), Irish (78.5%), and spoke English as their first language (84.8%). The mean age at follow-up was 46.6 years (SD=10.15), ranging from 25 to 74. All but one patient, who experienced ART as MTCT, were on ART (98.7%) at the time of follow-up assessment and 76/79 (96.2%) were virally suppressed. However, only 58/79 (73.4%) were continuously virally suppressed during the follow-up interval. Of the three participants who were not virally suppressed at T_2, two had low grade viremia (one of them had 42 copies/ml and one had 82 copies/ml). The other participant displayed high grade viremia at the follow-up assessment. This patient accessed ART intermittently to prevent MTCT and was off ART at follow-up.

Interestingly, all patients (100%) who were co-infected with viral hepatitis B (HBV) were retained in the study (10.1% of participants were HBV co-infected at T_2), while just over a half of those co-infected with viral hepatitis C (HCV) returned for follow-up (17.7% of participants were HCV co-infected at T_2). A big proportion 58/79 (72.2%) of those retained in the study never used illicit substances and 47/79 (59.5%) were employed at follow-up. A total of 22/79 (27.8%) screened positive for anxiety and nine of these (11.4%) also screened positive for depression. During the follow-up interval, two patients were newly diagnosed with Hypertension (HTN), five with Diabetes Mellitus (DM), and fourteen with hypercholesterolemia (including hypertriglyceridemia), which increased the total number of patients with HTN, DM, and hypercholesterolemia at follow-up to 10 (12.7%), 7 (8.9%), and 33 (41.8%), respectively.

Of the 25 participants who did not return for the follow-up assessment, seven died and 18 were lost to follow-up for other reasons. This constituted a total attrition rate of 24%. The attrition rate amongst those who met diagnostic criteria for HAND was 23% and amongst those who did not - 26%. Of those who died, only one (14%) had a normal cognitive status at baseline. This patient was also the only one who had a normal neurological examination at baseline. Notably, the other four (57%) of those patients who died met criteria for ANI and two (29%) met criteria for MND.

Those lost to follow-up (LTFU) were more likely than those who went on to have the T_2 assessments to be unemployed ($p = 0.0039$), to be past intravenous drug users (32% compared with 13.9%; $p = 0.0089$), and to be hepatitis C co-infected ($p=0.0024$). Those LTFU were over four times more likely to have a history of anxiety (12% vs 2.9%; $p = 0.0067$). They were twice as likely to have a history of illicit substance abuse (36% vs 17.3%; $p = 0.0134$) and twice as likely to be on methadone replacement therapy (32% compared with 15.4%; $p = 0.0215$). Those lost to follow-up spent an average of 1.6 years less in education than those who returned for follow-up assessment ($p = 0.0842$).

2.6.2 Symptoms Reported by Participants at Follow-up

The most commonly reported cognitive symptoms by participants at follow-up were: difficulties remembering names of friends, family members and work colleagues (66/79, 83.5%); difficulties managing schedules (51/79, 64.6%); poor concentration (46/79, 58.2%); misplacing phones, keys and wallets (42/79, 53.2%); and word finding difficulties (33/79, 42%) (Figure 2.1). Difficulties with

remembering names nearly doubled at T_2 (66/79 vs 39/79), compared with T_1 and so did reports of misplacing items (19/79 vs 42/79), while poor concentration trebled (15/79 vs 46/79).

Reporting of difficulties managing schedules remained nearly the same (48/79 vs 51/79). The symptoms relating to difficulties managing schedules included forgetting important dates and events, forgetting hospital appointments, missed medication and increasing reliance on family members and text reminders, lists and other memory aids. Thirty participants (38%) had been frequently told that they were repeating themselves. This was only a small increase when compared with reporting of this symptom at baseline: 24/79 (30%) vs 30/79 (38%). Approximately one third (25/79, 32%) reported difficulties with following directions and map-reading.

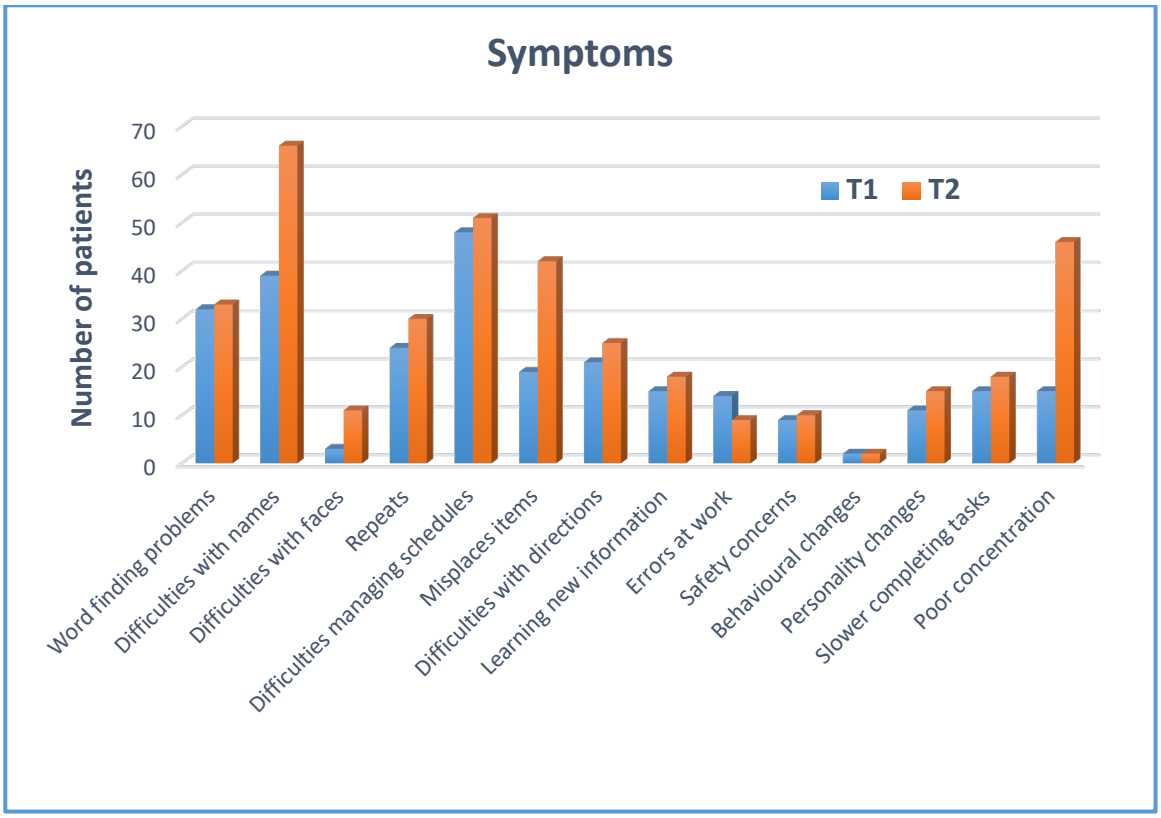


Figure 2.1. Symptoms reported by participants at follow-up assessment

Difficulties learning new information in college, new procedures, and policies in work, or learning to operate new personal/household gadgets was reported by 18 patients (23%). The same number of participants stated that they were slower at completing tasks. However, errors at work were reported less frequently at T_2 - 9/79 (11%) vs 14/79 (18%). Difficulty retaining new information and being less efficient, slower and errors at work have been the cause of a temporary suspension from the work placement in one case, led to transfer to a less demanding position in work in two cases, and led to job loss in one case. Behavioural changes and safety concerns at follow-up were reported as

frequently as at baseline 2/79 (2.5%) and 10/79 (12.6%), respectively. The reporting of personality change increased slightly: 11/79 (14%) vs 15/79 (19%).

2.6.3 Examination Findings

The most common findings on neurological examination were the cranial nerve abnormalities 35/79 (44.3%), which represented an increase when compared to findings at baseline ($p<0.0001$). The majority of the cranial nerve findings were minor eye movement abnormalities, such as slow saccades with broken pursuit movements with or without absent convergence. These were observed in 32 patients. The other cranial nerve abnormalities at T_1, as well as T_2, included facial asymmetry in two patients and visual field defect secondary to a stroke in one patient. Abnormal co-ordination in the form of moderate to severe action and intention tremor was displayed by 24 patients (30.4%) and constituted a fourfold increase ($p=0.003$), compared to respective findings at T_1. Slow and clumsy rapid alternating movements (RAM) increased from six observations at T_1 to ten observations (12.7%) at T_2.

Decreased sensation, hypertonicity, hyporeflexia and hyperreflexia were also observed in a higher number of patients twenty vs four (25.3% vs 5%; $p=0.0004$), six vs one (7.6% vs 1.3%; $p=0.0556$), sixteen vs eight (20.3% vs 10%; $p=0.0751$) and seven vs three (8.86% vs 3.8%; $p=0.1901$), respectively. Features of ideomotor apraxia and/or limb-kinetic apraxia were demonstrated by 12/79 (15.2%) patients at both assessments. Apraxia was examined by asking the patient to copy movements of the hands, mime using a toothbrush or a hairbrush, perform a salute, and mime blowing out a candle. Patients were often using their

finger as a substitute for the “object” and had most difficulties when copying movements of the hands (Figure 2.2).

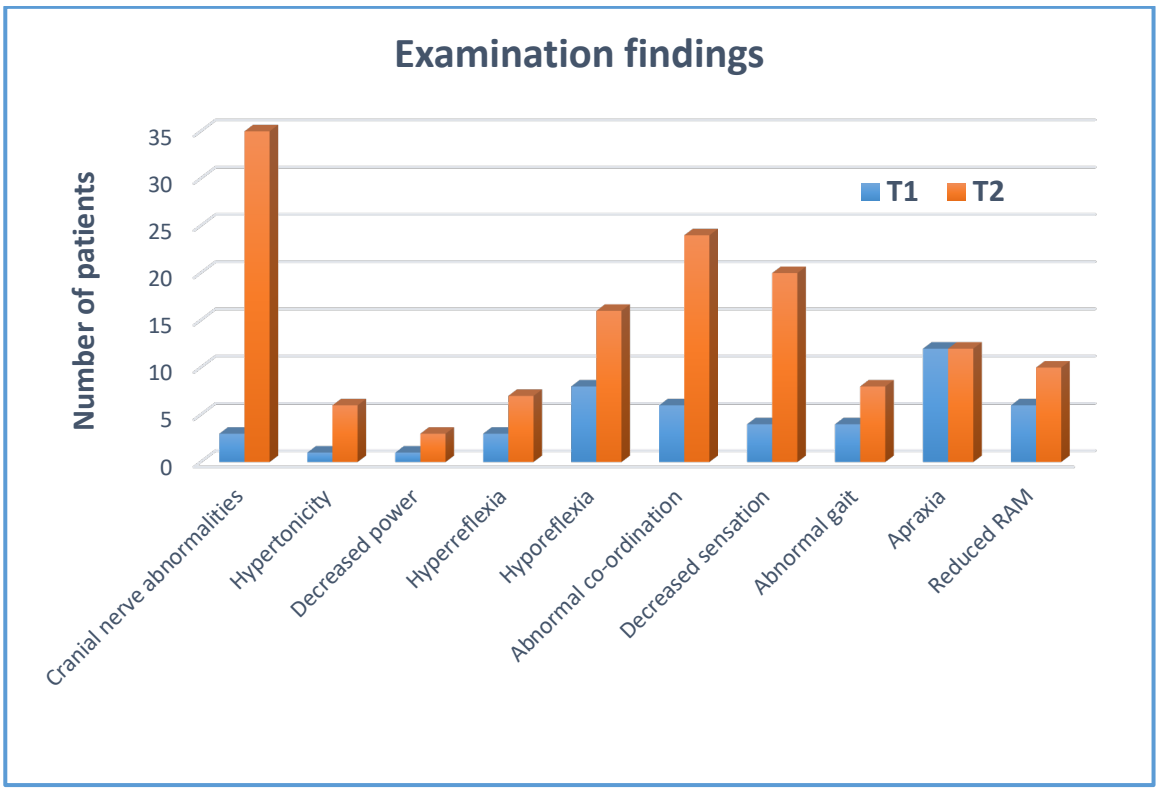


Figure 2.2. Neurological examination findings at follow-up assessment

2.6.4 Neurocognitive Change at Follow-up Assessed by HAND Diagnosis

The RBANS test results and functional status, as reported by patients or where available by a reliable informant, were used to establish the HAND diagnosis. Among the 79 participants who returned for follow-up assessment, 45 (57%) met criteria for HAND diagnosis at T_2. Of them, 20/79 (25%) met diagnostic criteria for asymptomatic neurocognitive impairment (ANI), 22/79 (28%) – for mild

neurocognitive disorder (MND), and 3/79 (4%) – for HIV associated dementia (HAD).

There were variable trajectories of change in HAND diagnosis observed at T_2 (Table 2.3). About two thirds of patients (52/79 or 66%) have remained stable and met diagnostic criteria for the same type of HAND at follow-up as at the baseline assessment, 12/79 (15%) showed an improvement to a lesser extent or no deficit on neurocognitive testing, and 15/79 (19%) performed worse at follow-up. Amongst those who improved, this improvement led to a diagnosis of HAND in remission, in nine participants – ANI in remission, and in three participants – MND in remission. Of those who performed worse, six participants had new or incident diagnosis of HAND, five of them met criteria for ANI, and one for HAD. The other participants who showed progression on HAND diagnosis (9/15), changed from asymptomatic impairment (ANI) to symptomatic (MND), as they reported having more difficulties in the activities of daily living, work or cognitive function at follow-up. Despite reporting more functional impairment, the overall total scores on neuropsychology tests did not change significantly in this subgroup.

Of those who did not meet criteria for HAND (34/79; 43%), nine performed within normal limits on all four neuropsychological tests. However, 25 participants displayed abnormalities in either one of the RBANS domains, or had an abnormal total score on MoCA, FAB, or ACE-r, or had a combination of the above. These abnormalities were classified as non-diagnostic.

To test whether the changes in HAND diagnosis at follow-up, described in Table 2.3, did or did not occur by chance, the Stuart-Maxwell test for more than 2x2 paired categorical data was used. The chi-square statistic was 8.45 with 3 degrees of freedom and the p-value was 0.0376, indicating that the changes in the type of HAND observed at T_2 did not occur by chance.

Table 2.3. HAND Diagnostic Profile Change at T_2

Study cohort diagnostic profile change at T2; T1 n = 104 and T2 n = 79								
T2 RBANS results / T1 total n=104		Total recruited at T2 n=79	Not HAND at T2	ANI at T2	MND at T2	HAD at T2	RIP	LTFU
Not HAND	38 36.5%	28 35.4%	22	5^		1^	1	9
ANI	43 – 41.3%	33 – 41.8%	9*	15	9^^		4	6
MND	21 – 20.2%	16 – 20.3%	3*		13		2	3
HAD	2 – 2%	2 – 2.5%				2		
Total		79	34/79 (43%)	20/79 (25%)	22/79 (28%)	3/79 (4%)	7	18
^ Incident diagnosis of HAND (ANI/MND/HAD); ^^ ANI progressed to MND; * HAND (ANI or MND) in remission; LTFU – lost to follow up other than RIP								

2.6.5 Break down of RBANS Results by Domains in HAND

On RBANS battery, attention (27/45; 60%), delayed memory (27/45; 60%), and visuospatial/constructional (26/45; 57.8%) domains were the most frequently affected in the patients who met criteria for HAND at follow-up. At T_2 assessment, immediate memory was impaired in a much smaller proportion than at T_1 and was abnormal in 51% (23/45). Language was abnormal in 48.9% (22/45) of patients with HAND (Figure 2.3).

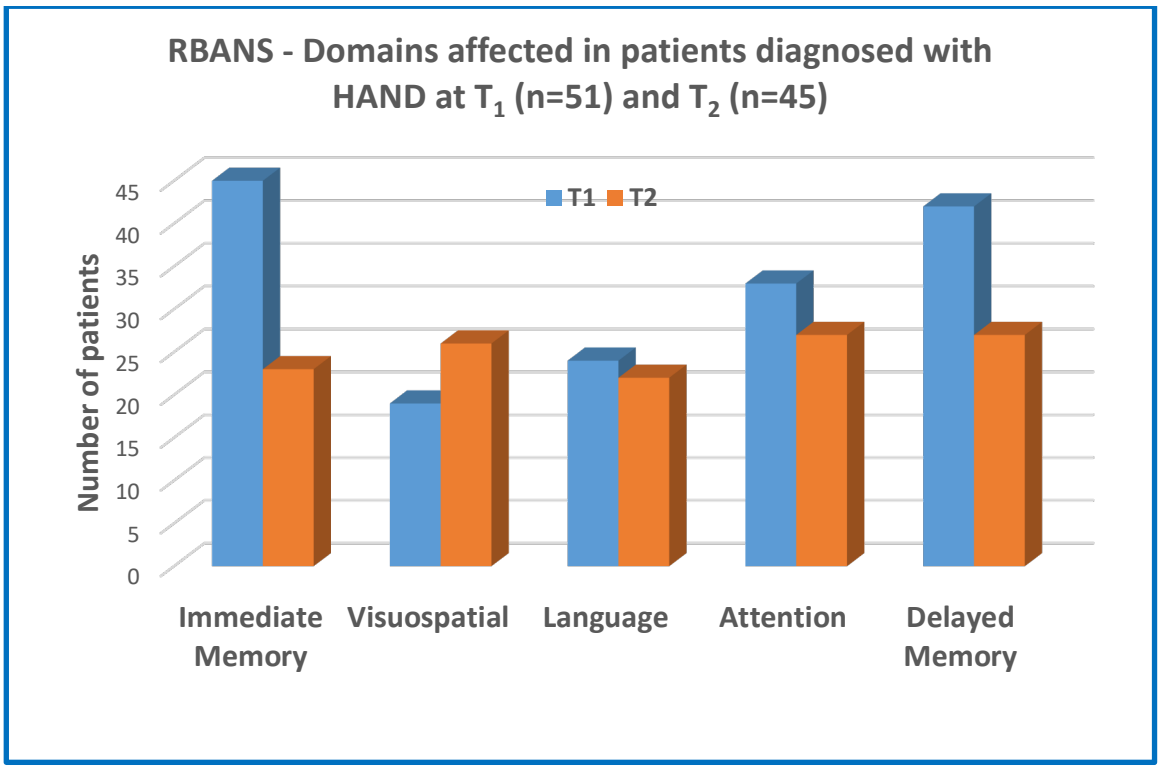


Figure 2.3. RBANS - Domains affected in patients diagnosed with HAND

In those with HAND, most RBANS domains were less frequently affected at follow-up than at baseline (immediate memory 51.1% vs 88.2%, p=0.0001; delayed memory 60% vs 82.4%, p=0.0154; attention 60% vs 64.7%; p=0.6368),

except for the visuospatial skills domain, which was abnormal in a greater number of patients at follow-up assessment (26/45; 57.8% vs 19/51; 37.3%; p=0.0457) and language, which at T_2 was affected in a slightly higher proportion than at T_1 (22/45; 48.9% and 24/51; 47%; p=0.8532). In the group that had non-diagnostic abnormalities, immediate memory was abnormal in one patient, visuospatial/constructional domain in two patients, attention in two patients, and delayed memory in one patient. There was no language impairment observed in the group with the non-diagnostic abnormalities.

The index scores of the five domains were compared across the three groups: those who performed normal, those with non-diagnostic changes and those who met criteria for HAND. The pairwise comparisons showed statistically significant differences between the index scores of patients with HAND and those with normal neuropsychological testing in all domains at both assessments, except for the visuoconstructional domain at T_1. Notably, there were significant differences between the index scores for those with HAND and those with non-diagnostic abnormalities in all domains at both assessments (Table 2.4 and Table 2.5). There were no significant differences between the index scores of patients with normal performance and those with non-diagnostic changes at baseline and at follow-up in any domain.

Table 2.4. RBANS Index Scores Results at T_1

RBANS Index Scores at T_1 (n=79)		Normal (n=7)		Non-Diagnostic Abnormalities (n= 21)		HAND (n=51)		Normal vs Non-Diagnostic Abnormalities		Normal vs HAND		Non-Diagnostic Abnormalities vs HAND	
								Difference	p-value	Difference	p-value	Difference	p-value
Immediate Memory	Mean (SD)	94.86	8.78	87.86	9.69	71.18	13.04	7.00	0.1029	23.68	<0.0001	16.68	<0.0001
	Median		90		85		76						
Visuospatial/ Constructional	Mean (SD)	98.00	8.23	98.90	11.31	90.43	16.57	-0.90	0.8484	7.57	0.2421	8.47	0.0338
	Median		96		100		92						
Language	Mean (SD)	95.86	5.84	95.14	5.23	81.84	15.94	0.71	0.7614	14.01	0.0258	13.30	0.0004
	Median		97		96		88						
Attention	Mean (SD)	95.71	10.66	96.57	9.12	78.35	15.19	-0.86	0.8378	17.36	0.0051	18.22	<0.0001
	Median		97		94		82						
Delayed Memory	Mean (SD)	96.00	5.69	87.10	15.17	66.47	16.01	8.90	0.1453	29.53	<0.0001	20.62	<0.0001
	Median		94		91		68						

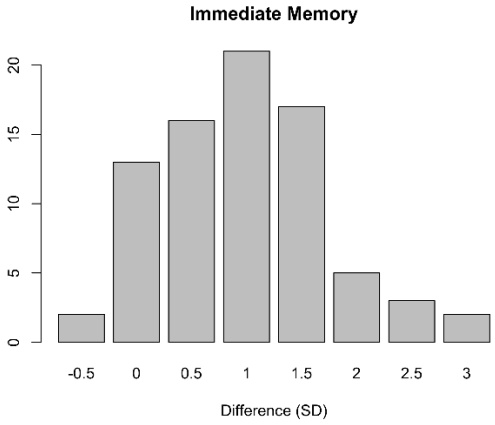
Table 2.5. RBANS Index Scores Results at T_2

RBANS Index Scores at T_2 (n=79)		Normal (n=9)		Non-Diagnostic Abnormalities (n= 25)		HAND (n=45)		Normal vs Non-Diagnostic Abnormalities		Normal vs HAND		Non-Diagnostic Abnormalities vs HAND	
								Difference	p-value	Difference	p-value	Difference	p-value
Immediate Memory	Mean (SD)	108.00	11.62	103.64	10.74	88.07	13.07	4.56	0.3141	19.93	0.0001	15.57	<0.0001
	Median		106		103		83						
Visuospatial/ Constructional	Mean (SD)	103.78	9.48	101.40	11.36	85.40	15.63	2.38	0.5789	18.38	0.0013	16.00	<0.0001
	Median		109		102		84						
Language	Mean (SD)	93.33	6.96	97.52	6.48	81.82	14.36	-4.19	0.1124	11.51	0.0233	15.70	<0.0001
	Median		92		97		85						
Attention	Mean (SD)	99.11	12.53	99.12	11.62	82.38	14.24	-0.01	0.9983	16.73	0.0019	16.74	<0.0001
	Median		97		100		82						
Delayed Memory	Mean (SD)	103.67	8.71	97.56	7.98	80.62	16.48	6.11	0.0660	23.05	0.0002	16.94	<0.0001
	Median		102		99		83						

2.6.6 Neurocognitive Change at Follow-up Assessed by RBANS Domains in the Entire Cohort

In this part of the analysis, a commonly used definition of a meaningful change at follow-up that is equal or greater than 0.5 standard deviation (SD) from the baseline score was adopted on the RBANS index scores of the five domains (243). Normative data for index scores, as well as standard deviations, are available for the RBANS test in the RBANS manual.

When applying this definition of a meaningful change, variable trajectories across different domains were also observed (Figure 2.4). On immediate memory, only two participants (2.5%) performed worse by 0.5 or more SD, 13 participants (16.5%) remained stable or had similar scores at the two time points and the majority 64 (81%) performed better. However, on the visuospatial/constructional domain 32 participants (40.5%) performed worse by 0.5 SD or more, 26 (32.9%) remained stable and 21 (26.6%) improved at follow-up. On the language domain, 13 subjects (16.5%) performed worse, 15 (19%) did better at follow-up and approximately two thirds (51/79; 64.5%) had stable scores. On attention, 12 participants (15.2%) did worse, 31 (39.2%) had better scores, and nearly half remained stable (36/79; 45.6%). Lastly, on delayed memory, only seven patients (8.9%) performed worse, 24 (30.4%) showed scores similar to those at baseline, and a little less than two thirds improved (48/79; 60.7%).


Immediate Memory
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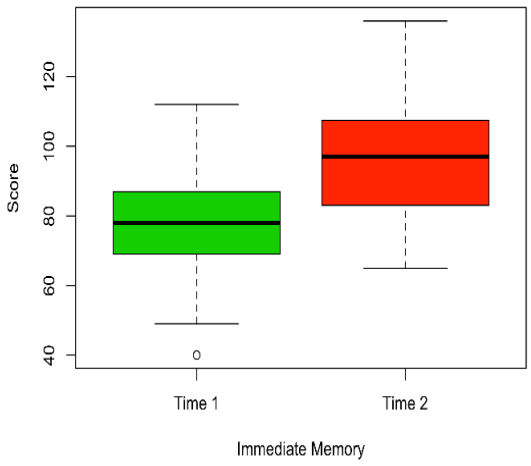
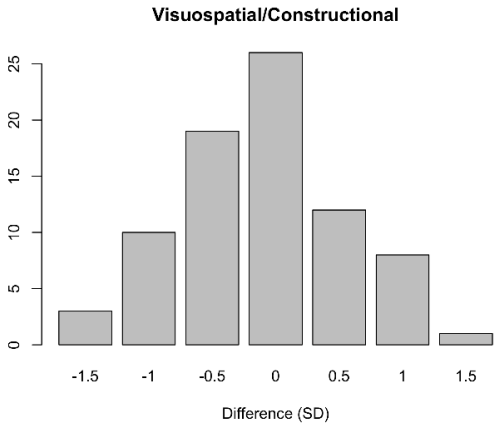
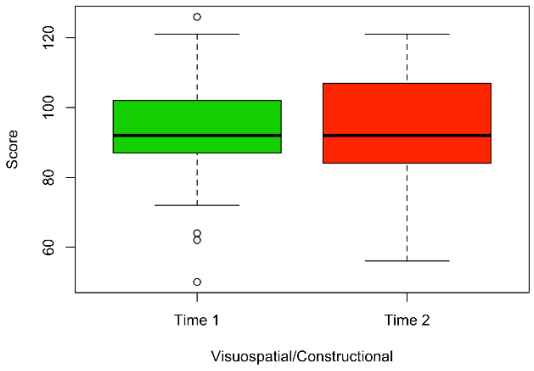
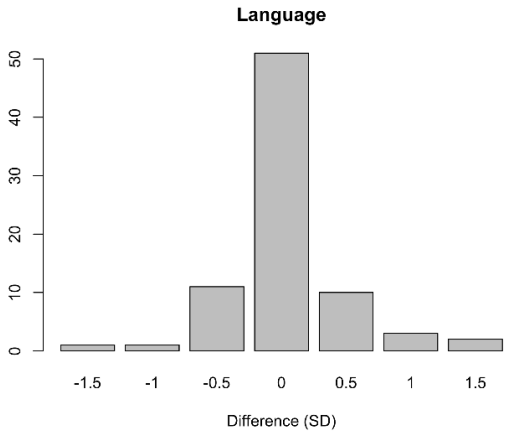
5
10
15
20
-0.5
0
0.5
1
1.5
2
2.5
3
Difference (SD)


Score
40
60
80
100
120
Time 1
Time 2
Immediate Memory


Visuospatial/Constructional
0
5
10
15
20
25
-1.5
-1
-0.5
0
0.5
1
1.5
Difference (SD)


Score
60
80
100
120
Time 1
Time 2
Visuospatial/Constructional


Language
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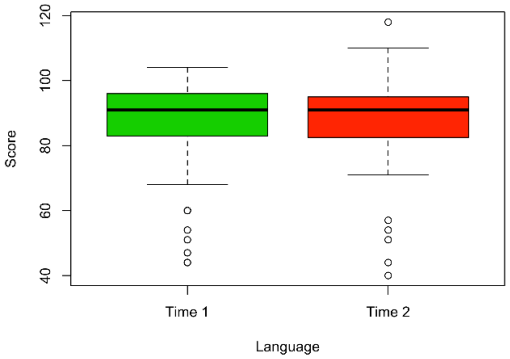
10
20
30
40
50
-1.5
-1
-0.5
0
0.5
1
1.5
Difference (SD)


Score
40
60
80
100
120
Time 1
Time 2
Language

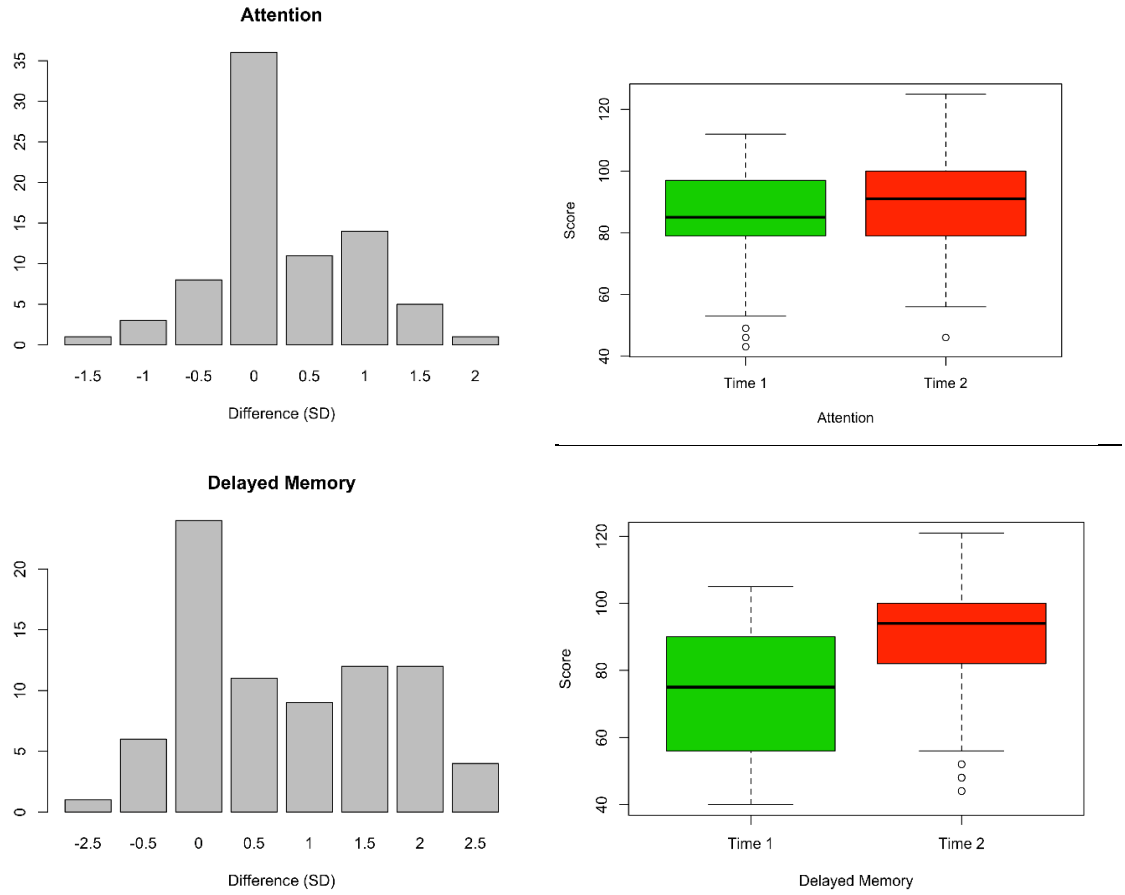


Figure 2.4. Distribution of differences in SD and RBANS Index Scores changes

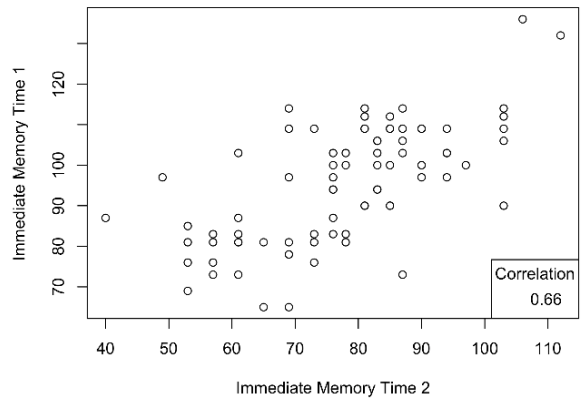
The distribution of changes in the RBANS index scores and differences of index scores between T_1 and T_2 in the Figure 2.4 show that, in general, as a group, this cohort remained stable on visuospatial/constructional (p=0.5783) and language (p=0.1552) domains. The distribution of changes in index scores and the comparison of index scores at T_1 and T_2 for attention, immediate memory and delayed memory domains showed a significant improvement at follow-up, with all p-values less than 0.0001 (Table 2.6).

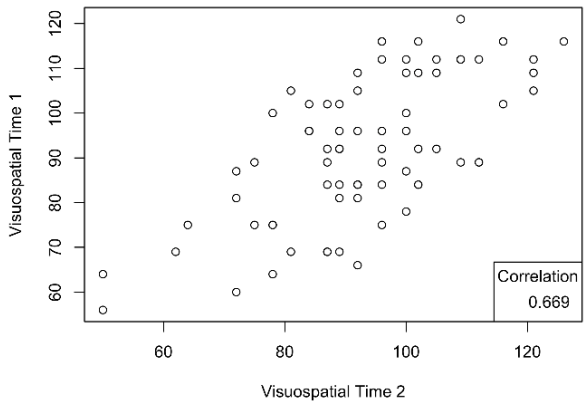
Table 2.6. RBANS Index Scores Differences T_1 vs T_2

RBANS Domains	Difference Mean Index Scores (T_1-T_2)	CI lower	CI upper	P-value	Difference Standard Deviation Scores	CI lower	CI upper	P-value
Immediate Memory	-17.557	-20.2882	-14.8257	2.20E-16	0.974684	0.803705	1.145662	2.20E-16
Visuospatial/ Constructional	0.797468	-2.04674	3.641677	0.5783	-0.10759	-0.25512	0.039929	0.1505
Delayed Memory	-14.038	-17.6629	-10.4131	3.4E-11	0.772152	0.556356	0.987948	4.58E-10
Language	-1.48101	-3.5351	0.573079	0.1552	0.037975	-0.06311	0.139059	0.4568
Attention	-4.8481	-7.64803	-2.04818	0.000916	0.259494	0.111763	0.407225	0.00078

2.6.7 Test-Retest Reliability

To evaluate for the test re-test reliability, for each of the RBANS domains, the Pearson correlation was calculated and compared with available normative data (Figure 2.5). As expected, the correlation coefficients in the current study were lower, with the exception of language domain, but comparable with those from the normative data: immediate memory 0.66 vs 0.72; visuospatial/constructional 0.67 vs 0.69; language 0.79 vs 0.42; attention 0.68 vs 0.77; and delayed memory 0.58 vs 0.67.





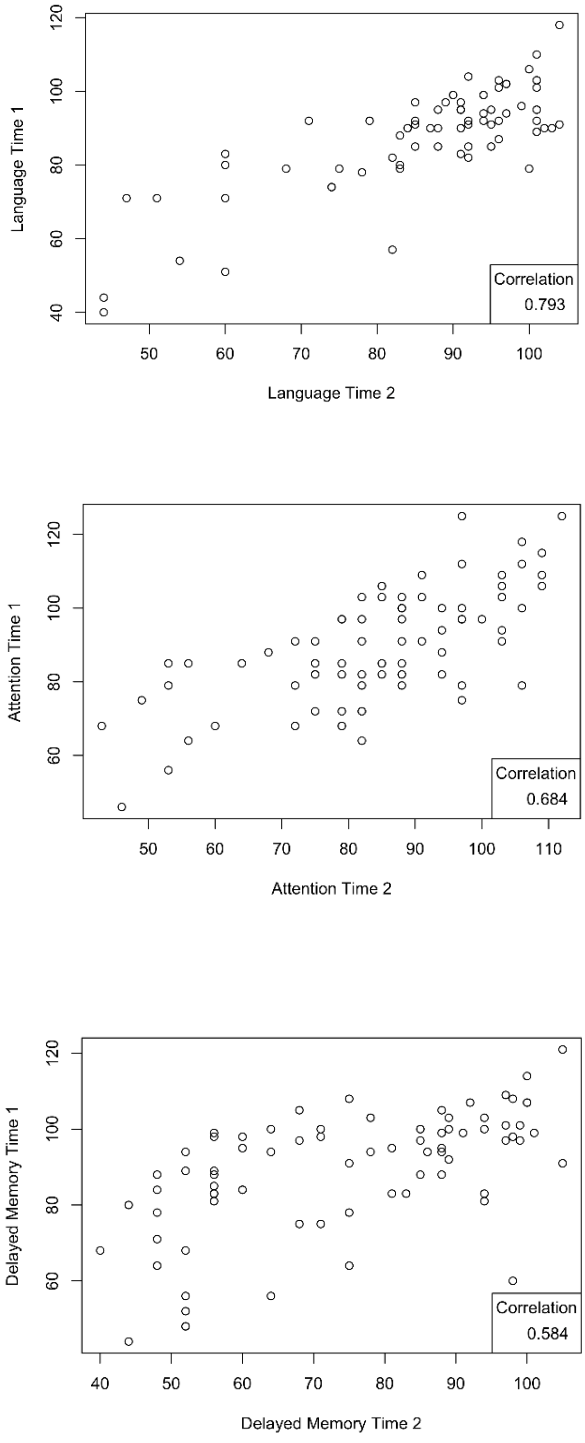


Figure 2.5. RBANS indexes test re-test correlation coefficients

2.6.8 Practice Effects

To account for the possible practice effects of retesting on the RBANS subtests, the RCI+PE algorithm described in the methods section above was applied to the raw scores of the twelve RBANS subtests. Without accounting for the practice effects, improvements in all the subtests were observed between T_1 and T_2 except for the figure copy (p=0.0005) and figure recall (p=0.4066) subtests, which worsened. The mean of the differences of all the improved subtests were statistically significant apart from coding (p=0.5049), picture naming (p=0.5587), and semantic fluency (p=0.5040).

When estimating for the short interval retest impact on test scores (which in theory is the strongest) by using normative data from Safaz et al., improvements in the digit span (p=0.2645), list recall (p=0.4916), and line orientation (p=0.5459) subtests stopped being significant. After accounting for the short interval re-test practice effect, the worsening in figure recall score became significant (p<0001) and deterioration in figure copy continued to be significant (p=0.0003). At the same time, for the coding (p=0.0001), semantic fluency (p=0.8552), and the list learning (p=0.9145) subtests subjects performed worse than expected when accounting for the possible practice effects, despite the actual improvement of the raw scores, with only coding showing a significant deterioration. For story memory, story recall, and list recognition the improvement, while less, continued to be significant.

When normative data obtained at twelve months interval by Duff and colleagues are used to estimate the impact of practice effect, figure copy (0.0074) and figure

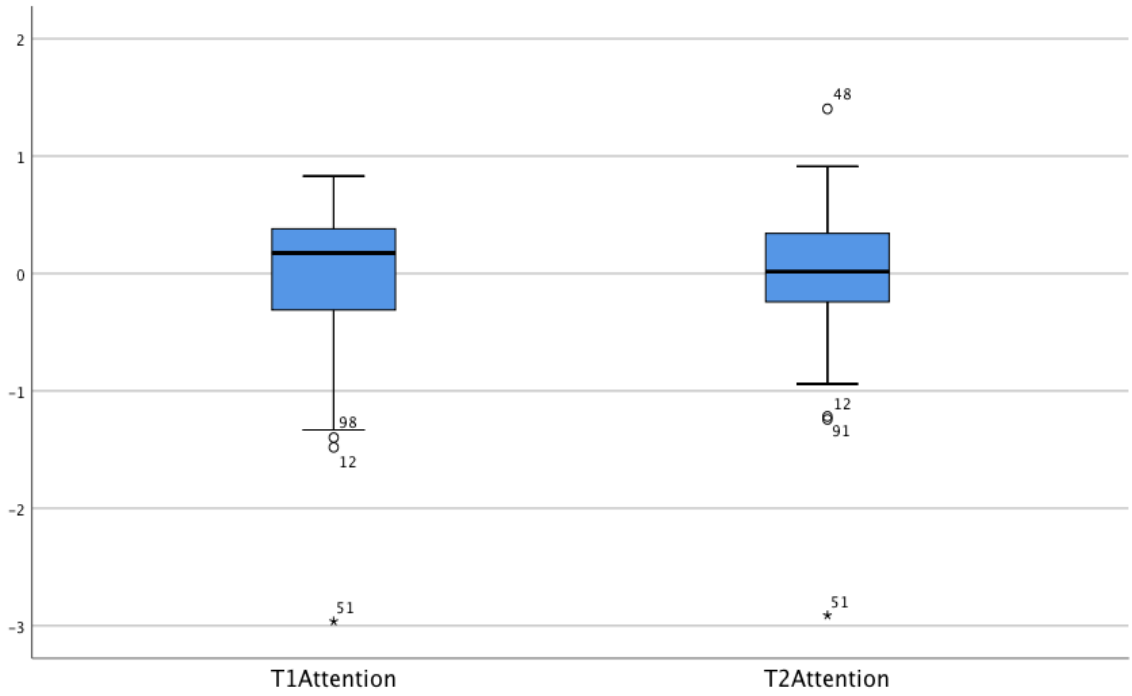
recall (0.0227) remain significantly worse, and the improvement in line orientation (p=0.0943) stops being statistically significant. Coding, picture naming, and semantic fluency all continued to show a statistically insignificant improvement. All other subtests showed improvements between T_1 and T_2, which were statistically significant (Table 2.7).

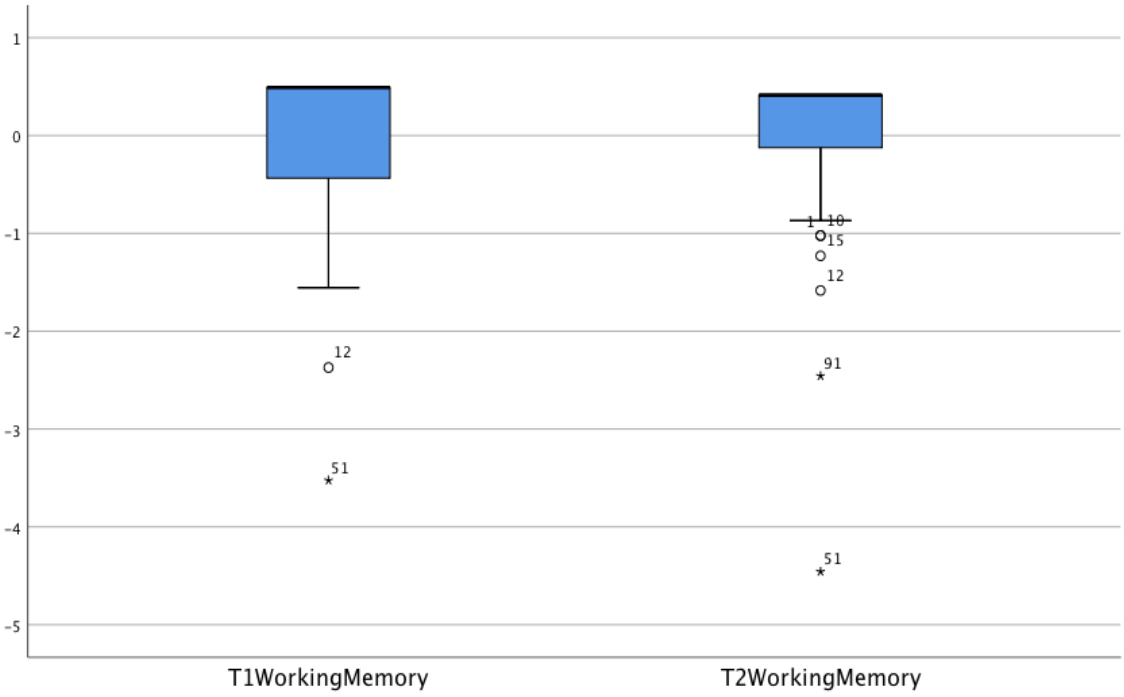
Table 2.7. RBANS Raw Scores Results and Assessment of Practice Effects Impact

Impact of adjusting for practice effects on RBANS subtest scores												
RBANS Subtest	**No adjustment for practice effect**				**Adjustment for practice effect using Safaz et al. 2014**				**Adjustment for practice effect using Duff et al. 2005**			
	Mean[1] of the differences	CI lower	CI upper	p-value	Mean[1] of the differences	CI lower	CI upper	p-value	Mean[1] of the differences	CI lower	CI upper	p-value
Digit span	-0.7342	-1.2730	-0.1953	0.0082	-0.1822	-0.5050	0.1406	0.2645	-0.4267	-0.6628	-0.1907	0.0006
Coding	-0.4937	-1.9606	0.9733	0.5049	0.5653	0.2886	0.8421	0.0001	-0.0804	-0.3241	0.1634	0.5135
List learning	-4.3038	-5.1574	-3.4502	0.0000	0.0132	-0.2314	0.2579	0.9145	-0.9667	-1.1507	-0.7826	0.0000
Story memory	-3.3671	-4.2732	-2.4610	0.0000	-0.4942	-0.8714	-0.1170	0.0109	-1.0945	-1.3221	-0.8669	0.0000
List recall	-1.8734	-2.4306	-1.3162	0.0000	-0.1223	-0.4745	0.2300	0.4916	-0.8732	-1.1315	-0.6148	0.0000
Story recall	-2.0380	-2.6212	-1.4547	0.0000	-0.5665	-0.8976	-0.2354	0.0010	-0.8822	-1.1184	-0.6459	0.0000
List recognition	-1.2532	-1.6675	-0.8388	0.0000	-1.4603	-2.0696	-0.8510	0.0000	-0.8994	-1.1921	-0.6067	0.0000
Figure recall	0.3418	-0.4737	1.1572	0.4066	0.6128	0.3355	0.8901	0.0000	0.2744	0.0393	0.5095	0.0227
Figure copy	0.8987	0.4075	1.3900	0.0005	0.3014	0.1437	0.4591	0.0003	0.3740	0.1033	0.6447	0.0074
Line orientation	-0.6962	-1.2089	-0.1835	0.0084	-0.0745	-0.3192	0.1701	0.5459	-0.1537	-0.3343	0.0270	0.0943
Picture naming	-0.0506	-0.2223	0.1210	0.5587	-0.0007	-0.1958	0.1943	0.9942	-0.2498	-0.5011	0.0015	0.0513
Semantic fluency	-0.2278	-0.9036	0.4479	0.5040	0.0200	-0.1972	0.2371	0.8552	-0.1433	-0.2976	0.0109	0.0681
Note 1. Mean of the differences - positive value means T_2 score < T_1 score												

2.6.9 Neurocognitive Change at Follow-up Assessed by z Scores in All Domains Tested

To do this analysis, first the z transformed value for each domain for each person was obtained, as described in the methods section of this chapter. The z scores for each of the seven domains at T_1 were compared to those at T_2 for the entire cohort (Figure 2.6). There were no significant changes observed at T_2 in most domains, except for the orientation, which worsened at T_2. Since there were many extreme values in the orientation domain, the medians were compared using the Wilcoxon Signed Rank Test for non-parametrical data for related samples. This change in the orientation domain was statistically significant (p=0.007).

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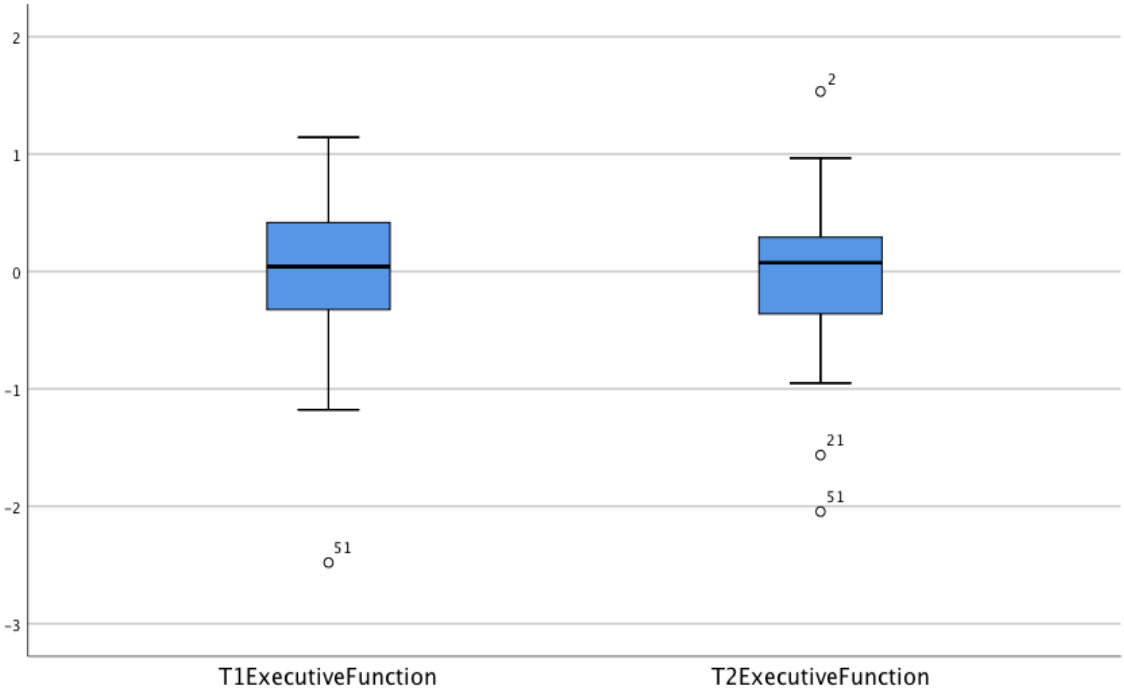
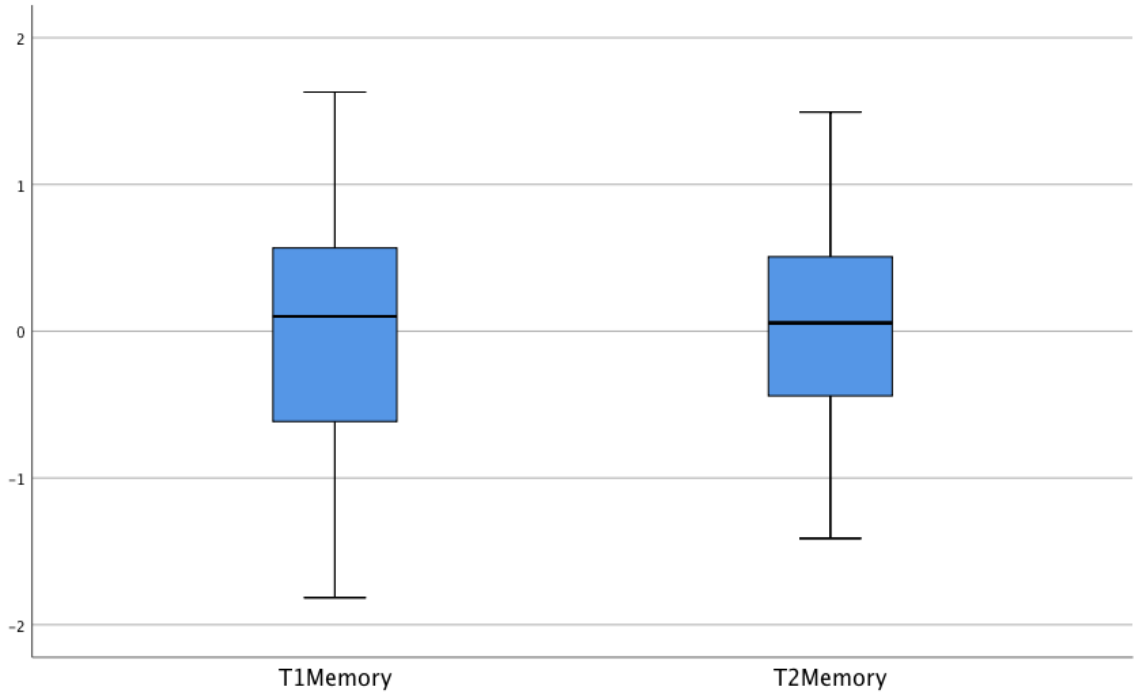
0
-1
-2
-3
-4
-5
12
51
1
10
15
12
91
51
T1WorkingMemory
T2WorkingMemory


2
1
0
-1
-2
-3
51
2
21
51
T1ExecutiveFunction
T2ExecutiveFunction


2
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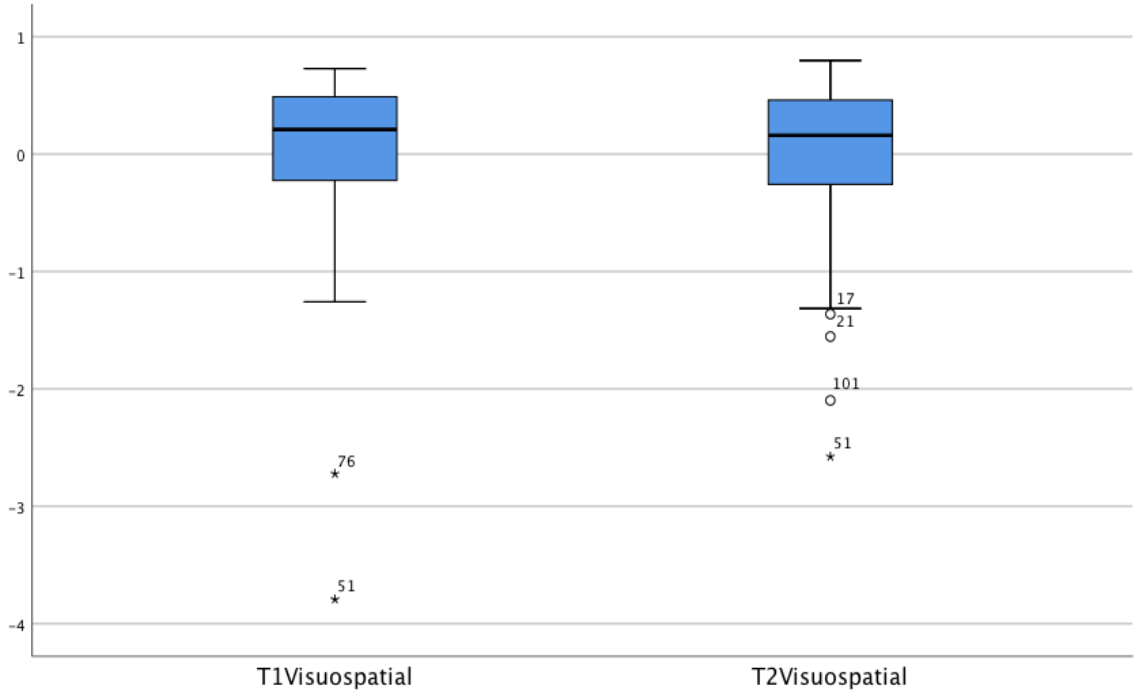
0
-1
-2
T1Memory
T2Memory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-1
-2
-3
-4
17
21
101
51
76
51
T1Visuospatial
T2Visuospatial

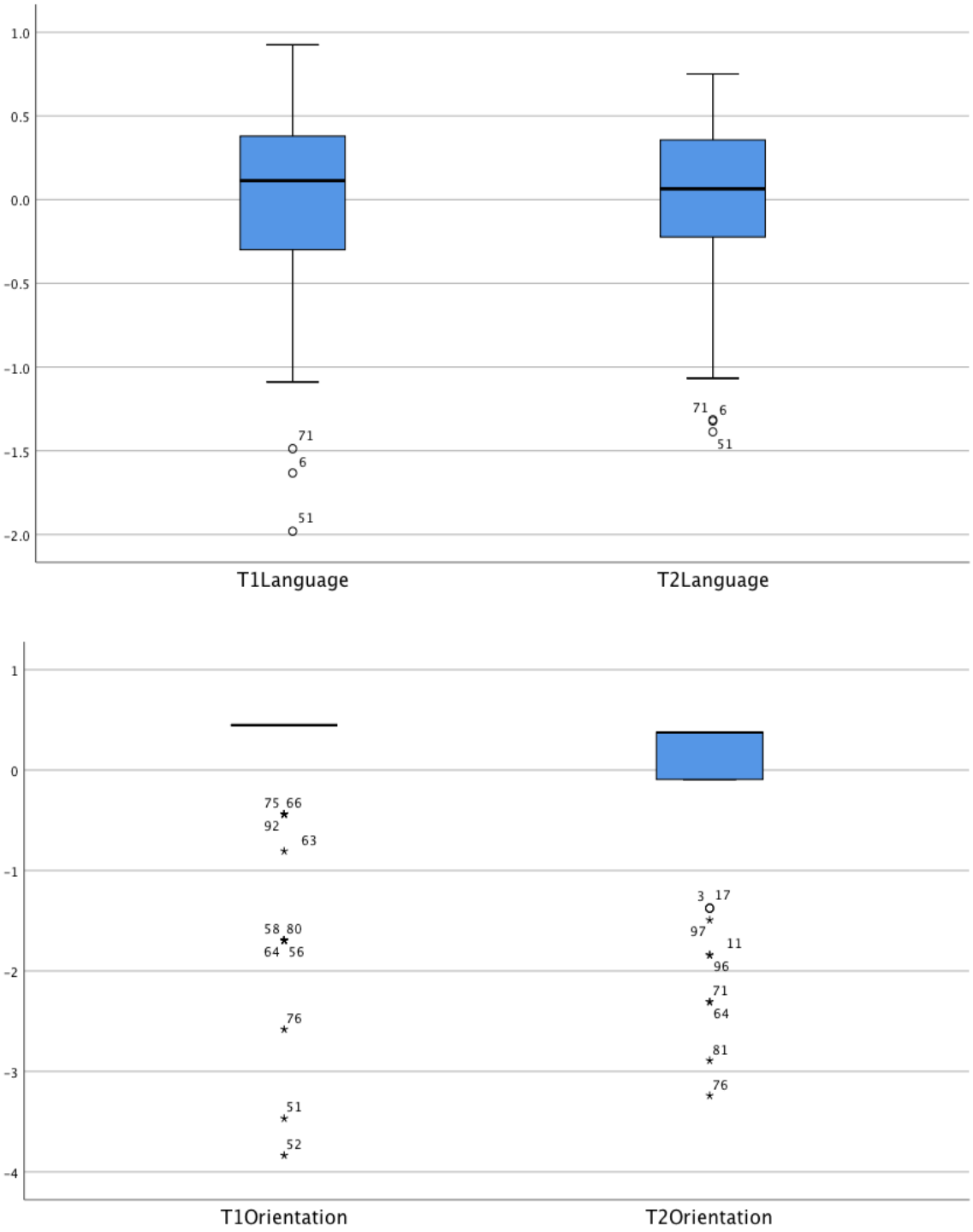
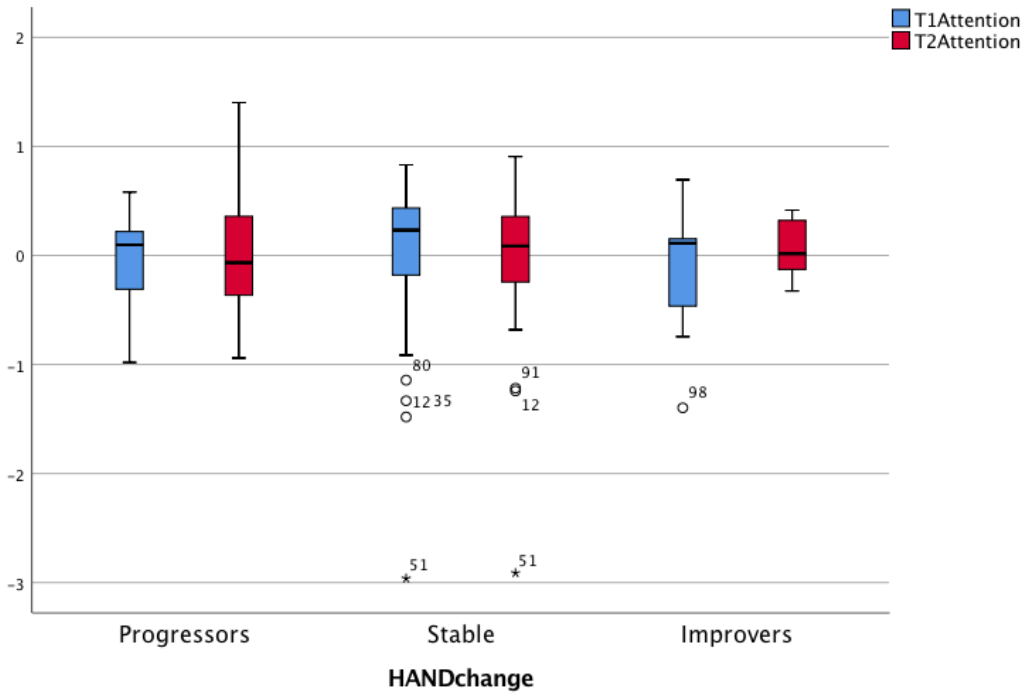


Figure 2.6. Comparison of *z* scores between T_1 and T_2

An ANOVA test for the difference between the z scores at T_1 and T_2 showed significant difference for the memory (p=0.021) and orientation (p=0.011) domains. However, after the Bonferroni correction for the multiple testing, these changes were not significant (cut off p<0.007).

Next, the change in each domain was assessed within the change of HAND diagnosis groups, i.e. each domain was compared at T_1 and T_2 for participants who progressed (15/79), for those who remained stable (52/79), and for those who improved (12/79). Most domains showed no significant difference between T_1 and T_2 when broken down into people who progressed, remained stable and improved based on HAND diagnosis, except for the language and orientation (Figure 2.7). The language in the improvers group was significantly worse at T_2, with a p-value of 0.005 on the paired sample T-test. The orientation was also significantly worse at T_2 in the improvers group, with a p-value of 0.021 on related sample Wilcoxon Signed Rank test for non-parametrical data. There was no statistically significant difference in orientation z scores in the group that progressed or the group that remained stable (p=0.067, p=0.162, respectively).


T1Attention
T2Attention
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1
0
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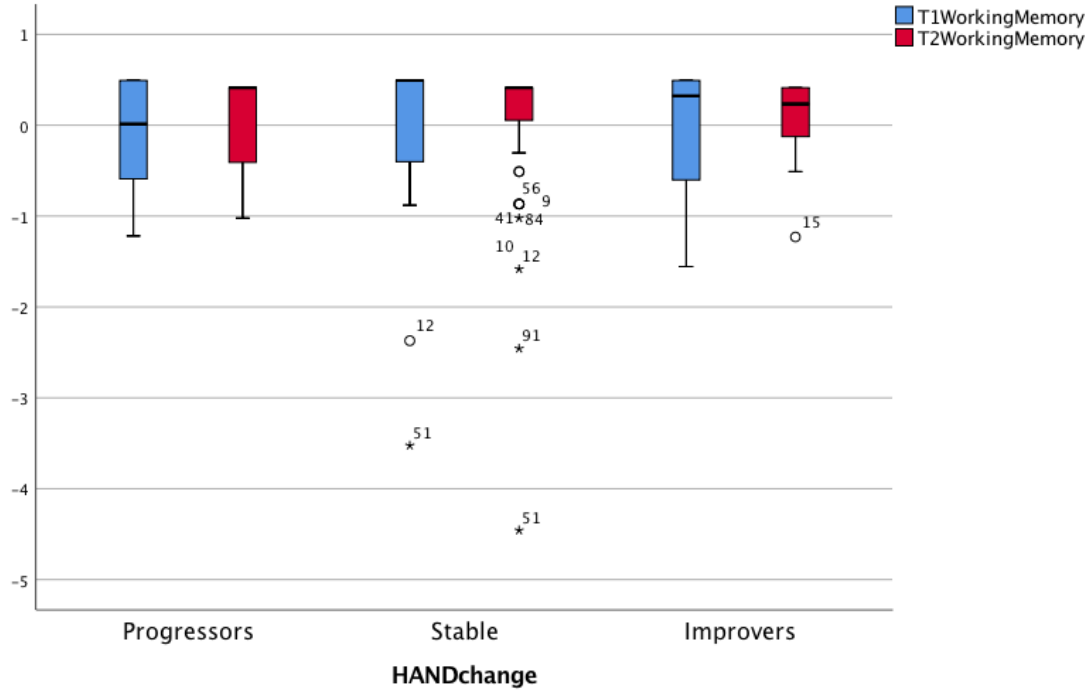
-2
-3
80
12 35
91
12
98
51
51
Progressors
Stable
Improvers
HANDchange


T1WorkingMemory
T2WorkingMemory
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-2
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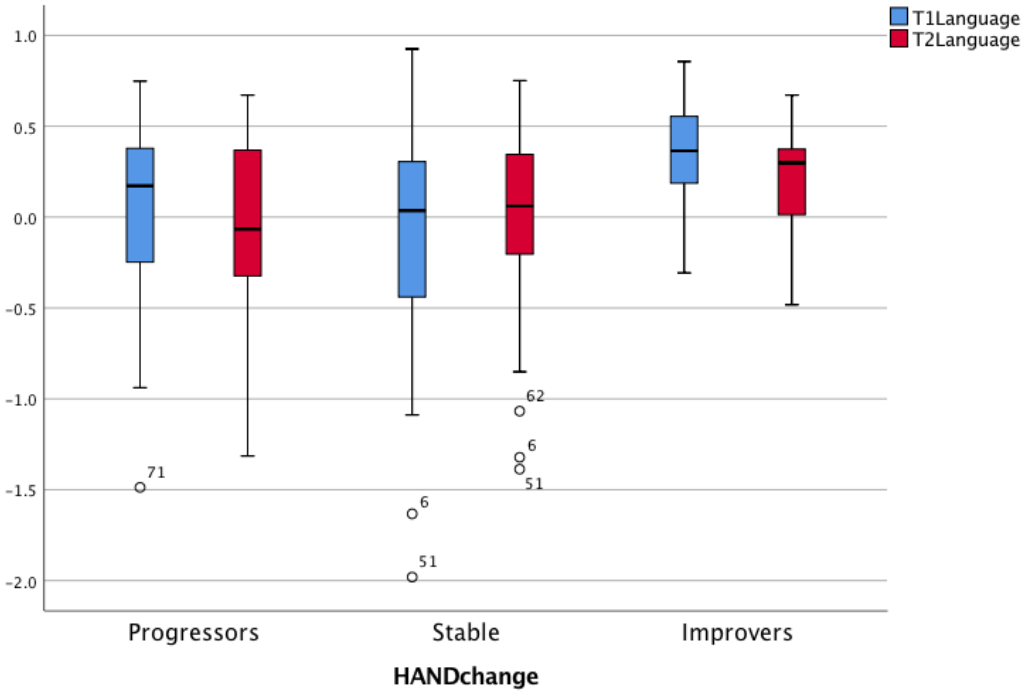
56
9
41
84
10
12
15
12
91
51
51
Progressors
Stable
Improvers
HANDchange


T1Language
T2Language
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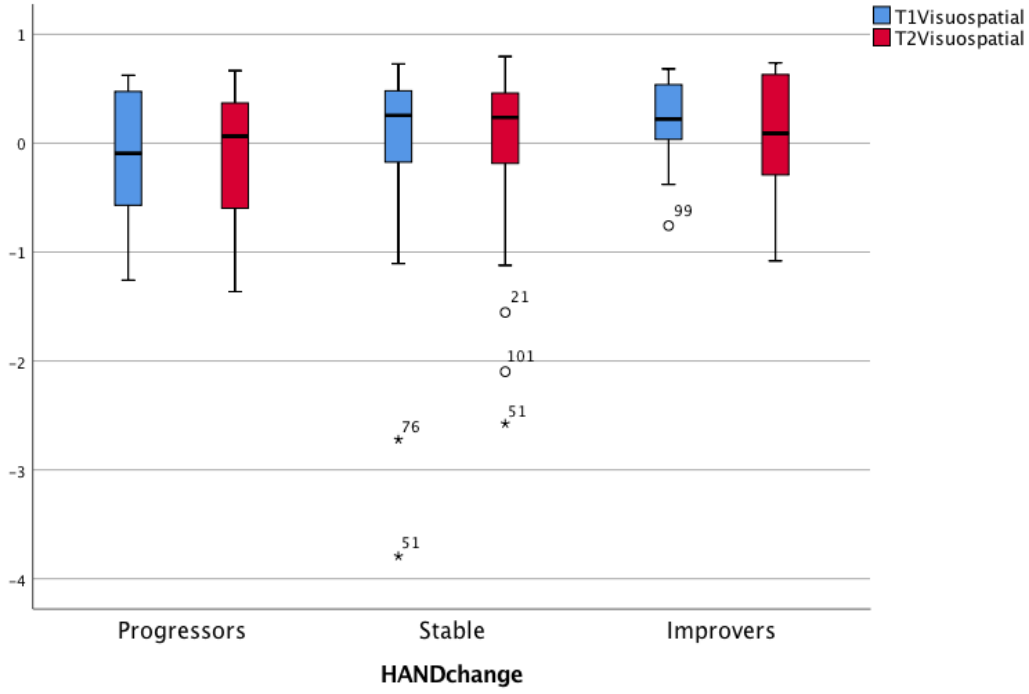
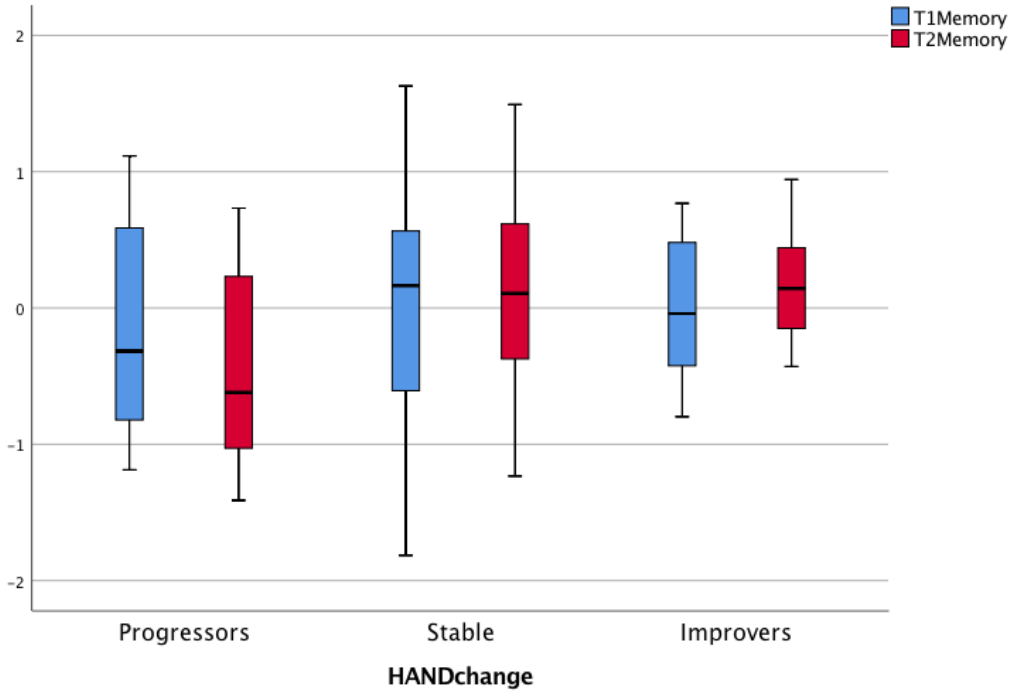
0.5
0.0
-0.5
-1.0
-1.5
-2.0
71
62
6
51
6
51
Progressors
Stable
Improvers
HANDchange


T1Visuospatial
T2Visuospatial
1
0
-1
-2
-3
-4
99
21
101
51
76
51
Progressors
Stable
Improvers
HANDchange


T1Memory
T2Memory
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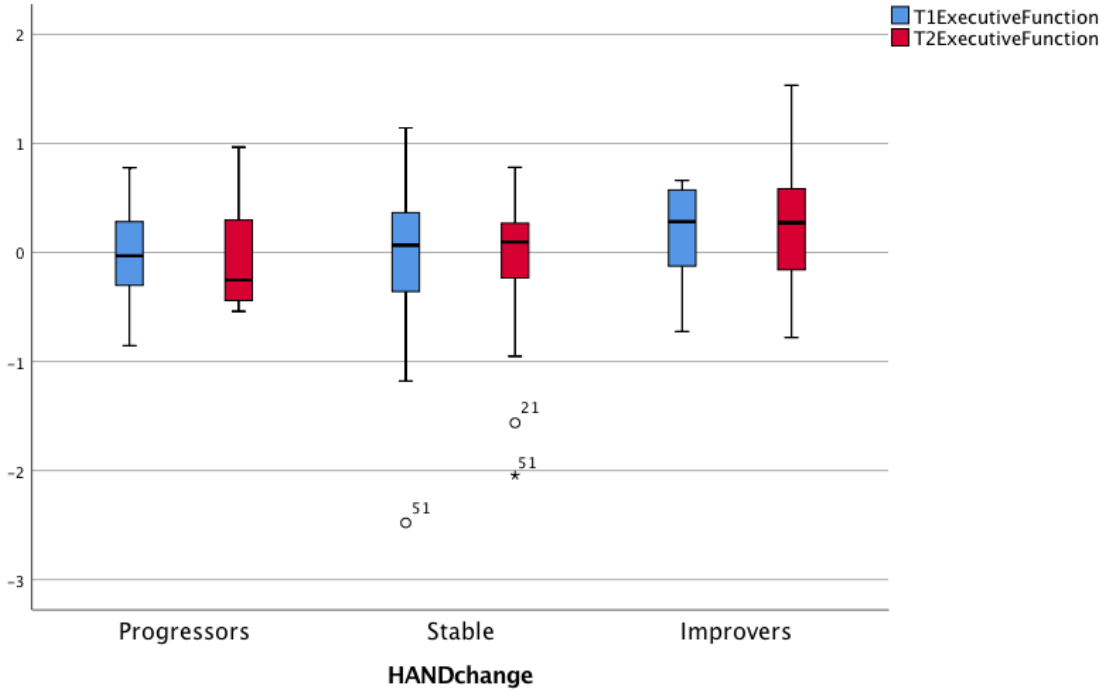
1
0
-1
-2
Progressors
Stable
Improvers
HANDchange


T1ExecutiveFunction
T2ExecutiveFunction
2
1
0
-1
-2
-3
21
51
51
Progressors
Stable
Improvers
HANDchange

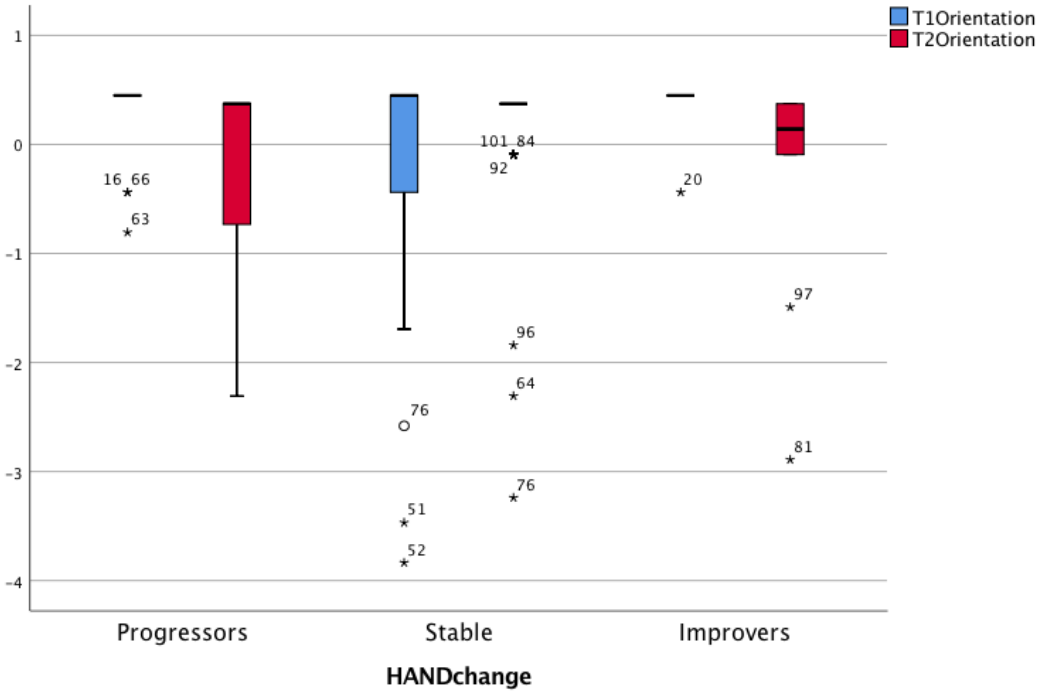


Figure 2.7. Comparison of *z* scores between T_1 and T_2 in Improved, Stable and Progressed group

Another analysis was carried out using ANOVA for parametric data and Kruskal Wallis test for non-parametric data to test for the differences in each domain across different HAND diagnoses (ANI, MND, HAD) at T_2. All domains show a significant difference between different HAND diagnoses (all p-values <0.001), except for working memory (p=0.331). In addition, orientation shows a p-value difference (p=0.029), which stops being significant after correction for multiple testing. This suggests that z scores in all domains, except for the working memory and orientation, can be predictive of HAND sub-type at T_2.

2.6.10 Predictors of Change in HAND Diagnosis at Follow-up

All the potential variables that could have influenced the change in HAND diagnosis between T_1 and T_2 were tested to determine if there was a statistically significant relationship between the change and that variable. The variables included were age, gender, education, presence of mental health comorbidities (psychosis, anxiety, depression), and medical comorbidities (DM, hypertension, hypercholesterolemia, CVD, HCV, HBV), history of drug and alcohol misuse, presence of other HIV neurological complication, positive screen for depression or anxiety or both on HADS screen, HIV disease variables (VL at baseline and follow-up, CD4+ count at baseline and follow-up, duration since diagnosis, and duration of ART treatment), and abnormal list recognition, list retention and story retention scores. Each variable was tested individually using chi square trend test (or linear by linear test), ANOVA, or where suitable Kruskal Wallis test and ordinal regression test. The chi square test revealed that the retention scores were significant predictors of change: List Retention score (p=0.038), Story Retention score (p=0.002) and Abnormal List Recognition (p=0.001). For any change in diagnosis group (improved, stable, or progressed), age at follow-up had the lowest p-value (0.057) when using the Kruskal Wallis test. Education attainment at T_2 had a significant relationship with change in diagnosis when using ordinal regression (p=0.049). Based on foregoing analyses, a multivariable regression model was developed, including the following variables: age, gender, years of formal education at follow-up, list retention score, story retention score, abnormal list recognition score, and duration of ART exposure at T_2.

In the multivariable analysis, the abnormal list recognition score is the most significant variable that predicts the change in HAND diagnosis (p=0.002). Having an abnormal score on list recognition test decreases the odds of improving in HAND diagnosis by 89.2%, when all the other variables in the model remain unchanged. Exposure to ART significantly improved cognitive performance at T_2 (p=0.049). Duration of ART exposure increases the odds of improving by 0.0003% for every extra day of ART exposure. Years of education, with a p-value of 0.07, is close to being statistically significant and increases the odds of improving by a factor of 1.15 for every added year spent in education.

2.6.11 Percent Retention Scores

Percent retention scores of the RBANS memory subtests were evaluated at baseline and at follow-up assessments. At follow-up, a total of 24/79 (30.1%) participants had an abnormal list retention score (LRS) and 7/79 (8.9%) had an abnormal story retention score (SRS). This is in contrast with a total of 32/79 (40.5%) having an abnormal LRS (p=0.1728) and 22/79 (27.8%) having an abnormal SRS at baseline (p=0.0022). At T_2 assessment, all of those who had an abnormal retention of story items met criteria for HAND.

In the HAND group at follow-up, in 40% of the subjects retention of list items was abnormal and 15.6% had an abnormal SRS. For those patients with HAND, at T_1, 47% had an abnormal retention of list items (p=0.4925) and 39.2% had an abnormal story retention (p=0.0107). At follow-up, most of those who had an abnormal story retention (5/7; 71.4%) also had an abnormal retention of list items, compared with 63.6% (14/22) at baseline (p=0.7103).

In the group with non-HAND abnormalities at T_1 there were eight (38%) participants who had an abnormal list retention and 2/21 (9.5%) had an abnormal story retention. When compared with the results at baseline, only 24% (6/25) had an abnormal retention of list items in the group with abnormalities that did not meet criteria for HAND at follow-up (p=0.3091) and none of these had an abnormal story retention score (p=0.1191). All of those who had normal performance at baseline, and at follow-up, had a normal list and story retention.

2.6.12 Abnormal List Recognition

There was a total of 16/79 (20.3%) participants who had an abnormal list recognition at T_2, all of them within the HAND group (16/45; 35.6%) compared with a total of 36/79 (45.6%) (p=0.0007) with 31 of them (60.8%) (p=0.0142) within the HAND group at baseline. In the HAND group, 61% (11/18) of those who had an abnormal list retention at T_2, also had an abnormal list recognition, in contrast with 75% (18/24) at T_1 (p=0.3375). At follow-up, in the HAND group, five patients (5/45; 11%) had an abnormal list recognition with only list retention being within normal limits, as opposed to 13/51 (25.5%) at baseline (p=0.0705). Abnormal list recognition score in this case is explained by poor learning efficiency rather than poor retention. None of the patients from non-HAND abnormalities (or non-diagnostic abnormalities) group at follow-up, and 5/21 (23.8%) in the non-HAND abnormalities group at baseline had abnormal LR (p=0.0106). Patients who performed normal at baseline and at follow-up had no difficulties with list recognition.

2.6.13 Cortical-Subcortical Deviation Score Results

Cortical-subcortical deviation scores were calculated at baseline and at follow-up in the 79 patients who underwent assessments at both time points, to characterise those with predominantly cortical or subcortical pattern of cognitive impairment.

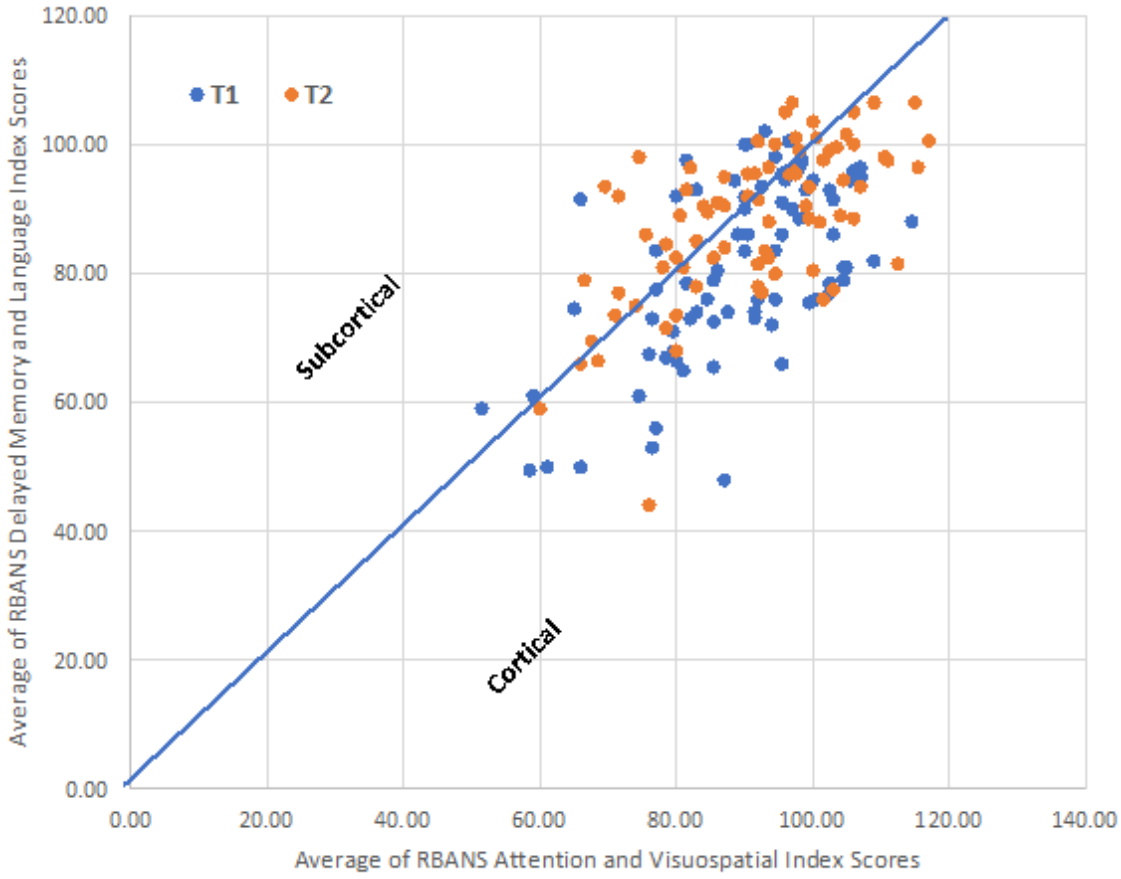


Figure 2.8. Cortical Subcortical Deviation Score change between T_1 and T_2 in those with HAND at T_2 (n=45)

At follow-up, 41.8% (35/79) of participants had a predominantly subcortical pattern of impairment, compared with 22.3% (20/79) at baseline (p=0.0088).

Amongst those who met diagnostic criteria for HAND at T_1, only 21.6% (11/51) had a subcortical type of impairment, while at T_2 within the HAND group, 48.9% (22/45) had a negative value cortical-subcortical deviation score, i.e. impairments on RBANS test were more in keeping with a subcortical pattern (p=0.0052) (Figure 2.8).

However, of those patients who did not meet criteria for HAND (non-diagnostic abnormalities group and those who performed normal on all tests), 32% had a predominantly subcortical pattern at baseline and 38% at follow-up. This difference was not significant (p=0.6255).

2.6.14 Results of Addenbrooke's Cognitive Exam – revised (ACE-r)

Performance on ACE-r test was considered abnormal when the total score gained was less than 88 out of a total 100 possible. Fewer subjects had an abnormal ACE-r score at follow-up (42/79; 53.2%) when compared with performance at baseline (49/79; 62%) (p=0.2646). At T_1 in the HAND group, 78.4% (40/51) had an abnormal ACE-r score, compared to 71% (32/45) at follow-up (p=0.4063). For patients with non-diagnostic abnormalities at T_1 (9/21; 42.9%) and T_2 (10/25; 40%), the proportion of those with an abnormal ACE-r score was lower at T_2 (p=0.8440).

The most commonly affected domains in HAND on ACE-r battery at follow-up were attention & orientation (26/45; 57.8%), memory (26/45; 57.8%) and fluency (26/; 57.8%) domains, with the visuospatial domain (25/32; 55.6%) following closely. On ACE-r, language was affected in the least number of patients with HAND (23/45; 51%).

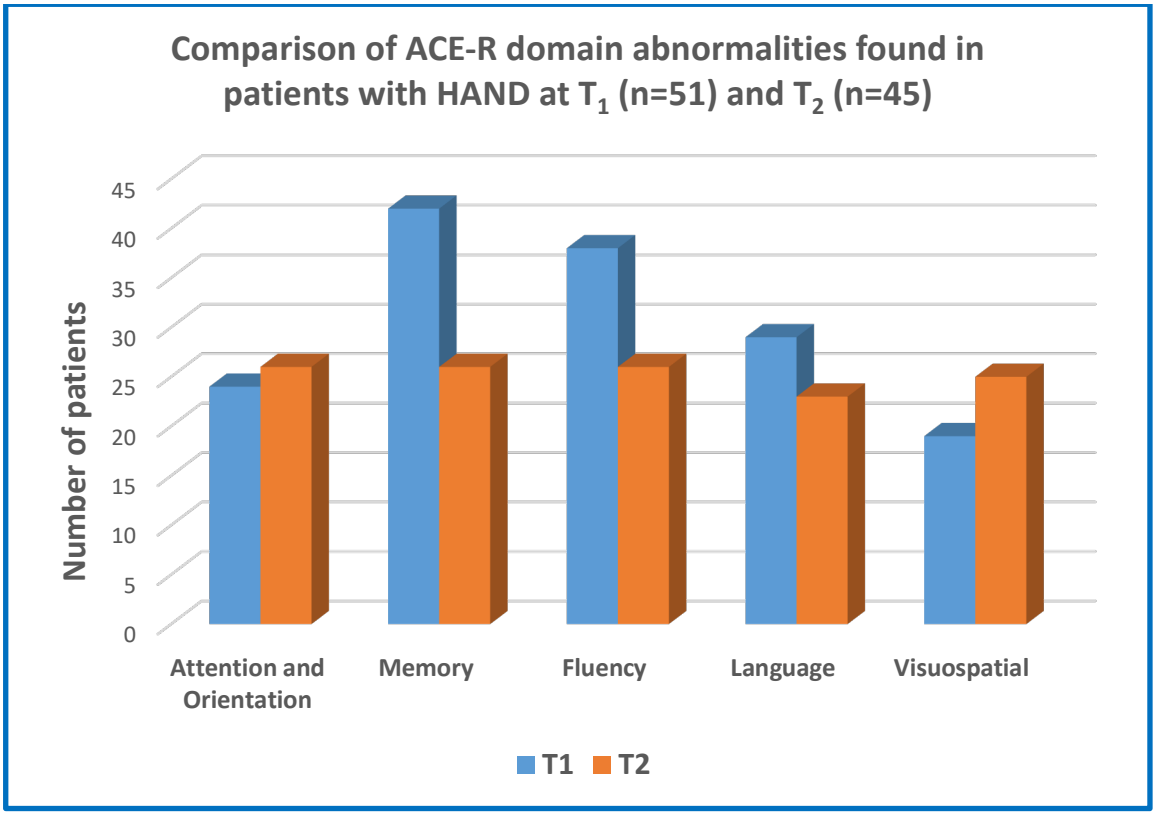


Figure 2.9. ACE-r domains affected in patients diagnosed with HAND

Attention & orientation and visuospatial domains were affected in a higher proportion of subjects with HAND at T_2, when compared with T_1 (26/45; 57.8% vs 24/51; 47%; p=0.2930 and 25/45; 55.6% vs 19/51; 37.3%; p=0.0741, respectively). Memory, fluency and language domains were affected in a smaller percentage at follow-up (26/45, 57.8% vs 42/51, 82.4%, p=0.0085; 25/45, 57.8% vs 38/51, 74.5%, p=0.0849; 23/45, 51% vs 29/51, 56.9%, p=0.5647, respectively), with only memory showing a significant difference in the proportion of subjects affected at follow-up (Figure 2.9).

2.6.15 Results of Montreal Cognitive Assessment (MoCA)

A total, adjusted for education MoCA score of less than 26 was considered to be abnormal. At follow-up assessment, 63.3% (50/79) of all participants had an abnormal MoCA test result, in contrast with 71% (56/79) at baseline (p=0.3044). In the HAND group, 73.3% (33/45) had an abnormal MoCA score at follow-up, compared with 82.4% (42/51) at baseline (p=0.2842). In the group with non-diagnostic abnormalities, 68% (17/25) had an abnormal MoCA score at T_2 and 66.7% (14/21) at T_1 (p=0.9262).

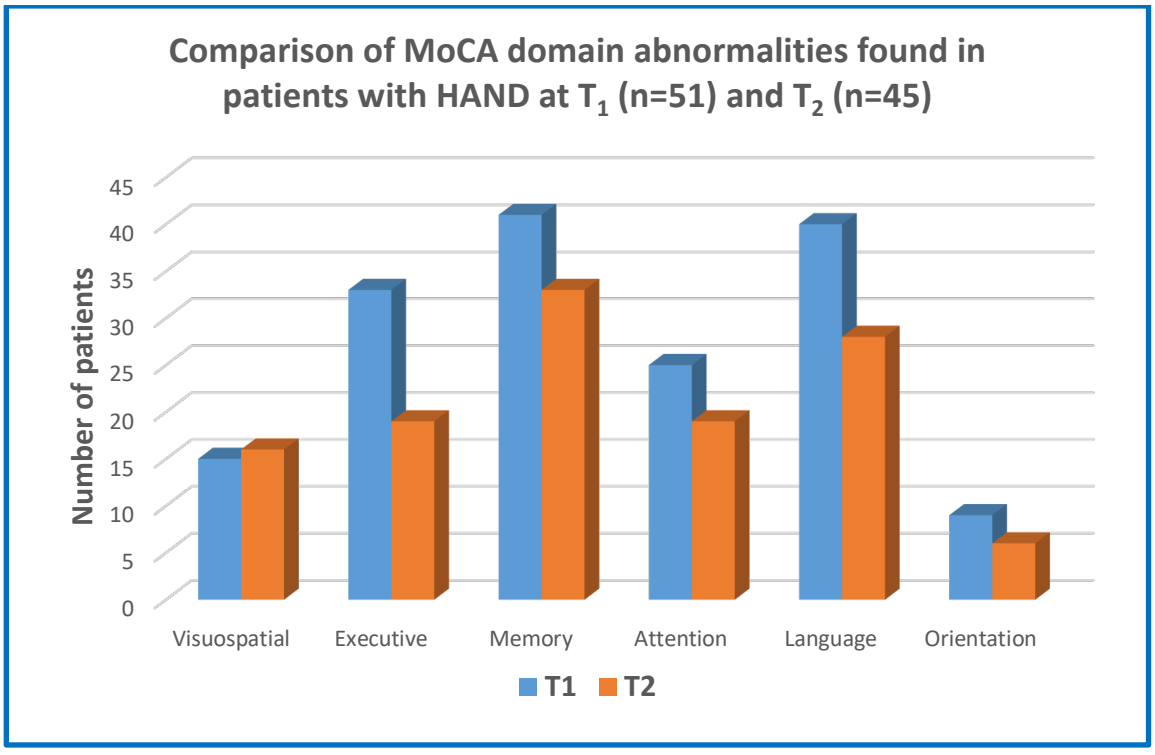


Figure 2.10. MoCA domains affected in patients diagnosed with HAND

The most commonly affected domains on MoCA, in the patients with HAND, at follow-up, were delayed memory in (73.3%) and language (62.2%) (Figure 2.10).

Executive and attention domains were affected in 42.2% each. At follow-up, a higher proportion of patients with HAND had an abnormal visuospatial domain (35.6% at T_2 vs 29.4% at T_1), but this difference is not statistically significant (p=0.5190). Memory, attention, language, orientation, and executive domains were affected in a smaller proportion of patients with HAND at follow-up (73.3% vs 80.4%, p=0.4114; 42.2% vs 49%, p=0.5068; 62.2% vs 78.4%, p=0.0831; 13.3% vs 17.6%, p=0.5642; 42.2% vs 64.7%, p=0.0281; respectively).

2.6.16 Results of Frontal Assessment Battery (FAB)

At follow-up, a total of 16/79 patients scored abnormal on the FAB test after the adjustment for age and education, as defined by Appollonio and colleagues, compared with 20/79 at baseline (20.3% vs 25.3%; p=0.4553). Of those who met diagnostic criteria for HAND, 26.7% (12/45) had an abnormal FAB, compared with 31.4% (16/51) at baseline (p=0.6151) (Figure 2.11). Only four (4/25; 16%) of those who had non-diagnostic abnormalities had an abnormal FAB test at follow-up, compared with 19% (4/21) at baseline (p=0.7913). Neither of these differences are statistically significant.

Those who had an abnormal FAB score at follow-up were more likely to complain of having safety issues (p=0.0009), personality change (p=0.0049), and being slower at completing tasks (p=0.0261). These participants were more likely than the rest of the cohort to display the following abnormalities on neurological examination: gait abnormalities (p=0.0019), slow rapid alternating movements (p=0.0124), apraxia (p=0.0056), and hypertonicity, which approached statistical significance (p=0.0613).

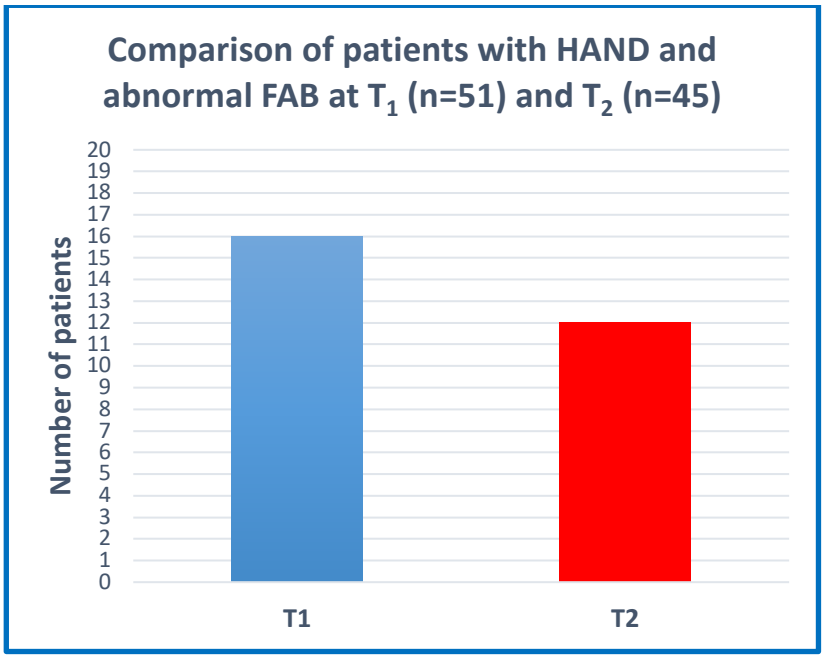


Figure 2.11. FAB in patients diagnosed with HAND

2.7 Discussion

2.7.1 Discussion of the Results

Of the original cohort of 104 well characterised HIV+ patients, 76% (79/104) underwent follow-up assessments in the current study. In general, this would be considered a good total retention rate. This is especially good when taking into account that the population studied in the current project would be regarded as a "high risk" for attrition, influenced by sociodemographic factors such as unemployment and low education level, unhealthy lifestyle factors such as smoking, high alcohol consumption, illicit drugs misuse, and high levels of psychological distress associated with underlying diagnosis of a chronic disease (244). Indeed, those who were lost to follow-up, including the seven patients who died, were more likely to be unemployed, to have spent less years in full time

education, to have a history of illicit drug use, to be past intravenous drug users and be on methadone replacement therapy, have a history of anxiety, and also be HCV co-infected.

The hypothesis predicted progression of cognitive impairment at follow-up assessments in this cohort. However, when change in neurocognitive status was assessed by applying HAND diagnosis criteria, variable trajectories were observed at T_2. Two thirds remained stable, 15% showed improvement to a milder HAND diagnosis or HAND in remission and 19% progressed to a more advanced HAND diagnosis or had an incident HAND diagnosis at follow-up. Despite the drop in the total number of cognitively impaired at follow-up, the proportion of HAND still remained high, a finding that is consistent with other longitudinal studies in the post-HAART era (211). Importantly, the number of more severe forms of HAND increased: 57% met diagnostic criteria for HAND at T_2 (25% - ANI; 28% - MND; 4% - HAD) vs 64.6% HAND at T_1 (41.8% - ANI; 20.3% – MND; 2.5% - HAD).

Notably, of those who progressed on HAND diagnosis, nine changed from ANI to MND, as they became symptomatic and reported more difficulties in work, activities of daily living or cognitive function impairments, despite the overall total RBANS score remaining stable and even apparent improvements being observed in some cognitive domains. In addition, those who progressed at follow-up, scored less on at least one of the cognitive domains (attention, delayed memory, visuospatial skills, or language domain).

Only one patient, which represents 1.2%, converted from “non-diagnostic changes” to HAD at follow-up. Of note, this patient was ART naïve at baseline and only commenced antiretroviral treatment 18 months after the baseline assessment. It is possible that being off treatment influenced conversion to HAD in this case. Incident HAD diagnosis at follow-up is a rare finding in other reported post-HAART follow-up studies. Becker et al. also reported only 1% conversion to HAD at one year follow-up in a study in 290 HIV+ individuals, with only 17 of them taking HAART (60).

Amongst those who improved, nine participants had ANI in remission and three had MND in remission. In general, both ANI and MND can be referred to as mild cognitive impairment (MCI) based on performance on neuropsychology tests. There is a growing body of evidence in the literature that those diagnosed with MCI at baseline neuropsychological testing contain both “true MCI”, who will eventually progress to dementia, and “false MCI”, who will perform within normal range at follow-up. Brooks et al. in their review identified 18 studies on clinical and community dwelling healthy samples, demonstrating conversion from MCI to “normality” at follow-up assessments in a varying proportion of participants, which can be as high as 92.6% in the amnestic type MCI and 48% in the mixed type MCI (245). Moreover, the usual psychometric criterion for MCI involves having a score of at least 1.5 SD below the mean for normal controls in at least one domain, while a performance which is 1 SD below the mean for normal controls in at least two domains is required to meet criteria for mild forms of HAND (ANI or MND). Hence, there is a chance that a proportion of those diagnosed with ANI and perhaps less of those with MND were misclassified at baseline as they

performed poorly for situational reasons, but performed within normal range at follow-up. Or indeed, with the commencement of ART treatment, there might be a degree of reversibility of the pathological processes/inflammation in the brain. The above might be particularly true in the context of clinically stable HIV disease as in the current study, where the majority of participants were on ART (98.7%) and virally suppressed (96.2%) at follow-up. Furthermore, similar to the current study, Dufouil et al. reported that 40% of MND had normal cognitive status or asymptomatic impairment at follow-up in their study (210). All of the above might explain some of the conversions from CI to normality in the current study. Alternatively, one could argue that ART improves cognitive status and delays progression to more severe impairments by controlling the HIV infection.

Findings in the current study are suggestive of overall stable cognitive impairment at follow-up. These are consistent with findings in the other studies discussed above and reporting stable cognitive status in 60% to 86%, improvement in 10% to 24%, and decline in 4% to 27%, although different methodological approaches were used in all of them, which makes comparisons difficult.

The multivariable analysis showed that having an abnormal list recognition at follow-up decreases the odds of improving in HAND diagnosis and longer duration of ART exposure increases the chances of improving. While, at baseline, lower education level predicted CI, at follow-up, every added year spent in education increased the odds of improving by a factor of 1.15. Educational attainment has been recognised as a protective factor in HIV populations and has been discussed in more detail in the introductory chapter.

In this study, history of depression/anxiety is defined as having a lifetime incidence of depression rather than the presence of depression or anxiety at T_1 or T_2. None of the participants expressed concern or was actively treated for a severe episode of depression at the time of T_2 assessment, although some 27.8% had a positive HADS screen for symptoms of anxiety and 11.4% also had a positive HADS screen for symptoms of depression. The progression of HAND was tested against HADS Anxiety subscale, HADS Depression subscale and, HADS Anxiety and Depression subscales combined as well as the life time history of depression. None of these variables was found to have a statistically significant relationship to HAND progression. Unlike in other discussed studies, other medical comorbidities and neurological complications of HIV, and HIV variables apart from ART treatment duration, did not influence HAND diagnosis change at follow-up. However, results in other reported studies were also inconsistent.

On follow-up assessment, an individual domain score change from baseline that was equal or greater than 0.5 standard deviations (SD) was also applied to further evaluate the course of HAND and its pattern. Again, heterogeneous trajectories across different RBANS domains were observed when this definition was adopted. As a group, this cohort showed statistically significant improvements of the mean index scores on immediate memory, delayed memory, and attention domains, with relative stability in language and insignificant worsening in the visuospatial skills domain. Other published studies also report improvements in executive functions and working memory. These improvements, however, were also described in HIV+ individuals not taking ART and have partly

been attributed to practice effects. Usually, there is an expectation that otherwise well subjects would show some degree of improvement in performance on repeat neuropsychology testing. These are more obvious in attention, learning, executive, and memory domains and occur due to prior exposure to the testing material and familiarity with the test environment and procedure. The current study did not have a comparable control group to be able to measure the true impact of practice effects.

When estimating the size of possible practice effects, a conservative approach was adopted and published normative data from two studies were used. When using Safaz et al. data (242), some of the results showing *significant improvements* became *insignificant improvements* (digit span, list recall and line orientation) or even converted to *worsening* (coding, list learning and semantic fluency), and *insignificant worsening* became *significant* (figure recall). Some *significant improvements* still remained *significant* (story memory, story recall and list recognition) and *significant worsening* in figure copy remained *significant*. While this is suggesting that some of the improvements can be attributed to practice effects, others, which were mainly observed in the memory subtests, exceeded the size of expected improvement and could be due to other test or subject related factors (tester agreement, reversible impairments, and test environment). This exercise suggests that subtle/subclinical impairments might be “hidden” in the apparent improvements of attention, learning efficiency and executive function subtests (i.e. frontostriatal/subcortical functions). However, the above control data were obtained in a healthy population at a 4-6 weeks re-test interval, which is significantly shorter than in the current study. Duff et al.

suggested that longer re-test intervals diminish the impact of practice effects, although they do not eliminate it completely. This might mean that by applying Safaz et al. data, the size of practice effects could be overestimated (235).

When applying Duff et al. published norms at a one year re-test interval (219), only the improvement in the line orientation subtest stopped being *significant* (i.e. improvement due to practice effect) and figure recall became *significantly worse* at T_2. However, the latter re-test norms were obtained in a much older population (mean age 72.89 years; SD 5.52) than the population in the current study. Since older subjects tend to show less improvements on re-testing, by applying Duff et al. published norms, there is a chance that the size of practice effects could be underestimated. This analysis emphasises the critical need for an appropriate control group in longitudinal studies that involve an evaluation of the longitudinal course of HAND with neuropsychology testing.

Although affected in less subjects than at T_1, delayed memory and attention still remained the most frequently affected domains on RBANS at T_2. However, significantly less subjects had an abnormal list recognition at follow-up, suggesting that memory impairment was due to poor retrieval rather than poor retention in a higher proportion. Similar to T_1, memory and attention domains as well as fluency were also more affected on ACE-r, but more subjects had impaired attention and visuospatial skills domain. MoCA results also show that memory and language/fluency were more frequently affected at T_2, with attention and executive function following after that. There was an increase in the number of patients who showed impairment on visuospatial/constructional domain on all tests in the battery. All these findings are suggestive that the mixed pattern of

both “cortical” and “subcortical” impairments in HAND shown in this cohort at baseline has been preserved at follow-up.

There were more people scoring normal on FAB at follow-up, but this difference was not statistically significant. Also, converting to normal on FAB at follow-up does not necessarily imply improved performance but might be a reflection of correction for age, as the corrected FAB score tends to be higher with increasing age, and some subjects might have scored within the normal range at T_2 simply because they grew older without having necessarily performed better at follow-up.

Inconsistencies were also observed between RBANS and total ACE-r, MoCA, and FAB results. However, Antinori et al. HAND diagnostic criteria were applied to separate RBANS test domains, whereas ACE-r, MoCA and FAB have a more simple scoring system that defines abnormal as the total test score being below a cut-off. Moreover, FAB is designed to assess a single, executive, domain, while HAND can involve impairment in multiple cognitive domains and RBANS allows for assessment of five domains. Furthermore, the improvements observed in the FAB, MoCA and ACE-r scores between T_1 and T_2 are small in number and may be simply the result of natural normal variability observed on repeated neuropsychological tests, as well as tester or test environment biases, or practice effects that have not been possible to account for.

Moreover, despite the heterogeneous changes shown on individual tests, no significant changes were observed at follow-up in any domain when raw scores of all the subtests from the battery were used in the *z* transformation, except for the

language and orientation, which got worse in the improvers group only. However, these findings are underpowered by the small number of participants in the improvers group. It should also be noted that orientation domain is not assessed by the RBANS test and only RBANS assessed domains participated when establishing the HAND diagnosis and grouping the subjects into “improvers”, “stable” and “progressors”. It is also possible that RBANS language subtests are lacking the more in depth language assessment provided by ACE-r which was included in the *z* transformation analysis. These might suggest that some of the improvers are not “true improvers” as while they improved in some domains, they got worse in domains other than those tested by RBANS. The lack of a comparable control group at baseline and follow-up could also have affected the interpretation of these results. All of the above emphasise the need for studies in larger HIV cohorts and need for a control group and validated assessment tools that include a wider range of domains with normative data for a wider age range.

When RBANS cortical-subcortical deviation scores (CSDS) were calculated, a higher proportion of negative value CSDS in those with HAND was observed at follow-up (48.9% at T_2 vs 21.6% at T_1), suggesting that at T_2 significantly more patients with HAND showed a “subcortical” pattern of impairment. It is possible that this difference might simply be a reflection of the “normal” variability in the performance on repeated neuropsychological testing, or a reflection of greater improvements in delayed memory (a “cortical” domain), which were not possible to reliably control for practice effects. Duff et al. found that 37% of community dwelling elderly had a CSDS of +10 and over, thus displaying a “cortical” profile and only 8% displayed “subcortical” profile and had a CSDS of -10 and less.

Although they cautioned clinicians about the low stability of this score due to "normal" variability in cognitive abilities and suggested to also take into account history, physical examination findings, and investigation results when making clinical decisions (246). Amongst those who did not meet criteria for HAND, this proportion at follow-up (38%) was not significantly different from that at baseline (32%). In addition, while statistically not significant, there was a higher proportion of those who had an impaired list retention with spared recognition on RBANS memory tests which have been linked to "subcortical" dementias. A selectively higher proportion of "subcortical" CSDS in those with HAND at follow-up, with higher proportion of "subcortical" type of memory impairment, together with increased number of patients who had an abnormal visuospatial skills domain (RBANS, ACE-r, and MoCA), and a high proportion of patients with impaired attention (RBANS, ACE-r), suggest that, despite the apparent stability, there might be a slow subclinical progression of the "subcortical" HAND pathology going on. However, the finding of worsened language domain z score in the "improvers" group suggests that a subclinical "cortical" degenerative process is also possible.

2.7.2 Strengths of the Study

In this study, a well characterised cohort of HIV+ individuals were followed and assessed clinically and with neuropsychology testing at two time points. There was a good retention rate in the follow-up study, which took place at a relatively long follow-up interval. The neuropsychology tests used are easy to administer and do not require specialist training, therefore can be easily applied in clinical conditions. To eliminate the inter-scorer bias, the RBANS figure copy and figure

recall subtests at baseline were re-scored by the candidate who assessed the cohort at follow-up using the new scoring criteria.

2.7.3 Limitations of the Study

The differential attrition rate in this study was 22.7% in the baseline HAND group and 26.3% in the Not-HAND group, which might introduce some retention bias. It is possible that there were more “decliners” amongst those who were lost to follow-up and these did not retain in the study due to cognitive impairment related poor judgement and decision making.

The neuropsychology assessments were performed by two testers: one at baseline (T_1) and the other at follow-up (T_2). Although all tests were administered according to the published guidelines at both assessments and every effort was made to reduce the variability, some differences in the administration technique are to be expected. This study did not have access to a comparable control group both at baseline and at follow-up, which at T_2 made it difficult to distinguish between “true improvements” and “normal/expected improvements” due to previous exposure to the testing material, and also to estimate what were “less than expected improvements”.

Test related factors such as testing environment could also bias the results. Follow-up assessments, unlike the assessments at baseline, were administered in one of the CRF clinical rooms outside the GUIDe clinic. This eliminated the “background noise” of a busy clinic but introduced the “novelty” of the testing room location, which was previously unknown to most patients. The latter could have biased the results of the orientation tasks. There was some “interference”

between the tests in the battery, which can bias the results of the subsequent test. For example, there were serial 7's backward tracking, a cube, and a clock drawing tasks in the ACE-r and the MoCA tests and patients had a chance to learn the strategy for the task in one test and apply it in the subsequent test.

Some noticeable differences between the findings of the clinical examinations conducted at T_1 and T_2 were observed, mainly in the areas of cranial nerve abnormalities. However, most cranial nerve findings at T_2 are accounted for by minor eye movement abnormalities. At T_1 these were not otherwise reported in the cranial nerve examination. Therefore, it is not possible to comment on this particular finding. Changes in sensation, coordination and motor function impairments were also observed. With respect to sensation, coordination and motor function abnormalities, these results should be interpreted with caution in the view of the fact that the assessments were carried out by two different clinicians who might have different approaches and thresholds to assessing marginal presentations of abnormality, i.e. no quantifying validated methods were used to standardise clinical examination findings. A tentative explanation of clinical examination findings corresponds with the view that HAND affects the cerebral and subcortical white and grey matter and this would be consistent with further impairments, in motor function, coordination and other functions.

2.8 Conclusion

Overall, this cohort showed stability, with less participants fulfilling the criteria for HAND at follow-up. Only one participant showed a clear decline on all tests in the battery and had a new incident diagnosis of HAD at follow-up. Despite the

apparent stability of HAND diagnosis, there was a heterogeneous evolution within each domain and each test in the battery.

As a group, this cohort showed a trend towards a higher proportion of "subcortical" pattern of dysfunction at follow-up. In addition, there were more participants displaying motor signs and minor eye movement disorder on the physical examination. However, there was also evidence of language decline in the "improvers" group. These findings suggest that a subclinical decline in the "subcortical" and less so in the "cortical" neurocognitive functions might be ongoing. This will be further evaluated for evidence of degeneration on neuroimaging (axonal loss and cortical atrophy) in the following chapter. However, overall, these results show that any putative neurodegenerative process has not been unmasked on follow-up neuropsychology testing, despite the worsening HAND characteristics in a proportion of the subjects. Nevertheless, the proportion of cognitively impaired in HIV remains very high, albeit stable, on HARRT. Continued detailed longitudinal assessments will be required to determine whether there is a continuous decline in cognitive function to further support the hypothesis of ongoing neurodegeneration in this well characterised, clinically stable cohort of HIV+ individuals.

3. Follow-up Neuro-Imaging in HIV Positive Patients with HIV Associated Neurocognitive Disorders

3.1 Introduction

The wide availability of HAART has led to increased life expectancy in HIV+ individuals and greatly reduced the number of those affected by severe forms of HIV related cognitive impairment such as HAD (162). However, more than half of people living with HIV and availing of effective combined antiretroviral therapy are still suffering from mild forms of HIV associated neurocognitive disorders (HAND) (35, 162, 247). While detailed neuropsychological assessment remains the "gold standard" for HAND diagnosis, additional biomarkers are needed to aid early diagnosis and to monitor the disease progression.

Since the introduction of HAART regimens, HIV disease plasma biomarkers, such as CD4+ cell counts, nadir CD4+, and HIV viral load (VL) can no longer be reliably used to predict HAND progression (248-251). Other markers such as CSF HIV RNA load or the presence of inflammatory cells and other chemokines in the CSF have been useful, but their retrieval is logistically difficult. In addition, some neuropsychological testing results could be subject to biases due to potential cultural differences and differences in education, as well as being dependent on the tester's technique and learned behaviours. All of these make direct comparisons and interpretation of neuropsychology measures difficult in both clinical and research conditions (252, 253). Neuroimaging, however, offers a non-invasive, objective, observer independent, *in vivo* picture of brain pathology.

MRI is relatively easy to acquire and causes minimal discomfort to the patient compared with the plasma or CSF markers acquisition. Obtained images can be stored and repeated at certain intervals to allow for follow-up comparisons and cross-sectional comparisons with healthy controls. Thus, different neuroimaging techniques, can be valuable tools for *in vivo* description of the evolving HAND neuropathological process, measure the disease progression, and serve as prognostic markers.

3.1.1 Outline of the Chapter

This chapter focuses on a description of the MRI findings in a subset of patients who participated in the baseline (T_1) and the follow-up (T_2) detailed neuropsychological assessments after an initial positive screening test for cognitive impairment (49, 192). A subset of that cohort who also participated in the baseline neuroimaging acquisition study (TP1) were re-enrolled in the follow-up neuroimaging study (TP2). The underlying hypothesis of the study was that HIV related brain injury will progress due to immune / inflammatory mediated neurodegeneration, despite ART treatment. Baseline and follow-up brain MRI imaging was acquired in this subset of participants to assess for the evidence of possible continuous inflammatory changes and / or possible progression of cortical grey matter atrophy in association with progressive cognitive decline.

This chapter provides a brief description of the MRI techniques used in the baseline and follow-up studies. A comprehensive review of the previous cross-sectional and longitudinal neuroimaging studies conducted, to date, in HIV

positive individuals is presented. The aims, objectives, study methods, and study results are also presented and discussed.

3.1.2 Description of Neuroimaging Techniques Used in the Current Study

Voxel Based Morphometry (VBM) and Diffusion Tensor Imaging (DTI) analyses, in addition to the standard structural MR acquisition, were used in the current follow-up study as well as in the baseline study.

3.1.2.1 Voxel Based Morphometry

Voxel based morphometry is an automated technique which uses high resolution structural T1-weighted MR images along with a voxel-wise parametric statistical approach to compare grey or white matter smoothed images between two groups. It allows assessment of regional grey and white matter volume differences between two groups of subjects or between the two brain hemispheres (254) and it is mainly used to detect focal atrophy. VBM has been used to identify brain regions that differ between healthy individuals and individuals with various conditions, such as Alzheimer's disease (255-259), frontotemporal dementia (260-263), other neurodegenerative conditions (264), motor neuron disease (265-269), epilepsy (270-274), multiple sclerosis (275, 276), movement disorders (277-279), psychiatric conditions (280-282), and more significantly for this project, HIV related brain disease (283).

The main advantage of this technique is that it can be used for the evaluation of anatomical differences of any brain regions of interest, as defined by the researcher. It was first designed and described by Wright and colleagues in 1995

(280). It was updated by Ashburner et al. in 2000 to include an improved automated segmentation and non-linear spatial normalisation (284). Later, in 2002, an optimised grey matter normalisation technique was introduced (285), followed by improved segmentation technique in 2005 (286), and deformation normalisation techniques in 2007 (287).

3.1.2.2 Diffusion Tensor Imaging (DTI)

DTI uses three-dimensional mapping of water molecule diffusion within brain white matter to assess and characterise microstructural changes of the white matter tracts. Diffusion is a random motion or transport of material (water molecule) over time from one location in space to another. In the biologic tissues, diffusion of water molecules can be hindered or restricted by different cellular structures (cell membranes and organelles). In the white matter, which has a “fibrous” structure (bundles of myelinated axonal fibres aligned in parallel), water diffusion is relatively unrestricted parallel to the neuronal axons, while it is greatly restricted in the direction perpendicular to the neuron fibres. Thus, water diffusion in the white matter is directionally dependent or anisotropic (288). DTI assesses the degree of anisotropy on a voxel by voxel basis and maps the white matter fibres/tracts direction (289). The diffusion anisotropy principle allows for the evaluation of the white matter tracts integrity (290).

The diffusion tensor, a term introduced by Basser (291), is an ellipsoid with eigenvectors defining directions of the three principal axes (x, y, z). Diffusion anisotropy is the extent to which the shape of the tensor ellipsoid deviates from that of a sphere. The measures of radii along each eigenvector are termed

eigenvalues (λ_1, λ_2, λ_3). The principal eigenvector is oriented in the direction of the maximum diffusivity, i.e. along the white matter tracts. Diffusivity along the principal axis is termed *Axial Diffusivity* (AD= λ_1) and its value reflects axonal integrity (292, 293). The diffusivities in the two minor axes are averaged to calculate the value of *Radial Diffusivity* (RD= $(\lambda_2+\lambda_3)/2$) which reflects diffusivity perpendicular to the white matter fibres. An increased RD value is an indicator of myelin loss (294-297). *Mean Diffusivity* (MD) derives from the sum of the three eigenvalues divided by three (MD= $(\lambda_1+\lambda_2+\lambda_3)/3$) and, when increased, reflects an increase in extracellular space due to an inflammatory process or neuronal atrophy (298-300). *Fractional Anisotropy* (FA) is a value between *zero* (which means that all eigenvalues are equal and the diffusion is *isotropic* or equally unrestricted or restricted in all directions) and *one* (which means that diffusion only takes place along one direction and is completely restricted along the other directions) (301). FA is calculated mathematically from the three eigenvalues and reflects axonal diameter, fibre density, and the integrity/coherence of the white matter tracts (302-305).

3.2 Findings of the Cross-Sectional Studies Using Different Neuroimaging Techniques to Characterise HIV Associated Brain Pathology

Different neuroimaging techniques that were used to characterise HIV associated brain pathology in the pre- and post-HAART era in the available cross-sectional studies will be discussed in this sub-chapter and presented in Table 3.1.

3.2.1 Standard Structural MRI

Structural MRI data acquisition in HIV+ individuals was initially validated in studies involving post-mortem imaging with morphopathological correlation (306-308) and in studies involving ante-mortem imaging, with subsequent post-mortem autopsy histopathological correlation (309). These studies demonstrated associations between the clinical stage of HIV disease, the pathological evidence of HIV infection severity, and the white and grey matter volumes estimated by neuroimaging. Standard clinical MR imaging prior to the introduction of ART showed evidence of global brain atrophy demonstrating sulci and ventricular enlargement with increased CSF volume (310-319), as well as white matter hyperintensities (309-312, 317, 318, 320). Although the standard clinical MR imaging findings correlated with neurocognitive status in the neurologically symptomatic HIV infected patients, Post and colleagues in 1991 found characteristic features in a minority of asymptomatic HIV+ individuals while nearly half of symptomatic patients had normal appearing standard MR (312). Dooneief et al. (1992) also concluded that, while the standard MRI appearance was able to distinguish HIV infected patients from seronegative individuals, it was not useful at differentiating symptomatic from asymptomatic HIV infected patients (321). This suggested that, while standard clinical MRI is revealing macroscopic brain changes (ventriculomegaly, brain atrophy) and white matter changes (parenchymal lesions) related to HIV infection, it may not be a sensitive enough tool to evaluate the microscopic white and grey matter changes in HIV related brain damage. Hence, additional techniques were needed.

3.2.2 Volumetric MRI Analyses Prior to Introduction of HAART

Quantitative MR data analyses have been shown to be more sensitive in detecting tissue volume loss in the symptomatic HIV+ patients when compared with asymptomatic HIV+ patients (322, 323). Volumetric analyses were performed in numerous studies and showed global cerebral white matter loss (309, 317, 322-324), cerebellar white matter loss (309, 311, 325), and subcortical grey matter atrophy (309, 313, 316, 322, 324), mainly of the caudate nucleus (319, 324, 326-329), and to a lesser extent cortical grey matter loss (309). Basal ganglia atrophy was strongly associated with HIV dementia (313, 316, 319). Paul et al. in their study showed significant correlation between the caudate nucleus size and performance on neuropsychology tests, while performance on motor tests correlated with the putamen size (329).

Interestingly, neuropathological studies also demonstrated cortical grey matter loss in HIV infected individuals measured by reduced neuronal numerical density in the superior frontal, parietal and calcarine cortices (330), as well as temporal cortex (331). However, despite the histological evidence of cortical grey matter loss, the neuroimaging studies in the pre-HAART era failed to demonstrate significant differences in the cortical grey matter volumes by serostatus or indeed any association of cortical volumes with cognitive performance (307, 332, 333). All of the above findings are consistent with the pre-HAART “subcortical” characterisation of HIV dementia.

3.2.2.1 Effect of HAART on disease progression and shifting from white matter to grey matter disease as shown by volumetric MR imaging

Volumetric imaging studies in the post-HAART era suggested a shift towards development of cortical brain injury in the setting of stable effective viral suppression. Cohen et al. (2010) and Thompson et al. (2015) demonstrated similar findings that show association between a longer HIV disease duration, cognitive impairment, and prefrontal and parietal lobe grey matter loss (249, 334). More recently, reduced thalamic and brainstem volumes as well as reduced cortical thickness in the orbitofrontal, primary motor and sensory cortices, cingulate gyrus, temporal and frontal lobes were reported by Sanford and colleagues in 2017 (335). In the post-HAART era, reduced grey matter volumes in the hippocampus and parahippocampal gyrus were described by Wade et al. (2015), Wilson et al. (2015), and Wang et al. (2015) (336-338).

Interestingly, Thompson et al. (2015) found no significant cortical thickness difference between those patients taking HAART and those who were not (334). However, this study was underpowered by small sample sizes (13 HIV+ subjects on HAART vs 13 HIV+ subjects not on HAART). Ances et al. (1999) and Cardenas et al. (2009) were also not able to show statistically significant differences between subcortical grey matter (amygdala, caudate and corpus callosum) thickness in HIV infected individuals on HAART and those not taking HAART (339, 340). Cardenas and colleagues (2009), however, concluded that ongoing atrophy in areas other than caudate was less severe and slower in those on HAART regimens (340).

3.2.2.2 HAART and age related changes on quantitative MR imaging

Towgood et al. (2012) found ongoing medial and superior frontal gyri grey matter reduction in treated HIV+ individuals with stable viral suppression (341). Becker et al. (2012), in the Multicentre Cohort Study (MACS), found ongoing HIV related grey matter changes in the posterior and inferior temporal lobes (right more than left), in the parietal lobes, and the cerebellum (342). Additional age related changes independent of HIV status in the grey and white matter of the superior and medial temporal regions, inferior frontal regions, and cingulate have been found in both of these studies.

3.2.2.3 Distinct morphometric patterns in HAND and in HIV unrelated mild cognitive impairment (MCI)

MRI morphometric analysis was also shown to be useful in differentiating HAND related changes in HIV infected people from changes related to MCI in seronegative individuals (343).

3.2.3 Diffusion Tensor Imaging

In addition to quantitative structural analysis, Diffusion Tensor Imaging (DTI) is widely used to assess the white mater integrity and microstructure through measurement of Fractional Anisotropy (FA) which, if reduced, is traditionally regarded as a marker of axonal loss and Mean Diffusivity (MD) which, when increased, reflects increased extracellular space due to inflammation and neuronal atrophy. The whole brain white matter FA was shown to be reduced in HIV+ individuals and was associated with cognitive status in the studies conducted prior to introduction of antiretroviral therapy (250, 344). In HIV infected individuals, FA and MD measures were found to be abnormal in centrum

semiovale, putamen and caudate (97), frontal and parietal white matter (345-347), corpus callosum (346, 348-351), internal capsule (346, 349), inferior longitudinal fasciculus (346), optic radiation (346, 350), and anterior commissure (349).

3.2.3.1 DTI parameter correlation with HIV infection markers and cognitive function

FA measures in the corpus callosum were significantly related to the duration of HIV infection prior to introduction of ART treatment (352). Diffuse white matter structural alterations, increased MD and radial diffusivity (RD), and reduced FA, which correlated with cognitive function were also observed in virally suppressed individuals in the post-HAART era by Underwood et al. (2017) (353). Towgood and colleagues (2012) concluded that FA and MD changes in the frontal and temporal white matter tracts observed in the asymptomatic HIV+ individuals with stable disease on HAART were consistent with ageing (341). In contrast, in an earlier study, Ragin et al. (2006) found correlations between DTI parameters of subcortical grey matter and plasma inflammatory markers such as Monocyte Chemoattractant Protein-1 (MCP-1), TNF-α and haematocrit in eleven participants stable on antiretroviral treatment, suggesting an inflammatory substrate of ongoing HIV related brain injury (97). The MD measures for centrum semiovale, caudate, and putamen correlated with values of MCP-1 in this sample. Measures of FA for centrum semiovale correlated with TNF-α and FA for putamen correlated with haematocrit level.

3.2.4 Structural and Metabolic Imaging

3.2.4.1 Positron Emission Tomography

Early Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) studies performed in HIV+ patients with ADC found altered regional glucose metabolic rates, showing an initial subcortical hypermetabolism (in the thalamus and basal ganglia) followed by cortical and subcortical grey matter hypometabolism, which was associated with functional decline and progression to dementia in nine of the twelve studied patients (354). Moreover, Brunetti et al. (1987) found that changes in frontal, parietal, temporal, and occipital regions were reversible in those treated with Zidovudine (355). However, this study only included four patients. Pascal et al. (1991) found significant glucose metabolic asymmetries in prefrontal and premotor regions in HIV+ patients when compared with seronegative individuals (356). Although these HIV+ individuals were asymptomatic initially and had normal quantitative MR imaging, half of them became symptomatic at 18-40 months follow-up, suggesting that metabolic (PET) scans can show pre-clinical pathophysiological changes in HAND. Although subtle, the FDG-PET changes are still observed in the HAART era in the frontal lobes in at least half of those with suppressed viral loads (357).

3.2.4.2 PiB PET imaging

Several studies suggested that in the post-HAART era, amyloid-β42 might play a role in the neuropathogenesis of HAND similar to that in Alzheimer's disease (167, 358, 359). Studies evaluating the mean cortical binding potential (MCBP) of amyloid-binding agent Pittsburgh compound B (^{11}C-PiB) in HIV+ patients, who

presented with HAND did not show increased fibrillar amyloid deposition even in those with low CSF amyloid-β42 (170). Thus, ^{11}C-PiB PET might be a useful tool in distinguishing HAND from Alzheimer's disease in older HIV+ individuals with cognitive impairment (360).

3.2.4.3 Magnetic resonance spectroscopy (MRS)

Another imaging technique used for *in vivo* assessment of brain metabolites in research studies is magnetic resonance spectroscopy (MRS), which can evaluate a number of metabolites: choline (Cho) – a membrane damage marker, N-acetyl aspartate (NAA) – a neuronal injury marker, and myo-inositol/creatine ratio (MI/Cr) – a marker of glial activation and astrogliosis. Significant reductions in NAA with increased Cho and MI/Cr were described in basal ganglia of ADC patients and were related to psychomotor slowing and cerebral atrophy (361, 362). However, similar changes were also found in the asymptomatic HIV+ individuals (329, 361, 363, 364). Decreased NAA/Cr ratio has been found to be reversible and correlates with the level of neuro-inflammation (363).

3.2.5 Structural and Functional Imaging

Functional MRI (f-MRI) has also been utilised in HIV+ individuals to assess neural networks and connectivity. Resting state f-MRI (RS fMRI) shows reductions in the frontostriatal connectivity, particularly between dorsolateral prefrontal cortex (DLPFC) and dorsal caudate as well as reductions in dorsal caudate connectivity to parietal and frontal regions when HIV+ patients were compared with HIV- individuals (365). Reduced connectivity was also shown between prefrontal cortex and bilateral precuneus, right superior frontal regions and right inferior

operculum (366). The latter finding was correlated with learning ability and memory. Despite some improvements in the network connectivity associated with insignificant improvements in cognitive function in clinically stable HIV+ individuals treated with HAART, significant ventral attention and default networks disruptions were still apparent on RS fMRI (252, 367). Jiang and colleagues (2016) found that, despite the comparable performance on standard neuropsychological tests assessing executive function, HIV+ individuals were slower than controls adapting to the task switching and this correlated with impaired fMRI signal at the dorsal anterior cingulate (ACC) (368). This finding suggests that subtle HIV related cognitive impairments can be missed on standard neuropsychology testing.

Castelo and colleagues (2006) showed altered integrity of hippocampal - prefrontal network during the episodic encoding tasks in HIV+ patients (369). Blood oxygenation level dependent functional MRI (BOLD-fMRI) also shows reduced activation in the normal attention networks with greater activation in the adjacent lateral prefrontal or contralateral regions in HIV+ individuals (370-372). These findings suggest that reduced efficiency in the normal attention networks due to HIV related brain injury requires adaptive usage of brain reserve to maintain normal task performance.

3.2.6 Neuroimaging Findings Correlated with the HIV Subtypes

While HIV clade B is the most studied subtype in HIV neuroimaging studies, Hoare et al. (2011) evaluated voxel-based FA and MD in HIV+ clade C (HIV-C) and Du Plessis and colleagues (2017) examined RS fMRI in HIV-C (367, 373).

The utility of MR imaging was explored in the evaluation of neurovirulence and possible specific neuropathological patterns of brain damage in different HIV clades by Ortega et al. (2013). Although the volumetric measures in the thalamus, hippocampus, corpus callosum, and cortical grey and white matter were significantly smaller in the HIV+ patients, there was no specific clade effect observed (69).

3.2.7 Neuroimaging Correlated with Host Characteristics such as APO Ɛ4 Allele Carriage

Other studies have looked at additional risk of HAND and the imaging markers of such risk in carriers of APO Ɛ4 allele. Chang et al. (2011) concluded that younger HIV+ carriers of APO Ɛ4 allele were at an additional risk of developing HAND and brain atrophy (374). Jahanshad et al. (2012) also showed that APO Ɛ4 HIV+ carriers have additional temporal and parietal network connections deficiencies (375).

Table 3.1. Findings of the Cross-Sectional Imaging Studies Using Different Imaging Techniques and Modalities

Authors / year	Participant characteristics	Imaging modalities	Findings
A. Pre-HAART imaging studies / imaging studies in untreated cohorts			
Rottenberg et al. 1987 (354)	12 HIV+ with ADC 18 HIV-	FDG-PET CT	Characteristic regional glucose metabolism alterations were observed in 9/12 HIV+ subjects. Relative subcortical (thalamus and basal ganglia) hypermetabolism was observed in early ADC. Disease progression was accompanied by cortical and subcortical grey matter hypometabolism.
Grafe et al. 1990 (306)	10 formalinised brains of deceased HIV+ patients	Post-mortem standard MRI	9/10 patients had pre-mortem cognitive changes. MR images showed atrophy in 8/10. Severe ventriculitis and focal gliosis were also seen.
Elovaara et al. 1990 (315)	72 HIV+ at various stages of the disease 34 HIV-	Standard MRI	Central and cortical atrophy, brain stem atrophy, and cerebellar atrophy was more frequent in HIV+. Subjects with cognitive deficits exhibited more severe central atrophy than cognitively intact patients. Minor brain atrophy and/or parenchymal lesions were found in 57% of cognitively intact HIV.
Post et al. 1991 (312)	119 HIV+ subjects (24 symptomatic, 95 asymptomatic)	Standard MRI	23 subjects (12/95 (13%) from the asymptomatic group and 11/24 (46%) from the symptomatic group) had abnormal images showing atrophy and/or white matter lesions. Asymptomatic group showed fewer, smaller, and/or less extensive abnormalities.
Pascal et al. 1991 (356)	15 HIV+ asymptomatic MSM 15 HIV- MSM	Standard MRI & FDG-PET CT	MRI showed no abnormalities on clinical or quantitative evaluation in HIV+ subjects. While significant glucose metabolism asymmetries were found in 10/15 HIV+ subjects on FDG-PET imaging, primarily in prefrontal (7/15) and premotor (4/15) regions.
Dal Pan et al. 1992 (313)	116 HIV+ 23 HIV- controls	Standard MRI & standardised planimetry	37/116 had HIV dementia, 40/116 had neurologic or neuropsychological abnormalities other than dementia, and 39/116 were neurologically intact. Imaging showed overall cerebral atrophy and selective caudate region atrophy which was associated with HIV dementia.
Dooneief et al.	129 HIV+ (75-MSM; 54-	Standard MRI	There was no differentiate found between asymptomatic and

Authors / year	Participant characteristics	Imaging modalities	Findings
1992 (321)	IVDU) 90 HIV- (38-MSM; 52-IVDU)		mildly symptomatic HIV-positive individuals regardless of risk group. Intracranial water percent may distinguish HIV-positive from HIV-negative individuals.
Broderik et al. 1993 (317)	45 HIV+ patients with AIDS (of them 10 had ADC)	Standard MRI & Volumetric analyses	Atrophy and abnormal signal intensity in the splenium was significantly associated with ADC. The presence of generalized deep white matter abnormalities did not differ significantly between patients with and without dementia.
Jernigan et al. 1993 (322)	98 HIV+ (31-symptomatic and 67-asymptomatic) 65 HIV-	Standard MRI & Volumetric analyses	Medically symptomatic but non-dementia HIV+ subjects showed significant increases in cerebrospinal fluid, reduced cerebral white matter, and cortical and subcortical grey matter volumes.
Heindel et al. 1994 (307)	17 HIV+ patients with AIDS	Post-mortem standard MRI	Significant relationships were observed between the severity of central nervous system HIV infection and the MRI volume estimates of grey matter and abnormal white matter.
Hassine et al. 1995 (308)	7 HIV+ asymptomatic 8 HIV- controls with similar cause of death 6 HIV+ who died of AIDS	Post-mortem standard MRI	Asymptomatic HIV+ showed significant brain atrophy compared to HIV- controls and significantly less atrophy than AIDS patients. Nonspecific MRI changes probably correspond, to scars of transient vascular inflammation found on post mortem examination.
Kieburtz et al. 1996 (319)	11 HIV+ patients with advanced disease	Standard MRI & Volumetric analyses	Caudate volume in patients with advanced HIV disease was associated with poor performance on neuropsychologic tests of complex motor and sequencing skills. Hippocampal volume was not related to impairment on neuropsychologic tests.
Salvan et al. 1997 (361)	112 HIV+ (35 neuroasymptomatic and 77 with ADC)	MRI (volumetrics using BFR) & MR Spectroscopy	14% of HIV patients with normal MRI displayed abnormal MRS, whatever their neurological status. A significant loss of NAA is more frequent in ADC patients. Only ADC patients display the double pattern - with a significant increase in choline signal and a significant loss of NAA. Cerebral atrophy, defined by bifrontal ratio, was related to the occurrence of NAA loss (in NAA and double patterns).
Jarvik et al. 1998 (310)	30 HIV+ patients with AIDS or ADC	Standard MRI	Findings: A - Multiple discrete high-signal foci in patients with toxoplasmosis and PML. B - Large, bilateral patchy / confluent high-signal areas within the white matter represented a white matter CMV or HIV encephalitis. C - Generalized enlargement of the cortical sulci and ventricles reflecting atrophic changes from the chronic HIV infection. D - Solitary high-signal-intensity lesions correlated with a non-viral opportunistic infection.

Authors / year	Participant characteristics	Imaging modalities	Findings
Stout et al. 1998 (324)	86 HIV+ (CDC stage: A-33; B-19; C-34) 23 HIV-	Standard MRI & Volumetric analyses	Participants whose systemic disease progressed to a higher CDC stage had significantly accelerated ventricular volume increases, white matter volume loss and caudate atrophy.
Ge et al. 2003 (323)	15 HIV+ (7-symptomatic and 8-asymptomatic) 10 HIV-	Standard MRI & Volumetric analyses	Volumetric analysis showed loss of brain tissue, that was more pronounced in neurologically symptomatic patients (p =.003) but not in asymptomatic patients (p =.23) when compared with control participants.
Archibald et al. 2004 (309)	21 HIV+ 19 HIV-	In-vivo structural MRI & Volumetric analyses	HIV+ subjects demonstrated CSF volume increase, associated with a significant reduction in the volumes of cerebral and cerebellar white matter, caudate nucleus, hippocampus, and, to a lesser extent, cerebral cortex. White-matter tissue showed signal change and volume loss. This white matter hyperintensities predicted diagnosis of HIV encephalitis at autopsy.
Elsheikh et al. 2010 (325)	Case report 10 years history of HIV+	Standard MRI & CT brain	Imaging revealed severe cerebellar atrophy, with no other cause identified, but chronic HIV infection.
Hoare et al. 2011 (373)	44 HIV+ (clade C) 10 HIV-	MRI & DTI analysis	The HIV+ group exhibited decreased FA in the corpus callosum, superior longitudinal fasciculus, and cingulum and sagittal stratum. These results provide evidence that white-matter integrity is compromised in individuals infected with clade C HIV.
Heaps et al. 2012 (333)	28 HIV+ (predominantly Clade C) 23 HIV-	Standard MRI & Volumetric analyses	HIV+ group had significantly lower total white matter (p<.01), thalamus (p<.01) and total grey matter (p<.01) volumes. No significant differences were observed between the groups in the caudate, corpus callosum, or total cortex.
Ortega et al. 2013 (69)	34 HIV+ HAART naïve (17 clade B and 17 clade C) 34 HIV-	Standard MRI & Volumetric analyses	HIV+ participants performed significantly worse on neuropsychological tests of processing speed and memory and had significantly smaller relative volumetrics within the thalamus, hippocampus, corpus callosum, and cortical grey and white matter compared to HIV- controls. However, no specific effect of HIV clade subtype was demonstrated.
Du Plessis et al. 2017 (367)	56 HIV+ HAART naïve (clade C)	RS fMRI	Results revealed ten regions in six RS networks where functional connectivity inversely correlated with worse performance. The networks affected included three independent attention networks: the default mode network, sensorimotor network, and basal ganglia. Connectivity in these regions did not correlate with plasma viral load or CD4 cell count.

Authors / year	Participant characteristics	Imaging modalities	Findings
B. Imaging studies in the ART/HAART treated cohorts			
Brunetti et al. 1987 (355)	4 HIV+ treated with Zidovudine	FDG-PET CT	Brain glucose metabolism was assessed using FDG-PET imaging before commencing Zidovudine treatment and at a later stage. Post-treatment imaging showed marked improvement in cortical glucose metabolism in all four patients.
Pavlakis et al. 1998 (363)	45 HIV+ children with AIDS treated with Zidovudine (2 HIV+ had follow-up studies) 18 HIV-	1.5T MRI & MR Spectroscopy	Subjects with progressive encephalopathy have lower NAA/Cr ratios than control subjects in the basal ganglia region. Two subjects also had reduced lactate peaks. After therapy, there was improvement in NAA/Cr and of the abnormal lactate peak. Authors suggest that decreased NAA/Cr in AIDS is reversible, and that brain lactate might correlate with inflammation.
Chang et al. 2001 (372)	11 HIV+ 11 HIV-	fMRI	HIV+ showed greater brain activation in some regions compared with control subjects while performing the same tasks. For the simpler tasks, HIV+ showed greater activation in the parietal regions. With more difficult tasks, HIV+ patients showed greater activation additionally in the frontal lobes. Reaction times during these tasks were slower but accuracy was similar in the patients with HIV compared with control subjects.
Pomara et al. 2001 (347)	6 HIV+ (stable on ART) 9 HIV-	MRI & DTI analyses	Abnormal fractional anisotropy was found in the white matter of the frontal lobes and internal capsules of the HIV-1 patients.
Filippi et al. 2001 (351)	10 HIV+ (4/10 on HAART with undetectable VL)	MRI & DTI analyses	DA in the splenium and genu was significantly decreased in the non-treated group. Patients with the highest diffusion constant elevations and largest anisotropy decreases had the most advanced HIV disease.
Ernst et al. 2002 (371)	10 HIV+ 10 HIV-	fMRI	HIV+ group showed greater magnitude of brain activation ($p \leq 0.001$) in the lateral prefrontal cortex, with normal performance on a battery of neuropsychological tests. HIV+ patients also showed increased activated brain volume in the lateral prefrontal cortex ($p = 0.007$). The increase in activated brain volume was independent of task difficulty.
Patel et al. 2003 (362)	15 HIV+ (14/15 on HAART; 7/15 neuro-symptomatic and 8/15 neuro-asymptomatic)	MR Spectroscopy	Whole brain NAA levels were significantly different between patients and healthy subjects after adjustment for age and gender. Intermediate negative correlations were found between the NAA level, the processing speed subtest score and the ADC

Authors / year	Participant characteristics	Imaging modalities	Findings
	13 HIV-		stage score.
Chang et al. 2004 (370)	18 HIV+ stable on ART 18 HIV-	4T MRI & fMRI	HIV+ subjects showed similar task performance (accuracies and reaction times) but decreased activation in the normal visual attention network (dorsal parietal, bilateral prefrontal, and cerebellar regions) and increased activation in adjacent or contralateral brain regions. Results suggest that HIV-associated brain injury leads to reduced efficiency in the normal attention network, thus requiring reorganization and increased usage of neural reserves to maintain performance during attention tasks.
Ragin et al. 2004 (344)	6 HIV+ (4-male, 2-female) – all on ART 8 HIV- (6-male, 2-female)	1.5T MRI & DTI analyses	Whole brain FA measures were significantly reduced in the patients with HIV vs controls and was significantly associated with severity of dementia. There were no significant differences between groups regarding whole brain ADC.
Thompson et al. 2005 (334)	26 HIV+ AIDS patients (13 on HAART) 14 HIV-	MRI & VBM	AIDS patients had 15% thinner primary sensory, motor, and premotor cortices. Prefrontal and parietal tissue loss correlated with cognitive/motor deficits. Frontopolar and language cortex thinning correlated with CD4+ count reduction. No significant thickness differences were found between the HAART and No-HAART groups.
Ragin et al. 2006 (97)	11 HIV+ (9-male, 2-female) all on ART	1.5T MRI & DTI analyses	Increased MD and reduced FA measures in centrum semiovale correlated with plasma monocyte chemoattractant protein-1 (MCP-1) and TNF- α, respectively. For caudate, MD was correlated with MCP-1. For putamen, correlations were identified between MD and MCP-1 and between FA and haematocrit.
Andersen et al. 2006 (357)	95 HIV+ on ART completed fatigue inventory, inflammatory and HIV markers 16/95 had imaging studies	FDG-PET CT (N=9 fatigue+; N=7 fatigue-)	Imaging showed minor abnormalities in the cerebral metabolic rate of glucose. These were not associated with fatigue but correlated with a short HIV history ($p = 0.058$), a low CD4 nadir ($p = 0.082$) and elevated tumour necrosis factor-α levels.
Castelo et al. 2006 (369)	14 HIV+ 14 HIV-	fMRI	HIV+ group demonstrated reduced signal intensity changes in the right inferior frontal gyrus, right posterior hippocampus, and left lingual gyrus despite no differences on NP tests performance. Additionally, the HIV+ group exhibited more activity within lateral frontal and posterior parietal regions.
Gabis et al. 2006 (364)	4 HIV+ children stable on ART	MRI & MR Spectroscopy	A low NAA to Cho ratio correlated with poor performance. Results suggested that lower NAA and higher choline values represent

Authors / year	Participant characteristics	Imaging modalities	Findings
	4 HIV-		neuronal dysfunction and inflammation that can be recognized before anatomic changes appear on MRI.
Paul et al. 2008 (329)	22 HIV+ (majority taking ART) 20 HIV-	MRI with Volumetric analyses & MR Spectroscopy	HIV+ performed significantly worse on NP testing. Caudate size correlated with performances on higher-order cognitive tests, and putamen size correlated with performances on motor tests. The Choline/creatine ratio was significantly higher and the NAA/Cho ratio was significantly lower in HIV patients. MRS differences are more pronounced than area size differences between HIV+ and HIV- individuals in mild stages of HIV-related cognitive impairment.
Gongvatana et al. 2009 (346)	39 HIV+ (49% with AIDS) 25 HIV-	MRI & DTI analyses	Cognitive impairment in the HIV+ group was related to WM injury in the internal capsule, corpus callosum, and superior longitudinal fasciculus. WM injury was not found to be associated with HIV viral load or CPE of ART used.
Cohen et al. 2010 (249)	69 HIV+ asymptomatic 13 HIV+ ADC (80% on ART)	MRI & VBM analyses	Patients with ADC had decreased total grey matter and parietal cortex volumes with increased total ventricular volumes when compared to asymptomatic group. Volumes of grey and white matter, of the parietal, temporal, and frontal lobes and the hippocampus, were most strongly associated with disease history factors, such as nadir CD4 and duration of infection. While basal ganglia volumes were related to plasma HIV RNA.
Muller-Oehring et al. 2010 (348)	21 HIV+ 19 HIV-	MRI & DTI analyses	DTI metrics revealed poorer fibre integrity of the corpus callosum in HIV+ than controls that was more pronounced in posterior than anterior regions.
Ances et al. 2010 (170)	10 HIV+ cognitively asymptomatic 20 HIV- healthy controls	PiB PET CT	All participants had PiB PET scan, concomitant neuropsychometric and CSF assessments. Regardless of CSF Amyloid-β42 level, none of the HIV+ participants, even with HAND, had fibrillar amyloid plaques as assessed by (11)C-PiB mean cortical binding potential (MCBP). In contrast, some community controls with low CSF Amyloid-β42 had high (11)C-PiB MCBP with elevated binding potentials within cortical regions.
Chang et al. 2011 (374)	69 HIV+ clinically-stable 70 HIV-	MRI with morphometric analysis & ApoE genotyping	HIV+ group had smaller volumes throughout the brain regardless of their HAND status. Compared to ApoE4- subjects, HIV- controls with ApoE4 had better memory and larger global brain volumes (cerebral white matter and cortex) while HIV+ subjects with the ApoE4 had poorer cognition (verbal fluency, learning,

Authors / year	Participant characteristics	Imaging modalities	Findings
			executive function and memory) and smaller cerebral and cerebellar white matter and subcortical structures.
Ances et al. 2012 (360)	16 HIV+ (11 cognitively normal and 5 with HAND) 19 AD clinic participants (8 cognitively normal and 11 with symptomatic AD)	PiB PET CT	Participants with symptomatic AD had lower CSF Amyloid-β42 levels ($p < .001$), and had higher CSF tau levels ($p < .001$) than other groups. Regardless of degree of impairment, HIV+ participants did not have increased (11)C-PiB levels. Mean and regional binding potentials were elevated for symptomatic AD participants ($p < .001$).
Ances et al. 2012 (376)	26 HIV+ on stable HAART 26 HIV+ naive to HAART 26 HIV-	MRI & VBM analyses	HIV associated volumetric reductions within the amygdala, caudate, and corpus callosum occurred despite HAART. HIV and aging independently increased brain vulnerability.
Becker et al. 2012 (342)	84 HIV+ 76 HIV- (Men aged 50 years and over)	MRI & VBM analyses	Age-related GM tissue loss was found in the superior temporal and inferior frontal regions. HIV-related GM loss was seen in the posterior and inferior temporal lobes, the parietal lobes, and the cerebellum.
Jahanshad et al. 2012 (375)	55 HIV+ (on HAART) 30 HIV- (all participants aged 60-80 years)	MRI & DTI analysis ApoE genotyping	HIV+ individuals showed frontal and motor connections disruptions. Those HIV+ who carried the apolipoprotein E4 allele (ApoE4) had additional network structure deficits in temporal and parietal connections. The ApoE4 carriers showed greater brain network inefficiencies the longer they were infected. Neural network deficiencies in HIV+ populations exceed those typical of normal aging, and were worse in those genetically predisposed to brain degeneration.
Ragin et al. 2012 (377)	43 HIV+ (23-ART naïve; 20-inintiated on ART) 21 HIV-	3T MRI & Volumetric analysis	HIV group showed reductions in total ($p = 0.0547$) and cortical ($p = 0.0109$) grey matter. The early HIV group also demonstrated reductions in measures for total and cortical grey matter as well as weaker performance on neuropsychological tests, with the most pronounced difference in psychomotor speed ($p = 0.001$).
Towgood et al. 2012 (341)	20 HIV+ (younger - age 20-40) 20 HIV+ (older - age 50-75) All asymptomatic on ART 20 HIV- younger men 22 HIV- older men	3T MRI VBM & DTI analyses	VBM revealed reduced GM volume on MRI in HIV+ participants within the medial and superior frontal gyri. Older participants had less GM volumes in frontal, temporal, insular regions, independent of the effect of HIV. DTI revealed reduced FA and increased MD in frontal and temporal white matter tracts, all consistent with ageing process.
Bonnet et al.	400 unselected HIV+ (200	MRI with Volumetric	Prevalence of CI was 59%, including 21% - ANI, 31% - MND, and

Authors / year	Participant characteristics	Imaging modalities	Findings
2013 (378)	of them underwent MRI acquisition; median age 47 years; 89% received HAART)	analysis	7% - HAD. The presence of CI was significantly associated with lower grey matter volume. Symptomatic neurocognitive disorders were mainly related to traditional HAND determinants and associated with grey matter atrophy at early disease stages.
Baker et al. 2015 (379)	64 HIV+ on HAART (HAART CPE ranking was assessed)	MRI with Volumetric analysis	Participants were divided into high (>/=7; n = 35) and low (<7; n = 29) CPE groups. No significant differences in brain integrity were observed between the two groups. Long-term HAART regimens with a high degree of CPE were not associated with significantly improved NP or neuroimaging outcomes in HIV+.
Ipser et al. 2015 (365)	15 HIV+ (86% on HAART) 15 HIV-	RS fMRI	Reductions in connectivity were observed between the dorsolateral prefrontal cortex (DLPFC) and the dorsal caudate, particularly in younger HIV+ participants (<50 years). Cognitive impairment, but none of the assessed clinical or immunological variables, was associated with reduced frontostriatal connectivity.
Wade et al. 2015 (336)	63 elderly HIV + (all on ART) 31 HIV-	MRI & shape based morphometry	Normal cognition-33, ANI-14, MND-16, HAD-0. HIV + participants had diffuse atrophy, particularly in the caudate, putamen, hippocampus and thalamus with enlarged ventricular spaces.
Wilson et al. 2015 (337)	17 HIV+ (all on ART) 17 HIV-	3T MRI & VBM analyses MEG	HIV+ participants had reduced grey matter volume in the postcentral gyrus, parahippocampal gyrus, and other regions. Left prefrontal and postcentral gyrus abnormal magnetoencephalography (MEG) activity was correlated with neuropsychological performance.
Wang et al. 2015 (338)	30 HIV+ (all on ART) 15 HIV-	3T MRI & DTI analyses; MR Spectroscopy	HIV+ group performed significantly worse on verbal learning and visual memory. Cognitive impairment was associated with impaired conductive function and metabolic changes in hippocampus and parahippocampal gyrus.
Tang et al. 2015 (349)	21 HIV+ with a history of heavy psychostimulant use 22 HIV-	3T MRI & DTI analyses	HIV+ group displayed decreased WM integrity, with significantly lower FA values for all white matter tracts included in the analysis (the genu of the corpus callosum, left and right anterior limbs of the internal capsule, and the anterior commissure).
Ann et al. 2016 (366)	24 HIV+ (12/24 with HAND and 12/24 cognitively normal) 11 HIV-	RS fMRI	The HAND group, who had impaired cognitive function at the time of MRI scan, showed significant decrease of RS functional connectivity between bilateral precuneus and prefrontal cortex compared with non-HAND group.
Arenas-Pinto et al.	146 HIV+ (75 on PI-	MRI with Volumetric	The proportion with symptomatic CI was no different in the PI-

Authors / year	Participant characteristics	Imaging modalities	Findings
2016 (380)	monotherapy; 71-triple ART) 78/146 were imaged	analysis & MR Spectroscopy	mono or triple therapy groups (13% and 18%, respectively; p = .41). There was no difference in any of the neuroimaging variables (p > .05). Symptomatic CI was only associated with higher global cortical atrophy score (p = .005).
Jiang et al. 2016 (368)	9 HIV+ (>=50 years) 4 HIV-	fMRI	NP and fMRI data were acquired while subjects were performing the face-gender or word-semantic task. HIV+ participants were significantly slower in adapting to change in task demand, and the NP impairments are quantitatively related to difference in fMRI signal at the dorsal anterior cingulate cortex.
Wang et al. 2016 (283)	26 HIV+ 26 HIV-	3T MRI; VBM & DTI analyses	GM volume was reduced mainly in the bilateral frontal cortices, bilateral anterior cingulate cortex, and left supplementary motor area in HIV+ group. Decreased FA was observed in the genu and body of corpus callosum, and bilateral anterior corona radiate. Areas of increased MD, RD, and AD in HIV+ patients were more extensive and observed in most of WM skeleton. Results indicated that structural brain alterations occurred early in HIV+ participants.
Heaps-Woodruff et al. 2016 (352)	25 HIV+ 21 HIV+/HCV+ 25 Healthy controls	MRI & DTI analyses	All diffusivity metrics from five sub-regions of the corpus callosum were compared across groups. HIV+ and HIV+/HCV+ groups had significantly lower FA values and higher MD and RD values compared to HIV- controls. No differences were present between the HIV+ and HIV+/HCV+ groups, i.e. no additive effect of HCV was found. Duration of HIV infection was significantly related to FA metrics in total corpus callosum.
Zhang et al. 2016 (343)	15 HIV+ (aged 60-70) 94% on ART and 80% undetectable VL 22 HIV- (aged 60-70) Datasets from 80 HIV- MCI cases were used for analyses	3T MRI & Volumetric analyses	Three regions were significantly different for HAND vs. MCI (right cerebellum VIIb, right cerebellum VIII, left precentral) and HAND vs. controls (left precuneus, right precentral, right cerebellum VIIb). Morphometric group analysis revealed that HAND had significantly faster tissue loss than MCI or controls in those regions.
Underwood et al. 2017 (353)	134 HIV+ stable on HAART 79 HIV-	MRI; VBM & DTI analyses	HIV+ group had lower GM but not WM volumes, observed in regions where structure generally did not correlate with cognitive function. Widespread abnormalities in WM microstructure were found, including reduced FA with increased MD and RD. Diffusion abnormalities correlated with cognitive function.

Authors / year	Participant characteristics	Imaging modalities	Findings
PET CT - positron emission tomography–computed tomography; FDG – fluorodeoxyglucose; PiB – Pittsburgh compound B; MCBP – mean cortical binding potential; MRI – magnetic resonance imaging; BFR – bifrontal ratios; VBM – voxel based morphometry; DTI – diffusion tensor imaging; FA – fractional anisotropy; MD – mean diffusivity; RD – radial diffusivity; AD – axial diffusivity; GM – grey matter; WM – white matter; fMRI – functional magnetic resonance imaging; RS fMRI – resting state fMRI; MRS – magnetic resonance spectroscopy; NAA - N-acetylaspartate; Cr – creatine; Cho – choline; MEG – magnetoencephalography; HCV – hepatitis C virus; CI – cognitive impairment; MCI – mild cognitive impairment; ADC – AIDS dementia complex; HAND – HIV associated neurocognitive disorders; ANI – asymptomatic neurocognitive impairment; MND – mild neurocognitive disorder; HAD – HIV associated dementia; NP – neuropsychology; ART – antiretroviral therapy; HAART – highly active antiretroviral therapy; PI – protease inhibitors; CPE – CNS penetration effectiveness; CSF – cerebrospinal fluid; ApoE – apolipoprotein E			

3.2.8 Neuroimaging Correlated with the Use of Different Antiretroviral Regimens

Available studies showed no beneficial effect on brain volumes and cognitive function in stable HIV+ participants taking high CPE HAART versus low CPE HAART (379), or in those taking triple ART versus PI monotherapy (380).

3.2.9 Neuroimaging in the Early HIV Infection Stages

More recent high resolution MR imaging together with additional volumetric and DTI analyses in patients recruited in the early stages of the HIV infection are showing that grey matter atrophy (bilateral frontal cortices, anterior cingulate cortices and left supplementary motor area) and FA alterations in the genu and body of corpus callosum and anterior corona radiate, together with diffuse MD, RD and AD alterations occur early in the course of HIV disease (350, 377, 378). Thus, high resolution MRI combined with morphometric and tract-based spatial statistics, not only allow us to gain valuable insights into the neuropathology of HIV related brain injury, but also act as early disease biomarkers.

3.3 The Role of Neuroimaging Studies in the Assessment of HAND Progression

Numerous cross-sectional studies using various neuroimaging techniques have enabled detailed description of diffuse and complex HIV related brain structural, metabolic and functional alterations. While this has provided sensitive disease biomarkers and early disease markers, the role of neuroimaging in the evaluation of the disease progression in longitudinal studies remained underexplored until

recently. Lately, there has been a large number of follow-up imaging studies published, exploring neuroimaging markers of HAND progression in the context of HAART therapy. These are listed in Table 3.2.

3.3.1 Longitudinal Imaging Studies Prior to the Introduction of HAART

3.3.1.1 Serial standard MRI findings prior to the introduction of HAART regimens

Most neuroimaging follow-up studies in the pre-HAART era used standard clinical MRI acquisition and reported stability at follow-up. Post et al. (1992) found deterioration on sequential standard MR imaging at 12-24 follow-up interval, in only three of the 31 participants and this correlated with clinical progression of the HIV disease (381). Later, Post et al. (1993) reported mild abnormalities on standard MRI which did not progress further at 24-42 months follow-up in 64 HIV+ individuals (382).

However, Hall et al. (1996) and Dooneief et al. (1996) reported progressive brain atrophy on follow-up standard MR imaging in those with declining CD4+ cell count and those with worsening cognitive status (327, 383). Although most often increasing brain atrophy at follow-up was observed in patients with AIDS, Raninko et al. (1997) reported mild slowly progressive atrophy in 33% of neurologically asymptomatic HIV+ patients (384).

3.3.1.2 Serial MRI volumetric analysis findings prior to the introduction of HAART

Stout et al. (1998) used region of interest (ROI) volumetric analysis, a more sensitive marker, to evaluate for longitudinal white and grey matter atrophy. They found progressive white matter and caudate grey matter volume loss between 12

to 30 months follow-up, which was related to CDC stage of HIV infection and the rate of CD4+ cell decline (324).

Although standard MRI longitudinal studies and studies with volumetric analyses did not entirely agree with each other, the most consistent finding prior to the introduction HAART was that of progressive brain atrophy (white matter and subcortical grey matter atrophy) in those with worsening immune status i.e. HIV infection progression in association with progressive cognitive impairment. This suggests that if left untreated, HIV infection ultimately progresses and leads to brain atrophy and a deterioration in cognitive status. The divergences in the findings are possibly created by selection biases, wide variability in methodology, and short follow-up intervals in some of these studies.

3.3.1.3 Longitudinal SPECT studies prior to the introduction of HAART regimens

Longitudinal single photon emission computed tomography (SPECT) studies in HIV+ cohorts on no antiretroviral therapy showed either no major neuropsychological and neuroimaging deterioration (385), or slow decline of the agent (99mTc-HMPAO) uptake at follow-up associated with progression of neuropsychological abnormalities (386). Interestingly, there was an initial high cortical and subcortical uptake of 99mTc-HMPAO at baseline, indicating increased blood flow as a result of possible inflammation, which correlated with impaired cognitive function (386). Thus, SPECT findings suggest that there is high level of inflammation in the early stages of the disease and possible degeneration later. This correlates with the finding of myelin pallor and retroviral particles in the giant multinucleated cells and perivascular infiltrates on

neuropathology samples (72, 73, 75, 80, 81, 84, 123, 387), which also support the theory of neuronal tissue damage in the presence of the HIV virus and inflammation.

3.3.2 Longitudinal Imaging Studies after the Introduction of HAART

The effectiveness of HAART on peripheral viral suppression with the resulting improvement in immune status, reduction of AIDS defining disease burden and life expectancy increase is undoubted (8, 20, 50, 51, 388). However, it remains to be evaluated how the introduction of HAART changed the course of brain pathology. Neuropsychological studies, which were discussed in detail in Chapter 2, indicate that neurocognitive impairment is still highly prevalent in the era of HAART. Although it is milder and more stable, there is evidence of progressive cognitive decline on psychometric tests in a subset of patients. Longitudinal neuroimaging could potentially provide us with an answer whether this decline is directly related to HIV infection of the brain or to other mechanisms, as well as serving as a biomarker of disease progression.

3.3.2.1 Standard MRI follow-up studies in the HAART treated HIV+ individuals

Heikinheimo and colleagues (2015) followed up 17 HIV+ patients who were treated with best available treatment (5-17 years on ART) for 23 to 30 years. While these patients showed typical age related cognitive decline, only two of them demonstrated subtle progression of brain atrophy on standard MR imaging (389).

3.3.2.2 Follow-up studies using morphometric analysis in the HAART treated HIV+ individuals

In their MRI studies with volumetric analysis, Ances et al. (2012), Zang et al. (2016), and Correa et al. (2016) found little or no difference in regional brain volumes at 6 months, 10 months, or at a median of 26.6 months follow-up, respectively (339, 343, 390). Haynes and colleagues (2016) also reported no longitudinal change in the ROI volume over 4.2 years in HIV+ participants treated with HAART when compared with seronegative individuals in both the younger and the older group (>50 years old) (391). In contrast, Clifford et al. (2017) followed up 39 older (over 60 years of age) HIV+ individuals who were virally suppressed for a median of three years with an average duration of HIV infection of 21 years and found more rapid average annualised rates of atrophy of cerebellum, brainstem, pallidum, caudate, total grey matter, and frontal lobes (392).

Pfefferbaum et al. (2014) followed up 51 HIV+ individuals (80% of them on HAART at baseline) and 65 seronegative controls for between six months and eight years and found acceleration of normal volume loss in the sensori-motor and frontal cortices, and hippocampus. Those who responded to HAART treatment by increasing CD4+ cell count had a slower atrophy progression of the frontal, temporo-parietal cortices, hippocampus and insula (393). Similarly, in an earlier study, Cardenas et al. (2009) compared 39 HIV+ individuals on HAART with 30 seronegative healthy controls and reported a higher rate of cerebellar, brainstem, thalamus, and caudate volume loss associated with lower CD4+

counts and a higher rate of total grey matter, frontal or parietal grey matter loss in those with detectable HIV RNA (394).

Sanford et al. (2018) reported that, although cortical thickness and subcortical volumes were smaller at baseline in HIV+ patients with controlled disease on HAART, when compared with seronegative individuals, the rate of brain volume changes over a two year period were similar between the groups (395).

The published longitudinal volumetric studies in the era of HAART suggest that, although at baseline those with a controlled HIV infection on HAART have smaller white and grey matter volumes than seronegative healthy controls, the rate of brain tissue volume loss is slowed down by ART treatment and is similar to the rate of volume loss in the seronegative controls. Moreover, accelerated brain tissue atrophy was correlated with the markers of HIV infection and its duration. The older age group of HIV+ patients on HAART is probably experiencing faster rates of brain atrophy, but this has to be yet further explored. It is important to note the heterogeneity of the study participants in these trials (i.e. different HIV infection stages, a mixture of ART treated and untreated patients, different disease duration, neurologically symptomatic and asymptomatic participants, and a wide age range), as well as different follow-up periods ranging from months to years even within one given trial, which make conclusions difficult to generalise.

3.3.2.3 Longitudinal DTI imaging

O'Connor et al. (2017) found microstructural white matter stability measured by DTI parameters at two and six months follow-up after commencement of ART treatment (396). Interestingly, in this sample, the FA was increased and MD, RD,

and AD were reduced in the seropositive group when compared with seronegative healthy control group, contrary to the findings of other studies that describe FA reductions (345, 351, 397-401). Such inconsistency has also been reported before in the HIV+ HAART naïve populations and reversed six months after the introduction of HAART (402). Authors postulated that discrepancies between the findings of different studies (most of them being cross-sectional studies) were probably arising from the wide methodological and sample variabilities.

Chang and colleagues (2008) in their one year longitudinal evaluation of DTI parameters in 39 HIV+ participants stable on HAART compared to 32 seronegative controls, found progression of MD increase in HIV+ group in frontal and parietal white matter, putamen and genu, with the greatest increase at follow-up being in the genu of corpus callosum ($p=0.0008$), which were correlated with the changes in cognitive status (345). Jones and colleagues (2018) at two year follow-up found reductions in FA values in the anterior thalamic radiations and the superior longitudinal fasciculus in the HIV+ patients, which correlated with greater individual variability on psychometric test performance (403).

In addition, Haynes et al. (2018) found greater increase in MD in the corpus callosum, the right posterior corona radiata and right posterior thalamic radiation in the HIV+ group at 4.2 year interval in 30 HIV+ virally suppressed individuals compared to 25 seronegative controls (391). The FA values, however, showed a greater decrease in the right anterior corona radiata, right anterior limb of the internal capsule, and the right genu and splenium of the corpus callosum in the older group (age=/> 50 years), in comparison with the younger group irrespective

of the HIV status. The HIV+ group had a greater cognitive impairment at follow-up which was associated with a greater increase in MD and a greater reduction in FA. Together, these findings suggest that HIV status and age are independently affecting DTI measures and cognition at follow-up. However, this study, as well as the other studies described above, is underpowered by the small number of subjects studied.

In contrast to the above findings, Correa and colleagues (2016) found no difference in DTI parameters at a median 26.6 months follow-up in 21 HIV+ individuals stable on ART (390). This study, however, had a shorter follow-up interval than the study reported by Haynes et al. Furthermore, Cole et al. (2018) evaluated DTI measures and cognitive performance in a more robust study in 123 HIV+ participants stable on ART (median age = 56.0 years) and in 78 matched controls (median age 57.2 years) at baseline and at a two year follow-up. They also found no difference in the rate of change in both cognitive status and neuroimaging measures between the groups at follow-up, although the HIV+ group scored lower on cognitive tests and had abnormal DTI parameters at baseline when compared with healthy controls (404). This suggests that effective treatment provided imaging and cognitive function stability over the two years interval.

The findings of longitudinal DTI studies are inconsistent, with some showing progression of the brain microstructural changes at follow-up and some showing stability in the stable HAART treated HIV+ cohorts. Such inconsistencies might be arising due to the differences in methodological approaches, variability of studied cohorts and variable follow-up intervals.

3.3.2.4 Longitudinal fMRI in HIV+ individuals treated with HAART regimens

Ernst et al. (2009) reported that, although HIV+ participants maintained neurocognitive task performance, at 12 months follow-up they showed declined efficiency with increased signal in the pre-frontal and posterior parietal cortices on fMRI. The control population, however, maintained task performance in association with decreased brain activation, which was concluded to be due to practice effects. This suggests that while slow cognitive decline can be missed on psychometric tests, fMRI shows increased use of brain reserve (405). Correa et al. (2017) also reported increased compensatory functional connectivity in fronto-parietal, visual pathways, and cerebellar networks on resting state fMRI (RS fMRI) in a cohort of virally suppressed HIV+ individuals at an average of 30 months follow-up (406). However, this study had no control group. Furthermore, Cole and colleagues (2018) reported no difference in the rate of change in RS fMRI between HIV+ individuals and seronegative controls at 24 months follow-up (404).

3.3.2.5 Longitudinal MR spectroscopy in HAART treated individuals

Congvatana et al. (2013) followed up 226 HIV+ individuals on HAART over 24 months (only 51% of participants were both plasma and CSF virally suppressed) and found progressive NAA decrease (a marker of neuronal integrity which is usually reduced in individuals with cognitive impairment) in the frontal grey and white matter in the symptomatic as well as the asymptomatic patients (407). Those symptomatic had more prominent progressive NAA reductions in the basal ganglia, while Cho, a marker of membrane turnover and MI, a marker of glial

proliferation, decreased contrary to the findings of cross-sectional studies which might suggest less active inflammation. These findings suggest continuous neuronal damage, despite the successful viral suppression. Cole et al. (2018), however, reported no significant difference in the rates of change in any neuroimaging variables including MRS, at 24 months follow-up when compared 123 virally suppressed HIV+ individuals with 78 closely matched seronegative controls (404).

3.3.3 Summary of Longitudinal Neuroimaging Studies in the Context of Treated HIV

The longitudinal imaging studies in those with controlled HIV disease suggest cognitive function stability with minor volumetric or diffusion tensor imaging changes over relatively short follow-up intervals. They show important white matter microstructural damage associated with white and grey matter volume differences between HIV+ cohorts and healthy controls at baseline. Those with detectable HIV RNA and older patients showed more significant disease progression at follow-up imaging. The fMRI and MR spectroscopy could be more sensitive to the slow insidious progression of brain injury in the context of treated HIV.

Table 3.2. Longitudinal Neuroimaging Studies in HIV-positive Individuals with Disease Related Cognitive Impairment

Author, year	Subjects description	Follow-up time	Imaging technique	Results
A. Pre HAART and non-HAART treated cohort studies				
Post et al. 1992 (381)	asymptomatic HIV+ (n = 20) neurologically symptomatic HIV+ (n = 11)	1-2 years	MRI (standard)	27 - no progression of MR (18 with minimally abnormal scans who remained asymptomatic with improved or static neuropsychologic performance) 1 - improvement 3 - MR and neurologic progression (with 1 – NP progression and autopsy confirmed presence of HIV-1 containing multinucleated giant cells in the brain)
Post et al. 1993 (382)	asymptomatic HIV+ (n=64) symptomatic HIV+ (n=10)	24-42 months	MRI (standard)	Most asymptomatic subjects (80%) had persistently normal MR images. Half of symptomatic and 20% of asymptomatic subjects had mild unchanged abnormalities. The imaging abnormalities were usually minor and static.
Dooneief et al. 1996 (383)	HIV+ HIV- Gay men and IVDU	1 year	MRI (standard)	Those HIV+ subjects with CD4 count < 200 cell/ml at baseline, had an increased burden of white matter hyperintensities. Atrophy increased in association with declining CD4 count and/or neurologic deterioration.
Hall et al. 1996 (327)	asymptomatic HIV+ (n=15) 32 with AIDS	30 months	MRI, bicaudate/brain ratio (BCR) and bifrontal/brain ratio (BFR)	Increase in atrophy correlated with worsening in cognitive function. Changes were more pronounced in the symptomatic group.
Raininko et al. 1997 (384)	61 HIV+	2.5-66 months	MRI (standard)	(39%) showed the development and/or progression of atrophy mild developing/progressive atrophy was found in 33% of asymptomatic or neurologically intact subjects
Stout et al. 1998 (324)	86 HIV+ 23 HIV-	6 monthly 2-5 f/ups	MRI, ROI volumes	Reduction of white matter volume and caudate nucleus volume was accelerated in CDC stage C disease and correlated to rate of CD4+ decline.
Christensson et al.	24 HIV+	6-46	SPECT	High cortical and subcortical 99mTc-HMPAO uptake correlated with

Author, year	Subjects description	Follow-up time	Imaging technique	Results
1999 (386)	24 controls	months		low performance in cognitive dysfunction tests, due to possible inflammatory reaction in the brain. Significant decline in 99mTc-HMPAO uptake over time and progression of neuropsychological abnormalities.
Samuelsson et al. 2006 (385)	28 early stage HIV	7 years two yearly	SPECT MRI	No major deterioration in the neurological, psychological performance, or neuroimaging examinations.
B. HAART treated cohort studies				
Chang et al. 2008 (345)	HIV+ (n=39) ART stable HIV- (n=32)	12 months	3T MRI DTI	Increase in MD in frontal and parietal WM, putamen, and genu. Changes in global cognitive deficit score correlated with changes in MD in the genu and FA in the parietal and frontal WM and putamen. Greater than normal age-related inflammatory changes (MD) in the genu of HIV patients
Cardenas et al. 2009 (394)	39 HIV+ patients on ART; 30 HIV- controls	24 months	MRI, VBM ROI morphometry	Faster rates of volume loss in cerebellum, brainstem, thalamus, and caudate, in those with poorer immune function (lower CD4). Amongst those with detectable HIV RNA, higher log viral loads were correlated with greater volume loss in total GM, and individually within frontal, and parietal GM regions.
Ernst et al. 2009 (405)	31 HIV+ stable ART 32 HIV-	12 months	fMRI	Declined efficiency in HIV+ patients – compensatory increase in fMRI signals in the prefrontal and posterior parietal cortices for the more difficult tasks in HIV+, whereas HIV- controls showed decreased brain activation due to practice effect. Significant interactions between HIV status and time of study in left insula, left parietal, left temporal, and several frontal regions (left and right middle frontal gyrus, and anterior cingulate).
Lentz et al. 2011 (408)	9 HIV+ (2 on ART at baseline and 7 eventually on ART) 9 HIV- controls	2 months 6 months	MR Spectroscopy	Changes in lipid membrane metabolism (initial increase in Cho levels) in the frontal cortex and white matter during the first year of HIV infection – a marker of inflammation. CD16+ monocytes were associated with lower NAA levels – a marker of neuronal damage. (No treatment effect was found).
Ances et al. 2012 (376)	HIV+/HAART+; n = 26 HIV+/HAART-; n = 26 HIV- ;n = 26 12 HIV+/HAART- had longitudinal	6 months after c/o HAART	MRI, VBM	Significant reductions in subcortical brain volumes (amygdala, caudate, and corpus callosum) compared with HIV- participants. However, HAART did not affect brain structure as regional volumes were similar for HIV+/HAART- and HIV+/HAART+. No association existed between NP performance and VBM.

Author, year	Subjects description	Follow-up time	Imaging technique	Results
	assessment after starting HAART			
Wright et al. 2012 (402)	HIV+/HAART+; n = 21 HIV+/HAART-; n = 21 HIV- ;n = 21 10 HIV+/HAART- had longitudinal assessment after starting HAART	6 month after c/o HAART	MRI, DTI	The HIV+/HAART- participants had significantly lower MD, AD, and RD in all corpus callosum regions and the centrum semiovale compared to HIV- and HIV+/HAART+ individuals. In some HIV+ subjects, initiation of HAART led to significant increases in MD, RD, and AD but not FA. Changes in DTI parameters in the WM observed after the HAART commencement could reflect reduced neuro-inflammation.
Gongvatana et al. 2013 (407)	226 HIV+ on stable CART, (138 of them asymptomatic)	24 months	MR Spectroscopy	Significant annual decreases in brain metabolite levels in all regions examined, including NAA (2.95 %) and Cho (2.61 %) in the FWM (frontal); NAA (1.89 %), Cr (1.84 %), Cho (2.19 %), and Glx (6.05 %) in the MFC (mid frontal cortex); and Glx (2.80 %) in the BG (basal ganglia). Similar metabolite decreases were observed in the asymptomatic and those with subclinical impairment. Neurocognitive decline was associated with longitudinal decreases in Glx in the subcortical structures: FWM and the BG, and in NAA in the BG.
Pfefferbaum et al. 2014 (393)	51 HIV+ 80% on ART at baseline 65 HIV- controls	6 months to 8 years	MRI, VBM ROI	Expected age-related decrease in volume. Acceleration of the normal ageing trajectory, including neocortex (the frontal and sensorimotor neocortices, and hippocampus), and the thalamus with longer disease duration. Those with increasing CD4 counts exhibited slower expansion of Sylvian fissure volume and slower declines of frontal and temporoparietal cortices, insula, and hippocampus tissue volumes.
Heikinheimo et al. 2015 (389)	80 HIV+ on best available treatment	1986-1990; 1997; 2013	1.5 T MRI, f/up 3 T MRI, BCR	HIV-seropositive patients, while on best-available treatment, showed no evidence of HIV-associated neurocognitive disorder on neuropsychological and neuroradiological evaluations.
Correa et al. 2016 (390)	21 HIV+ HAART (CD4 >200 cell/ml and VL <50copies/ml)	Median 26.6 months	1.5T MRI, VBM cortical thickness, and DTI	There were no significant differences in cortical thickness, deep grey matter structures volumes or diffusivity parameters between scans at the two time points (P-value set at< 0.05).
Zhang et al. 2016 (343)	Ageing HAND (n=15) 95% on ART and HC (=21) (age 60-70); demographically matched MCI cases	At least 10 months	3T MRI, VBM ROI	The change between two time points and same ROI was small. The cerebellum VIIb was the only region that significantly distinguished HAND from MCI and HAND from controls.

Author, year	Subjects description	Follow-up time	Imaging technique	Results
	(n=80); and HC (n=26)			
Clifford et al. 2017 (392)	HIV+(n = 38) virally suppressed (39% HAND), and age-matched HIV-HC (n = 24) Mean age 63	Over 21 years	MRI, VBM	HIV serostatus was associated with more rapid average annualized rates of WM atrophy in the cerebellum (0.42% vs. 0.02%, P = 0.016), caudate (0.74% vs. 0.03%, P = 0.012), frontal lobe (0.48% vs. 0.01%, P = 0.034), total cortical grey matter (0.65% vs. 0.16%, P = 0.027), brainstem (0.31% vs. 0.01%, P = 0.026), and pallidum (0.73% vs. 0.39%, P = 0.046).
Correa et al. 2017 (406)	HIV+ (all virally suppressed on HAART with no dementia)	Average 30 months	1.5T MRI, VBM, DTI, RS-fMRI	No significant differences in cortical thickness, deep GM volumes, or diffusivity variables were identified at the two time points. At follow-up, HIV+ had compensatory increased areas of functional connectivity in visual pathways, fronto-parietal and cerebellar resting state networks ($p < 0.05$).
O'Connor et al. 2017 (396)	9 HIV+ 12 HIV- controls	Baseline, 3 and 6 months after ART initiation	MRI, DTI	FA and MD measures were very stable during 6 months, with intra-class correlation coefficients all >0.96. The high longitudinal reliability of DTI WM microstructure measures makes them promising disease-activity markers
Cole et al. 2018 (404) COBRA study	123 HIV+ (median age = 56.0 years) virally suppressed and 79 HIV- controls (median age = 57.2 years)	24 months	MRI, DTI, RS-fMRI, MR spectroscopy, arterial spin labelling	At baseline, HIV+ had poorer global cognitive performance (P<0.01), lower grey matter volume (P=0.04), higher white matter hyperintensities load (P=0.02), abnormal white-matter microstructure (P<0.005) and greater 'brain-predicted age difference' (P=0.01). Longitudinally, there were no significant differences in rates of change in any neuroimaging measure between PLWH and HIV-negative controls (P>0.1).
Haynes et al. 2018 (391)	15 HIV+ (aged 20-40 years) 15 HIV+ (aged >/= 50 years) all stable on HAART, 9 HIV- (aged 20-40 years) 16 HIV- (aged >/= 50 years)	4.2 years	MRI, VBM, DTI	DTI showed that the HIV+ group had a greater increase in MD, but there were no group differences in volume change using VBM. The change in cognitive performance was correlated with change in the DTI measures, and this effect was stronger for the HIV+ participants.
Jones et al. 2018 (403)	38 HIV+ 26 HIV-	24 months	MRI, DTI	Increases in IIV (Intra Individual variability of NP performance - greater dispersion) were related to lower FA values in the anterior

Author, year	Subjects description	Follow-up time	Imaging technique	Results
				thalamic radiations (ATR) and the superior longitudinal fasciculus (SLF) in HIV+ group.
Sanford et al. 2018 (395)	48 HIV+ stable on HAART 31 HIV-	24 months	MRI, VBM	Cortical thickness and subcortical volumes were smaller in HIV-positive individuals compared with controls. However, changes in brain volume over time were similar between the groups.
Underwood et al. 2018 (409)	139 HIV+ virally suppressed (CHARTER cohort)		MRI, VBM	T1-predicted age exceeded chronological age changes greater those with confounding comorbidities and prior AIDS.

3.4 Aims

The follow-up MRI study had two aims:

- Aim 1: To determine whether or not there is progression of neuro-degeneration at follow-up in a well characterised group of 50 HIV+ patients by comparing the baseline and follow-up VBM data.
- Aim 2: To evaluate whether or not there is progression of white matter damage and role of persisting neuro-inflammation at follow-up in a well characterised group of 50 HIV+ patients using the baseline and follow-up DTI data analysis.

3.5 Objectives

The objectives of this study were to:

- Recruit the 50 subjects who participated in the baseline "MRI Brain Imaging in HAND" study for follow-up MRI data acquisition (49)
- Compare participants' baseline VBM data with follow-up VBM data and with the VBM imaging data acquired from the control group to assess for progression of grey matter tissue loss
- Compare participants' baseline DTI data with follow-up DTI imaging and with the DTI imaging data acquired from the control subjects to assess for evidence of persistent inflammatory changes and progressive white matter damage.

3.6 Ethical Approval

The researcher adhered to the Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki while acquiring the MRI and other clinical and demographic data for the study titled “Follow-up brain MR imaging in a sub-cohort of well characterised HIV+ patients”. The study was approved by the Combined Ethics Committee of the Adelaide and Meath Hospitals Incorporating the National Children’s Hospital (AMNCH) and St. James’s Hospital (SJH) (REC Reference: 2014-09 List 33 (9) 2014-10 list 37 (8)). Participation in the study was voluntary and it was explained to patients that their refusal to participate or withdrawal from the study would not affect their ongoing medical care. All participants were provided with the participant information leaflet, containing the description of the study aims, study objectives, inclusion and exclusion criteria, and risks associated with MRI data acquisition. All potential participants were given the opportunity to ask any questions about the study. A written informed consent was provided by all subjects before their participation (Appendix 7). All patients were provided with a phone number to contact the researcher if they had any further questions or if they wished to cancel or re-schedule their appointment for MRI data acquisition.

3.7 Methods

3.7.1 Study Design

This was an observational prospective follow-up multimodal MR imaging study.

3.7.2. Study Site

Neuroimaging data acquisition took place at the Centre for Advanced Medical Imaging (CAMI) in SJH for all study participants at baseline and follow-up, and for all healthy controls using the same scanner (3 Tesla, Achieva, Philips Medical Systems) at both time points.

3.7.3 Study Population

The potential participant population included the 50 patients who attend the HIV services in SJH and who underwent a detailed Neurocognitive assessment (at baseline T_1 and follow-up T_2) and had MR imaging acquired at baseline – Time Point 1 (TP1), following a positive screening test for cognitive impairment. During the recruitment for the follow-up Neuroimaging assessment, patients who met all of the inclusion and none of the exclusion criteria, were approached during their routine clinic appointment with HIV services and were asked to volunteer to participate in the follow-up study for Time Point 2 (TP2) MR data acquisition.

3.7.4 Inclusion and Exclusion Criteria

To be eligible for follow-up MRI acquisition, apart from the inclusion criteria outlined in Chapter 2 for neuropsychology follow-up assessment, potential participants had to meet the following additional inclusion criteria:

- Have had an initial MR imaging acquired at TP1
- Have undergone follow-up (T_2) detailed neuropsychological assessment
- Be willing to participate in the follow-up MRI study.

Exclusion criteria for the follow-up MRI study participation are outlined below:

- Patients who did not have the detailed Neurocognitive and Neuroimaging assessment at baseline
- Patients who were not eligible for follow-up Neuroimaging assessment or who were eligible but did not participate in the follow-up detailed Neurocognitive assessment study
- Patients who were acutely unwell or unfit to partake in the MR imaging acquisition study.

Additional exclusion criteria for the follow-up MRI study included other contraindications for MRI acquisition for standard safety reasons:

- Pregnancy
- Cerebral aneurysm clip
- Cochlear implant
- Presence of metal in the eye or orbit
- Implanted spine stabilization rods
- Other metallic implants
- Implantable cardiac devices (MRI incompatible Pacemaker or ICD) in situ
- Implanted insulin pump
- Implanted analgesics or other medicines pump
- Implanted nerve stimulators
- Severe claustrophobia
- Patients who weigh more than 250 kg.

3.7.5 MRI Data Acquisition

Magnetic resonance (MR) data were acquired on a 3 Tesla Philips Achieva system using an 8-channel receive-only head coil. The same scanner was used at both time points (TP1 and TP2), using identical imaging protocol for both time points. To allow for tissue volumetric analysis, high resolution T1-weighted images were acquired using a 3D volumetric fast gradient echo sequence with spatial resolution = 1 x 1 x 1 mm^3, field-of-view (FOV) of 256 x 256 x 160 mm, TR/TE = 8.5/3.9 ms, flip angle = 8°, SENSE factor = 1.5. Diffusion tensor images (DTI) were acquired using a spin-echo planar imaging (SE-EPI) sequence with a 32-direction Stejskal-Tanner diffusion encoding scheme: FOV = 245 x 245 x 150 mm, 60 slices with no inter-slice gap, spatial resolution = 2.5 x 2.5 x 2.5 mm^3, TR/TE = 8200 / 59 ms, SENSE factor = 2, b-values = 0, 1100 s/mm2, with SPIR fat suppression and dynamic stabilization in an acquisition time of 5 min 53 s. MRI protocols are presented in Appendix 8.

Following the file conversions and quality assessments, imaging data were deemed suitable for quantitative analyses for 50 healthy controls, as well as for 49 HIV seropositive patients who had high-quality baseline scans at time point-one (TP1). In addition, 42 of them had high-quality follow-up (TP2) MR data, while seven patients were lost to follow-up (two patients died, two did not consent to follow-up, one patient moved to another city and was no longer attending GUIDe services in SJH, and two had claustrophobia). For comparative analyses, only the 42 HIV positive patients who had both TP1 and TP2 scans were included.

3.7.6 Demographic and Clinical Data Collected and Recorded

The baseline “MRI Brain Imaging in HAND” study database was updated at TP2 to include age at follow-up and the time interval between the scans for those participants who agreed to participate in the study. In addition, clinical data (any changes in medical history, ART therapy, HIV viral load, CD4 count at follow-up) were also updated.

3.7.7 Missing Data

While 50 patients were recruited and had MR data acquisition at TP1, and 43 of them were re-enrolled and had their MRI acquired at TP2, imaging data acquisition was incomplete for one of the participants at TP1. Therefore, this participant’s imaging data were excluded from the analysis and data acquired from 42 patients was analysed.

3.7.8 Data Protection

Participants’ demographic and clinical data was pseudo-anonymised using the unique study number assigned at baseline and stored in a password protected Microsoft Excel 2013 database. Imaging data was also pseudo-anonymised using a unique number assigned by CAMI. MR images were stored on a password protected hard drive device. All collected and stored data were treated in accordance with Data Protection Act of 1988 & Data Protection Amendment Act of 2003.

3.7.9 Grey Matter Analyses

Voxel based morphometry (VBM) was performed to assess grey matter regional density alterations and to evaluate patterns of grey matter pathology. The FMRIB's software library (FSL) (410) was used to perform VBM analyses (285). Following brain extraction and tissue-type segmentation, grey matter partial volume images were aligned to the MNI152 standard space using affine registration. Grey matter partial volume estimates were non-linearly co-registered to a study specific template, modulated by a Jacobian field warp and smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. For comparison of the study groups with concomitant controlling for the age and gender (411, 412), the threshold free cluster enhancement (TFCE) method (413) and permutation based nonparametric inference were used. Statistical significance was set at $p < 0.05$ family wise error (FWE).

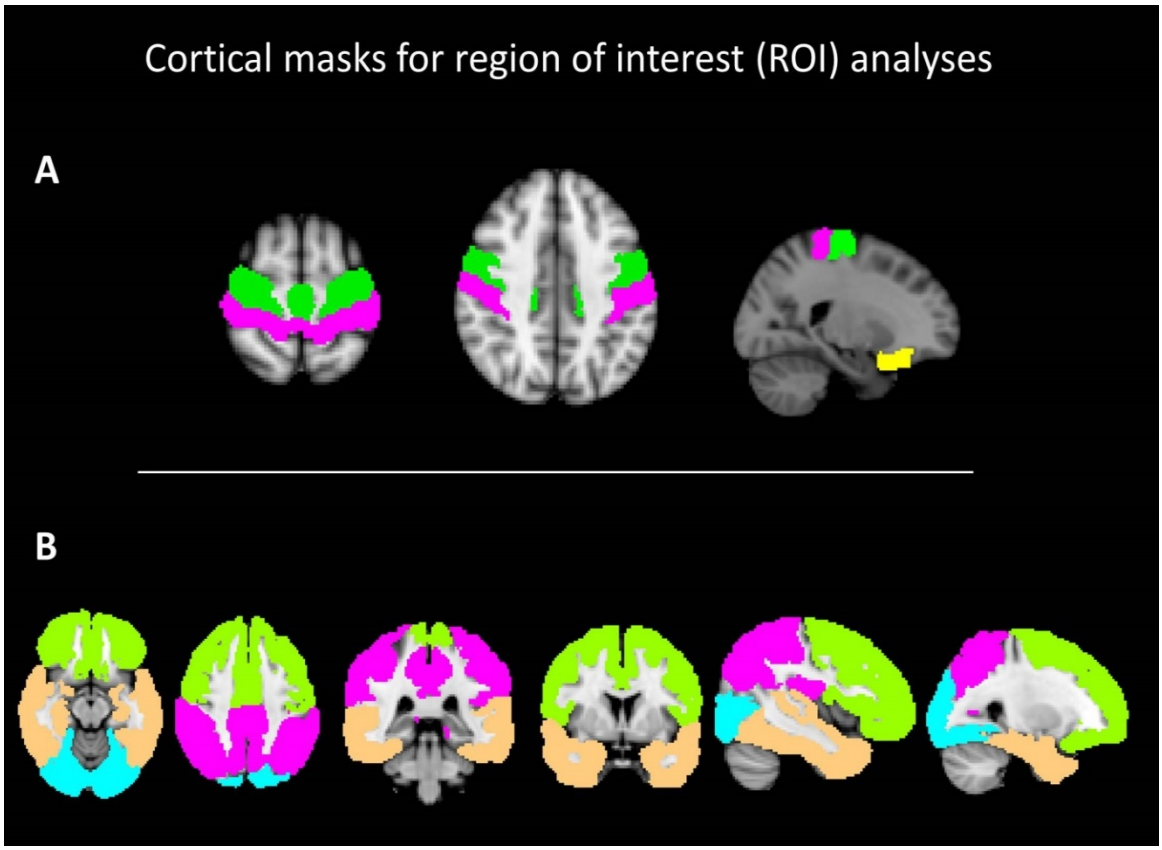
3.7.10 White Matter Analyses

Pre-processing of raw diffusion tensor imaging (DTI) datasets included eddy current corrections, motion corrections, and brain-tissue extraction using FMRIB's software library (FSL) (414). The axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA) maps were generated subsequent to diffusion tensor model fitting. Tract based spatial statistics (TBSS) and permutation based nonparametric inference were used to compare the study groups' white matter profiles. The threshold free cluster enhancement (TFCE) method was applied (413, 415). Design matrices included age and gender as covariates. Statistical significance was set at $p<0.05$ FWE.

3.7.11 Region of Interest (ROI) Analyses

In addition to the above standard “whole-brain” analyses, supplementary region of interest (ROI) analyses were performed using atlas based cortical grey matter and tract based white matter segmentation. Cortical grey matter ROIs included the bilateral precentral gyrus, bilateral postcentral gyrus, bilateral orbitofrontal cortex masks, and bilateral masks for the occipital, parietal, frontal and temporal lobes. Cortical masks for these ROIs were defined based on the Harvard-Oxford Cortical (HO) Atlas (416, 417) and the Montreal Neurological Institute (MNI) Atlas (418). For each ROI mask, the 2mm 25% threshold maps of the original atlas labels were used. The resulting ROI masks are shown in Figure 3.1.

Subsequently, the average T1-singal intensity values were retrieved from the above seven ROI masks for each study participant. For each study group, partial volume estimates were plotted in box plots to illustrate the group level longitudinal grey matter alterations in the cortical regions, described above. In addition, analyses of covariance (ANCOVA) were performed correcting for age. These exploratory statistics were carried out with IBM’s SPSS Statistics Version 22. Assumptions of normality, linearity, and homogeneity of variances were verified. Regional mean grey matter partial volume estimates were included as the dependent variable, study group allocation – as the categorical independent variable, and age at the time of MR imaging acquisition was used as the covariate. Following Bonferroni corrections for multiple testing for the seven ROIs, a p-value ≤ 0.0071 was considered significant.



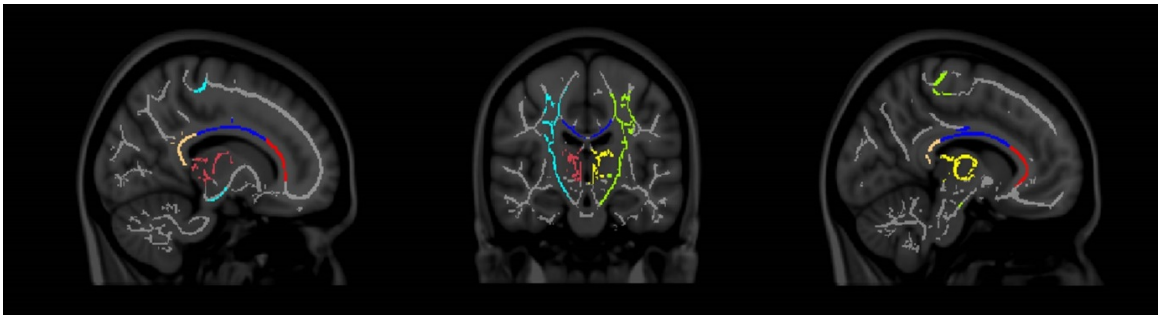
A – Masks defined by the Harvard-Oxford cortical atlas for the precentral gyrus (green), postcentral gyrus (purple) and orbitofrontal lobe (yellow). **B** – ROIs defined based on the MNI atlas for the frontal (lime), parietal (purple), occipital (aquamarine) and temporal lobes (gold).

Figure 3.1. Atlas-based region of interest (ROI) masks

Similar to the cortical ROI masks, tract based white matter masks were created for corticospinal tracts (CSTs), genu, body and splenium of corpus callosum and for thalamic white matter regions (Figure 3.2). These regions were defined using the JHU DTI based white matter atlas (419) which consists of 48 white matter tract labels created from the manual segmentation of DTI maps obtained from brain images of 81 subjects.

Average AD, FA, RD, and MD values were retrieved from the above white matter masks. Intergroup ANCOVAs were performed correcting for age and illustrative

box plots were generated. The aforementioned statistical analyses were carried out with IBM's SPSS Version 22. Assumptions of normality, linearity, and homogeneity of variances were verified. Average regional FA, AD, RD, or MD values were included as dependent variable, study group allocation - as categorical independent variable, and age at the time of MRI scan was used as covariate.



Atlas based white matter ROIs for the corticospinal tracts (green – left CST and aquamarine – right CST), genu (red), body (blue) and splenium (gold) of the corpus callosum, and thalamic white matter tracts (yellow – left thalamic tracts and pink – right thalamic tracts).

Figure 3.2. Atlas based white matter ROIs

3.7.12 Role of the PhD Candidate and Acknowledgements

The candidate performed a comprehensive literature review on available neuroimaging studies in PLWH with disease associated cognitive impairment. The candidate identified, recruited and re-enrolled all of the follow-up study participants and consented them before the follow-up MRI data acquisition. The

candidate created and maintained the follow-up study database and updated it with the follow-up neuropsychology and clinical data.

The processing and visualisation of the MRI imaging data was carried out with the assistance and support of The TCD Computational Neuroimaging Group led by Dr Peter Bede, with additional statistical analyses performed by the PhD candidate.

3.8 Results

3.8.1 Characteristics of MRI Study Participants

Of the 104 subjects enrolled in the initial / baseline "Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment", 50 participants also underwent brain imaging acquisition at baseline – TP1 (49, 420). Of these, 43 agreed to a follow-up MRI scanning at the time of, or soon after their follow-up neuropsychological assessment (T_2). However, one patient had an incomplete MR data acquisition at TP1. For this reason, following the pre-processing standardised MRI data quality assessments, this patient's data were excluded from the follow-up study analyses.

Of the 50 patients scanned at TP1, seven were lost to follow-up (two patients died, two did not consent to a follow-up MRI acquisition due to work or family commitments, one patient moved home and transferred care to another hospital, one patient declined the opportunity of follow-up scanning due to claustrophobia, and one patient's scanning was discontinued within the first 10 seconds due to

severe claustrophobia). All of the above led to a total attrition rate of 14.3%. Attrition rate amongst those who met diagnostic criteria for HAND at TP1 (43/49) was 14% (6/43) and amongst those who did not – 16.7% (1/6).

The characteristics of the 49 participants who completed the baseline MR scanning and the 42 participants who partook in the follow-up MR study as well as the seven patients who were lost to follow-up are described in Table 3.3. The majority of follow-up study participants were male (81.0%), Irish (66.6%), and spoke English as their first language (73.8%). The mean age at follow-up was 48.6 years (SD 10.28), ranging from 31 to 75 years. The mean follow-up interval was 41.23 months (SD 7.58), ranging from 29 to 59 months. All but one patient, who received ART as MTCT, were on ART (97.6%) at the time of follow-up study (TP2), and 40/42 (95.2%) were virally suppressed. However, only 33/42 (78.6%) were continuously virally suppressed during the follow-up interval. Of the two participants who were not virally suppressed at TP2, one had low grade viremia, and the other participant displayed high grade viremia at the time of follow-up assessment. The latter received ART, as MTCT and was not taking ART at the time of TP2 assessment.

Eight of the nine viral hepatitis C (HCV) co-infected patients who enrolled in the baseline study returned for follow-up. At TP2, 19.0% of participants were HCV co-infected. Three quarters (3/4) of the viral hepatitis B (HBV) co-infected patients who participated in the baseline MR acquisition were retained in the follow-up study representing 7.1% of those who were followed up. The majority of those who retained in the study – 73.8% (31/42) never used illicit substances and 54.8% (23/42) were employed at the time of follow-up. A total of 13/42 (31.0%)

screened positive for anxiety and this number is similar to that at TP1, while six of them (14.3%) also screened positive for depression which is double as much as at TP1. During the follow-up interval, one patient was newly diagnosed with hypertension (HTN), two with diabetes mellitus (DM), ten with hypercholesterolemia (including hypertriglyceridemia), and six with depression, which increased the total number of patients with HTN, DM, hypercholesterolemia, and depression at follow-up to 7 (16.7%), 4 (9.5%), 15 (35.2%), and 20 (47.6%), respectively.

Table 3.3. Characteristics of Patients Who Participated in the Baseline (TP1) and Follow-up (TP2) MRI Studies

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
Demographics									
Age (in years)	Mean (SD)	45.1	(10.68)	45.4	(13.26)	45.1	(10.38)	48.6	(10.28)
	Median (range)	44.3	(27-71)	48.4	(27-65)	44.2	(27-71)	47.3	(31-75)
Gender	Male	40	(81.6%)	6	(85.7%)	34	(81.0%)	34	(81.0%)
	Female	9	(18.4%)	1	(14.3%)	8	(19.0%)	8	(19.0%)
Country of birth	Ireland	34	(69.4%)	6	(85.7%)	28	(66.6%)	28	(66.6%)
	Europe	4	(8.2%)	1	(14.3%)	3	(7.1%)	3	(7.1%)
	Africa	10	(20.4%)	0	(0.0%)	10	(23.8%)	10	(23.8%)
	South America	1	(2.0%)	0	(0.0%)	1	(2.4%)	1	(2.4%)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
English as a first language	Yes	38	(77.6%)	7	(100.0%)	31	(73.8%)	31	(73.8%)
Handedness	Right	46	(93.9%)	6	(85.7%)	40	(95.2%)	40	(95.2%)
	Left	3	(6.1%)	1	(14.3%)	2	(4.8%)	2	(4.8%)
Employment history	Employed	27	(55.1%)	4	(57.1%)	23	(54.8%)	23	(54.8%)
	Student	3	(6.1%)	0	(0.0%)	3	(7.1%)	3	(7.1%)
	Unemployed	16	(32.7%)	2	(28.6%)	14	(33.3%)	14	(33.3%)
	Retired	3	(6.1%)	1	(14.3%)	2	(4.8%)	2	(4.8%)
Years of education	Mean (SD)	13.6	(3.8)	14.71	(3.3)	13.4	(3.9)	13.7	(3.9)
	Median (range)	14	(7-23)	15.0	(9-18)	13.5	(7-23)	14.0	(7-24)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
HIV disease related characteristics									
Mode of transmission	Heterosexual	19	(38.8%)	1	(14.3%)	18	(42.9%)	18	(42.9%)
	MSM	21	(42.9%)	4	(57.1%)	17	(40.5%)	17	(40.5%)
	IVDU	7	(14.3%)	2	(28.6%)	5	(11.9%)	5	(11.9%)
	Other	2	(4.1%)	0	(0.0%)	2	(4.8%)	2	(4.8%)
Time since diagnosis (years)	Mean (SD)	10.43	(7.83)	12.3	(10.38)	10.13	(7.44)	13.57	(7.42)
	Median (range)	9.1	(10.1-28.6)	10.7	(2.6-28.1)	8.3	(1.0-28.6)	11.91	(4.3-32.4)
Nadir CD4 count	Mean (SD)	220.06	(179.6)	265.71	(111.10)	212.08	(188.9)	212.08	(188.9)
	Median (range)	191.0	9-848	274.0	(112-388)	167.0	(9-848)	167.0	(9-848)
Current CD4 count	Mean (SD)	596.35	(282.91)	567.57	(210.56)	601.14	(294.37)	638.88	(258.66)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
	Median (range)	565.0	(147-1292)	600	(283-936)	553.5	(147-1292)	658.50	(164-1394)
Virally suppressed	Yes	44	(89.8%)	6	(85.7%)	38	(90.5%)	40	(95.2%)
	No	1	(2.0%)	0	(0.0%)	1	(2.4%)	1	(2.4%)
	Not on ART at time of assessment	4	(8.2%)	1	(14.3%)	3	(7.1%)	1	(2.4%)
Antiretroviral therapy	Naïve	2	(4.1%)	1	(14.3%)	1	(2.4%)	0	(0.0%)
	MTCT	2	(4.1%)	0	(0.0%)	2	(4.8%)	1	(2.4%)
	On ART	45	(91.8%)	6	(85.7%)	39	(92.9%)	41	(97.6%)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
Interval between TP1 and TP2 assessments (months)	Mean (SD)	n/a		n/a		n/a		41.23	(7.58)
	Median (range)	n/a		n/a		n/a		41.22	(29.17-58.64)
Continuous viral suppression between TP1 and TP2	Yes	n/a		n/a		n/a		33	(78.6%)
Exposure to HAART (total years)	Mean (SD)	6.66	(5.03)	6.26	(6.39)	6.72	(4.85)	10.15	(4.85)
	Median (range)	6.13	(0.0-17.0)	3.40	(0.2-15.7)	6.34	(0.0-17.0)	9.87	(2.8-20.8)
History of CNS Opportunistic Infections									
Hx of Cryptococcal Meningitis	Yes	1	(2.0%)	0	(0.0%)	1	(2.4%)	1	(2.4%)
Hx of CNS TB	Yes	2	(4.1%)	0	(0.0%)	2	(4.8%)	2	(4.8%)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
Hx of Toxoplasmosis	Yes	1	(2.0%)	0	(0.0%)	1	(2.4%)	1	(2.4%)
Hx of Encephalitis	Yes	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Hx of PML	Yes	1	(2.0%)	0	(0.0%)	1	(2.4%)	1	(2.4%)
Other Comorbidities									
Epilepsy	Yes	3	(6.1%)	0	(0.0%)	3	(7.1%)	3	(7.1%)
Stroke	Yes	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Hx of Depression	Yes	14	(28.6%)	0	(0.0%)	14	(33.3%)	20	(47.6%)
Hx of Bipolar Disorder	Yes	1	(2.0%)	1	(14.3%)	0	(0.0%)	0	(0.0%)
Hx of Anxiety	Yes	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Hx of Schizophrenia	Yes	1	(2.0%)	0	(0.0%)	1	(2.4%)	1	(2.4%)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
Hx of Psychosis	Yes	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Neuropathy	Yes	1	(2.0%)	0	(0.0%)	1	(2.4%)	2	(4.8%)
Hypertension	Yes	8	(16.3%)	2	(28.6%)	6	(14.3%)	7	(16.7%)
Hypercholesterolemia	Yes	5	(10.2%)	0	(0.0%)	5	(11.9%)	15	(35.7%)
Diabetes Mellitus	Yes	3	(6.1%)	1	(14.3%)	2	(4.8%)	4	(9.5%)
Hx of Hepatitis B	Yes	4	(8.2%)	1	(14.3%)	3	(7.1%)	3	(7.1%)
Hx of Hepatitis C	Yes	9	(18.4%)	1	(14.3%)	8	(19.0%)	8	(19.0%)
Hx of Syphilis	Yes	8	(16.3%)	3	(42.9%)	5	(11.9%)	5	(11.9%)
Medications									
Benzodiazepines	Yes	8	(16.3%)	0	(0.0%)	8	(19.0%)	9	(21.4%)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
Methadone Replacement Therapy	Yes	6	(12.2%)	1	(14.3%)	5	(11.9%)	8	(19.0%)
Anti-depressants	Yes	5	(10.2%)	0	(0.0%)	5	(11.9%)	5	(11.9%)
Anti-seizure	Yes	2	(4.1%)	0	(0.0%)	2	(4.8%)	2	(4.8%)
Anti-psychotics	Yes	2	(4.1%)	1	(14.3%)	1	(2.4%)	1	(2.4%)
Lifestyle History									
Smoking history	Smoker	21	(42.9%)	2	(28.6%)	19	(45.2%)	20	(47.6%)
	Non-smoker	17	(34.7%)	2	(28.6%)	15	(35.7%)	15	(35.7%)
	Ex-smoker	11	(22.4%)	3	(42.9%)	8	(19.0%)	7	(16.7%)
Alcohol history	Non-drinker	7	(14.3%)	2	(28.6%)	5	(11.9%)	5	(11.9%)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
Units per week	1-20 units	32	(65.3%)	5	(71.4%)	27	(64.3%)	27	(64.3%)
	>21 units	7	(14.3%)	0	(0.0%)	7	(16.7%)	7	(16.7%)
	Former drinker	3	(6.1%)	0	(0.0%)	3	(7.1%)	3	(7.1%)
Illicit Substance Use	Never	37	(75.5%)	6	(85.7%)	31	(73.8%)	31	(73.8%)
	Current non IV use	6	(12.2%)	0	(0.0%)	6	(14.3%)	6	(14.3%)
	Current IV use	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
	Previous use	6	(12.2%)	1	(14.3%)	5	(11.9%)	5	(11.9%)
Family history of neurodegenerative disorders	Yes	7	(14.3%)	1	(14.3%)	6	(14.3%)	6	(14.3%)

Characteristics of Patients that had MRI acquisition		All subjects assessed at TP1; n = 49		Subjects lost to follow-up; n = 7		Subjects assessed at TP1 and TP2; characteristics at TP1; n = 42		Subjects assessed at TP1 and TP2; characteristics at TP2; n = 42	
Hospital Anxiety and Depression Scale									
Positive screen for anxiety	Yes	14	(28.6%)	1	(14.3%)	13	(31.0%)	13	(31.0%)
HADS Anxiety score	Mean (SD)	7.51	(4.53)	5.43	(4.04)	7.86	(4.56)	7.55	(4.53)
	Median (range)	7.00	(0-18)	5.00	(0-13)	8.00	(0-18)	7.50	(0-15)
Positive screen for depression	Yes	4	(8.2%)	1	(14.3%)	3	(7.1%)	6	(14.3%)
HADS Depression score	Mean (SD)	5.59	(4.22)	5.29	(3.30)	5.64	(4.39)	5.57	(4.56)
	Median (range)	5.00	(0-19)	5.00	(1-11)	5.50	(0-19)	5.00	(0-18)

3.8.2 MRI Study Participants Diagnostic Profile Change

The diagnosis of HAND at TP2 was established as described in Chapter 2 and HAND diagnosis changes for those who participated in the follow-up brain imaging study at TP2 are presented in Table 3.4. Of the two participants who died after the baseline study and did not participate in the follow-up study, one had ANI and the other one presented with non-diagnostic abnormalities at TP1. Additionally, four participants who had ANI, and one participant who had MND at baseline (TP1), were lost to follow-up.

At TP2, most diagnostic changes were observed in the TP1 ANI group, one third (7/23) had mild symptomatic impairment and were diagnosed with mild neurocognitive disorder (MND), one third (8/23) remained asymptomatic and performed within non-diagnostic range (had either changes in one domain on RBANS or abnormal ACE-r, MoCA or FAB tests) on follow-up neuropsychology testing, and one third (8/23) were stable (ANI) at follow-up. Most participants who were diagnosed with MND at baseline (10/13) had symptomatic cognitive impairments within the MND range at follow-up, and only three (3/13) reported resolution of symptoms, with two of them performing within normal range on tests at follow-up. While the one patient who had HAD at TP1 performed better at the TP2 assessment, they still remained severely functionally impaired and performed within the same diagnostic range (HAD) on neuropsychology tests. There were no new cases of HAD at TP2 in the imaging study. At TP2, there was a higher number of participants with symptomatic mild cognitive impairment, specifically MND - 17/42 (40.5%), as opposed to 13/42 (31%) at TP1.

Table 3.4. HAND Diagnosis Change for MRI Study Participants at Follow-up (TP2)

MRI study cohort diagnostic profile change at TP2; TP1 n=49 and TP2 n=42								
TP2 RBANS results / TP1 total n=49		Total recruited at TP2 n=42	Not HAND at TP2	ANI at TP2	MND at TP2	HAD at TP2	RIP	LTFU
Not HAND	6 (12%)	5 (11.9%)	4	1^			1	
ANI	28 (57%)	23 (54.7%)	8*	8	7^^		1	4
MND	14 (29%)	13 (31%)	3*		10			1
HAD	1 (2%)	1 (2.4%)				1		
	TP2 Total	42	15/42 (35.7%)	9/42 (21.4%)	17/42 (40.5%)	1/42 (2.4%)	2	5
^ Incident diagnosis of HAND (ANI/MND/HAD); ^^ ANI progressed to MND; * HAND (ANI or MND) in remission; LTFU – lost to follow-up other than RIP								

3.8.3 Study Participants and Control Group Demographic Characteristics

For the purpose of this study, the MRI data of 50 healthy controls was used. These were healthy controls gender, age, and handedness matched with the study participants. Exclusion criteria for the control group included incidental intracranial findings, previous cerebrovascular event, neurosurgery, uncontrolled hypertension, type I diabetes, smoking and alcohol or illicit drug misuse, as well as the contraindications for MR scanning. The demographic and clinical profile (gender, age at the time of imaging acquisition, and handedness) of healthy controls and patients who were included in the neuroimaging data analyses are summarised in Table 3.5.

Table 3.5. Demographic and Clinical Profile of MRI Study Participants

Participants	Healthy controls n=50	HIV+ patients TP1 n=49	HIV+ patients TP2 n=42
Age years (SD)	49.18 (± 11.1)	45.1 (± 10.68)	48.6 (± 10.28)
Gender Male / Female	40 / 10	40 / 9	34 / 8
Handedness Right / Left	46 / 4	46 / 3	40 / 2
TP1 – TP2 interval months (SD)	N / A	N / A	41.23 (± 7.58)
N/A – not applicable; TP1 – time point 1; TP2 – time point 2			

3.8.4 Symptoms of HIV Positive Participants at Follow-up MRI Acquisition

Difficulties reported by the MRI study participants at TP1 and TP2 assessments are summarised in Figure 3.3. The most significant changes from TP1 to TP2 were observed in reporting concentration difficulties ($p<0.0001$), difficulties with names ($p=0.0001$) and misplaced items ($p=0.007$).

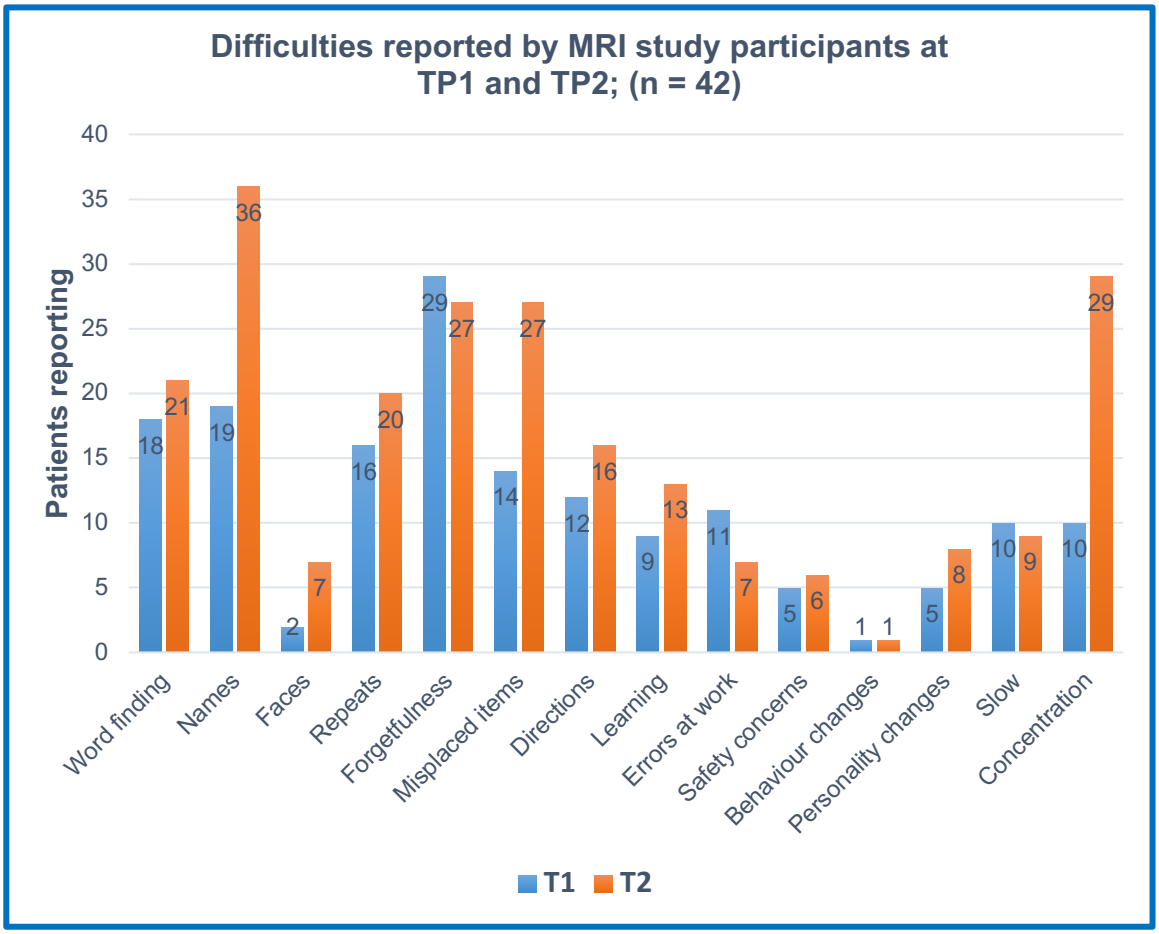


Figure 3.3. Symptoms reported by study participants

3.8.5 Clinical Signs of HIV Positive Participants at Follow-up MRI Acquisition

Clinical signs at TP1 and TP2 for 42 MRI study participants are shown in Figure 3.4. Although there were more subjects who had abnormal tone, power, reflexes, sensation, gait, and reduced speed and amplitude of rapid alternating movements at TP2 than at TP1, these differences were not statistically significant.

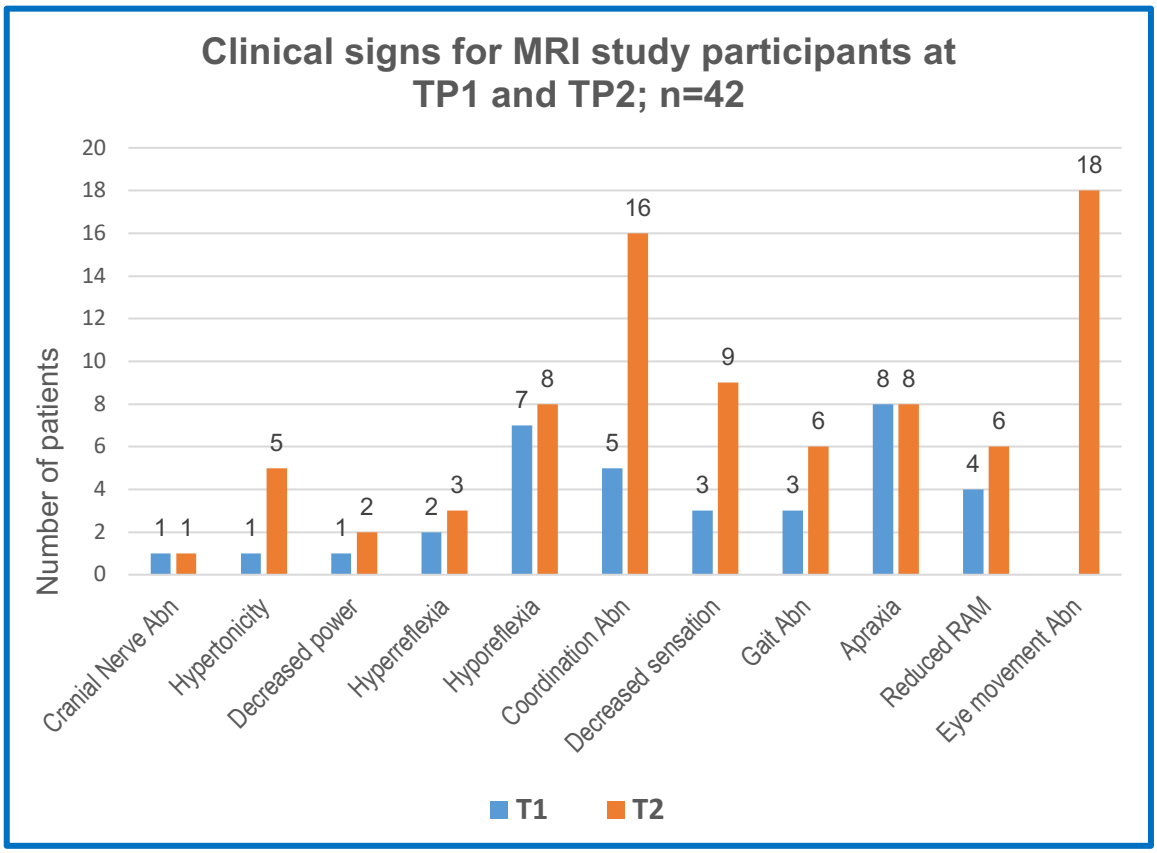


Figure 3.4. Clinical examination findings in MRI study participants at TP1 and TP2

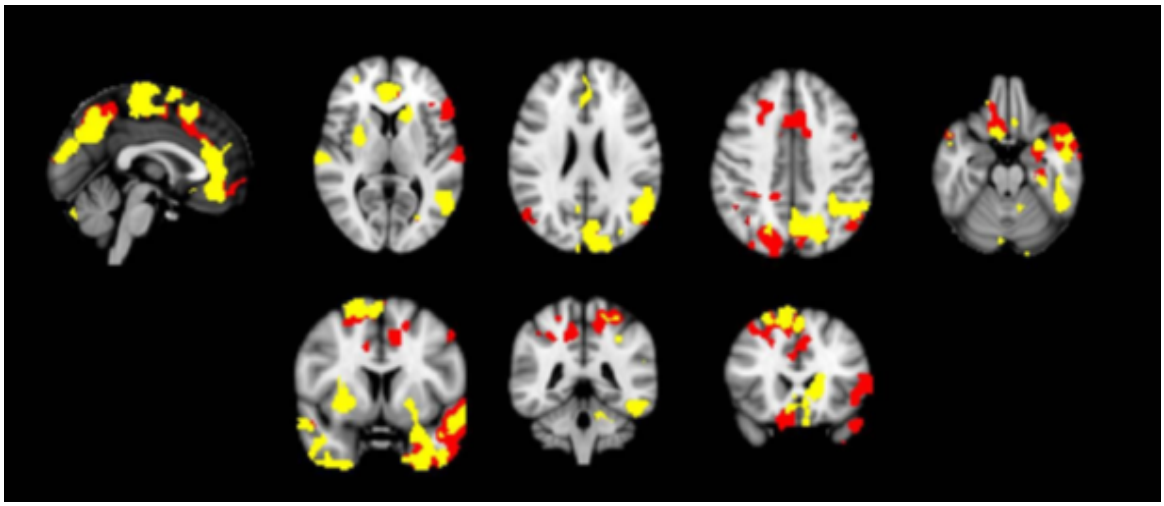
More participants presented with abnormal coordination at TP2 compared with TP1, and this difference was statistically significant (p=0.003). Minor eye

movement abnormalities (broken pursuits and / or absent convergence), which were only recorded at TP2, were observed in nearly a half (18/42; 42.86%) of the TP2 participants.

3.8.6 Whole Brain Analyses

3.8.6.1 Whole brain voxel based morphometry (VBM) analyses

Whole brain grey matter analyses using voxel based morphometry did not reach statistical significance when comparing HIV positive patients to healthy controls irrespective of the time point data utilised (Figure 3.5). Direct comparison of the MR data acquired at first (TP1) and second (TP2) time points did not reach statistical significance either.



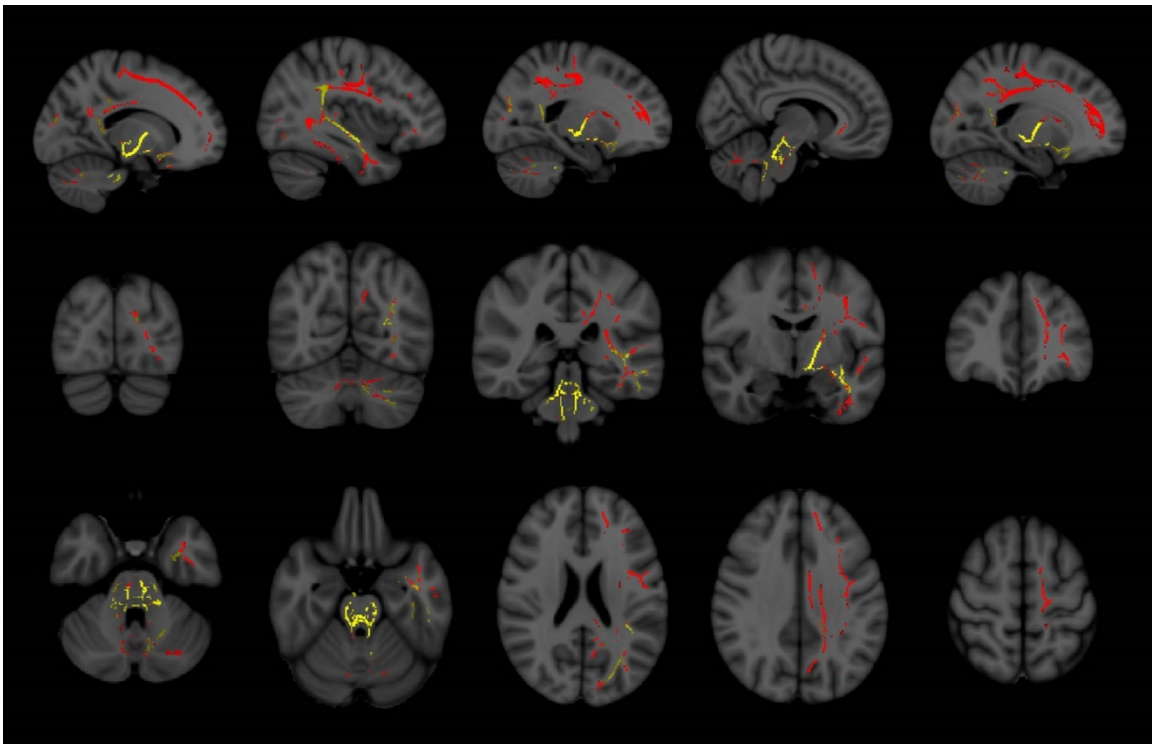
Whole brain cortical density changes in comparison with controls at baseline (yellow) and additional changes at follow-up (red) did not reach statistical significance (statistical significance set at p-value < 0.05 FWE TFCE corrected for age).

Figure 3.5. Whole brain cortical density changes

3.8.6.2 Whole brain white matter diffusion tensor imaging (DTI) analyses

3.8.6.2.1 Axial diffusivity analyses (AD)

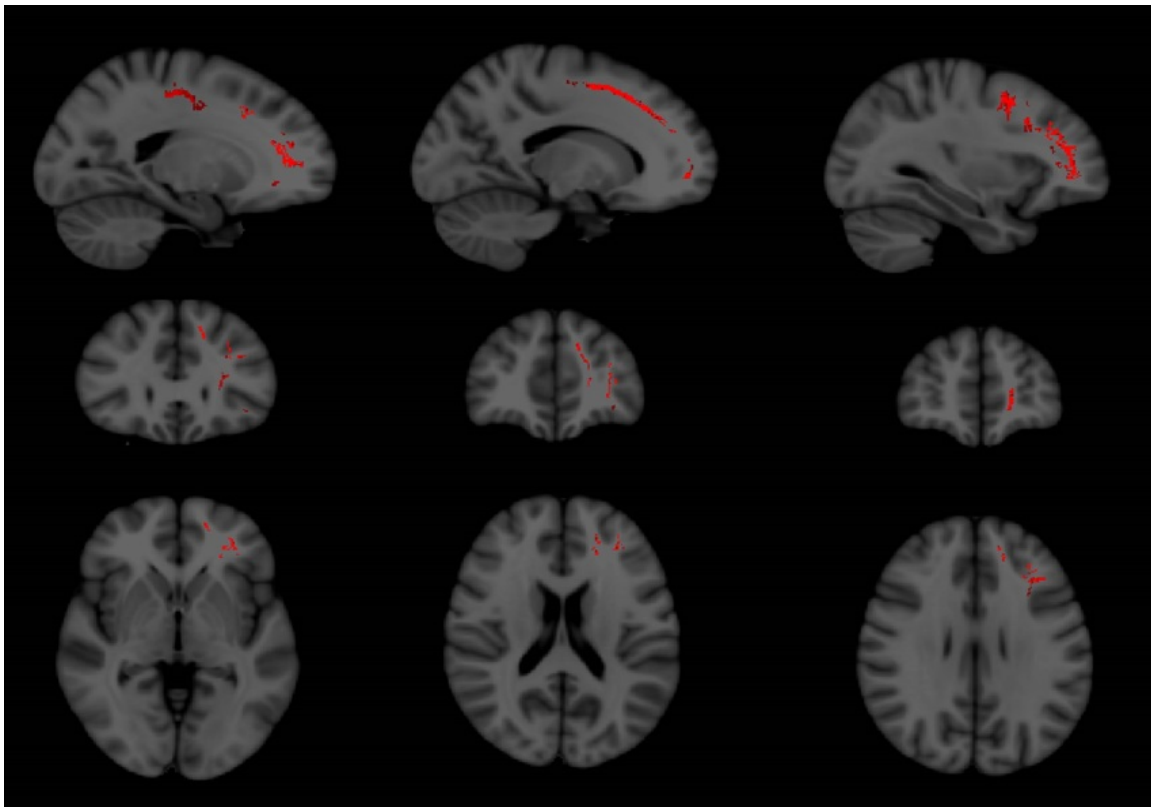
When whole brain DTI datasets at TP1 and TP2 were compared to those of healthy controls (HC), for AD measure, progressive multi-lobar, cerebellar, and brainstem white matter alterations were identified on follow-up scans (Figure 3.6).



White matter AD alterations compared with controls at baseline (yellow), and additional white matter regions altered in contrast to controls on follow-up (red) ($p < 0.05$ FWE TFCE corrected for age).

Figure 3.6. Whole brain white matter AD alterations at TP1 and TP2

A trend of progressive, mainly left frontal ($p=0.008$), white matter degeneration was also detected when the TP1 and TP2 AD maps of the 42 HIV+ patients were compared (Figure 3.7).

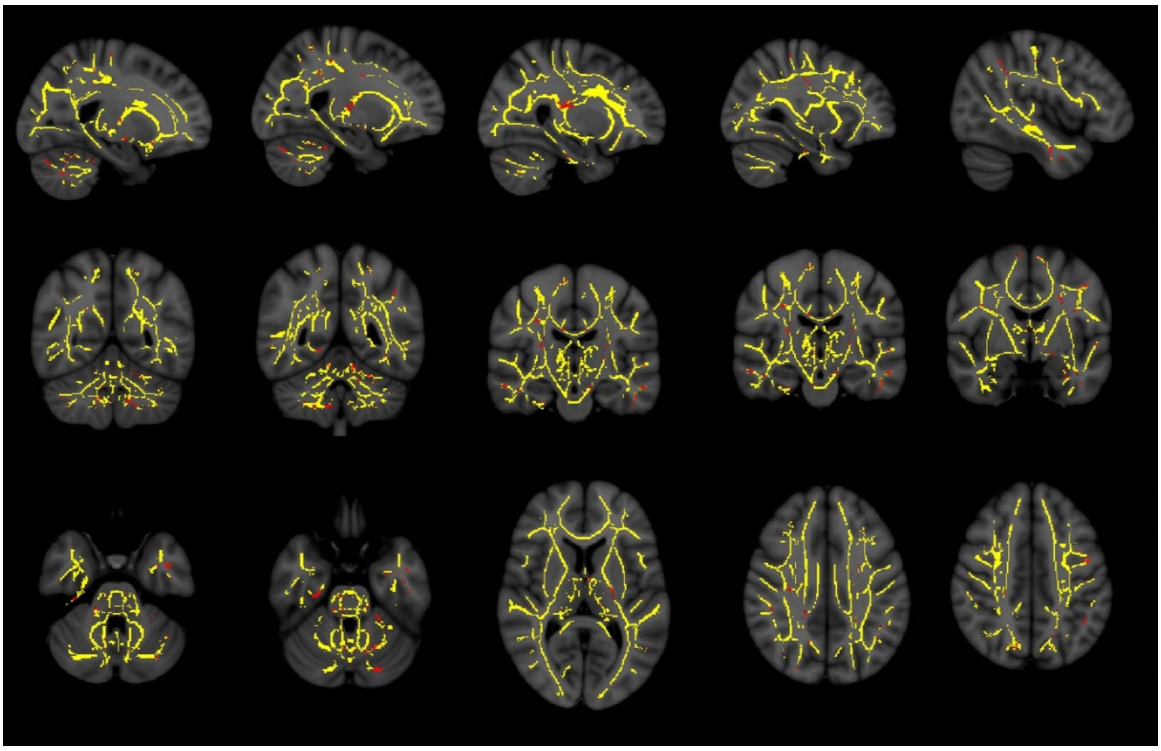


Whole brain AD alteration trend identified between TP1 and TP2 at $p < 0.07$ FWE TFCE corrected for age.

Figure 3.7. Regions of increased AD at TP2 compared to TP1

3.8.6.2.2 Fractional Anisotropy analyses (FA)

Widespread multi-lobar FA reductions were identified at baseline at $p<0.01$ FWE, when the TP1 scans of HIV+ patients were compared to those of healthy controls. At follow-up, on TP2 scans, FA changes showed only minimal progression involving additional regions of corpus callosum, bilateral temporal lobes, CSTs, and cerebellar hemispheres. The new changes did not reach statistical significance (Figure 3.8).

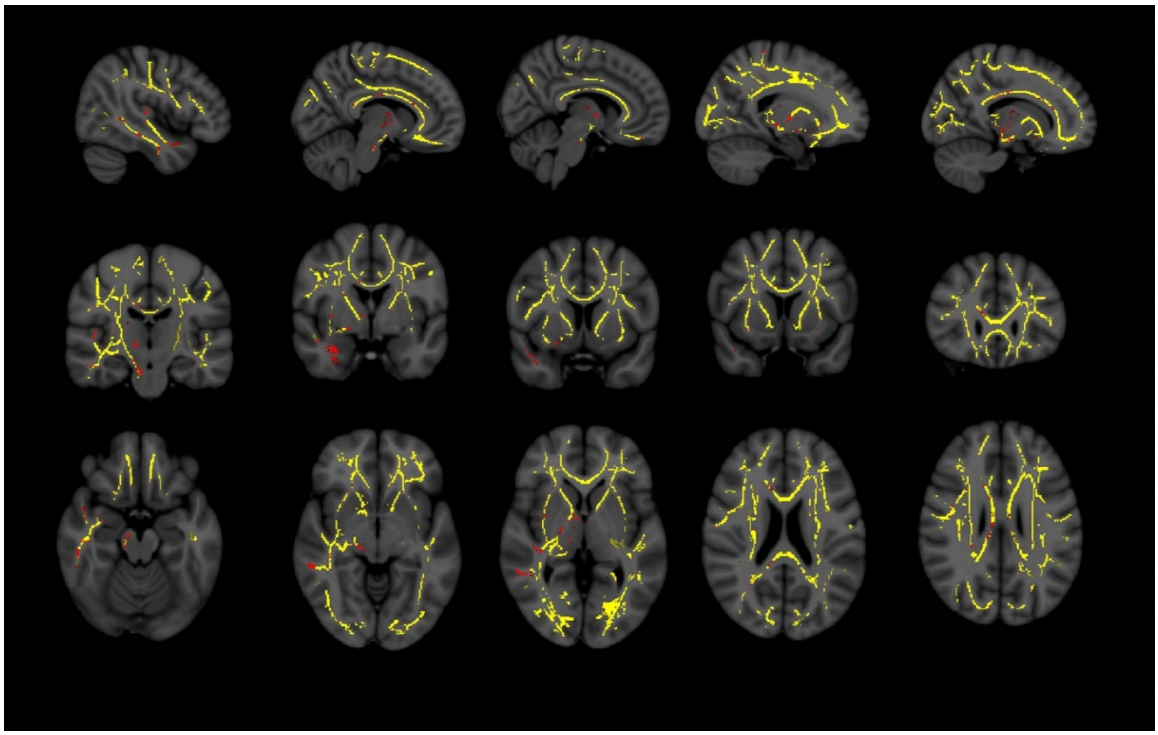


Regional white matter FA reductions in the HIV cohort compared with controls at TP1 (yellow) and additional regions affected on follow-up scanning at TP2 (red) at $p < 0.01$ FWE TFCE corrected for age.

Figure 3.8. Whole brain FA reductions at TP1 and TP2

3.8.6.2.3 Mean diffusivity analyses (MD)

Similar to the statistical FA maps, in the HIV cohort compared with healthy controls (HC), increased MD was detected extensively in the fronto-parietal and occipital regions with the relative sparing of the anterior temporal lobes and cerebellum at baseline using a $p<0.01$ FWE TFCE threshold (Figure 3.9). Additional right anterior temporal (this reached statistical significance at TP2 when compared with HC, with $p<0.0001$), as well as midbrain and thalamic, mean diffusivity changes were detected on follow-up scanning.

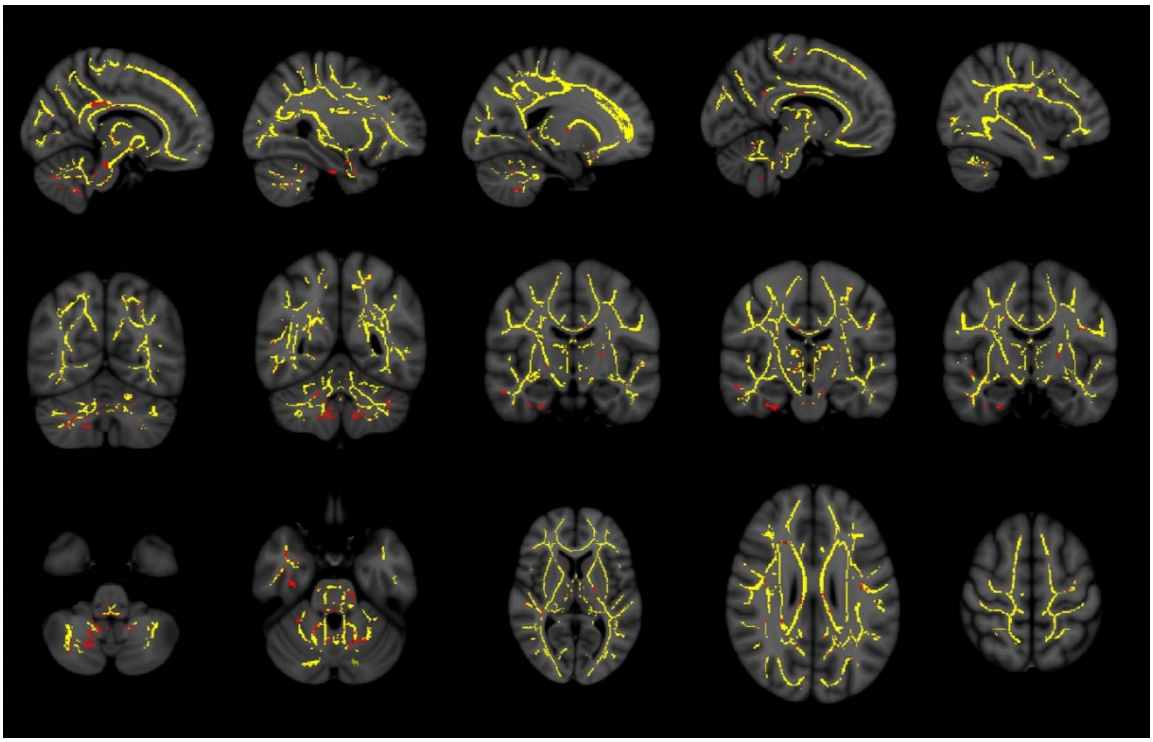


Whole brain white matter regions of increased MD in the HIV cohort at TP1 (yellow) at p < 0.01 FEW TFCE corrected for age, and additional white matter regions affected on follow-up scanning at TP2 (red).

Figure 3.9. Regions of increased MD at TP1 and TP2

3.8.6.2.4 Radial diffusivity analyses (RD)

Increased RD was detected in all cerebral lobes and the cerebellum at baseline on TP1 scans compared with controls at p<0.01 FWE TFCE. This was complemented by additional, although not statistically significant, white matter RD changes in the cerebellar, mesencephalic and mesial (right more than left) temporal lobe regions on the follow-up scanning (Figure 3.10).



Regions of increased RD in the HIV cohort at TP1 (yellow) at p < 0.01 FWE TFCE corrected for age, and additional white matter regions affected on follow-up scanning at TP2 (red).

Figure 3.10. Regions of increased RD at TP1 and TP2

3.8.7 Region of Interest Analyses

Grey matter VBM ROI and white matter DTI ROI analyses were carried out.

3.8.7.1 Grey matter VBM ROI outcomes

VBM intergroup differences for the chosen seven cortical regions of interest (ROIs) did not reach statistical significance at any time point (Table 3.6). Changes in the frontal and temporal lobes, although close to being statistically significant when comparing TP2 with the healthy control group (p=0.067, p=0.067, respectively), were not significant when controlling for age and multiple

testing (p=0.067 and p=0.06, respectively); (*P-value* was considered statistically significant when less than 0.0071 following the Bonferroni correction).

Table 3.6. Intergroup Differences for Partial Volumes in the ROIs Studied

Region of interest	Intergroup ANCOVA (age corrected) *p-value**
Precentral gyrus	0.075
Post central gyrus	0.409
Orbitofrontal gyrus	0.55
Frontal lobe	0.067
Temporal lobe	0.06
Occipital lobe	0.507
Parietal lobe	0.094
**p-value* set at p < 0.0071 following Bonferroni correction for multiple testing	

Although statistical significance was not reached, as shown in Table 3.6, the estimated marginal means (means that were adjusted for other variables in the model) suggested progressive temporal, frontal and parietal lobe grey matter degeneration (Figure 3.11). However, the p-values for the grey matter changes between TP1 and TP2 did not reach statistical significance p=0.907, p=0.825, and p=0.396, respectively.

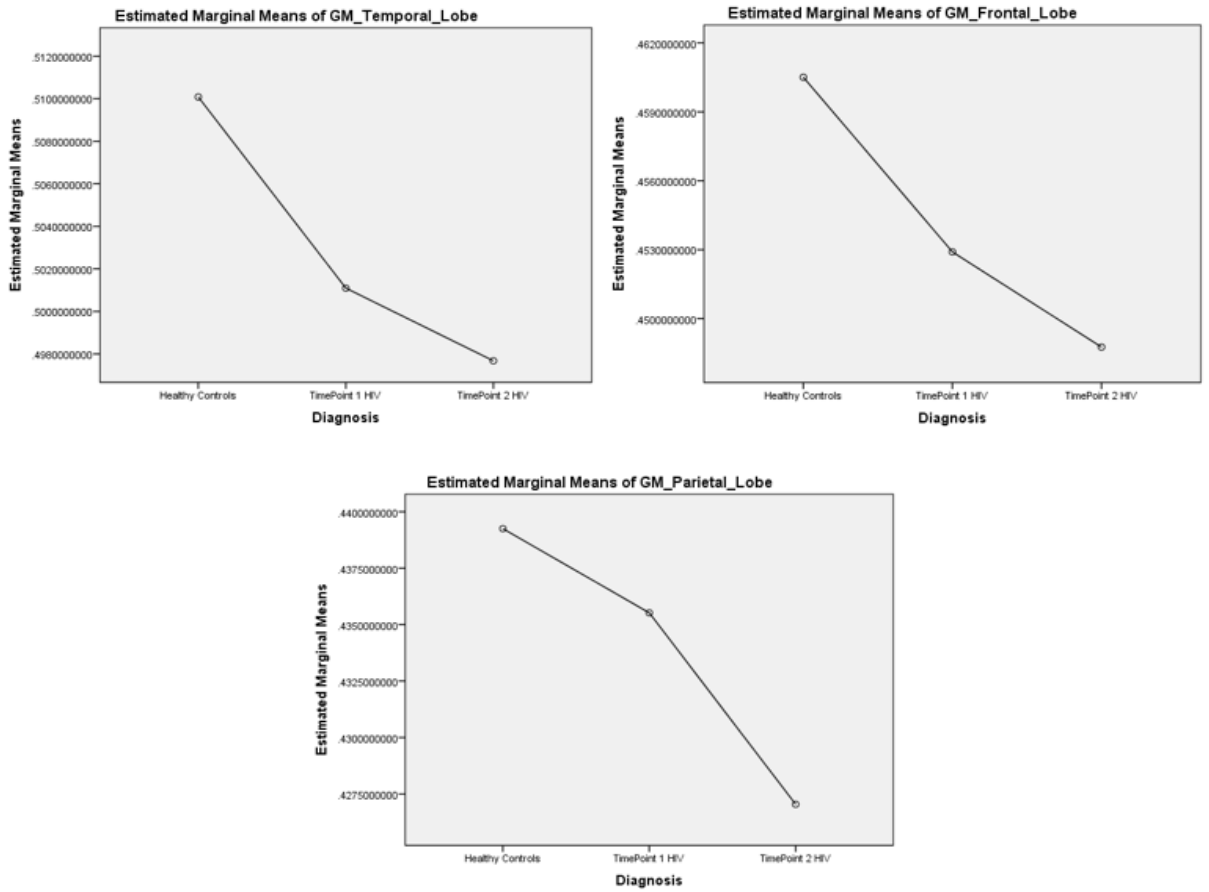


Figure 3.11. Age adjusted estimated marginal means of temporal, frontal and parietal lobe partial volumes in healthy controls and HIV+ cohort at baseline (TP1) and follow-up (TP2) scanning

The box plots of the grey matter profiles for the 42 HIV+ participants at TP1 and TP2, compared with controls, are presented in Figure 3.12.

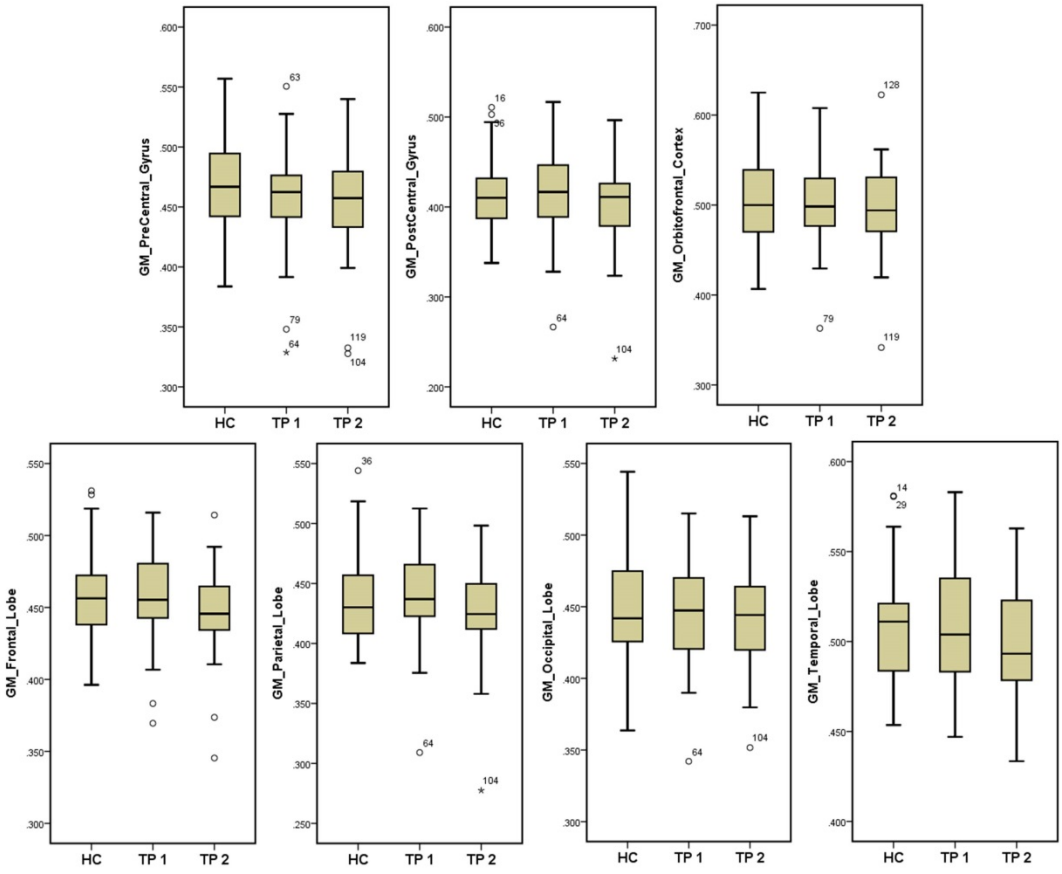


Figure 3.12. ROIs grey matter profile at baseline (TP1) and follow-up (TP2) compared with HC

3.8.7.2 White matter DTI ROI outcomes

3.8.7.2.1 ROI axial diffusivity (AD) analyses

The ROI AD analyses showed that, at TP2, age corrected ROI AD alterations in the HIV+ group did not reach statistical significance when compared with the control group following corrections for multiple testing (p-value significant when

less than 0.0071) with the exception of the left CST (p=0.003) (Table 3.7). Box plots for the ROI AD profiles of HIV+ and control groups are presented in Figure 3.13.

Table 3.7. AD Intergroup Differences in Tract Based White Matter ROIs

Region of interest	Intergroup ANCOVA (age corrected) *p-value**
AD Genu of the corpus callosum	0.819
AD Body of the corpus callosum	0.501
AD Splenium of the corpus callosum	0.023
AD Left corticospinal tract	0.003
AD Right corticospinal tract	0.498
AD Left thalamic white matter skeleton	0.754
AD Right thalamic white matter skeleton	0.684
**p-value* set at p < 0.0071 following Bonferroni correction for multiple testing	

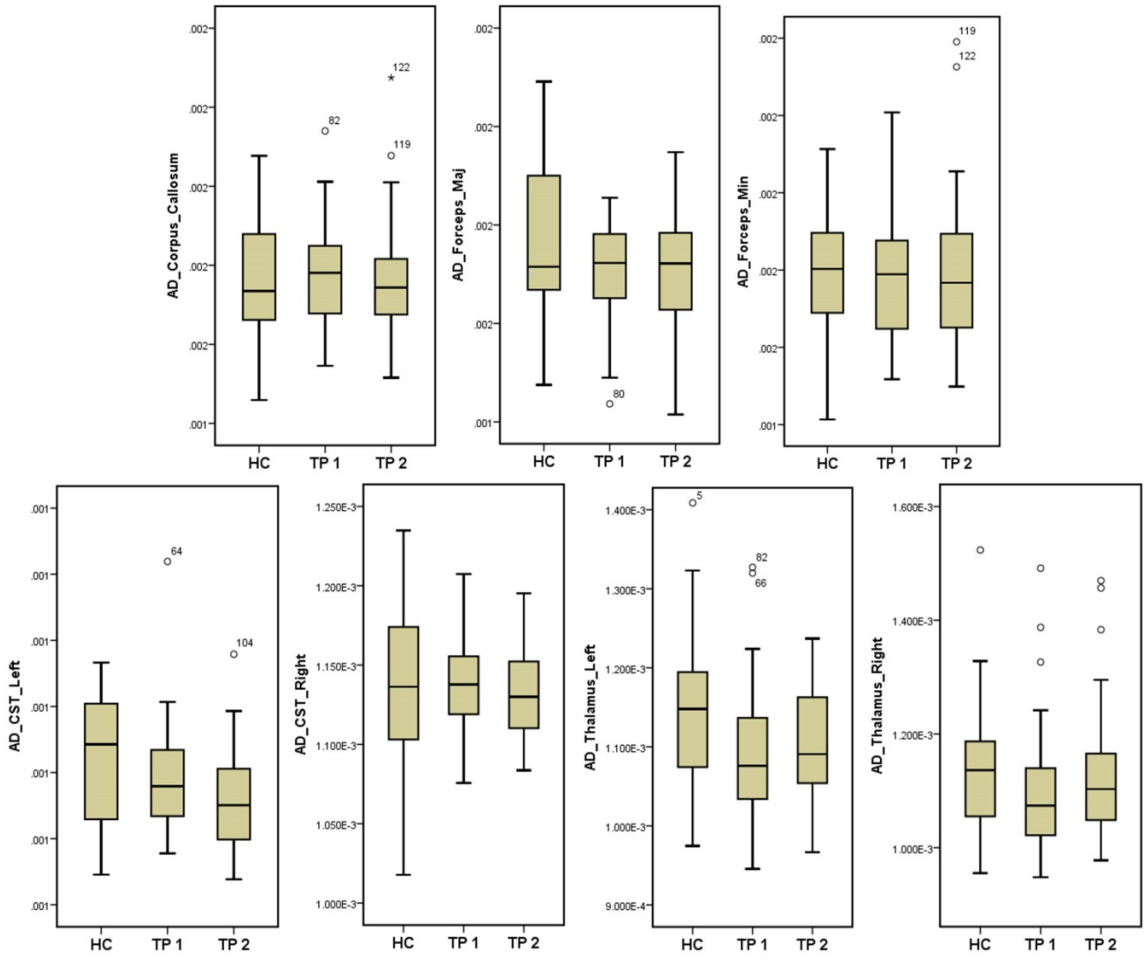


Figure 3.13. AD profiles of tract based ROIs at baseline (TP1) and follow-up (TP2) compared with HC

3.8.7.2.2 ROI fractional anisotropy (FA) analyses

Intergroup comparisons of tract based FA values reached statistical significance in all of the ROIs (segments of the corpus callosum, corticospinal tracts and thalamic white matter skeleton). These results are presented in Table 3.8. Post hoc testing in all of these ROIs revealed significant FA reductions in the HIV+ group at baseline (TP1) when compared with healthy controls, with no further significant changes detected on follow-up (TP2) scanning (Figure 3.14 & 3.15).

Table 3.8. FA Intergroup Differences in Tract Based White Matter ROIs

Region of interest	**Intergroup ANCOVA (age corrected)** *p-value**
FA Genu of the corpus callosum	$p < 0.00001$
FA Body of the corpus callosum	$p < 0.00001$
FA Splenium of the corpus callosum	$p < 0.00001$
FA Left corticospinal tract	$p < 0.00001$
FA Right corticospinal tract	$p < 0.00001$
FA Left thalamic white matter skeleton	$p < 0.00001$
FA Right thalamic white matter skeleton	$p < 0.00001$
**p-value* significant at $p < 0.0071$ following Bonferroni correction for multiple testing	

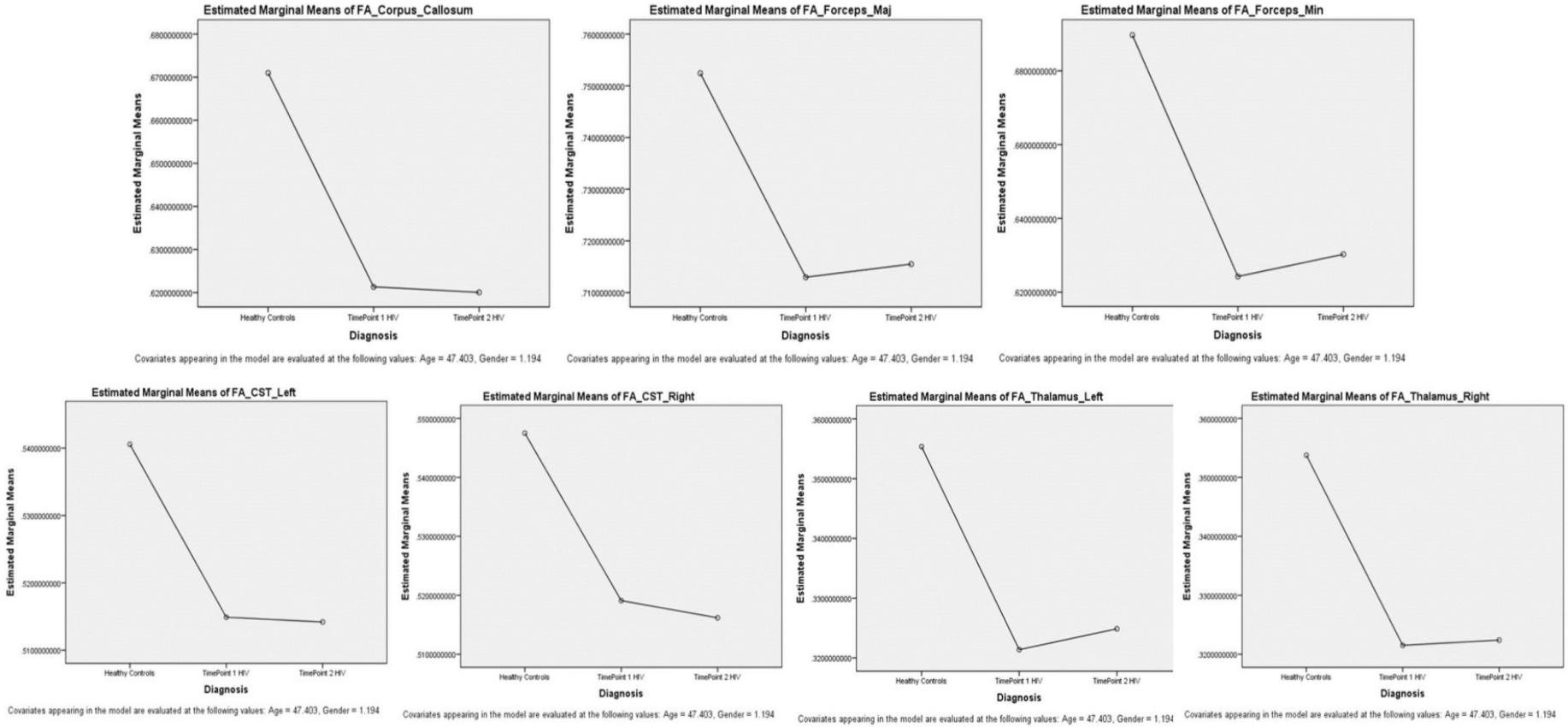


Figure 3.14. Age adjusted FA estimated marginal means for seven white matter ROIs in healthy controls and HIV+ participants, at baseline (TP1) and follow-up scanning (TP2)

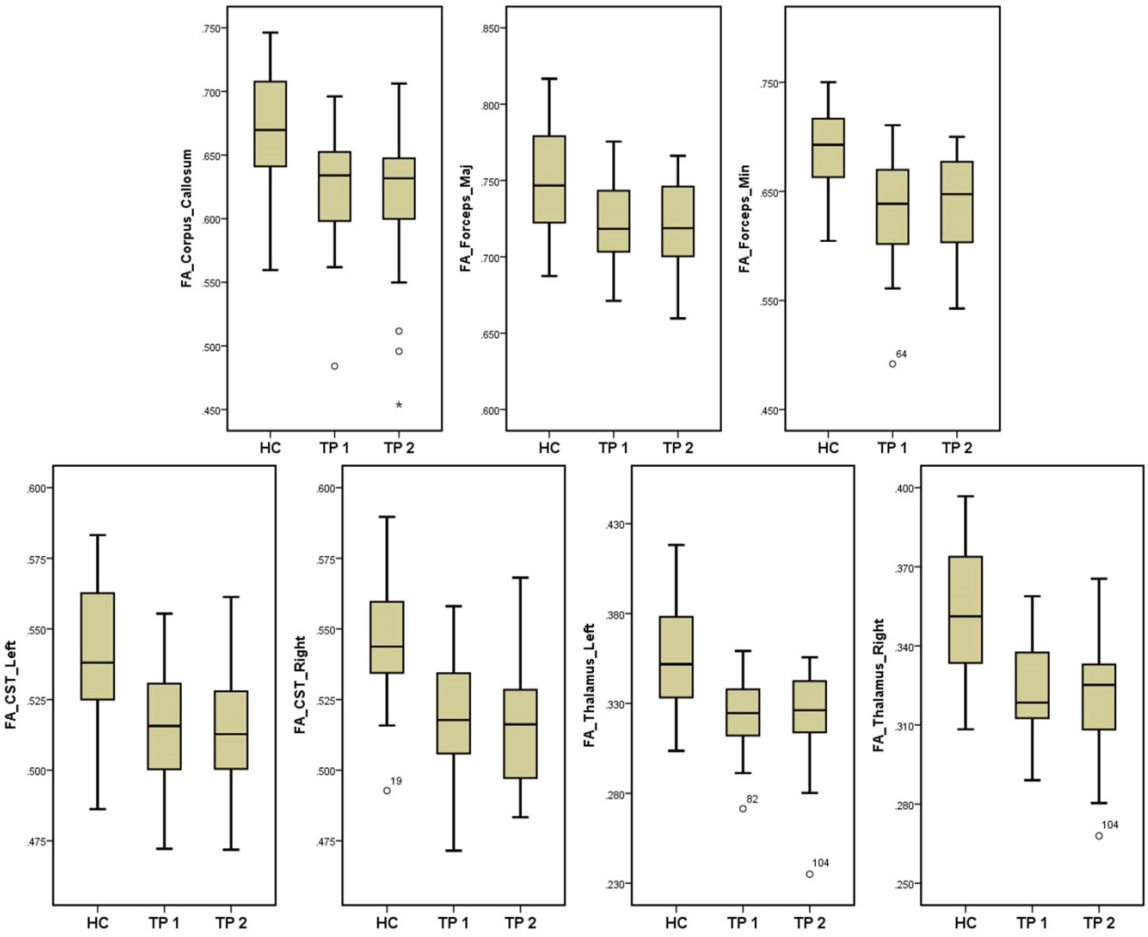


Figure 3.15. Regional FA reductions, at baseline (TP1) and follow-up (TP2) compared with HC

3.8.7.2.3 ROI radial diffusivity (RD) analyses

Increased RD was detected in most ROIs with sparing of both thalami (Table 3.9). Post hoc analyses showed that all these changes were present at baseline (TP1) when comparing HIV+ group with the control group, with no further statistically significant changes observed at follow-up (TP2) (Figure 3.16).

Table 3.9. RD Intergroup Differences in Tract Based White Matter ROIs

Region of interest	Intergroup ANCOVA (age corrected) *p-value**
RD Genu of the corpus callosum	p < 0.00001
RD Body of the corpus callosum	p < 0.00001
RD Splenium of the corpus callosum	p < 0.00001
RD Left corticospinal tract	p < 0.00001
RD Right corticospinal tract	p < 0.00001
RD Left thalamic white matter skeleton	p = 0.076
RD Right thalamic white matter skeleton	p = 0.041
**p-value* significant at p < 0.0071 following Bonferroni correction for multiple testing	

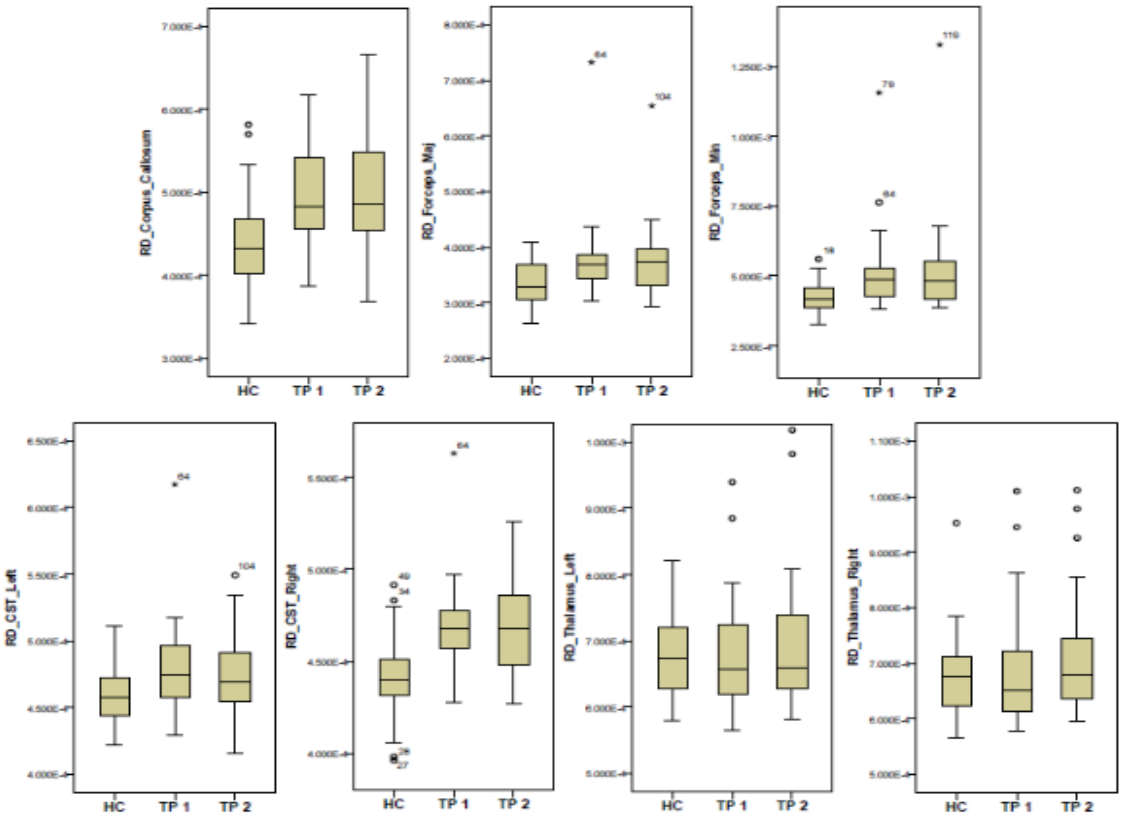


Figure 3.16. Regional RD changes, at baseline (TP1) and follow-up (TP2) compared with healthy controls (HC)

3.8.7.2.4 ROI mean diffusivity (MD) results

Although MD analyses showed intergroup differences with increased values in all ROIs, these were only statistically significant in the right CST, in the genu and the body of the corpus callosum with p<0.00001, p=0.001, and p<0.00001, respectively, following corrections for age and multiple testing (Table 3.10). Post hoc analysis indicated that all of these differences were present at baseline (TP1) and there were no significant changes between TP1 and TP2 with p-values of p=0.788, p=0.985, p=0.981 in the right CST, the genu and the body of the corpus callosum, respectively. There were no significant MD changes between TP1 and TP2 in the other ROIs studied.

Table 3.10. MD Intergroup Differences in Tract Based White Matter ROIs

Region of interest	**Intergroup ANCOVA (age corrected)** *p-value**
MD Genu of the corpus callosum	p = 0.001
MD Body of the corpus callosum	p < 0.00001
MD Splenium of the corpus callosum	p = 0.046
MD Left corticospinal tract	p = 0.064
MD Right corticospinal tract	p < 0.00001
MD Left thalamic white matter skeleton	p = 0.229
MD Right thalamic white matter skeleton	p = 0.154
**p-value* significant at p < 0.0071 following Bonferroni correction for multiple testing	

3.9 Discussion

These multiparametric quantitative MRI analyses suggest that HIV is mainly associated with considerable and widespread white matter pathology. However,

white matter DTI metrics alterations do not demonstrate significant focal progression at follow-up, which might suggest stability on HAART, or that this was too short a follow-up interval for a more substantial change to take place in the context of controlled disease. It must be noted that follow-up period is significantly shorter (mean 41.23 months; SD 7.58; range 29.17-58.64 months) than the period between the HIV diagnosis and TP1 imaging (a mean 10.43 years; SD 7.83; range 10.1-28.6 years). With regards to neurodegeneration, the mean 41 months interval is probably too short to capture significant changes, although a trend of neurodegeneration was observed on VBM ROI frontal, parietal, and temporal cortical grey matter volumes.

These results agree with other recently published studies, suggesting that white matter disease remains the main determinant of the HIV related cognitive impairment in the HAART treated patients with well controlled HIV disease (345, 353, 409). However, one could argue that SJH study group was clinically heterogeneous, with only 78.6% of participants having sustained viral suppression during the entire follow-up period. Nevertheless, only one patient had high grade viremia during this period while not taking HAART. While poor disease control could have an impact on DTI and VBM results, its magnitude at a group level analyses is uncertain in this particular case.

3.9.1 VBM Results Discussion

The VBM findings indicated that no significant cortical atrophy was present in this HIV+ cohort when compared with healthy controls and corrected for age. The grey matter volume alterations did not reach statistical significance on follow-up scans either, nor did them when TP1 scans were directly compared with TP2

scans. While there was no significant cortical atrophy observed, estimated marginal means (means adjusted for age) of cortical volumes showed a trend of insidious antero-lateral (frontal, parietal and temporal lobes) cortical grey matter loss with a bigger difference between TP1 and TP2 in the parietal and frontal lobes. While findings of frontal, parietal and temporal grey matter volume alteration is consistent with the findings of other studies (283, 341, 342), some authors distinguished between the disease related (medial and superior frontal gyri and postero-inferior temporal lobe) grey matter loss versus the age related (inferior frontal and superior temporal lobes) independent of the HIV related grey matter atrophy (341, 342).

3.9.2 DTI Results Discussion

The divergent patterns of FA, AD, MD, and RD alterations highlight the value of multiparametric diffusion tensor imaging analyses, in contrast to relying on a single diffusivity metric. While all four diffusivity measures captured widespread white matter integrity alterations, axial diffusivity revealed relatively focal patterns of WM damage at $p<0.05$ FWE. At baseline, whole brain AD maps highlighted the brain stem, left corticospinal tract, left temporal lobe, and splenium of corpus callosum pathology, which progressed to include cerebellar, parietal and dorsolateral prefrontal regions on follow-up imaging. However, the progression of AD alteration between TP1 and TP2 was only statistically significant for the left frontal lobe ($p=0.008$) when the two time point scans were directly compared. While reduced AD has traditionally been associated with axonal damage (292, 297, 421-423) and more so in the “acute” phase of a CNS disease, the increased AD has been described to reflect axonal fragmentation and degeneration in the

"chronic" stages or a degenerative CNS disease (424, 425). The concordance of AD alterations to morphopathological changes have been increasingly called into question (293, 297, 426, 427).

Radial diffusivity (RD), is often considered as a myelin related measure (294-297), but this is now widely regarded as a simplistic interpretation (421). In this cohort, RD was significantly increased in most of the brain regions at baseline, with insignificant further increase in the right temporal and cerebellar lobes and showed "stability" or insignificant "reduction" in the other brain areas at follow-up.

FA and MD are composite proxies of white matter integrity, which are defined based on all three eigenvalues (288, 297). Irrespective of the histopathological interpretation of the four diffusivity metrics, the divergent patterns show the value of evaluating multiple white matter DTI measures, instead of merely relying on FA, which in our case was already significantly reduced in all brain regions on whole brain analyses at TP1 and only showed further statistically nonsignificant changes in the cerebellum at TP2. Consistent with the whole brain analyses, ROI analyses confirmed significant FA reduction in all seven white matter ROIs at baseline, compared to controls, with no additional statistically significant changes on follow-up, but nonsignificant further alterations in CSTs and corpus callosum.

3.9.3 Follow-up Clinical, Neuropsychology and MRI Data Results

Minimal clinical/neuropsychological progression discussed in Chapter 2 can be influenced by many factors and other biases (practice effects that have not been possible to account for, variability of testers' technique, natural variability on neuropsychology tests performance when these are applied repeatedly, test

environment and personal factors, and possibly compensatory mechanisms, or so called cognitive reserve). However, imaging DTI and VBM data, are objective, observer independent and most importantly free of practice effect and other personal/situational biases. Therefore, it was expected that DTI and VBM analysis would capture preclinical or subclinical changes that have not been apparent on follow-up clinical or neuropsychological testing.

At follow-up, DTI analyses showed further white matter involvement in the cerebellum (progressive insignificant bilateral MD increase and right sided insignificant RD increase with reduced FA and increased AD) and right temporal lobe (progressive insignificant RD, MD and AD increases). Clinically, imaging findings in the cerebellum were complemented by more patients presenting with abnormal co-ordination ($p=0.003$) at follow-up than at baseline. The minor progressive MD and RD increases could signify continuous inflammation / increased tissue water and demyelination, while increased AD could possibly reflect axonal degeneration associated with it (288). However, as previously mentioned, this could be a rather simplistic interpretation of the DTI imaging changes that may not translate to these suggested histological changes.

In the left frontal lobe, there was a significant further AD reduction ($p=0.008$) on direct comparison of TP1 and TP2 scans, which was associated with MD reduction rather than further MD increase between TP1 and TP2 ($p=0.049$). While progressive AD reduction could signify further axonal damage, the MD reduction as opposed to increase could be interpreted as instituted gliosis (288). However, this could be a misinterpretation as MD is defined based on all three eigenvalues and depends on changes in any of them. Histopathological

correlation of such findings would be helpful in clarifying them further. Interestingly, at follow-up, more participants reported concentration difficulties ($p<0.0001$), and misplacing items ($p=0.007$), and as a group, they performed significantly worse ($p=0.022$) on the RBANS figure copy test, which is considered a “subcortical” RBANS sub-test mainly assessing fronto-parietal pathways.

Significant widespread FA reductions were found on whole brain DTI analysis and in all of the ROIs studied, to signify extensive white matter integrity disruption. Further insignificant progression was seen in the left cerebellum, corpus callosum, and both CSTs on the TP2 scans. While on the follow-up clinical examination, more participants displayed pyramidal signs such as hypertonicity and hyperreflexia, these changes also did not reach statistical significance ($p=0.063$ and $p=0.5$, respectively). Progressive alterations were seen in all diffusivity metrics in the midbrain. At follow-up assessments, nearly half of the study participants were noted to have minor eye movement disorder. However, eye movement abnormalities were not recorded at baseline, so it was not possible to make any comparisons with the baseline occurrence of this sign.

3.9.4 Study Strengths

This is a very well characterised sub-cohort of patients both at baseline and at follow-up. The majority of TP1 participants (over 85%) returned for the follow-up MR data acquisition. Most study participants were on ART treatment and had well controlled HIV disease, with the majority having sustained viral suppression throughout the entire follow-up period (78.6%). The study group was age, gender, and handedness matched with the healthy controls. The statistical MR data

analyses included controlling for age to reliably distinguish between disease related changes and the changes due to normal ageing.

3.9.5 Study Limitations

Clinically, this was a heterogeneous group, with three participants not being on antiretroviral therapy at baseline and one not being on continuous ART treatment at follow-up. Just over 21% of participants were not continuously virally suppressed during the follow-up period. At the group level, these factors could have possibly influenced some of the minor progressive changes observed at follow-up. Additionally, one patient had a diagnosis of HAD with associated extensive white matter disease at baseline, and one patient had a diagnosis of PML, which could also influence our findings.

While the socioeconomic background including education attainment and employment were characterised for the HIV+ cohort, these data were not available for the healthy control group. However, it is known that none of the healthy control group participants had any conditions that would increase cardiovascular risk factors, such as type I diabetes, uncontrolled hypertension, previous stroke, or previous neurosurgery, or any incidental brain MRI findings. Healthy controls were non-smokers and did not report alcohol or illicit drug misuse.

The HIV clade was not recorded in this study. However, nearly three quarters or 73.7% are originally from Ireland or another European country. Therefore, it can be assumed that at least these participants are infected with HIV clade B subtype.

The mean interval between baseline and follow-up MR data acquisition was 41.23 months. This is probably not long enough for a controlled chronic disease to cause significant white matter and grey matter disease. However, the findings suggest overall stability of brain disease in well controlled patients over a relatively short period of time. In addition to being relatively short, the follow-up interval ranged widely from 29.17 to 58.64 months. The lack of a control group for neuropsychology analyses made it impossible to adequately control for the practice effects and, therefore, to accurately correlate changes seen on MR imaging to those observed on neuropsychology testing at follow-up.

3.10 Conclusion

In this cohort, AD provides the most focal changes at baseline and captures progressive changes on follow-up imaging. The other three diffusivity metrics, FA, MD, and RD, demonstrate widespread and significant changes already at baseline, with limited progression over time. Widespread diffusivity alterations (AD, RD, MD) have been previously described in well controlled cohorts (376, 391, 397, 399, 400). The direct “group level” comparison of the HIV+ participants images at the two time points (TP1 compared to TP2) did not show statistically significant changes in most of the metrics studied, which is consistent with other longitudinal studies (390, 404). However, a trend for progressive alteration during the follow-up period was identified for whole brain AD at $p<0.07$ TFCE FWE, which only reached statistical significance in the left frontal lobe white matter (TP1 vs TP2; $p=0.008$). Consistent with other studies in treated HIV positive cohorts (376, 390, 393, 395), current study did not show significant HIV disease

related cortical atrophy, but subtle progressive fronto-parieto-temporal cortical grey matter loss was detected.

Overall, this study analyses showed significant extensive white matter disease in the HIV+ cohort, compared with healthy controls at baseline already, with no significant change between TP1 and TP2, and a trend for insidious, although statistically not significant fronto-parietal and less of a temporal cortical grey matter loss. The imaging findings at TP2 were clinically complemented by more patients having a “subcortical”, rather than “cortical”, pattern of changes on follow-up neurology and neuropsychology assessments.

4. Healthcare service utilisation in HIV positive patients with a positive screen for cognitive impairment

4.1 Introduction

4.1.1 Outline of the Chapter

This chapter is concerned with evaluating hospital service utilization and health care costs of people living with HIV (PLWH) who are attending HIV services at St. James's Hospital (SJH) in Dublin. The results reported earlier by McNamara et al. (192) and by other researchers (428) suggest that HIV+ patients with disease related cognitive impairment (CI) generally have poorer health outcomes than those without. The hypothesis underlying this project is that patients with CI have higher morbidity and mortality and consequently higher healthcare costs and non-healthcare related resource utilisation. This chapter examines this relationship in more detail by analysing data on hospital resources used by a subgroup of PLWH attending HIV services at SJH randomly selected from the sub-cohort of 604 patients who participated in "The prevalence of a positive screen for cognitive impairment" study between December 2010 and February 2013 (192).

The first part of the chapter presents background information on this subject by reviewing the available literature. This is followed by a description of the hypothesis, aims and objectives of the study. A detailed outline of study approach includes the methodology followed to gather data from hospital information systems and hospital accounting management systems, along with the statistical methods used to analyse the data. Results of the analysis are then presented

followed by further interpretation and discussion. Lastly, to the extent permitted by the strength of the data, conclusions are drawn and further research is suggested together with practical changes that could be made to address the needs of patients with HIV presenting with cognitive impairment.

4.1.2 Background and Context

SJH is the largest teaching hospital in Ireland. Its HIV services provide care for approximately 2,200 HIV infected individuals. A previous cross-sectional study carried out at SJH by McNamara et al. between December 2010 and February 2013 showed that, out of 604 randomly selected patients who were tested using the Brief Neuro-Cognitive Screen (BNCS), 51.5% (311/604) screened positive for cognitive impairment (CI+) and 48.5% (293/604) screened negative for cognitive impairment (CI-) (192). The BNCS is a tool that has been developed and validated by Ellis and colleagues (2005) to estimate the frequency of HIV associated neurocognitive disorders (429). It is a brief and reliable tool (with 65% sensitivity and a specificity of 72%) that is easy to use and does not require the assistance of neurology healthcare professionals to be administered. (A copy of BNCS is presented in Appendix 1). Following the BNCS screening, 104/311 CI+ patients underwent detailed neurocognitive assessment using a comprehensive neurocognitive assessment battery. A follow-up study of the 104 patients who participated in “The Detailed Neuropsychological Assessment Study of Patients who Screened Positive for Cognitive Impairment” (49) has been described in Chapter 2. This chapter evaluates hospital resource utilization and costs data for 200 patients who were randomly selected from the 604 patients screened

between December 2010 and February 2013, 100 patients who screened positive for CI (CI+ group) and 100 who screened negative for CI (CI- group).

4.1.3 Growing Global Scale and Cost of Healthcare Services Provided to People Living with HIV

With the advent of HAART in 1995, the number of HIV related deaths fell substantially (20). According to UNAIDS, in 2016, an estimated 1.0 Million died from HIV/AIDS, down from a peak of an estimated 1.9 Million deaths in 2005, although, for women of reproductive age (15 to 49 years), it was still the leading cause of death (388). In addition, according to the same source, 1.8 Million people became newly diagnosed with HIV in 2016. Together, these data mean that the number of people living with HIV continues to grow, leading to increasing demand on health care providers. In 2016, it was estimated that, globally, 36.7 Million (30.8 to 42.9 Million) people were living with HIV, 34.5 Million of them adults. This is comparable to the 45.8 Million people estimated to be living with dementia in 2015 (430). Worldwide, the annual cost of caring for PLWH is estimated to be $35 Billion by 2031 (431). This figure probably underestimates the annual cost if countries are going to achieve the UNAIDS 90/90/90 target for treating HIV, where, by 2020, 90% of people with HIV know their HIV status, 90% of people who know their status are on HAART, and 90% of those on HAART are virally suppressed (432). A more recent study found that, in order to reach these UNAIDS targets, a further $52.5 Billion per annum will be required by 2020 (433).

Moreover, despite the effective treatment being available and falling death rates, the disability-adjusted life years (DALY) burden of HIV/AIDS disease increased from a ranking of thirty-third in 1990 to fifth in 2010 for all age groups. For those

aged 30-44 years in both genders, HIV/AIDS was the leading cause of DALY in 2010 (3).

The availability of HAART has also had an impact on epidemiology of HIV in Ireland. The most recent data available from the HSE indicates that in 2016, there were 508 newly diagnosed cases of HIV, an increase of 5% compared with 2015 (434). However, after excluding those people who had previously been diagnosed in other countries, the number of new diagnoses actually fell by 6% in 2016. Notable for 2016, the Central Statistics Office - Vital Statistics (CSO) reported only eight deaths due to HIV or AIDS (435). Similar to the global trend, a falling death rate means people are living with HIV longer and the growing HIV population in Ireland is increasing the demand on HIV services. A study published in 2012 found that, since the 1980s, when HIV diagnostic testing began, about 6,600 people had been diagnosed with HIV in Ireland (436). A study by Brennan et al. estimated that there were 3,820 (3,668 to 4,134) HIV patients in care in Ireland in 2012 (437).

The wide availability of effective treatment is affecting the HIV population age profile also. It was estimated that in the US by 2015, half of HIV positive individuals would have been over 50 years of age (438). Furthermore, it is projected that, globally, 73% of the HIV population will be over 50 by 2030 (439). Brennan et al. estimated that, in Ireland, the proportion of PLWH aged 50 years or older would increase from 18% in 2012 to 30% in 2020 (437). Even though the ageing of people with HIV in Ireland is less than estimated in other studies, it still represents a significant shift. The growth of the HIV population and increasingly older age profile of people with HIV could also affect the prevalence of HAND, as

well as the normal challenges of ageing being possibly magnified for some of them (440).

While early research by Valcour et al. (2004) found that older people living with HIV were more susceptible to HAND than younger people, this may be explained by longer exposure to HIV infection pre-HAART rather than age (441). More recent research (2012) concluded that it is too early to determine the extent, if any, that normal ageing and HIV interact to accelerate cognitive decline (159). Similarly, recent research by Sheppard, published in 2015, found that, while being HIV infected conferred a fivefold risk of developing a neurocognitive disorder over a one year period, development of cognitive impairment was not found to be associated with age (191). Other research by Akay et al. (2014) suggests that the development of HAND may be associated with longer exposure to certain HAART regimens (145). While the debate around the determinants and direction of causality continues, the fact remains that people with HIV are living longer than ever before and the number of PLWH is growing.

4.1.4 Why HAND Might Lead to Higher Healthcare Costs

After the introduction of HAART, there was a 60-80% decline in the number of HIV/AIDS related deaths (20). A concomitant fall in the most severe forms of HIV associated cognitive impairment, or HIV associated dementia (HAD) was also observed (442). HAD has become rare only affecting between 2 and 4% of HIV infected individuals (53). However, milder forms of HIV related neurocognitive impairment remain quite common affecting about 30% of people with HIV without AIDS and up to about 50% of those with AIDS (443, 444). This association has also been confirmed in longitudinal studies of large HIV positive cohorts such as

CHARTER (2010 and 2011) and MACS (2016) (55, 56, 445), meaning that the number of PLWH and concomitant HIV associated cognitive dysfunction continues to rise.

HIV infected people with associated poor performance on neuropsychological tests are frequently found to have difficulties in everyday functioning such as cooking and shopping, difficulties in managing finances, and medication management (446). This poorer level of functioning negatively affects adherence to treatment, with patients following more complex treatment regimens being the most impacted (447). Research by Thaler et al. (2015) also found that increases in HIV related neurocognitive intra-individual variability were associated with poorer overall medication adherence (448). Moreover, in another study, Thaler and colleagues (2015) found that participants with poor neurocognitive status confounded by substance misuse demonstrated the poorest medication adherence (449). In addition, research by Hinkin et al. (2004) studying 148 HIV infected adults aged between 25 and 69 years found that, while overall older adults were more adherent to their medication, those with cognitive dysfunction had a 2.5 times greater risk of poor adherence (450). Subsequent research by Ettenhofer et al. (2009) involving 431 HIV seropositive adults also demonstrated that, while mean adherence rates to HAART regimens were higher in older individuals (>/= 50 years), concomitant impairment of executive, processing speed and motor functioning domains was associated with poorer adherence to medications in the older HIV infected population only (451). This suggests that HIV seropositive patients with associated CI are at a greater risk of being noncompliant with treatment regimens, hence at risk of developing ART

resistance (452, 453) and unstable disease, which ultimately would lead to higher health care resource utilisation and costs.

Moreover, impaired neuropsychological performance has been associated with poor work performance where poor memory, executive and intellectual functioning was most strongly associated with employment status (454). In addition, people who have recovered from neurocognitive dysfunction found it harder to return to work after an episode of disability (455). Research published in 2010 by Chernoff et al. concluded that poor executive function test performance was a statistically significant predictor of employment re-entry failure in 174 HIV infected participants enrolled in a vocational rehabilitation programme (456). Similarly, Woods and colleagues (2011) found that HIV seropositive individuals with neurocognitive deficits were more likely to be unemployed and rely on family and social supports (457). Apart from general social supports relating to employment status, these people were shown to benefit from targeted supports. For example, medication specific social supports were found to improve medication adherence in HIV infected individuals with comorbid alcohol or other drug dependencies (458). All of the above indicate that HIV associated cognitive dysfunction might contribute to increased health care related costs and hospital resource utilisation, as well as a non-health care related financial burden on society.

4.1.5 Estimate of Additional Costs of Treating PLWH who are also Cognitively Impaired

A search of the literature examining the costs of caring for people with HIV who were also cognitively impaired did not find any published research in Ireland or

outside the country at the outset of the current study, although a US study that looked at in-hospital related costs in PLWH who also have HAND was published in April 2018 and will be discussed in this review. The reasons for limited research in this area remain unclear.

Brennan et al. (2015) published research into the costs of providing ambulatory care to people with HIV in Ireland, reporting estimated costs of €973 per patient/month (2012 prices; CI 95%; €938 - €1,008), with the cost of HAART medications accounting for 88% of the total costs (459). This is comparable with international estimates of medication costs ranging from 60% (Italy) and up to 84-89% (Canada, Germany) of the total HIV care costs, including inpatient care (460-462). Interestingly, Brennan and colleagues in their study in Cork University Hospital (CUH, Ireland) found that patients aged over 50 years had slightly lower monthly costs of €928 per month vs €980 per month for younger PLWH. This was explained by older patients being on less costly drug regimens and having persistently controlled disease, hence, less outpatient appointment visits. This study also found female patients to cost more on average than male patients and this was mainly attributable to women of reproductive age being on more expensive HAART regimens than men and also due to ante-natal clinic attendance for those women who were pregnant during the study period.

In contrast to the finding that the costs of care for older patients are less in the Irish (UCH) study, a study published in 2015 from a Western Canadian clinic showed the opposite. The average cost per patient per month was CAN$1,325 for older patients (> 50 years), compared with CAN$1,075 for younger patients (463). Contrary to the Irish study, the reasons for higher cost in the older patient

group in the Canadian study included that the drug costs used for older patients were higher, and that older people attended more clinic visits and were on HAART more continuously.

Brennan and colleagues (2016) also examined inpatient resource utilisation and found that being categorised as non-stable HIV infected individual (stable patients were virally suppressed and on HARRT for the entire study period, were diagnosed over one year prior to the study, were not attending antenatal care and had the lowest CD4 count ≥ 350 cell/ml during the study period) conferred a fivefold risk of HIV related/associated inpatient admission and a higher risk of using non-HIV OPD services (437). However, this study had a number of limitations, one of them being limited information on comorbidities, including cognitive status, therefore, it was not possible to make inferences, with regards to the impact of HIV related cognitive impairment on healthcare costs.

4.1.6 Conclusions

A review of the literature found that the added healthcare costs of HAND have not been assessed. However, in one US study, which was published after the data on hospital resource utilisation for the current study was collected, Patel et al. (2018) evaluated inpatient outcomes and health care related costs from 2005 to 2014 in HIV infected individuals who were also diagnosed with HAND. It found that these patients had a longer length of stay with an increased cost per inpatient stay and a higher risk of mortality (464). However, this study only evaluated the in-hospital / inpatient aspect of health care costs of PLWH and HAND. Research, to date, has tended to examine the overall costs of providing care to people with HIV without discriminating between different cognitive status sub-groups, and usually

discriminating by immune status, age and gender. The available cost data is insufficient and healthcare financial and administration systems are not designed to capture the specific costs of HIV infected people with disease associated cognitive dysfunction or HAND. Taking into account that HIV infected patients with poorly controlled disease (i.e. non-stable) are using the inpatient and outpatient resources more intensely, as shown by Brennan et al. research, and this sub-group of PLWH are more likely to develop HAND, we could speculate that PLWH who have HAND might be more health care resource demanding at both, in-patient and out-patient levels than those with normal cognitive function. Also, HAND could well be one of the reasons for increased DALY in PLWH of working age and, thus, contribute to the non-health care related societal financial burden.

The current study is the first to look at cost and resource utilisation of PLWH through the lens of cognitive impairment in Ireland and the first reported one in Europe. From the literature, it is clear that the health system response to caring for people with HIV varies across different countries and unlike other areas of medicine the concept of international best practice is still emerging. Consequently, the measured costs of care today probably do not reflect the costs of providing best practice care in the future. Some available research suggested a range of interventions that have the potential to compensate for, slow down, or even reverse the neurocognitive dysfunction and these interventions might require additional resources in the future if proven to be effective (some of these interventions will be briefly discussed in the discussion and conclusion chapter). Despite these promising interventions, the cornerstone of treatment for HIV is adherence to HAART (452, 465) and achieving this requires a range of supports

and interventions that ensure patients comply with treatment and remain virally suppressed (466).

4.2 Aims and Objectives

This chapter has the following aims:

- Aim 1: To determine whether HIV patients screening positive for cognitive impairment (CI+ group) require a larger quantum and a wider range of hospital services, and incur higher healthcare related costs than patients who screen negative for cognitive impairment (CI- group).
- Aim 2: To identify the additional services and supports to meet the needs of HIV patients screening positive for cognitive impairment (CI+) that may add to patient non-health related costs.

4.2.1 Objectives

The objectives of this study were:

1. To compare the hospital services utilisation (outpatient - OP, day case - DC and inpatient - IP) of 100 HIV infected subjects screening positive for cognitive impairment (CI+ group) with 100 HIV infected subjects who screened negative for cognitive impairment (CI- group) for the years 2011-2016
2. To compare the costs of providing hospital services (OP, DC, and IP) to these two groups for the year 2014 and 2015

3. To evaluate the role of cognitive impairment as a determinant of added healthcare costs and report on the additional supports such as those provided by Social Work Department required for these patients
4. To compare other poor outcomes such as Did Not Attend (DNA) rates, Emergency Department (ED) attendance rates, medication non-compliance, death (RIP) rates, and poor virologic control in CI+ group versus CI- group.

4.3 Methodology

The methods followed during the course of this research are set out below.

4.3.1 Study Population

The study population was a subset of patients attending HIV services in St. James's Hospital who previously participated in "The Prevalence of a positive screen for Cognitive Impairment" Study, conducted at SJH between December 2010 and November 2013, during which 604 participants were screened with the use of Brief Neurocognitive Assessment Screening tool (BNCS) (192). For the purpose of this study, 100 participants who screened positive for CI and 100 participants who screened negative for CI were randomly selected. (It was believed that 100 subjects would be representative of the original 311 CI+ and of the original 293 CI- participants.) Due to the random nature of the selection for participation in the cost and hospital utilisation study, any matching for co-morbidities was virtually excluded.

4.3.2. Randomisation Tool for Entry into the Study

In March 2017, an online randomisation software application "GraphPad" was used to generate two sets of 100 random numbers. One set of 100 random numbers was created for the CI+ group to randomly select 100 participants out of 311 who screened positive for CI. Another set of 100 random numbers was created for the CI- group to select 100 participants out of 293 who screened negative for CI. The randomisation calculator is freely accessible via the application link provided below.

https://www.graphpad.com/quickcalcs/randomize1.cfm

4.3.3 Study Design

This was an observational retrospective review study.

4.3.4 Ethics

Approval to use patient data contained in the hospital's patient administration system (PAS), Electronic Patient Records (EPR) and finance system was obtained from the St. James's Hospital Research and Innovation Office (Reference number 5081).

4.3.5 Data Collection

For each of the 200 patients randomly selected from the aforementioned screening study, the following information was collected from the hospital patient administration system (PAS) and electronic patient records (EPR) for the six year period from 1st January 2011 to 31st December 2016:

- Age at time of enrolment into the study
- Gender
- Other demographic and social history
- Number of Emergency Department (ED, also referred to as AE) attendances
- Number and duration of hospital inpatient episodes (IP)
- Number of Day Case episodes (DC)
- Number of outpatient clinic attendances (OP) and types of services attended (e.g. specialty)
- Number of Medical Social Work (MSW) clinic attendances and types of services/supports accessed
- Number of Did Not Attend (DNA) OP appointments
- Transfer of Care (TOC) to other services, if known
- Number of deaths (RIP), if any
- HIV viral load (VL) at baseline, at follow-up and during the follow-up period
- Treatment compliance issues
- History of depression, viral hepatitis B / C, hypertension, Diabetes Mellitus, hypercholesterolemia, and stroke.

For each of the study subjects, the results of the baseline BNCS assessment (positive or negative screen for CI) was obtained from the prevalence study. For these 200 subjects, information on clinical data at baseline was used from the original study and clinical information at follow-up was updated from the EPR. In addition, the HIV services Medical Social Work Department provided a more detailed breakdown of the type of supports provided and services accessed by

the study subjects. These data were analysed to determine the differences in clinical outcomes and hospital services utilisation between the two groups.

4.3.6 Hospital Patient Cost Data

Additionally, for 2014 and 2015 only, data reporting costs of the inpatient episodes of care, day case episodes, and outpatient clinic visits was obtained for these 200 patients. Hospital costs information was only available for 2014 and 2015 and was provided from the SJH patient costing system. The costing data for each individual participant was accessed by the hospital finance department through Power Performance Manager (PPM), an activity based costing tool. A software application called *"Qlik Sense"* was then used to interrogate and visualise the data from the costing system. The calculation of costs per case is adjusted by reference to the relative cost weight of each Diagnosis Related Group (DRG). The hospital estimation of the price of an episode of care encompasses all costs appropriately associated with the delivery of that care. These include:

1. Pay related costs (e.g. medical, nursing, administration etc.)
2. Non-pay costs (e.g. drugs, medical supplies, laboratory equipment etc.)
3. Costs of diagnostics (e.g. medical services, theatres, radiology department, laboratories etc.).

The cost of individual patient episodes of care is estimated by directly linking the costs incurred to patient clinical data for the specific episode (e.g. laboratory and radiology tests, inpatient bed days). However, this system does not have the

capacity to provide a breakdown of each component that comprises the total cost of the individual episode of care.

4.3.7 Missing Data and Scope of Analysis

The utilisation and cost analysis was carried out for 100 patients in each group. Over the study period, a number of these patients left the care of the hospital for the following reasons:

- Six participants died during the study period
- Ten participants transferred their care (TOC) to other services/hospitals at some point during the follow-up interval, due to moving home (sometimes to other countries)
- Fourteen participants were lost to follow-up (LTFU). Some of the LTFU participants accessed Private SJH OP Services. However, these data were not accessible to the researcher.

It is acknowledged that the size of the study cohort fell over the course of the study period for the reasons cited above. Once these patients had left the care of SJH or left the SJH Public services to access the Private services, data pertaining their utilisation of healthcare resources or the costs incurred by other health care providers (e.g. ED, OP, IP attendances at other hospitals or care provided by private clinics) were not accessible.

4.3.8 Data Protection

All patient related information was treated in a strictly confidential manner and in accordance with Data Protection Act from 1988 and Data Protection Amendment Act from 2003.

4.3.9 Statistical Analysis

The statistical analysis of the hospital utilisation data and hospital patient cost data was performed using *IBM® SPSS® Statistics V24*. For the analysis of the two year hospital cost data, a Mann-Whitney U test was used due to the data being not normally distributed. When the six years hospital resource utilisation data were analysed, a Mann-Whitney U test was used for the continuous variables analysis where the data was not normally distributed and a Chi-squared test was used for the categorical data analysis (467).

4.3.10 Role of the PhD Candidate

The candidate created and maintained the hospital services and healthcare cost database for the 200 patients. The candidate collected the information from the PAS and EPR hospital systems regarding the number of ED, OP, IP, DC, DNA episodes of care and detailed breakdown of types of services accessed per speciality over the six year follow-up. The information on clinical data at baseline, as well as the results of the baseline BNCS assessment (positive or negative screen for CI), was used from the original prevalence study. The clinical (comorbidities, number of deaths, TOC, LTFU, compliance issues) and laboratory (HIV RNA load or VL) information during the six year follow-up was updated by the candidate from the EPR system. The hospital Financial Department provided

the financial data on costs over a two year period. In addition, the GUIDe Medical Social Work Department provided a detailed breakdown of the type of supports accessed by the study subjects during the six year follow-up. The candidate performed the statistical analyses of the data.

4.4 Results

This Section presents the results of the data analysis of the two groups of patients. It provides:

1. A general description of the study cohort describing the demographic differences between the CI+ and CI- groups
2. A comparison of the differences in health care related hospital services utilisation between the two groups during the six year period from January 2011 to December 2016
3. A comparison of the differences in other outcomes between the two groups (DNA, RIP, treatment non-compliance, VL at baseline, follow-up and during the follow-up period, history of depression)
4. A comparison of the differences in non-health care related hospital services utilisation between the two groups during the six year period from January 2011 to December 2016
5. A comparison of the costs of providing hospital based care (IP, OP, DC) to the two groups during 2014 and 2015.

4.4.1 Description of the Study Cohort

A general demographic and clinical description of the study population as well as the study outcome measures are provided in Table 4.1.

Table 4.1. General Description of the Study Population and Results for the Six Year Follow-up Period from January 2011 to December 2016; n=200

Description		**Positive Screen for CI (n=100)**		**Negative Screen for CI (n=100)**		***p-value***
Patient demographic characteristics						
Age at baseline (years)	Mean (SD)	39.0	(9.79)	40.9	(11.00)	0.1985
	Median(range)	38.0	(18-76)	41.0	(19-65)	
Male gender YES	(%)	71		91		<0.001
Years of education	Mean (SD)	13.67	(4.04)	15.22	(3.75)	0.0054
Unemployed YES	(%)	41		21		0.002
Country of Birth:						
Ireland	(%)	62		86		<0.001
Europe	(%)	6		7		0.775
Africa	(%)	31		3		<0.001
Other	(%)	1		4		0.175
Behavioural characteristics						
Smoker	(%)	51		37		0.047
Illicit drug use - IVDU	(%)	3		1		0.314
Illicit drug use - Other	(%)	17		20		0.586
Alcohol use > 10 units/week	(%)	20		31		0.751
Other clinical characteristics / History of						
Hepatitis B	(%)	10		10		1.000
Hepatitis C	(%)	21		14		0.194
Hypertension	(%)	10		15		0.286
Hypercholesterolemia	(%)	15		30		0.011
Diabetes Mellitus	(%)	3		4		0.701
Stroke	(%)	1		0		n/a
Depression	(%)	14		15		0.841
Hospital service utilization						
ED presentations per patient	Mean (SD)	0.86	(1.83)	0.53	(1.48)	0.042
	Median (range)	0	(0-10)	0	(0-10)	
Total admission episodes per patient (IP and DC)	Mean (SD)	4.44	(4.35)	2.47	(3.57)	0.044
	Median (range)	4	(16-28)	3	(0-26)	
Total in-hospital care days per patient (IP and DC)	Mean (SD)	16.11	(43.59)	6.72	(3.5)	0.032
	Median (range)	4	(12-338)	3.5	(0-111)	
Day Case episodes per patient	Mean (SD)	3.36	(2.74)	2.89	(2.60)	0.166
	Median (range)	3	(0-18)	3	(0-15)	
Inpatient episodes (IP) per patient	Mean (SD)	1.08	(2.71)	0.58	(1.51)	0.345
	Median (range)	0	(0-18)	0	(0-11)	

Description		Positive Screen for CI (n=100)		Negative Screen for CI (n=100)		*p-value*
Patients availing of IP care	(%)	34		26		0.218
IP days per patient	Mean (SD)	12.75	(43.04)	3.83	(13.98)	0.302
	Median (range)	0	(0-336)	0	(0-96)	
IP episodes Length of Stay; (n=108 IP for CI+ group and n=58 IP for CI- group)	Mean (SD)	11.8	(9.46)	6.60	(4.50)	<0.001
	Median (range)	5	(2-40)	3.5	(1-17)	
OP number of attendances – HIV services / GUIDE	Mean (SD)	25.08	(9.32)	21.46	(10.68)	0.051
	Median (range)	24	(2-67)	22	(1-52)	
OP number of attendances – Medical Social Work	Mean (SD)	2.26	(3.72)	1.71	(3.01)	0.148
	Median (range)	1	(0-21)	0	(0-13)	
OP number of attendances – other than GUIDE services	Mean (SD)	7.58	(20.12)	6.11	(14.82)	0.198
	Median (range)	2	(0-177)	1	(0-117)	
OP number of attendances – all services	Mean (SD)	34.92	(25.08)	29.28	(20.69)	0.049
	Median (range)	30	(2-217)	26	(1-144)	
OP number of DNAs	Mean (SD)	9.84	(8.28)	6.32	(5.13)	<0.001
	Median (range)	8	(0-50)	5.5	(0-22)	
Clinical outcomes						
Noncompliance with treatment	Yes / number	24		11		0.016
Virally suppressed at baseline	No / number	29		26		0.635
Virally suppressed at follow-up	No / number	7		4		0.352
Viral load detectable between baseline and follow-up time	Yes / number	42		31		0.106
LTFU	Yes / number	8		6		0.306
Transfer of Care	Yes / number	4		6		0.419
RIP	Yes / number	5		1		0.212
Age at RIP	Mean (SD)	38.50	(8.66)	55.90	(n/a)	(n/a)
	Median (range)	36.45	(29.9-52.7)	n/a		

Statistical significance is presented in the table, where appropriate. At baseline, the CI+ group were, on average, two years younger than the CI- group, but this difference was not found to be statistically significant (p=0.199). There was a higher proportion of female patients in the CI+ group, and this was found to be statistically significant (p<0.001). Female patients had a 3.2 higher risk of having a positive screen for cognitive impairment (95% CI: 1.609 – 6.454). Those patients from CI+ group were more likely to have a lower educational attainment

(p=0.0054), to be unemployed (p=0.002), to be African in origin (p<0.001), and to be a current smoker (p=0.047) at the time of screening for cognitive impairment. All of these are consistent with the original findings in “The Prevalence of a positive screen for Cognitive Impairment” study, i.e. did not happen by chance during the randomisation process (192). Patients from CI- group, however, were more likely to suffer from hypercholesterolemia (p=0.011).

4.4.2 Health Care Related Hospital Services Utilization

In terms of the use of hospital services over the six year period, the absolute values of all the measures of hospital services use (OP, CD, and IP) were found to be higher for the group screening positive for cognitive impairment. These differences were found to be statistically significant for accessing in-hospital services – total number of in-hospital episodes of care which includes all Inpatient episodes (IP) and Day Case episodes (DC) combined (p=0.044), total admission days (p=0.032), as well as Outpatient visits for all specialities combined (OP) (p=0.049) (Table 4.1).

On average, over the six year period, the CI+ group had:

- 0.33 more ED visits per patient than the CI- group and this was found to be significant (p=0.042)
- significantly more OP DNAs per patient, 9.84 vs 6.32 (p<0.001)
- 3.62 more GUIDe OP visits per patient which approached significance (p=0.051)
- 0.55 more MSW visits per patient (p=0.148)
- 0.47 more Day Case episodes of care per patient (p=0.166)

- 0.5 more IP admission episodes per patient (p=0.108)
- 5.2 days longer length of inpatient stay per admission episode (p<0.0001)
- 8.92 more days spent in hospital per patient during the IP episodes of care (p=0.302).

As a group, for all of the types of hospital based care evaluated in the current study, the CI+ group patients accessed hospital services more often than the CI- group patients. Figure 4.1 provides a general overview of the use of hospital services by the study population over the six-year period. The differences are most pronounced for the number of inpatient days (1275 IP days for CI+ group vs 383 IP days for CI- group). A more detailed breakdown of hospital services use is presented below.

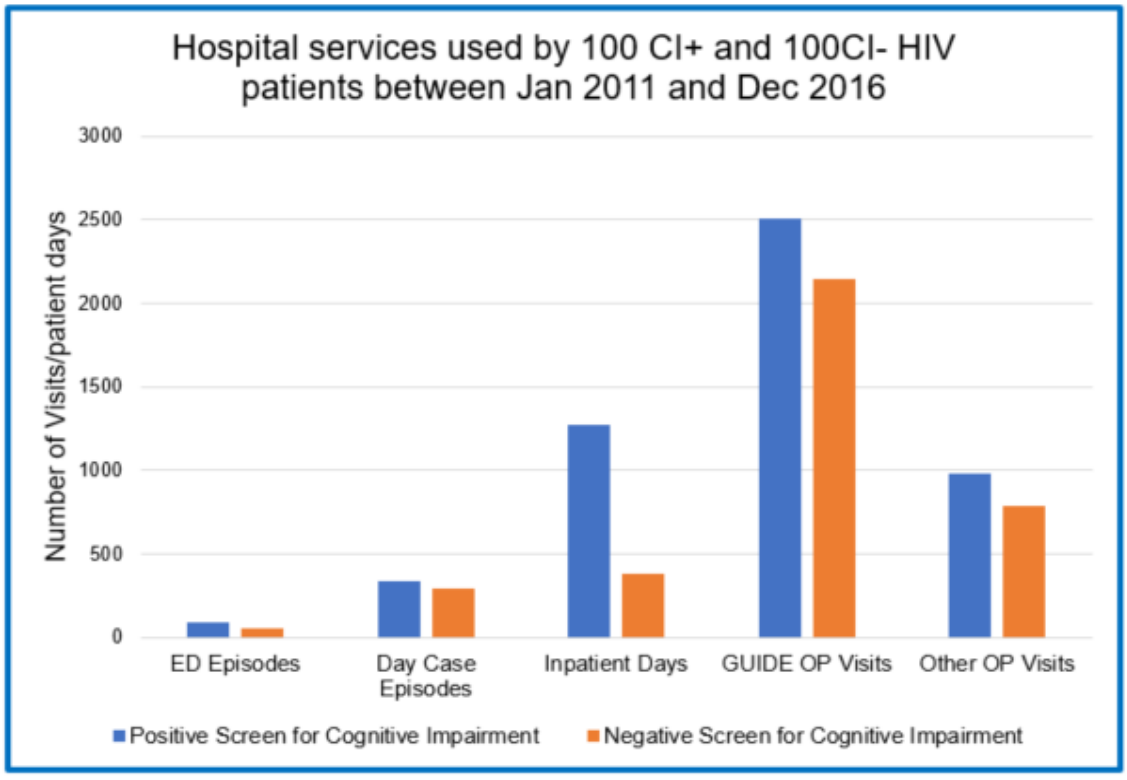


Figure 4.1. Overview of hospital services used by 200 study subjects

4.4.2.1 Emergency Department (ED) attendances

St. James's Hospital operates the busiest emergency medicine (EM) service in the country and it is open 24 hours per day, seven days per week. The data from the hospital's PAS system showed that over the six year period, the majority of the patients (141/200) in the study did not attend the ED at all. However, for the patients who screened positive for cognitive impairment, the rate of ED presentations was higher and this was found to be significant (p=0.042), with 36 patients attending ED at least once over the study period, compared with 23 patients in the group of patients who screening negative for cognitive impairment.

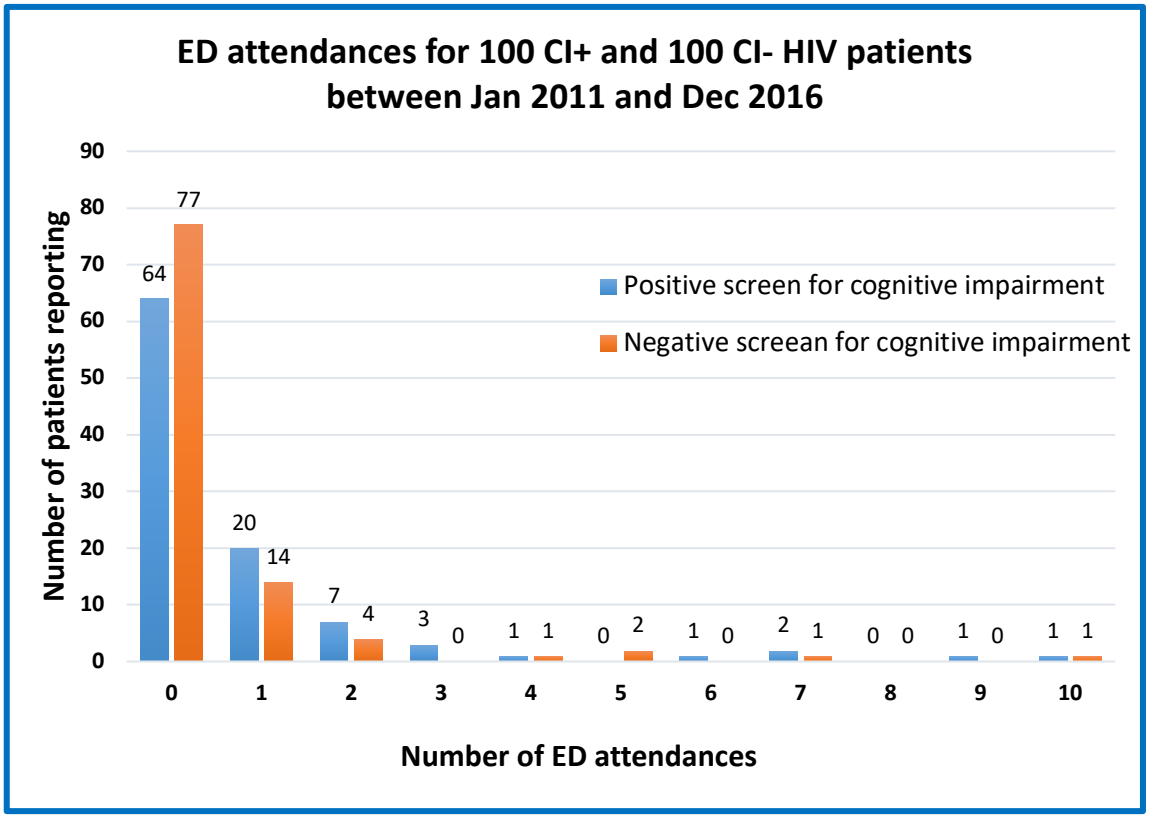


Figure 4.2. Number of ED presentations between Jan 2011 and Dec 2016

Figure 4.2 shows that patients from CI+ group were almost twice as likely as those from CI- group to have made multiple visits to the ED over the six year period. Sixteen of those patients who screened positive presented two or more times to the ED, compared with nine patients from CI- group.

4.4.2.2 Hospital Day Case Episodes

Patients cared for by the hospital are often admitted to a hospital Day Ward for a Day Case episode of care. Day Case (DC) admissions can be for a wide range of procedures and interventions including, for example, minor surgical interventions, cardiovascular procedures, medication administration/infusions, or for diagnostic tests or procedures.

The patient data from the hospital PAS and EPR systems indicated that over the six year period there were 336 DC episodes of care attributable to the 100 patients from CI+ group and 289 DC episodes for the 100 patients from CI- group (47 fewer episodes). Figure 4.3 presents the DC episodes frequency distribution and shows that, overall, the majority of patients in both groups had at least one Day Case episode of care. For the CI+ group, 92 participants were admitted for DC care on at least one occasion, compared with 76 patients in the CI- group. However, from Table 4.1, it can be seen that when the data for both groups were analysed and compared, the difference between the two groups was not statistically significant ($p=0.166$).

While the groups were equal in terms of patients having just one Day Case admission (11 each), the CI+ group had more patients who had multiple Day

Case episodes, 81% vs 65% (p=0.012) and the odds of being admitted for two or more DC episodes of care were 1.24 times greater for CI+ group participants (95% CI: 0.8121 – 1.9122). The number of patients admitted for DC care on multiple occasions (between 2 and 5 times) was 67 for the CI+ group and 54 (p=0.0607) for the CI- group. Similarly, the number of patients having between 6 and 20 DC visits was 14 for the CI+ group and 11 for the CI- group (p=0.5223).

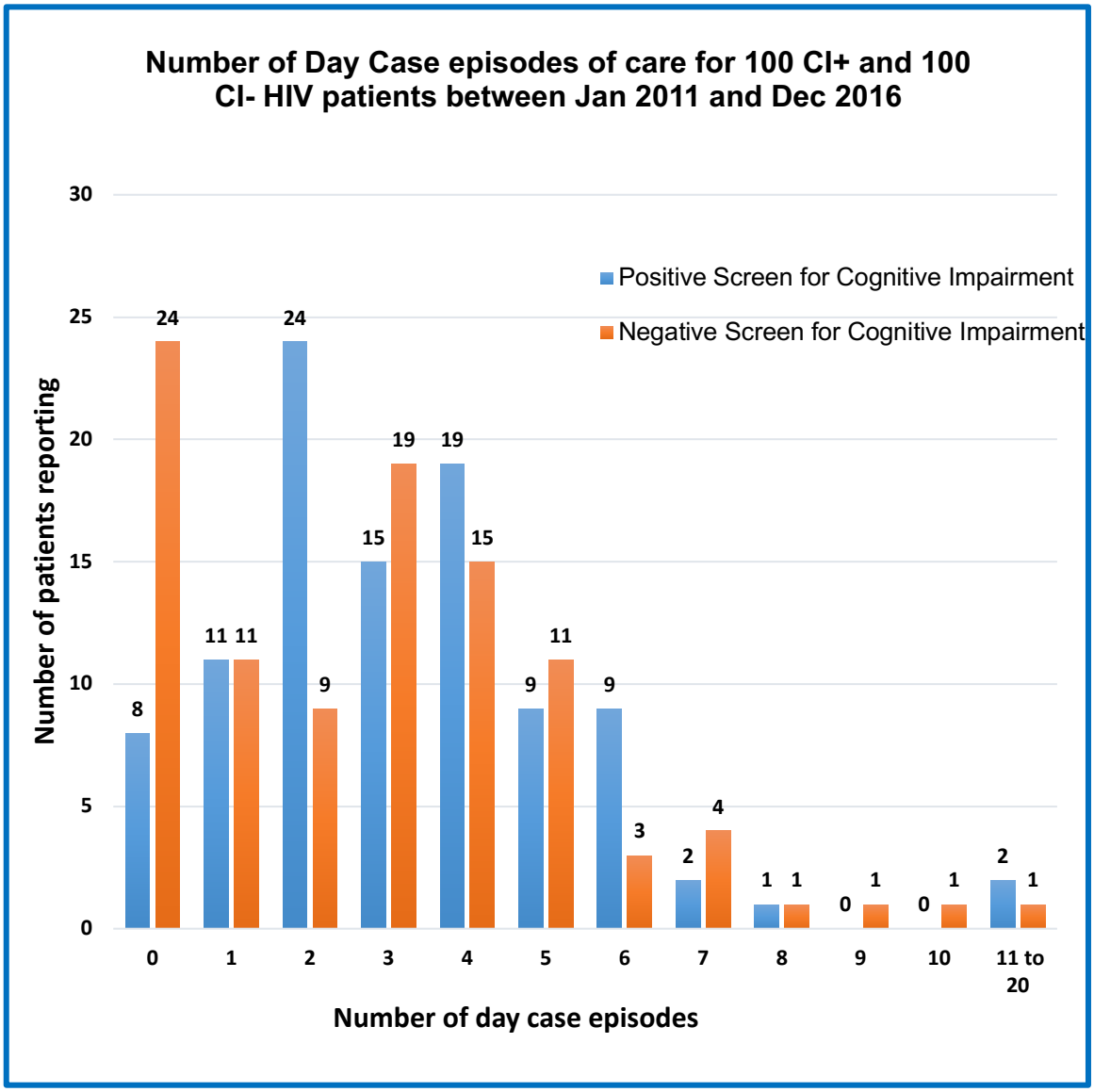


Figure 4.3. Day Case episodes between Jan 2011 and Dec 2016

4.4.2.3 Hospital Inpatient Episodes

Patients who screened positive for cognitive impairment had almost twice as many IP episodes of care over the six year period of the study (108 IP admissions in the CI+ group versus 58 in CI-), although the number of IP episodes per patient for the groups compared was found not to be significant (p=0.345). The odds of a CI+ patient being admitted for an IP episode of care was 1.3 times greater than for a CI- patient (95% CI: 0.7314 – 2.3380). Figure 4.4 shows the frequency distribution of the number of IP episodes of care.

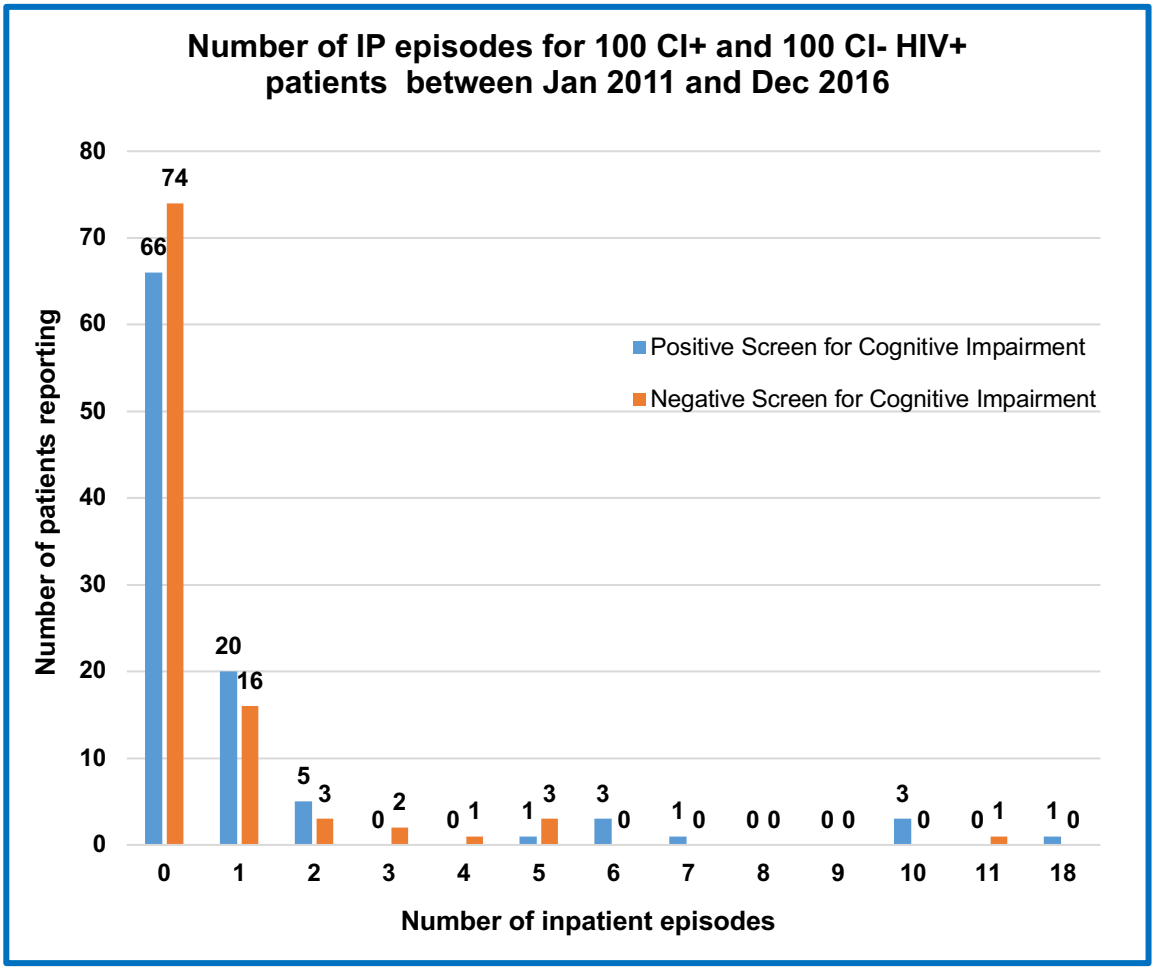


Figure 4.4. Inpatient episodes of care between Jan 2011 and Dec 2016

The majority (70%) of participants had no inpatient stays over the six year period, but the CI+ group had a higher proportion of patients who had at least one IP episode of care (34% versus 26%), though this difference was not found to be statistically significant (p=0.218). Once admitted, the average length of stay (ALOS) of patients screening positive for cognitive impairment was almost twice as long (11.8 versus 6.6 days) and this was found to be a significant difference (p<0.0001).

The impact of more admissions and longer lengths of stay means that the group that screened positive for cognitive impairment accounted for a larger number of inpatient days. In total, over the six year period, the group screening positive accounted for 1,275 inpatient days, compared with 383 days for the group screening negative, which is over 3.3 times as much. However, the analysis of IP days per patient data found this difference not to be statistically significant (p=0.302).

4.4.2.4 Hospital Outpatient Clinic attendances

The hospital EPR/PAS provided information on the number and type of outpatient services attended by the 200 study subjects between January 2011 and December 2016. In total, the 200 study subjects attended 6,533 outpatient clinics with 3,543 (54.2%) of these being attended by patients screening positive for cognitive impairment and 2,990 (45.8%) by those screening negative, and the frequency of all types of OP visits per patient was found to be significantly higher for CI+ group (p=0.049). Most (72.5%) of the outpatient visits were for clinics run by the GUIDe service. The GUIDe clinics attendance rates per patient were

higher in the CI+ group than in the CI- group and this difference approached statistical significance (p=0.051). GUIDe clinic visits accounted for 4,654 of the total OP visits and of these 54% (2,508) were attended by the subjects who had previously screened positive for cognitive impairment and 46% (2,146) were attended by subjects screening negative. Figure 4.5 provides a breakdown of the outpatient services most frequently accessed during the six years by the study subjects who screened positive for CI. After the GUIDe OP clinics (71% of attendances), the next most commonly attended services were Medical Social Work (6%), Haematology (4%), Hepatology (2%), and Clinical Nutrition (2%).

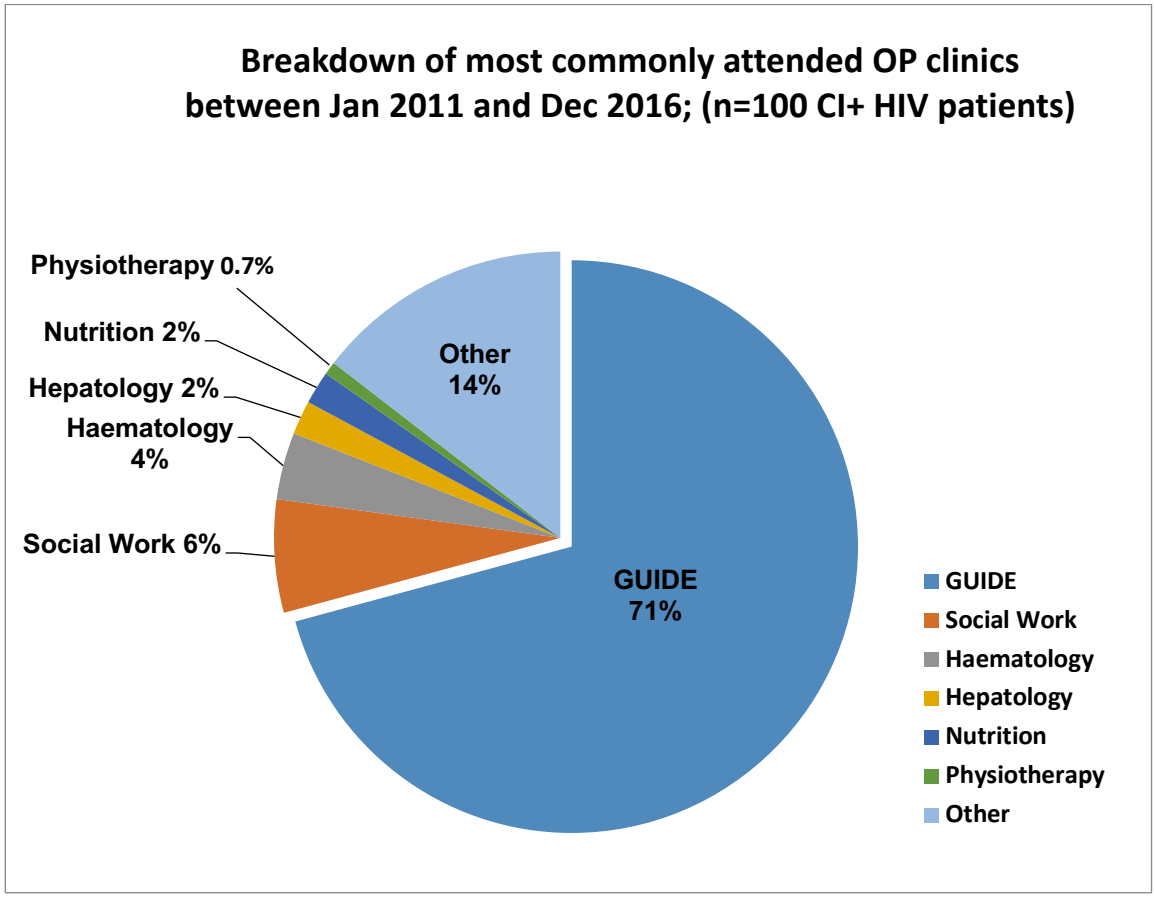


Figure 4.5. OP clinics attended by CI+ group between Jan 2011 and Dec 2016

Figure 4.6 provides a breakdown of the outpatient services most frequently accessed by the study subjects who screened negative for CI during the six years. The breakdown of services accessed by the CI- group is similar to that of the CI+ group, with the GUIDe clinics accounting for 72% of attendances, followed by Medical Social Work (6%), Haematology (3%), Hepatology (2%), Physiotherapy (2%), and Clinical Nutrition (1%).

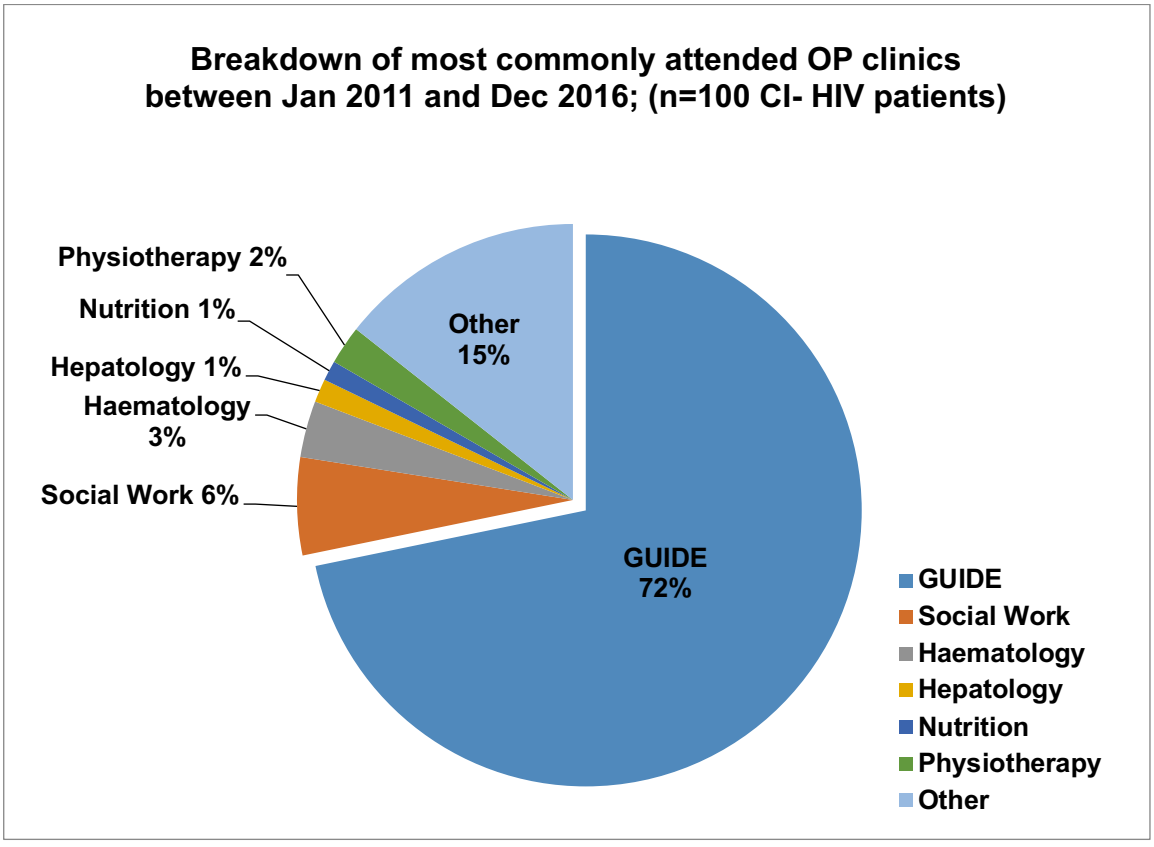


Figure 4.6. OP clinics attended by CI- group between Jan 2011 and Dec 2016

In Figure 4.7, a breakdown of the outpatient clinics attended over the six year period for all outpatient services excluding the GUIDe service is provided. By far, the biggest service accessed by all patients after the GUIDe clinics are outpatient services provided by the GUIDe department Medical Social Work service (MSW). Over the six year period, the 200 study subjects accessed the outpatient MSW service 397 times, which amounts to nearly a quarter of all the non-HIV services attended (22.5%). The next most commonly attended OP services are Haematology and Hepatology clinics for both CI+ and CI- groups.

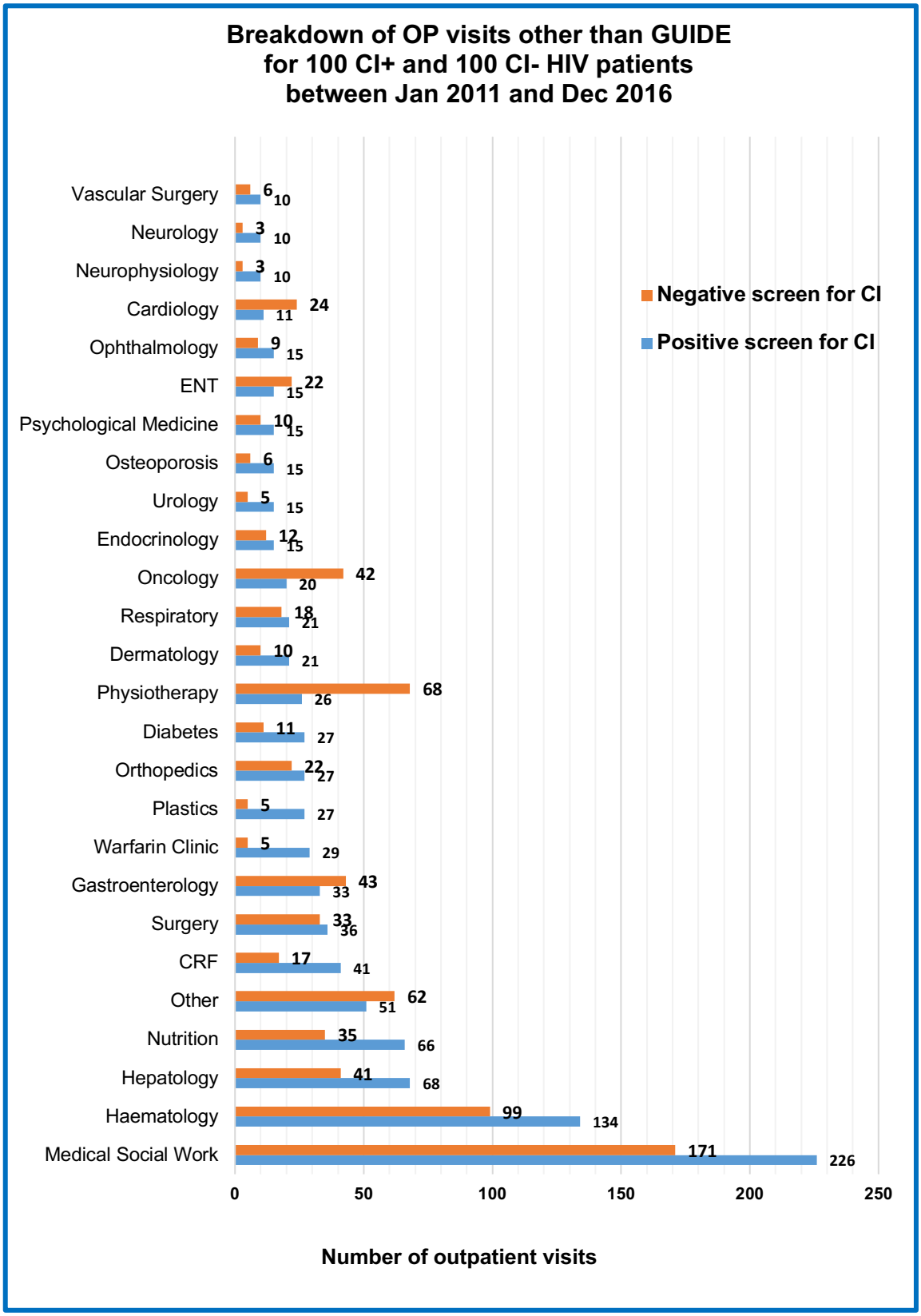


Figure 4.7. Outpatient attendances between Jan 2011 and Dec 2016

In Figure 4.7, it is shown that CI+ group attended more clinics for most specialities shown except for the Gastroenterology, Cardiology, ENT, Physiotherapy and Oncology services. The “Other” category of outpatient services in Figure 4.7 includes 131 visits for the following services: Anaesthesiology, General Medicine, Geriatric Medicine, Gynaecology, Surgery, Maxillofacial Surgery, Nephrology, Occupational Therapy, Psychiatry, Pain Management, Palliative Care, Radiation Oncology, Rheumatology, and Cardio-Thoracic Surgery.

4.4.3 Clinical Outcomes

Table 4.1 presents the results of clinical outcomes during the six year follow-up period. Analysis did find a significant association between cognitive status and noncompliance with the prescribed treatment, 24 in CI+ group vs 11 in CI- group ($p=0.016$). The risk of being noncompliant was 2.18 times greater for a patient from CI+ group (95% CI: 1.130 – 4.211). However, the analysis showed that for both groups, CI+ ($p<0.001$) and CI- ($p=0.002$), noncompliance increased the risk of having a detectable viral load during the follow-up period, but this risk was marginally higher for the CI- group (2.81 vs 2.61) (Table 4.2).

More participants in the CI+ group had detectable HIV RNA (VL>40 copies/ml) during the study period (42 vs 31; $p=0.106$). Although the latter difference was not found to be significant, the odds of having a detectable VL during the follow-up period was 1.6 times greater for participants from the CI+ group (95% CI: 0.902 – 2.881) and this parallels with the increased risk of noncompliance in the CI+ group. In addition, more CI+ participants had a detectable VL at baseline (29 vs 26; $p=0.635$) and at follow-up (7 vs 4; $p=0.352$). These differences were not

statistically significant, although suggestive of a higher likelihood of unstable disease control in the CI+ group.

Table 4.2. Detectable VL and Medication Noncompliance in CI+ and CI- Participants during the Follow-up Period

Group	X^2	*df*	*P*
CI+	17.907	1	<.001
Odds Ratio	8.757 CI (2.915 - 26.306)		
Risk	2.616 CI (1.757 - 3.896)		
CI-	10.061	1	0.002
Odds Ratio	7.62 CI (1.870 - 31.318)		
Risk	2.814 CI (1.699 - 4.662)		

There was an attrition rate of 17% in the CI+ group and 13% in the CI- group over the follow-up period. Overall, however, the differential attrition rate between the groups was not found to be statistically significant (p=0.4294). More of the group that screened positive for cognitive impairment were lost to follow-up (8 vs 6; p=0.306), although they could have availed of HIV services elsewhere. Contrary to LTFU numbers, more of the CI- group participants informed the medical team of transferring their care (TOC) to other hospitals by requesting appropriate information to be released to the new service provider (6 vs 4; p=0.419). These differences are not significant, and in fact, LTFU and TOC together led to loss of 12 participants (12%) in each group by the end of the follow-up period. More

patients from CI+ group died during the study period (5 vs 1), which increased attrition for the CI+ group.

While the difference in the death rate between the two groups was also not statistically significant (p=0.212), the odds of RIP in the CI+ group is 5.21 times (95% CI: 0.598 – 45.426) greater compared with the CI- group. The mean age of the five patients who died in the CI+ group was 38 years and the one deceased patient from the CI- group died at the age of 56 years.

The number of participants who were diagnosed with Depression and availed of specialised treatment prescribed by a Psychiatry Specialist or their Primary Care Physician (i.e. GP) was nearly equal in both groups (14 patients in the CI+ group and 15 in the CI- group) (p=0.841).

4.4.4 Non-Health Care Related Hospital Services Use

The SJH Medical Social Work Department (MSW) has a dedicated service for the GUIDe clinic users and offers a wide range of different supports to people living with HIV. MSW was the second most common service accessed after the GUIDe clinics by both CI+ and CI- groups (6% of all attendances for each group). However, the CI+ group accessed this service more frequently than the CI- group.

During the study period, there were 226 MSW clinic attendances registered for CI+ group versus 171 for CI- group. Despite this difference, the statistical analysis of frequency of MSW visits per patient did not show significance (p=0.148) between the groups compared (Table 4.1). The frequency of different

types of MSW services and supports accessed was analysed in more detail and is presented in Table 4.3 and Figure 4.8.

Table 4.3. Frequency and Type of MSW Supports Accessed between Jan 2011 and Dec 2016

Services / supports accessed		**Positive Screen for CI (n=100)**		**Negative Screen for CI (n=100)**		***p-value***
Medical Social Work OP attendances per patient	Mean (SD) Median (range)	2.26 1	(3.72) (0-21)	1.71 0	(3.01) (0-13)	0.148
					X^2	***p-value***
Housing & accommodation	Yes /number	15		6		0.038
Financial/employment/social welfare/mortgages	Yes /number	27		15		0.037
Counselling /adjustment to illness	Yes /number	42		37		0.470
Child welfare and protection	Yes /number	4		1		0.184
Mental health/suicide & self-harm	Yes /number	6		3		0.249
General support/family & relationships issues	Yes /number	32		26		0.159
Addiction support	Yes /number	3		2		0.651
HIV status disclosure	Yes /number	6		7		0.774

The differences in accessing MSW services that relate to *Housing and Accommodation* as well as *Financial / Employment / Social Welfare / Mortgages* supports were statistically significant with p-values of 0.038 and 0.037, respectively, with the CI+ group participants accessing these supports more frequently. Although most of the other MSW supports were also accessed more frequently by the CI+ group participants, these differences were not significant with p-values p=0.470, p=0.184, p=0.249, p=0.159, and p=0.651 for supports provided in the area of *"General Counselling and Adjustment to Illness", "Child*

Welfare and Protection", "Mental health, Suicide and Self-harm", General Emotional support – Family and Relationships issues" and *"Addiction support"*, respectively. Contrary to the other types, support in the area of *"Diagnosis Disclosure issues"* was sought more often by CI- group than CI+ group participants, although this difference was minor – seven versus six patients, respectively (p=0.774).

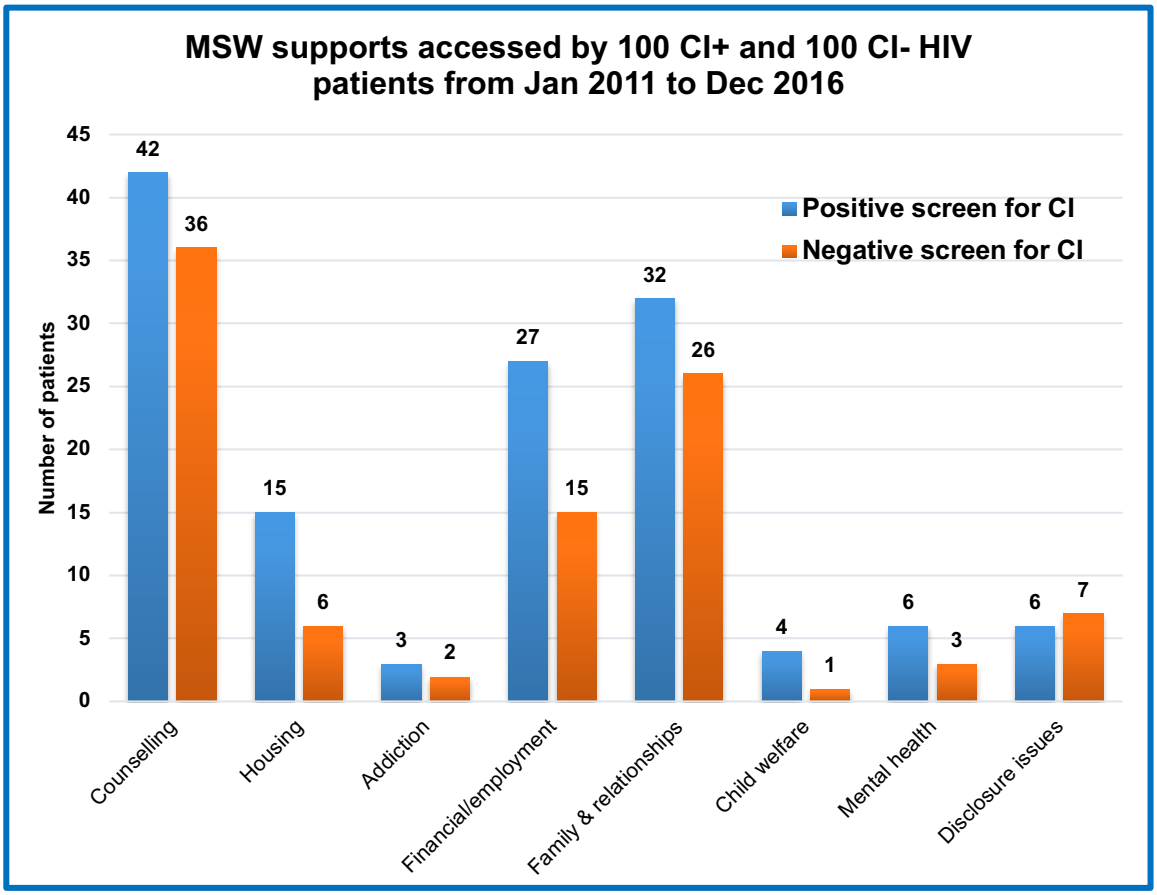


Figure 4.8. Type of supports provided by MSW from Jan 2011 to Dec 2016

4.4.5 Comparison of the Costs of Hospital Based Care – OP, IP, and DC Episodes of Care Provided to the two Groups during 2014 and 2015.

Costs of providing hospital based care for 2014 and 2015 for 200 patients were available from the hospital financial system. The summary of the cost data for hospital based Inpatient, Day Case, and Outpatient episodes of care are presented in Table 4.4.

Consistent with the data reported earlier in this chapter, the CI+ group are bigger users of all hospital services and account for greater costs, with the exception of Day Case episodes costs for 2014, which were found to be higher in the CI- group. Over the two year period (2014 and 2015 combined), the total costs for the CI+ group amounted to €508,818, compared with €233,855, for the CI- group, with an average cost per patient per annum for CI+ being nearly double the cost of a CI- patient in 2014 (€2,733/patient/year versus €1,405/patient/year, respectively) and 2.5 times higher in 2015 (€2,355/patient/year versus €933/patient/year, respectively). For 2014 and 2015 combined, the difference in total cost between the groups amounted to €274,963. Most of this difference (90.5%) is accounted for by the difference in the Inpatient care costs (€248,572). The same trend is observed for 2014 and 2015 separately, with Inpatient services accounting for a greater difference observed in 2014 (95.3% or €126,517) than in 2015 (85.8% or €122,054).

Table 4.4. Hospital Inpatient, Day Case and Outpatient Costs for 2014 and 2015 in 100 CI+ and 100 CI- HIV Patients

Year / Cognitive screen status n=100 in each column	2014 CI- group	2014 CI+ group	2015 CI- group	2015 CI+ group	2014 + 2015 CI- group	2014 + 2015 CI+ group
Day case episodes Total	109	106	76	122	185	228
Total annual/biannual cost DC (€)	22,859	13,535	19,951	26,238	42,811	39,774
Ave. cost per episode DC Total (€)	210	128	263	215	231	174
Ave. cost per patient DC Total (€)	229	135	200	262	428	398
Annual/biannual cost – DC GUIDe (€)	15,964	10,132	18,530	21,379	34,479	31,511
Ave. per episode DC – GUIDe (€)	161	102	250	184	411	286
Ave. per patient DC – GUIDe (€)	160	101	185	214	345	315
OP visits	328	431	292	378	620	809
Total annual/biannual cost (€)	66,839	82,396	69,168	83,040	136,007	165,436
Ave. cost per OP visit Total (€)	204	191	237	220	219	204
Ave. cost patient OP Total (€)	668	824	692	830	1,360	1,654
Annual/biannual cost – OP GUIDe (€)	61,528	65,079	59,910	67,102	121,438	132,181
Ave. per episode OP – GUIDe (€)	214	190	235	210	449	400
Ave. per patient OP – GUIDe (€)	615	651	599	671	1,214	1,322
Inpatient episodes	6	12	1	13	7	25
Total annual/biannual cost (€)	50,842	177,359	4,196	126,250	55,037	303,609
Total inpatient Days	43	206	2	168	45	374
Ave. cost per IP episode	8,474	14,780	4,196	9,712	7,862	12,144
Ave. cost per patient (€)	508	1,774	42	1,263	550	3,036
ALOS (Days)	7.17	17.17	2.00	12.92	6.43	14.96
Annual/biannual cost – IP GUIDe (€)	3,348	24,461	0	41,100	3,348	65,561
Ave. per episode IP – GUIDe (€)	3,348	4,892	0	5,871	3,348	10,763
Ave. cost per patient – IP GUIDe (€)	33	245	0	411	33	656
Total Cost (€)	140,540	273,290	93,315	235,529	233,855	508,818
Mean cost per patient Total (€)	1,405	2,733	933	2,355	2,339	5,088
Mean cost per patient GUIDe Total (€)	808	997	485	1,296	1,592	2,292

Statistical analysis of the data was carried out to evaluate whether or not these results were statistically significant.

4.4.5.1 Unique visit cost analysis

The hospital cost data was first analysed to determine whether or not any observed difference in costs could have arisen due to the differences in *unique visit* costs, rather than differences in the intensity of patient's hospital service usage. Therefore, before exploring the cost per patient, analysis was conducted to ensure that *unique visit* costs (including unique cost of IP, DC and OP episodes) did not differ across patient's cognitive status. It was expected that the cost of an episode of care for a CI+ participant would not differ significantly from the cost of an episode of care for a CI- patient. As all data proved to be not-normally distributed, a Mann-Whitney U test was performed to determine if there was a statistically significant difference in the *unique visit* costs.

Table 4.5. Comparison of Unique Visit Costs across CI Status

Type of visit	2014		2015		2014 + 2015	
	U	***p***	***U***	***p***	***U***	***p***
IP/DC/OP combined	120647.0	.829	89425.5	.16	422319.5	.837
Cost per IP episode	31.0	.640	6.00	.901	20247.5	.485
Cost per DC episode	5534.0	.593	3993.5	.101	83.0	.859
Cost per OP episode	68387.0	.427	52062.0	.204	242109.0	.259

Analysis was performed to compare: cost per visit (2014 and 2015 combined), cost per visit for year 2014 and cost per visit for year 2015, cost per visit for each episode type (IP, DC, or OP) for (2014 and 2015 combined), cost per visit for

each episode type (IP, DC, or OP) for 2014, and cost per visit for each episode type (IP, DC, or OP) for 2015. Results are presented in Table 4.5 and show that there were no statistically significant differences between the *unique visit* costs, in general, or per episode type (IP, DC, OP) in either of the two years, 2014 or 2015, or for the two years combined (2014 + 2015) across the patients' cognitive status.

4.4.5.2 Individual patient cost evaluation

The costs incurred per individual patient were compared (total cost per patient for year 2014, 2015 and 2014 + 2015 combined, cost per patient incurred during IP episodes, DC episodes and OP visits for year 2014, 2015 and 2014 + 2015 combined). The cost per patient was significantly higher for the CI+ group for IP episodes of care (p=0.017) and Day Case visits (p=0.047) only in 2015. These results are reported in Table 4.6 below.

Table 4.6. Comparison of Costs Incurred per Individual Patient

Type of episode	**2014**		**2015**		**2014 + 2015**	
	U	***p***	***U***	***p***	***U***	***p***
Total cost (IP+DC+OP)	4388.0	.134	4534.5	.254	4427.0	.161
Cost per IP episodes	4849.0	.304	4649.0	.017	4695.0	.092
Cost per DC episodes	4676.0	.412	4213.5	.047	4298.5	.084
Cost per OP episodes	4401.0	.142	4702.5	.466	4576.0	.300

4.4.5.3 Analysis of frequency distribution of episodes of care

Analyses (Mann Whitney U test) were then performed to see if there was a statistically significant difference in the frequency of attendances for hospital episodes of care (total number of visits, IP, DC and OP) per patient between the two groups for 2014, 2015, or 2014 and 2015 combined, to explain the difference in the cost per patient. Subsequent analysis showed that the difference in the frequency distribution of IP admissions and DC episodes of care was statistically significant between the two groups of patients in 2015 (p=0.016 and p=0.019, respectively), with the total number of IP episodes being 13 in CI+ group versus one in CI- group and the total number of DC episodes being 122 for CI+ patients versus 76 for CI- patients. These results are reported in Table 4.7 below and the total number of IP, OP, and DC episodes for each year for CI+ and CI- groups are reported in Table 4.4.

Table 4.7. Comparison of Frequency Distribution of Hospital Episodes of Care in 100 CI+ and 100 CI- HIV Patients for Years 2014, 2015, and 2014+2015 Combined

Comparison of frequency distribution of IP, DC, OP episodes						
Type of Episode	**2014**		**2015**		**2014 + 2015**	
	U	***p***	***U***	***p***	***U***	***p***
IP episodes	4849.0	.304	4647.0	.016	4693.0	.090
DC episodes	4737.5	.495	4105.0	.019	4225.5	.053
OP episodes	4133.5	.032	4231.0	.056	4141.0	.035

Moreover, the total number of OP visits in 2014 was greater for the CI+ group (431 versus 328) and analysis of the frequency distribution of attendances for OP visits was found to be statistically significant for year 2014 (p=0.032). This was

also true for the data for both years combined (total number of OP visits was 809 versus 620, respectively), with the frequency distribution of attendances of OP visits showing a statistically significant difference (p=0.035). However, in 2015, this difference for OP attendances was just close to being significant, with a p-value of 0.056.

4.4.5.4 GUIDe service costs for year 2014 and 2015

Over the two year period, the CI+ group had more episodes of inpatient care, 25 (78%) versus 7 (22%) (12 for CI+ and 6 for CI- in 2014, and 13 for CI+ and one for CI- in 2015) (Figure 4.9). Twelve of 25 IP episodes for CI+ group (48%) were under the care of GUIDe services, which accounted for 37% of all IP episodes for 2014 and 2015. For CI- group, one of seven (14.3%) IP episodes were under GUIDe services, which accounted for 3% of all IP episodes over the two year period.

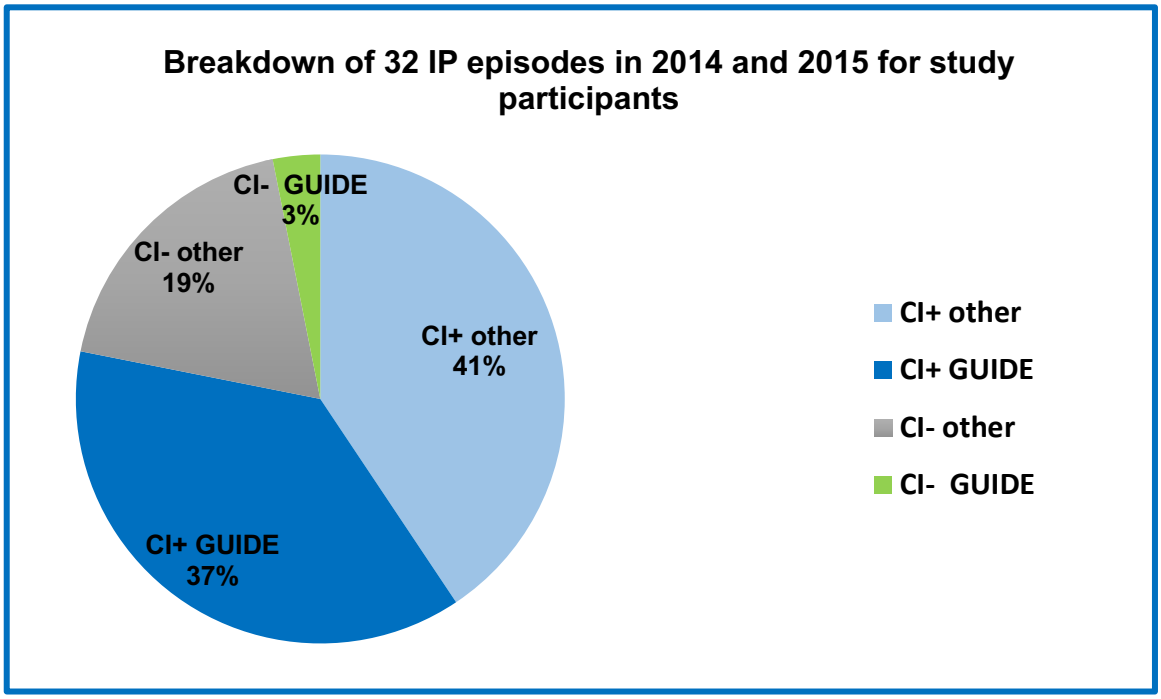


Figure 4.9. Breakdown of IP episodes in 2014 and 2015

The cost of IP care under GUIDe services accounted for 21.6% (€65,561) of the total inpatient cost incurred for 2014 and 2015 combined for the CI+ group and constituted 6.1% (€3,348) of the total inpatient cost for the CI- group. For 2014, 13.8% (or €24,461) of the total IP cost for the CI+ group patients and 6.56% (or €3,348) of the total IP cost for the CI- group patients were attributed to IP care provided by GUIDe service. For 2015, 32.6% (or €41,100) of total IP cost for the CI+ group patients and 0% or €0 for the CI- group were accounted for by the GUIDe service. The average cost per IP visit under GUIDe care for the CI+ group was €4,892 for 2014 and €5,871 for 2015. For the CI- group, the average cost of an inpatient stay under GUIDe services amounted to €3,348 in 2014 and there were no inpatient stays associated with the GUIDe services in 2015 for this group (Table 4.4 and Figure 4.12).

Figure 4.10 shows the breakdown of outpatient clinic visits in 2014 and 2015 for the CI+ and CI- groups and the share of these visits accounted for by the GUIDe service. Overall the CI+ group accounted for 56% of the 1,429 visits and over 82% of these visits were GUIDe OP clinic attendances. In the CI- group, a higher proportion (86.4%) of the attended OP clinics was for GUIDe services.

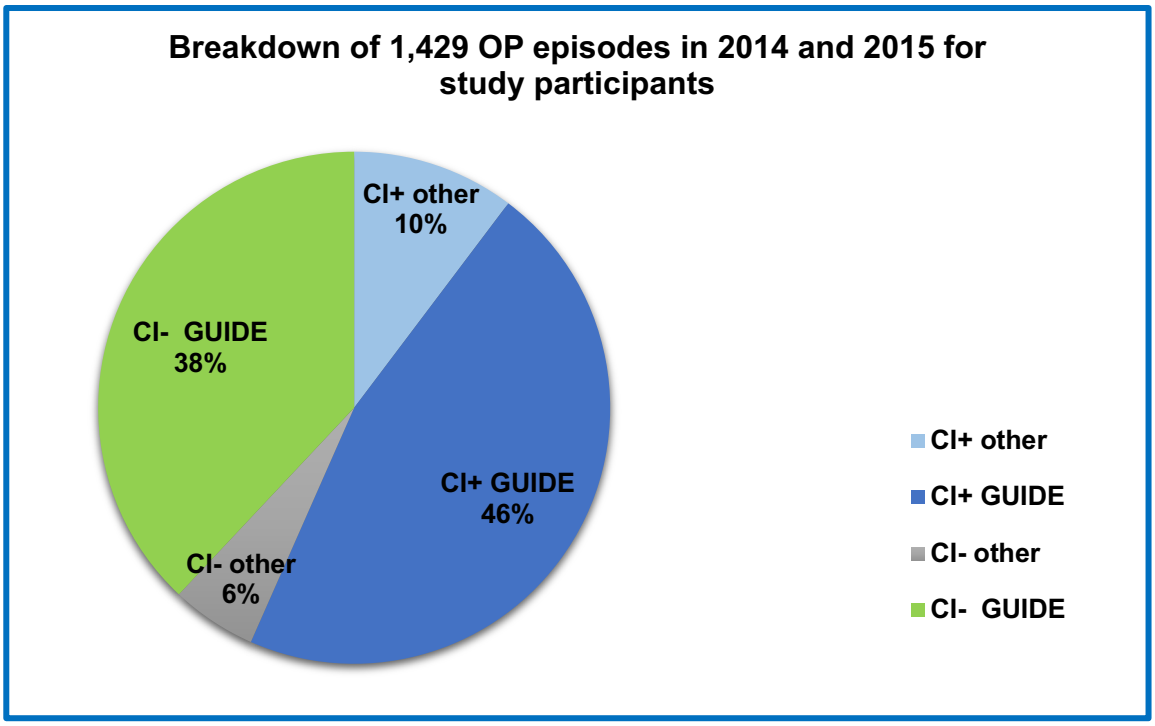


Figure 4.10. Breakdown of OP visits in 2014 and 2015

The cost of OP GUIDe visits for the CI+ group amounted to €132,181 or 80.4% of total OP costs for 2014 and 2015 combined when compared with the CI- group (€121,438 or 89.3%). For 2014, the cost for OP GUIDe visits were €65,079 or 80% of total OP costs for the group for CI+ patients (average cost per visit €190) and €61,520 or 92% of total OP cost for the group of CI- patients (average cost per visit €214); and €67,102 or 80.8% of total OP cost for the group of CI+

patients (average cost per visit €210) and €59,910 or 86.62% of total OP cost for CI- patients (average cost per visit €235) for 2015 (Table 4.4 and Figure 4.12).

Figure 4.11 provides a breakdown of the 413 Day Case episodes of care provided to both groups in 2014 and 2015. The CI+ group patients accounted for 55% of the Day Case admissions and most (94%) were under the care of GUIDe service. Similarly, for the CI- group 93% of the DC episodes were under the care of GUIDe service.

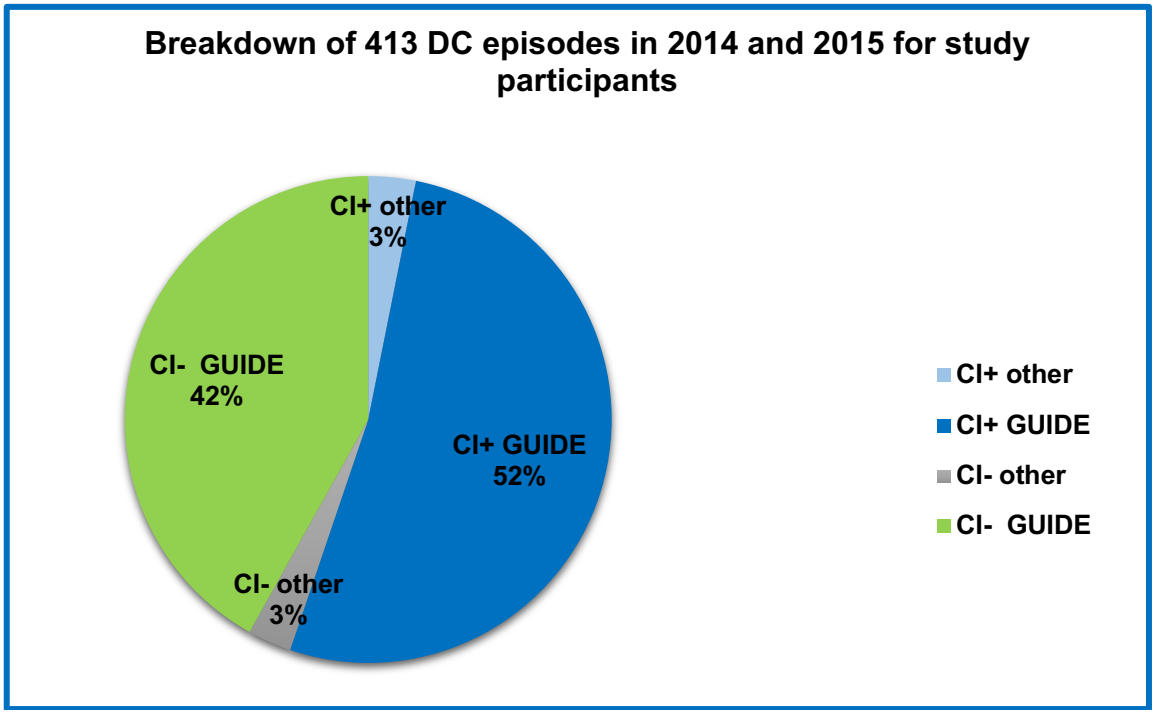


Figure 4.11. Breakdown of day case episodes in 2014 and 2015

The cost of DC episodes under the care of GUIDe services was calculated to be €66,005 or 79.9% of total incurred DC costs for 2014 and 2015 combined, of which only 47.7%, or €31,511, were attributable to the CI+ group. For 2014, this

accounted for €10,132 – 74.86% of DC costs for CI+ patients (average cost per DC visit €102) and €15,964 – 70% of DC costs for CI- patients (average cost per DC visit €161). In 2015, the cost of DC episodes under GUIDe services was €21,379 or 81.48% of DC costs for CI+ (average cost per DC visit €184) and €18,530 or 92.9% of DC costs for CI- patients (average cost per DC visit €250) (Table 4.4 and Figure 4.12).

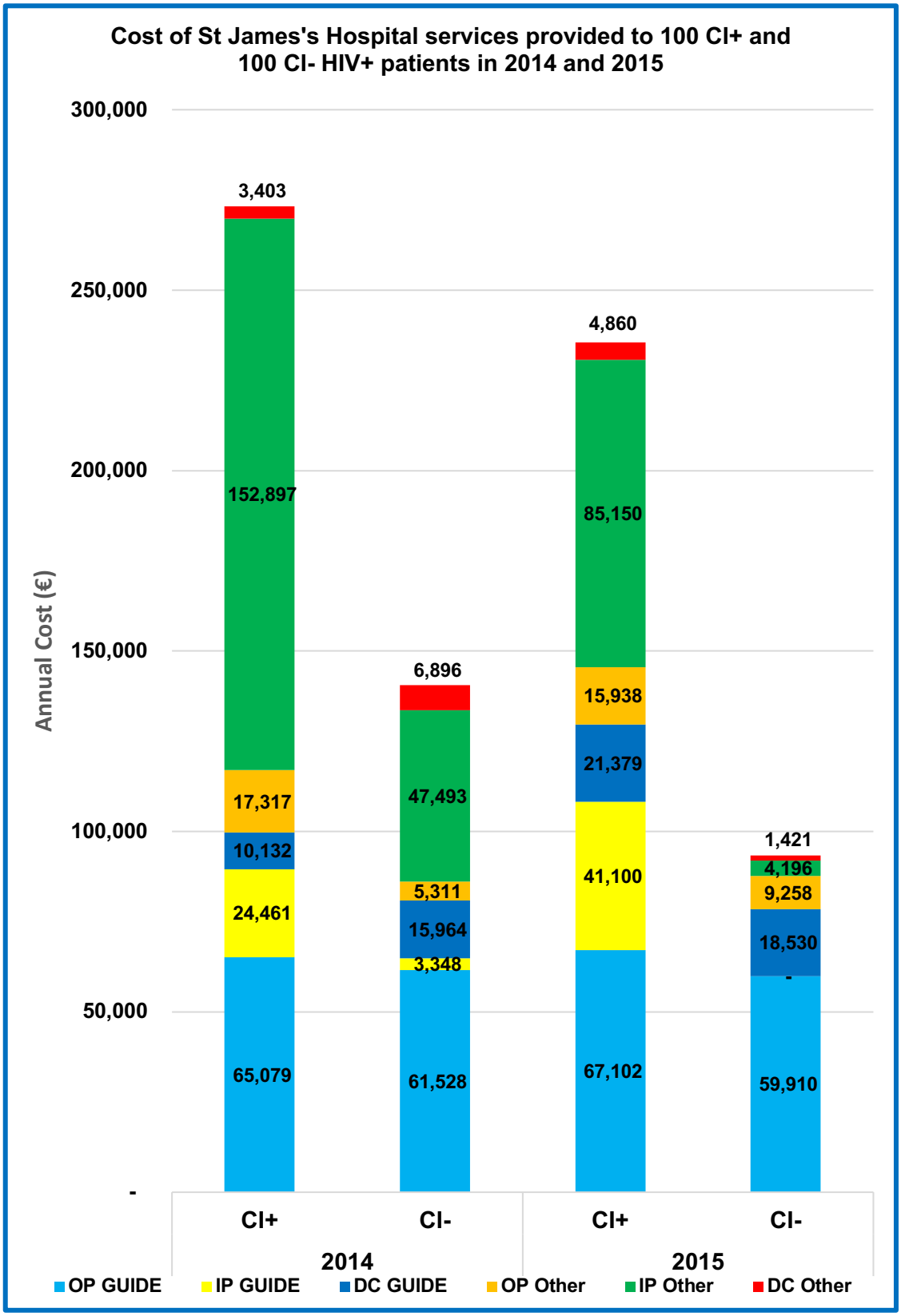


Figure 4.12. Cost distribution for IP, DC and OP episodes for 2104 and 2015

The overall cost of CI+ group participants in 2014 amounted to €273,290, of which €99,672 (36.5%) was accounted for by GUIDe services. In 2015, overall costs totalled €235,529, of which €129,581 (55%) was accounted for by GUIDe services. For the CI- patient group in 2014, the overall cost was €140,540, of which €80,840 (57.5%) was for services provided by GUIDe. In 2015, the total cost incurred by the CI- group was €93,315, of which €78,440 (84%) was accounted for services provided by GUIDe (Table 4.4).

4.5 Discussion

Overall, in this study, the differential attrition rate between the groups, 17% and 13% in the CI+ and CI- group, respectively, was not found to be statistically significant (p=0.4294). While the difference in the death rate between the two groups was also not statistically significant (p=0.212), the results suggest a fivefold greater risk of death for PLWH who also have cognitive dysfunction over the six year study period. This agrees with the recent report by Patel et al., who found a higher risk of in-hospital mortality in HIV positive patients with HAND (464). Importantly, Gebo et al. in their study found that “decedent” patients contributed to significantly higher health care related costs when compared with “non-decedent” patients. This was explained by either poorly controlled HIV disease and related complications or another serious illness that required multiple hospital admissions for medical/surgical interventions prior to death (468). In our study, the mean age of the CI+ group patients who died was 38 years and the only deceased patient, from the CI- group, died at the age of 56 years. However,

due to the small numbers, the age at death difference between the groups was not possible to compare reliably.

The six year data analysis shows higher utilisation of all services in the CI+ group and these differences were statistically significant for the total number of OP visits, with the difference for GUIDe visits approaching statistical significance (p=0.051), while this number was not significantly different for other attended OP clinics. This suggests poorer HIV disease control over the six years and a clinical need for closer monitoring and hence, higher number of GUIDe OP attendances. However, the 2014 – 2015 cost data shows that, while higher for CI+ group, the differences in expenditures on GUIDe Outpatient service were not significantly different for 2014 and 2015 (€65,079 and €61,520 for CI+ and CI- groups for 2014, respectively; or €67,102 and €59,910 for CI+ and CI- groups for 2015, respectively), despite the number of visits being significantly higher, at least for the year 2014, and approaching significance for the year 2015. This led to a higher average costs per GUIDe OP visit for a CI- group patient during both years (€190 for CI+ group versus €214 for CI- group, for 2014, and €210 for CI+ group versus €235 for CI- group, for 2015). While there was not enough information in the data gathered from the financial system to explain this paradoxical finding, one explanation could possibly be that more CI- patients were taking ART at the time, which increased the average cost of GUIDe OP visit for a patient who screened negative (CI-), compared with a patient who screened positive (CI+) for cognitive impairment. Although the average cost per GUIDe visit for CI+ patient was lower, the overall expenditures for the group for OP GUIDe visits were higher.

Over the six years, the CI+ group also had a significantly higher number of total in-hospital episodes of care (IP and DC combined), as well as the total number of admission days (IP and DC combined). However, when the six year IP and DC episode data were analysed separately, these differences were not statistically significant for each of the type of care provided. However, cost data analysis for 2014 and 2015 showed that, for 2015, both IP and DC care was accessed by CI+ patients significantly more often and this led to significantly higher costs incurred by the hospital while providing in-hospital care to this group.

An interesting finding during the 2014 and 2015 cost data analysis is that in 2014, the CI+ group of patients had significantly more OP episodes than the CI- patients, which did not lead to statistically significant differences in costs per patient between the CI+ group and the CI- group. However, in 2015, there was a shift towards significantly more DC and IP episodes of care in the CI+ group. Moreover, for the CI+ patients, the cost incurred for DC under GUIDe services in 2015 was double the cost in 2014 (€10,132 and €21,379 in 2014 and 2015, respectively), and the IP cost under GUIDe services for 2015 was close to being double the cost for 2014 (€24,461 and €41,100 in 2014 and 2015, respectively). The costs for the CI- group, in 2015, for IP episodes for all specialities significantly dropped when compared with 2014 (from €50,842 in 2014 to €4,196 in 2015; and from €3,348 to €0 for IP GUIDe services alone). For the CI- group, there was also a minor drop in the overall total cost for DC episodes from €22,859 to €19,951, with a minor cost increase for DC episodes under the care of GUIDe services (from €15,964 in 2014 to €18,530 in 2015). This suggests a shift from less acute and less costly care in the setting of the GUIDe outpatient

department to a more acute but also more resource intense in-hospital (Day Case and Inpatient) care for the CI+ group, and overall stability for the CI- group.

As well as having more inpatient episodes over the six years, the CI+ group patients also stayed in hospital for longer, almost 13 days compared with 3.8 days on average for the CI- group. The average length of stay (ALOS) was found to be five days longer for a CI+ patient and this was a statistically significant finding ($p<0.001$). This is consistent with the data published recently by Patel et al. (2018) who also reported longer in-patient stay for PLWH who have HAND (464).

The above described findings suggest that cognitive status in PLWH can possibly be a useful clinical marker of the overall health and wellbeing, as well as HIV disease control. Indeed, the current study results show that the odds of having detectable viral load during the follow-up period were greater in the CI+ group. While having a detectable VL was strongly associated with treatment non-compliance in both groups, the non-adherence risk was found to be 2.18 times higher in the CI+ group. Disproportionately greater difficulties in adhering to treatment in the CI+ group is consistent with previous findings showing that HIV seropositive individuals with disease related cognitive dysfunction have higher rates of non-adherence to ART (446, 447, 451).

As well as poor adherence to prescribed treatment, in the current study, the group that screened positive for cognitive impairment was also found to have poorer engagement with services as they displayed significantly higher DNA rates than the CI- group ($p<0.001$). Poor engagement with services can also

compromise treatment compliance, as GUIDe clinic attendance ensures regular ART prescription and dispensing. Disengagement with HIV services and treatment non-adherence may lead to ART resistance, especially notable for NRTIs and NNRTIs (452, 453, 469, 470). The development of ART resistance can ultimately lead to loss of disease control and subsequently increased health care costs. Studies have shown that all non-drug related health care costs increase with HIV disease progression and are inversely proportionate to immune status / CD4 counts (465, 468, 471). Moreover, Gonzalo et al. (2009) and Stoll et al. (2002) independently showed that the introduction of HAART is cost saving for healthcare providers and leads to major economic benefits for society and government, provided that patients are compliant with their prescribed treatment. This is consistent with the current study finding of a lower overall OP GUIDe expenditure in the CI- group, who are more likely to be on ART and be compliant with it, despite the higher cost per OP visit. Compliance also leads to individual patient gains, a longer life and a better quality of life (472, 473).

Both, frequent OP appointments and a higher number of in-hospital care episodes are markers of higher healthcare costs (471). Moreover, these suggest that CI+ patients might have poorer HIV disease control and/or multiple co-morbidities for which they require more inpatient and outpatient care. Poor HIV disease control may also contribute to impaired cognitive status.

There was a large number of other specialty clinics attended by both groups of patients. While the proportion of non-GUIDe OP clinics attended over the six years was nearly equal for both groups (29% and 28% for CI+ and CI- group, respectively), the cost data for the two years examined show much higher total

expenses incurred by the CI+ group while under the care of services other than GUIDe when compared to CI- group (IP, DC and OP combined). These were nearly three times higher for the total non-GUIDe costs for 2014 (€173,618 and €59,700, for CI+ and CI- group, respectively) and over seven times higher for 2015 (€105,948 and €14,875, for CI+ and CI- group, respectively). The 2014 and 2015 cost analysis showed a greater proportion of costs incurred by services other than GUIDe for the CI+ group for IP episodes of care (€152,897 and €47,493 for the CI+ and CI- groups, respectively in 2014 and €85,150 and €4,196, for the CI+ and CI- groups, respectively in 2015) and for DC episodes for 2015 (€4,860 and €1,421 for the CI+ and CI- groups, respectively), but the opposite was found for DC episodes for 2014 (€3,403 and €6,896 for the CI+ and CI- groups, respectively). While using the data available, it was not possible to extrapolate how much of the costs were due to diseases that are HIV-unrelated, this is consistent with previous findings that with the introduction of HAART, HIV infected people live longer and hence, become more likely to suffer from HIV-unrelated comorbidities. Current study data suggest that CI+ patients access non-GUIDe services more frequently than CI- group, therefore, they might have other serious illnesses and / or a higher burden of disease other than HIV. Serious comorbidities such as cardiovascular or cerebrovascular disease and malignancies have been shown in other studies to increase non-ART related costs in the HAART era (468).

While GUIDe OP clinics are the most frequently attended type of OP clinics (71% and 72% for CI+ and CI- group, respectively), the next most attended medical specialities for both groups are Haematology and Hepatology (4% vs 2% for

Haematology services for CI+ and CI-, respectively; and 2% vs 1% for Hepatology services for CI+ and CI-, respectively). This might reflect the fact that at the start of the HIV epidemic patients with Haemophilia were at a greater risk of acquiring HIV infection and SJH is the country's main centre for Haemophilia care. However, as a matter of fact, both the GUIDe and Haematology services are provided to patients with HIV and Haemophilia in the Haematology clinics, which is not reflected by the hospital PAS data. In addition, a large proportion of SJH HIV seropositive cohort are also Hepatitis B or C virus co-infected, and those with chronic decompensated liver disease, as well as those who have chronic non-viral hepatitis related liver disease, are referred to Hepatology services for specialist care. While there were more attendances for Neurology OP services in the CI+ group than in CI- (ten vs three, respectively), with similar number of attendances for Neurophysiology clinics (ten vs three, respectively), these were unexpectedly low. However, 32 of the 100 CI+ patients participated in the detailed neuropsychology studies and were followed by Neurology Research Registrars, which might explain the small number of Neurology OP visits and the higher number of CRF visits for this group. The analysed data, however, did not include the number of referrals for Neurology review or indeed other services during the IP episodes of care, which could yield a higher total number of Neurology services provided to HIV+ people.

Interestingly, the second most attended hospital OP service for both groups was a non-medical one. The Medical Social Work OP service attendance accounted for 6% of all OP attendances for each CI+ and CI- group, which suggests that PLWH encounter significant psychosocial difficulties irrespective of cognitive

status. MSW Department at SJH offers a wide range of supports and services to PLWH who attend the GUIDe clinic. While CI+ availed of greater number of most MSW services available, they accessed the *Housing & Accommodation* and *Financial / Employment / Social Welfare / Mortgages* supports significantly more often. This finding concurs with previous research findings of greater employment difficulties and therefore more financial issues and dependency on unemployment or disability benefits and other social welfare schemes (454, 457). In addition, housing status is an important facilitator of optimal engagement with HIV health care services and medication adherence (474). The finding of excessive MSW supports also suggests that, at a hospital level, the non-healthcare related costs are also higher for the CI+ group. In earlier chapters of this work, it was also noted that PLWH and HAND frequently complained of memory and concentration difficulties and had difficulties meeting the requirements of their job, which led to transfer to a less demanding position or, sometimes, to loss of employment.

4.5.1 Strengths of the Study

This is the first study known to look at both inpatient and outpatient healthcare and non-health care related costs in people living with HIV through the lens of cognitive dysfunction. It was a large study population, with an equal number of subjects in both groups, with small overall and differential attrition rates. The study analysed data obtained from hospital administrative systems over a six year period, which is long enough to provide an adequate picture of differences in hospital resource utilisation between the two groups. In addition, the data obtained from PAS and EPR systems regarding all types of hospital care attendances for 2014 and 2015 was consistent with the data extracted from

hospital's financial system, which gives added confidence in the robustness of the data collected from both systems. The two year cost data captured all costs related to IP, DC and OP episodes of care, and these would constitute the majority of costs of medical care for a patient attending hospital services. This permits adequate analysis of hospital cost differences between the two groups.

4.5.2 Limitations of the Study

This study looked at hospital services utilisation and costs in 100 CI+ and 100 CI- patients, which makes for only 1/3 of the originally screened cohort and represents ~7% of the total HIV+ cohort attending SJH. While these 200 patients were randomly selected and were considered to be fairly representative of the original 604 sub-cohort screened for CI in the prevalence study, they may not be representative of the entire SJH cohort of HIV+ patients. While it is acknowledged that more data is needed to confirm our findings and the study would have benefitted from a larger sample of patients, it nevertheless underpins the association between cognitive impairment and higher hospital services utilisation and costs. The study looked at the GUIDe service and MSW supports in some detail. However, a detailed analysis of the co-morbidities that were associated with the utilisation of non-GUIDe services, or the reasons of ED, IP, DC episodes was not undertaken, as this was beyond the scope of this project. Comorbidities and other relevant clinical data were characterised in more detail in the original "Prevalence of the Positive screen for Cognitive Impairment" study (192). Another limitation is that the current study only looked at viral suppression as a marker of disease stability over the six year period.

In addition, the hospital's patient costing system is a new system and data was only available for 2014 and 2015. Therefore, the cost analysis was limited to these two years only. The resource utilisation analysis only looked at resources/services provided by SJH. Some patients did not incur costs during these two years or were lost to follow-up, but might have been attending health care services in other institutions, which was not possible to account for. Due to the limitations of the hospital costing system, the cost information collected excluded the costs associated with ED visits, Social Work appointments, and diagnostics not associated with the OP, DC, and the IP episodes mentioned above. From the data obtained from the financial system, it was also not possible to infer how much of the resources spent on GUIDe services were due to ART drug cost, blood tests etc. in either the CI+ or CI- groups. The availability of such information for SJH would add insight into the factors affecting the high care costs of PLWH who have cognitive impairment (CI+), compared with those who are not cognitively impaired (CI-).

4.6 Conclusions and Further Suggestions

Overall, this study showed that CI+ patients are more hospital resource demanding for both HIV and non-HIV related health care services. Although the average cost per OP and DC episode for GUIDe services was lower for a CI+ group patient, they utilised these services more frequently, and hence were more resource consuming. Lower average cost per visit in the CI+ group for OP and DC services may probably be explained by more of these patients not being on ART at the time. However, study participants from the CI+ group required health

care providers' attention at all levels of hospital care (IP, OP, DC, and ED) more frequently.

PLWH who screened positive for CI have poorer health related outcomes. They are more likely to be treatment non-compliant, hence more likely to have uncontrolled disease (i.e. detectable plasma HIV VL) and be at a higher risk of death, as opposed to those who screen negative for cognitive impairment. As well as being treatment non-compliant, this group of patients are also more likely to fail to attend their routine appointments with their main health care provider (the hospital). They are, however, more likely to attend emergency services more often. All of these factors contribute to making this group of patients more "resource intense" users of hospital services. They frequently fail to engage with a primary care physician (General Practitioner) in the community also. The reason for the poor engagement with services and non-adherence might lie in the internal or perceived external HIV stigma (475). But, in this study, engagement with services and compliance issues were seen more frequently in the CI+ group, suggesting that these might be partly a reflection of their cognitive dysfunction, i.e. poor judgement and decision making, poor organisational skills and poor memory. These hospital variables (DNA rate and medication non-compliance) could be used as a marker of existing or emerging cognitive impairment in PLWH to identify those who need extra-treatment, treatment adjustments or other system's support to achieve better disease control. In many countries, some of the social supports that might benefit people with HIV and HAND cannot be accessed due to resource constraints and not all of those with a clear need can

be helped. These systemic barriers to care are often found to have a disproportionate impact on people from lower socioeconomic backgrounds (476).

When it comes to non-health care resource utilisation, the CI+ group of patients more often availed of the hospital MSW supports available to them. When CI+ group was looked at through the prism of MSW services accessed, it emerged that this group had significantly more employment and financial difficulties and were more frequently accessing *Financial / Employment / Social Welfare / Mortgages* and *Housing & Accommodation* supports. Employment difficulties and reliance on family and social payments in PLWH and disease related cognitive impairment have been shown in prior studies and have been discussed in the literature review. Results of the current study show that HIV+ patients who screened positive for CI are also intensive users of non-health care related hospital resources. Findings captured during the MSW data analysis are probably just the tip of the iceberg in terms of societal financial burden, as it was not possible to capture the resources and services accessed by these people in the community, including community Social Welfare supports and access to specialised community services (specialised liaison nurse, community clinics / nurse, care assistance, physiotherapy, occupational therapy and others).

Overall, PLWH who have a positive screen for cognitive impairment proved to be more resource demanding in all aspects of hospital care (both health related and non-health related), although the findings were not always statistically significant. This study was a snapshot of a larger cohort and may not generalise across the entire SJH HIV positive population. The issue of HIV related cognitive dysfunction and HAND increasing the health care utilisation and costs needs to be explored

in the larger SJH HIV+ patient population and over a longer time interval. The reasons for longer hospital admissions and more frequent ED presentations as well as more frequent OP, DC visits in this population also need to be explored to be better informed regarding the role that disease related CI, HIV alone, and other psychosocial issues play in higher hospital care costs. Due to the original study design these were not looked at in the current study and this is recognised as a study limitation. However, despite its limitations, this research, further supports current clinical guidelines, advising that, for all PLWH, focus needs to be on disease stability and putting supports in place that enable engagement with HIV services and adherence to treatment.

5. Seizures in HIV: The Case for Special Consideration

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Abstract

Purpose: This study aimed to determine the rate, cause, and management of seizures in the context of potential ART-ASD interactions in a cohort of HIV+ individuals.

Methods: Records of 604 HIV+ patients were reviewed and those reporting epilepsy/seizure diagnosis were further evaluated.

Results: This cohort exhibited a seizure rate of 2.4%. HIV+ patients treated for epilepsy displayed low serum ASD levels and failed to achieve seizure control. They were more likely to disengage from Neurology follow-up.

Conclusion: For HIV+ patients presenting with seizures/epilepsy the ASD prescription and the provision of supplementary support services needs to be carefully considered.

This paper was published in Epilepsy and Behavior Journal in 2018 (Appendix 9).

5.1 Introduction

In Ireland newly diagnosed cases of HIV have been reported at an annual rate that ranges from 7.0 to 7.5 per 100,000 (477). Despite the introduction of highly active anti-retroviral therapy (HAART), 40-60% of HIV infected individuals develop neurological complications (36, 39, 42). The frequency of new seizures in the HIV+ population is estimated to be between 4-11% in the populations studied (478). To date the literature on the epidemiology of seizures and epilepsy in HIV has not generated reliable per patient year incidence estimates. Also no prevalence rates have been determined that can easily separate recurrent provoked seizures from unprovoked attacks (epilepsy). The data we have so far suggest a prevalence of all seizures of about 6% in a reasonably large HIV+ cohort with approximately half of these identified as being unprovoked attacks (42).

These data compare with a point prevalence of 0.8% for epilepsy in Ireland in the general population (479), although there is an expected 10% life time risk of a seizure of any type in the general population (480). Taking into account the wide variations in prevalence estimates for epilepsy due to differences in definitional and methodological approaches, a reasonable estimate of the prevalence of seizure disorders in people with HIV in Ireland is at least 3 times that of the general population.

Acute symptomatic seizures can be the presenting feature of HIV infection (481). The main causes of seizures in HIV infected individuals include: the expected background genetic and environmental risk of epilepsy in that population; HIV infection itself or its CNS complications such as cerebral toxoplasmosis, tuberculoma, cryptococcal meningitis, PML, CNS lymphoma, syphilitic meningitis and HIV associated dementia (478, 481-484). HIV-related seizures may also be provoked by concurrently administered drugs (485). Both HIV and seizures (including epilepsy and provoked seizures) may necessitate long term treatment with both antiretroviral therapy (ART) and anti-seizure drugs (ASD), which can lead to potentially serious ART-ASD interactions (484, 486).

The aim of this study was to determine the rate, type, cause and practiced treatment of new onset seizures (NOS) and epilepsy in a cohort of HIV infected individuals attending St James's Hospital (SJH) in Dublin and to inform best practice seizure management in the context of potential ASD-ART interactions.

5.2 Methods

A dedicated HIV clinic in SJH is attended by a population of approximately 2,200 HIV+ patients. A subset of 604 HIV+ individuals accessing this service previously participated in a Cognitive Impairment (CI) Prevalence Study, conducted between January 2011 and November 2013. Participants were screened for HIV related CI using the Brief Neurocognitive Assessment Screening (BNCS) tool (192). In the current study we further evaluated in detail the occurrence of seizure/epilepsy as another frequent neurological comorbidity in this population. For this purpose the Electronic Patient Records (EPR) of the 604 participants in the Cognitive

Impairment Prevalence Study were reviewed for the diagnosis of “seizure”, “seizure disorder” and “epilepsy”; as well as for neurology/epilepsy clinic attendances, EEG evaluations and ASD prescriptions. In those patients who met our search criteria, medical notes and EPR notes were further reviewed to obtain more detailed information about their history of seizures and epilepsy. This included recording the frequency of acute seizures and epilepsy in this cohort of patients, cause of seizures and their management.

Our assessment of patient adherence to prescribed treatment relied on the review of clinical notes. Adherence to ART was easier to ascertain as the ART therapy is observed in the HIV clinic. ASD therapy compliance on the other hand is usually interrogated with the patient (and with a family member or a caregiver where available) during the clinic appointment and is reconciled with the dispensing pharmacy. Checking serum ASD drug levels was also a helpful tool, however, the results were interpreted with caution in the context of possible drug-drug interactions.

5.3 Results

5.3.1 Demographic and clinical characteristics of HIV+ patients with NOS

Out of 604 HIV+ patients, a total of 15 (2.4%) had a history of epilepsy or single provoked or unprovoked seizure at some stage in their life. The rate of epilepsy or seizures in those who screened positive for CI (311/604) was higher at 3.2% (10/311) than the 1.7% (5/293) for those who screened negative for CI. The male to female ratio amongst those who had seizures/epilepsy was 11:4. This ratio

was consistent with the gender distribution in the Cognitive Impairment Prevalence Study cohort with the majority of participants being male (78.8%) (192). In four of 15 patients the history of epilepsy or seizures predated the diagnosis of HIV, of which two had established childhood onset epilepsy. Seizures re-occurred after the HIV diagnosis in one of the patients with childhood onset epilepsy and the other one experienced increased seizure frequency after the HIV diagnosis was established.

NOS, epilepsy diagnosis, or adulthood re-occurrence of seizures after or around the diagnosis of HIV was documented in a total of 13 patients with a mean latency of 69 months (5.8 years). A total of 14 out of 15 cases underwent further detailed analysis with one case excluded from the detailed analysis due to the lack of further information. (This participant had a single provoked event in childhood in the setting of a medication adverse event.) The mean age at NOS was 36 years, ranging from 13 to 57 years. Further clinical characteristics of HIV+ patients with seizures are presented in Table 5.1.

Table 5.1. Demographic and clinical characteristics of HIV-positive patients with seizures

Demographic and clinical characteristics of HIV-positive patients with seizures	
Seizure onset relative to HIV Diagnosis (n=15)	
Before HIV Diagnosis	**Total 4**
Acute Symptomatic Seizure in childhood	1
Childhood Epilepsy*	2*
One year before HIV Diagnosis	1

Demographic and clinical characteristics of HIV-positive patients with seizures	
At/After HIV Diagnosis	**Total 12**
New Onset Seizure	11
Childhood epilepsy with seizure re-occurrence*	1*
Gender (n=15)	M:F 11:4
Way of infection (n=15)	
Heterosexual	5
MSM	3
IVDU	7
Age at NOS / seizure re-occurrence (n=14)**	
Mean	36
Range	13 – 57
Seizure onset latency after HIV Diagnosis (range; mean) (n=14)**	0 – 168 months (14 years); 69 months (5.8 years)
0 (childhood with subsequent poor seizure control)	1
0 (12 months prior to HIV diagnosis)	1
At HIV diagnosis	2
1-6 months after HIV diagnosis	2
6-12 months after HIV diagnosis	1
36-168 months (3-15 years) after HIV diagnosis	7

Demographic and clinical characteristics of HIV-positive patients with seizures	
CD4+ lymphocyte count at the time of NOS (n=14)**	
<200 cell/mm3	
>200 cell/mm3	5
NK	5
N/A	3
	1
HAART treatment at NOS (n=14)**	
No	8
Yes	3
NK	2
N/A	1
* In one case of known childhood epilepsy, seizures restarted in adulthood 5 years after the HIV diagnosis. The age at seizure re-occurrence was used for the purpose of this analysis. ** The childhood single seizure in acute settings was excluded from further analysis due to no available information. NOS – New Onset Seizure; NK - Not known due to presentation at an overseas health care centre; N/A not applicable; IVDU – intravenous drug user; MSM – men having sex with men.	

5.3.2 Seizure semiology, etiology and evaluation

Repeated seizure episodes led to the diagnosis of epilepsy in 6/14 (43%) patients necessitating ongoing ASD therapy. One of these had previously been diagnosed with Idiopathic Generalized Epilepsy (IGE) (or *Generalized Genetic Epilepsy (GGE)* according to the new ILAE classification of the epilepsies) (487). Seizures were the presenting symptom of HIV in three cases (21%). This includes one case where the seizure occurrence was reported one year prior to the HIV diagnosis. In five patients (36%) seizures were the presenting feature of and were

caused by a CNS opportunistic infection (one of them at the time of HIV diagnosis). Other causes included: benzodiazepine withdrawal (4 patients). Alcohol withdrawal was documented during the first two presentations with seizure re-occurrence in adulthood in one of the patients with a previous history of childhood epilepsy. However, this patient developed further events while abstinent and was eventually diagnosed with epilepsy. No cause other than HIV was found in three patients, two of whom had focal gliosis shown by MRI (Table 5.2).

With regards to seizure type, one patient had *generalized onset tonic-clonic seizures*. *Focal onset seizures* (*aware* or *with impaired awareness*) were documented in four patients. In six patients seizure type was classified as probable *focal onset to bilateral tonic-clonic seizure*; two patients had *unknown onset tonic-clonic seizures* and in one patient the seizure type was not possible to classify due to the lack of further information - *unclassified seizure* (488). Status Epilepticus (SE) was documented in a total of three patients (21%) (one – non motor SE and two – motor SE).

EEG reports were available for nine of the patients. Only one of them had evidence of epileptiform discharges. A total of four patients showed focal dysfunction and one patient had generalized slow activity. Three patients showed no abnormality on EEG. Brain MRI or written reports were available for review for eleven patients (Table 5.2). Seven of them had focal brain lesions (FBL) and two had diffuse lesions shown by MRI (Table 5.2; Figure 5.1).

Table 5.2. Seizure Semiology and Etiology, Diagnostic Evaluation and Neurology Specialist Involvement.

Seizure semiology and etiology, diagnostic evaluation and Neurology specialist involvement. (n=14)	
Seizure type	
Focal onset seizure +/- to bilateral tonic-clonic	**Total 4**
- Focal aware	1
- Focal with impaired awareness	3
Focal to bilateral tonic-clonic*	6
Generalized Genetic Epilepsy (IGE)	1
Unknown onset tonic-clonic	2
Unclassified**	1
Seizure occurrence	
Acute symptomatic seizures	8
Recurrent seizures/epilepsy	6
EEG findings	
Generalized epileptic discharges	1
Focal dysfunction	4
Generalized slow activity	1
No abnormality	3
EEG not available	5

Seizure semiology and etiology, diagnostic evaluation and Neurology specialist involvement. (n=14)	
Brain MRI findings	
Focal brain lesions' (FBL) etiology	**Total FBL 7**
- CNS Toxoplasmosis	3
- TB meningitis	1
- PML	1
- Focal gliosis (unspecified)	2
Diffuse brain lesions	2
Normal MRI	2
MRI not available / performed elsewhere ***	3
Possible etiology of seizure	
CNS complication	**Total CNS complications 5**
- CNS Toxoplasmosis	3
- TB meningitis	1
- PML	1
Other:	**Total other 9**
Benzodiazepine withdrawal	4
Previous history of epilepsy	2
No other cause apart from HIV	3
Neurology Specialist Involvement	
Neurology review at NOS	13
Neurology/Epilepsy follow-up	8
Disengaged from Neurology/Epilepsy follow-up	5****

Seizure semiology and etiology, diagnostic evaluation and Neurology specialist involvement. (n=14)
* Patients presented with witnessed episodes of tonic-clonic motor activity with impaired awareness and focal brain lesion on brain imaging or focal EEG changes ** No further information was available to be able to classify seizure type for one participant ***Two patients (one with IGE) initially presented elsewhere and information regarding imaging was not available; one patient had normal CT brain at seizure presentation ****2 of 5 patients who disengaged with epilepsy service still had documented seizures IGE – Idiopathic Generalized Epilepsy FBL - Focal brain lesions

A total of thirteen patients were reviewed by the Neurology service at the time of seizure presentation. Eight of them attended neurology/epilepsy services for follow-up with a subsequent high rate (5/8) of disengagement from these services.

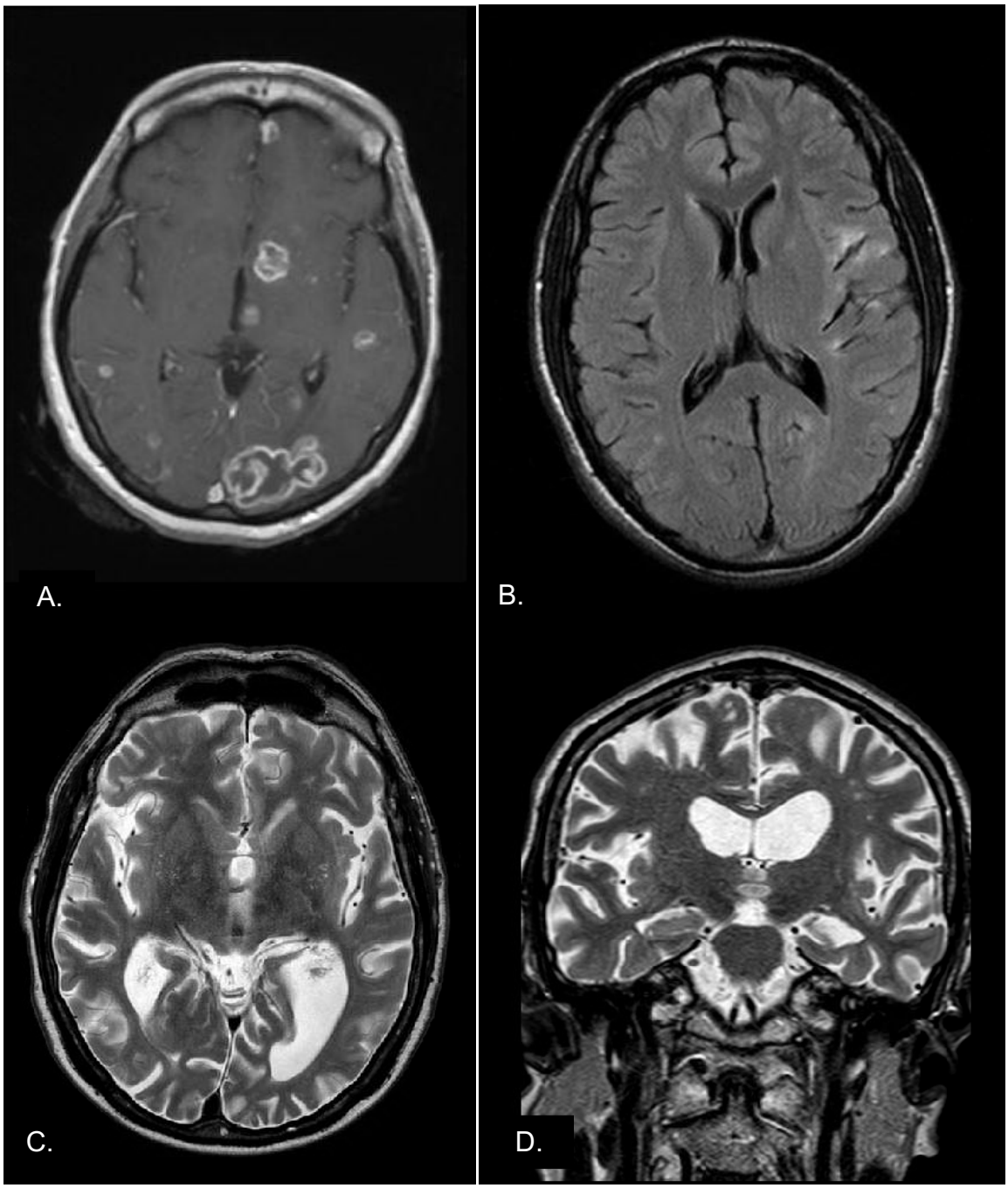

Figure 5.1. MRI images: A. Case I - Axial T1 MRI image enhanced with contrast showing multiple ring enhancing lesions in a patient presenting with status epilepticus in the context of HIV and Cerebral Toxoplasmosis; B. Case II - Axial T2 FLAIR image showing multiple high signal intensity foci in keeping with gliosis in the site of a previous infection in a patient treated for seizures on background of HIV and ongoing treatment for TB meningitis; C. and D. Case III - Axial and coronal T2 images showing focal atrophy of the left temporal lobe and hippocampus on background global atrophy in a patient with refractory epilepsy in the context of HIV and PML.

5.3.3 ASD treatment, side effects and potential ASD-ART Interactions

ASD treatment was commenced in 10 patients: six of them were diagnosed with epilepsy and four presented with acute symptomatic seizures (Table 5.3).

Table 5.3. ASD Treatment, Side Effects and Potential ASD-ART Interactions

no	Engagement with Neurology/ Epilepsy services	ASD/ Serum level	ASD Side effect	SZ free	Possible ART/ASD interactions	CSF penetration	Viral load while on ASD*
1	Disengaged	LTG-	-	NO	Abacavir – LTG ↓ Efavirenz – no Lamivudine – no	High High Low	-
2	Disengaged	LTG-	-	NO	Efavirenz – no Tenofovir – no Emtricitabine	High	-
3	n/a	VPA	-	YES	↑ Lopinavir – VPA ↑ Ritonavir – VPA Tenofovir – no Emtricitabine	High High	-
4	Disengaged	PHT↓ LEV	Mood	YES	Atazanavir – PHT ↓ Ritonavir – PHT Tenofovir – PHT Emtricitabine	High	2,200- <50 (copies/ml)

no	Engagement with Neurology/ Epilepsy services	ASD/ Serum level	ASD Side effect	SZ free	Possible ART/ASD interactions	CSF penetration	Viral load while on ASD*
5	n/a	LEV		YES	Efavirenz – no Emtricitabine Tenofovir – no	High	-
6	Regular follow-up	VPA↔↓ LEV LTG-		NO	Tenofovir – no Emtricitabine Ritonavir – LTG↓; VPA Darunavir – VPA	High	ND
7	Regular follow-up	VPA↓↓ LEV LTG- PG ZOS↓↓	Mood Cog	NO	Darunavir – VPA; ZOS Etravirine Ritonavir – LTG↓; ZOS; VPA	High	<40/ND
8	Disengaged	LEV-	Mood	NO	Darunavir – no Raltegravir – no Ritonavir – no Tenofovir – no Emtricitabine	High	<40/ND
9	Regular follow-up	VPA LTG↓↔ LEV↔ LAC↓	 Pers	NO	Efavirenz – VPA; LAC↓ Abacavir – LTG↓ Lamivudine – no Dolutegravir – no	High High Low	ND

no	Engagement with Neurology/ Epilepsy services	ASD/ Serum level	ASD Side effect	SZ free	Possible ART/ASD interactions	CSF penetration	Viral load while on ASD*
10	n/a	LEV	-	-	Zidovudine – no Lamivudine – no Lopinavir – no Ritonavir – no	High Low High High	<40 - 4000 (copies /ml)

* Viral Load results were not available prior to 2012

ASD – Anti-seizure Drug; ART – Antiretroviral Therapy; VL – Viral Load; ND – HIV RNA not detected; n/a - not applicable; no – no reported potential ART-ASD interactions; n/k – not known

- information not available

↔ ASD serum level within normal range

↓ ASD serum level low/lowered by potential interaction

↑ ASD serum level high/increased by potential interaction

Cog – cognitive symptoms; Mood – mood disturbances; Pers – personality changes; SZ – seizure; LEV – Levetiracetam; LTG – Lamotrigine; LAC – Lacosamide; PHT – Phenytoin; PG – Pregabalin; VPA – Sodium Valproate; ZOS - Zonisamide

The most commonly prescribed ASD was Levetiracetam (prescribed 1st in 4 cases and 2nd or 3rd in 3 cases), which also had the highest rate of adverse events (4/7), followed by Lamotrigine (prescribed 1st in 2 cases, 2nd in one case and 3rd in 2 cases) and Sodium Valproate (prescribed 1st in 4 cases, never as a second agent). Seizure freedom after introducing the first ASD was achieved in only three cases. In all three cases patients presented with acute symptomatic seizures in the setting of a CNS opportunistic infection.

5.4 Discussion

Seizures and epilepsy are important CNS complications of HIV infection. Seizure prevalence in our HIV cohort is higher than in the general population (2.4% versus approximately 1.5% if we include symptomatic seizures and epilepsy). This difference is lower than in other reported studies (478, 484). Interestingly, we found a higher seizure prevalence rate (3.2%) in the HIV+ individuals who screened positive for cognitive impairment. Most of our patients (14/15) had at least one seizure after acquiring HIV infection. However, two of them had been previously diagnosed with epilepsy in childhood. After their diagnosis of HIV, one of these two patients had seizure reoccurrence and the other had increased seizure frequency. Notably, both had seizures refractory to ASD therapy, which could potentially be due to unfavourable ASD-ART interactions. Furthermore, eight of our patients had no other obvious risk factors for epilepsy apart from HIV and its CNS complications. This was further supported by lesional neuroimaging findings consistent with changes described in HIV encephalitis/encephalopathy or CNS opportunistic infection in seven of them.

HIV stage and immune status at NOS is important. Five of our patients had an AIDS defining CNS opportunistic infection at the time of seizure presentation. A further two patients had advanced HIV disease with CD4+ counts below 200 cells/mm3. Slightly less than half (6/14) of those who underwent detailed analysis experienced the first seizure close to the time of HIV diagnosis (+/- 12 months) when infection is more likely to be uncontrolled (Table 1). Eight patients experienced only one seizure, half of these at the time of a concomitant CNS

opportunistic infection. This is highly relevant as it highlights the importance of prompt diagnosis and treatment of opportunistic infection in those presenting with seizures in the context of a known or new HIV diagnosis. These patients may only require short term ASD therapy, thus avoiding potential long term ASD side effects and/or ASD-ART interactions.

Patients who were diagnosed with epilepsy (6/14) were less likely to have had CNS opportunistic infections. Only one patient with recurrent seizures was diagnosed with PML. Two patients had a preexisting epilepsy diagnosis. The remaining three had FBL reported on MRI and for two of these the FBL description was consistent with the MRI changes reported in HIV encephalitis/encephalopathy. These findings suggest that the direct effect of HIV on the CNS may also be responsible for recurrent seizures in this category of patients. It was also found that these patients were more likely to present with seizures later in the course of their HIV disease: three years or more after their HIV diagnosis.

ART-ASD interactions can lead to increased serum drug levels in either drug class and increase the risk of toxicity (489). ART-ASD interactions can also cause reduced drug serum levels with resulting consequences; such as reduced ASD levels and poor seizure control, or reduced ART levels with resulting poor virologic suppression and disease progression (490-492). The latter is expected with the use of enzyme-inducing, older generation ASDs (phenobarbital, phenytoin and carbamazepine) (493-495). The direct effects on HIV viral replication that could potentially lead to increased viral load by commonly used ASDs such as valproate have also been described (483, 496).

In our study, of the three most prescribed ASDs LEV proved to be effective, provided the patient was compliant with the treatment and did not develop side effects. However, LEV had the highest incidence of side effects reported by patients (4/7) and had to be discontinued in two cases due to mood/personality disturbance. Levetiracetam has the least ASD-ART interactions reported in the literature but its propensity to cause intolerable neuropsychiatric side effects has been documented (483, 497). Therefore, careful consideration needs to be given to LEV prescription in this category of patients as HIV infected individuals may develop depression as part of their primary diagnosis. In this study LEV has been associated with virologic control failure in two cases. However, this has not been attributed to a possible ASD-ART interaction but to poor ART compliance.

LTG failed to demonstrate acceptable seizure control in our cohort. This can be explained by either the small dose prescribed or ASD-ART interactions not being explored. Prescription of higher doses in these patients would have been appropriate but had not been given enough consideration. Overall, VPA showed poor seizure control in our cohort. However VPA – ART interactions were possible and persistently low serum levels were recorded despite the high doses being prescribed. It was not possible to evaluate its effect on virologic control due to the lack of data prior to 2012. Also, most patients who had been on VPA therapy had it discontinued by 2013.

Although the majority of patients were reviewed by neurology/epilepsy services in the acute settings and follow-up appointments were arranged, there was a high rate of disengagement with epilepsy/neurology services observed in our cohort (5/8). This is despite patients being contacted by one of the Epilepsy Specialist

Nurses to confirm the follow-up appointments. Additionally, an appointment letter is issued to the patients' home address and a text message reminder is sent to the patients' mobile phone a week before any SJH follow-up appointment to ensure attendance. This experience suggests that this particular group of patients and especially those with concomitant cognitive impairment may need supplementary counselling and support services to optimize their clinical care.

This study highlights the importance of both HIV and Neurology Specialist involvement in the evaluation and treatment of this category of patients and perhaps the need for integrated Neurology service in the HIV clinic for relevant patients.

5.5 Study limitations

Our study has a number of important limitations. This is a small cohort retrospective chart review. Although an initial group of 604 HIV+ patients were looked at, only a small number proved to have suffered from seizures/epilepsy. Selection bias is also possible. However, the 604 patients who consented to participate in the initial Cognitive Impairment Prevalence Study were judged to be fairly representative of the 2,200 HIV+ patients attending the HIV services at SJH (192). It was difficult to draw meaningful conclusions with regards to ASDs effectiveness, adverse events and possible interactions for a number of reasons including: the small subject numbers, the retrospective nature of the study, the heterogeneity of antiepileptic drugs used in such a small cohort and the limitations of the clinical data available. Finally, many of the patients had poor

engagement with the neurology/epilepsy services and adherence to ASD therapy was difficult to ascertain or manage.

5.6 Conclusion

While comparisons to the general population are difficult, it does appear that the prevalence of both symptomatic seizures and epilepsy (recurrent unprovoked attacks) is greater in HIV+ individuals. However, the rates found in our cohort (2.4%) appear lower than other published estimates. An interesting finding is that the rate appears higher in those who screened positive for cognitive impairment (3.2%); a finding that requires further study. With respect to Status Epilepticus and seizure freedom rates, again the HIV+ population fairs worse than the general population. Finally, an important conclusion is that HIV alone is not a major cause of seizures/epilepsy in this study population. The majority of seizure cases presented with focal or diffuse lesions shown by MRI and most of these were caused by opportunistic CNS infections.

It is in the area of treatment that most work needs to be done. ART-ASD interactions were not given adequate consideration in this cohort and might be the cause of ineffective treatment and poor outcomes. Recently, evidence based recommendations on ASD treatment in the context of ART therapy have been jointly developed by the American Academy of Neurology and the International League Against Epilepsy (498). These published guidelines should be carefully considered when it comes to making treatment decisions.

6. Conclusions and Future Directions

6.1 Current Knowledge

The literature review confirmed a broad spectrum of research that has demonstrated the impact of the HIV disease on the central nervous system (CNS). In the early years of the HIV/AIDS epidemic, it was shown that the HIV virus can cross the blood brain barrier soon after the virus transmission (86) and either immediately infect the brain, or re-infect the brain as the disease progresses and cause disease related cognitive impairment (84, 85), as well as other neurological disorders. In the pre-HAART era, longitudinal studies with relatively long follow-up periods demonstrated a gradual development of deficits in the memory, attention, speed of information processing, executive function, and language domains associated with the progression of HIV infection.

The introduction of antiretroviral therapy in the mid 1990's led to a substantial fall in HIV/AIDS related mortality. Coupled with this fall in mortality, was a fall in the number of people suffering from the most severe form of HIV associated neurocognitive impairment or HIV associated dementia (HAD). Nevertheless, milder forms of HIV associated neurocognitive disorders (HAND) are still estimated to affect between 30% and 50% of PLWH. While the plasma HIV viral load shows reductions within weeks after initiation of HAART treatment, a longer interval is observed before the virus is effectively suppressed in the central nervous system or CSF. Notably, CNS viral suppression efficacy varies for different antiretroviral agents mainly due to variability in CSF/CNS penetration

(64, 110). While the majority of treated HIV+ individuals show stable cognitive function or even improved neuropsychological test performance at relatively short interval follow-up (4, 113), gradual progression of cognitive impairment has been observed in a small proportion of HIV+ individuals receiving HAART treatment. Following the available literature review, the hypothesis of this work is that HAND is a progressive disorder due to neuro-degeneration resulting from ongoing neuro-inflammation triggered by HIV virus after entering the CNS. Therefore, it was hypothesized that study subjects would show progression of cognitive decline on neuropsychology testing, and progressive neuro-degenerative changes on MRI imaging despite the effective ART treatment.

6.2 Neuropsychology Assessments Follow-up

For the past few years, the effects of HIV on cognition in the post-HAART era have been studied in a cohort of patients attending a specialised HIV unit in an area of high social deprivation in the centre of Dublin, Ireland. Of the original sub-cohort of 104 subjects who underwent the baseline detailed neuropsychological assessment between August 2011 and November 2013 (49), 79 (76%) returned for a follow-up assessment after a mean interval of 3 years. This is a good retention rate when compared with prior follow-up studies in PLWH, which show high dropout rates (209, 210). In this cohort, the subjects lost to follow-up (LTFU) were more likely to be unemployed, have spent fewer years in formal education, and have a history of illicit drug use. This finding is consistent with prior studies

showing the impact of poor socioeconomic factors on high attrition rates in population-based longitudinal studies (244).

For the 79 subjects assessed twice, it was hypothesised that progression of cognitive decline due to continuous inflammatory changes and neurodegeneration would be found at T_2 neuropsychological assessment. Applying the Antinori et al. HAND criteria and RBANS test, the results of current study, however, showed progression of cognitive impairment in only 19% (15/79) of the subjects, whereas 66% remained stable, and 15% showed an improvement either to a milder form or a remission of HAND. These findings are consistent with the findings of other studies conducted following the early introduction and sustained use of HAART (71, 162, 166). While only a small proportion of those who were assessed at T_2 were observed to display progression of cognitive dysfunction, there was an increase in the proportion of more severe forms of HAND (MND and HAD) at follow-up, when compared with baseline (22.8% versus 32.0%, respectively).

At follow-up, neuropsychological testing of this cohort found statistically significant improvements in the RBANS immediate memory, delayed memory and attention domains. This is consistent with the findings of similar studies and are thought to be, at least partially, due to the practice effects as demonstrated by the attempted corrections using published control data. The current neuropsychology follow-up study (T_2), as well as the initial cross-sectional study (T_1), did not have a control group that could have been used to adequately estimate the effects of prior exposure and familiarity with the test environment and content of the tests. Interestingly though, as a group, this cohort showed statistically significant

reductions in the figure copy subtest scores, which is suggestive of disruptions in the fronto-parietal connections. This was supported by significant Axial Diffusivity (AD) measure changes on the DTI analysis in the frontal white matter at follow-up when imaging data at TP2 was compared with that at TP1 in the Neuroimaging follow-up study (TP2). (The baseline and follow-up Neuropsychological assessments are referred to as T_1 and T_2, respectively, and the Neuroimaging baseline and follow-up data acquisition are referred to as TP1 and TP2, respectively).

However, when the subtests of the entire neuropsychological battery were grouped into seven domains, z transformed, and the results at the two time points (T_1 and T_2) were compared at the whole group level, most domains did not show significant differences. Interestingly, language and orientation domains were found to be significantly worse in the group that showed an improved HAND diagnosis. Perhaps, the language subtests of the RBANS test, which was chosen to adjudicate the HAND diagnosis, were less sensitive than other language subtests in the battery to detect a decline. Also, there are no RBANS subtests with published norms to assess orientation, which means that orientation domain was not accounted for when the HAND diagnosis was established at either time point. These findings highlight the importance of multi-domain assessment, particularly when given the fact that some of the tools in any chosen battery may not be sensitive enough for a certain domain evaluation or may not exist at all.

The RBANS test data was also used to calculate the cortical-subcortical deviation score (CSDS). This found that at T_2, a higher proportion of subjects than at T_1 (48.9% vs 21.6%) had subcortical type of impairments. While this result should be

viewed cautiously, as it might just simply reflect normal variability or the vulnerability of CSDS to practice effects and other factors, it is suggestive of a possible progression of the subcortical type of impairments versus more stability of the cortical type of impairments. This is consistent with the findings of more significant white matter disease in the MRI study in a subset of 42 of the 79 subjects. In addition, at follow-up, a higher proportion of patients had pyramidal and cerebellar signs on neurological examination, which corroborated with progressive, although insignificant, microstructural changes in the CSTs and cerebellum, as shown by DTI analysis. Another interesting finding was the minor eye movement abnormalities in a high number of patients who returned for follow-up which was supported by the whole brain DTI findings showing further brainstem Axial Diffusivity (AD) changes at TP2.

The neuropsychology profile of cognitive impairment at follow-up remains mixed, a dysexecutive and amnestic one, despite the high variability shown on neuropsychological performance. Multivariate analysis showed that having an abnormal list recognition score at follow-up reduced the odds of having an improved HAND diagnosis by nearly 90%. This RBANS subtest can potentially be used as a clinical marker of established cognitive impairment. However, further longitudinal follow-up would be required. Conversely, having an added year of education increased the odds of improving at follow-up, while a lower educational attainment was found to be predictive of CI at baseline. These findings support the findings of previously discussed other studies and are suggestive of neuroprotective effect of cognitive reserve in the HIV+ population. Notably, every extra day of exposure to ART treatment significantly increased the odds of

improving by 0.0003%, suggesting that effective treatment can stabilise the HIV related cognitive dysfunction.

6.3 MRI Follow-up

A subset of the study cohort (42/79) and 50 healthy control subjects were included in the brain MRI study. It was hypothesised that, at follow-up, more changes consistent with a degenerative cortical and / or subcortical process would be found. Data from MR images of 42 subjects undertaken at TP1 were compared with the MR scans acquired at TP2. The study participants' scans at the two time points were also compared with the scans of 50 healthy controls. Multiparametric analysis, including whole brain and ROI VBM and DTI, was carried out. Analysis of the MRI data showed some minor deterioration in both white and grey matter between the TP1 and TP2, but none of the differences observed were found to be statistically significant, except for the AD measures of the left frontal lobe white matter. There were, however, statistically significant differences between the healthy control group and HIV group at TP1 in most of the white matter diffusivity measures, suggesting that extensive widespread white matter disease took place before the baseline assessment but did not progress significantly at the time of follow-up.

In addition, although nonsignificant, frontal, parietal, and temporal cortical grey matter thinning was found on VBM data analysis at baseline when comparing the HIV+ cohort with the healthy controls and adjusting for age. A trend for minimal,

statistically insignificant progression of cortical thinning was also observed between TP1 and TP2. This was more pronounced in the parietal and frontal lobes, and less pronounced in the temporal lobes. While there was an insidious statistically nonsignificant progression of cortical grey matter loss, the findings of this study suggest that white matter disease remains one of the main determinants of the HIV related cognitive impairment. Although widespread white matter changes are present at baseline and at an average re-test interval of 40 months, no significant progression of white matter disease was evident in this cohort of patients, with the exception of the left frontal lobe AD metrics. This suggests continuous axonal / white matter degeneration in this region. In addition, it also corroborated with poorer performance on RBANS figure copy subtest. Further neuropsychology and neuroimaging longitudinal follow-up of this cohort is required to evaluate the aforementioned DTI axial diffusivity metrics and fronto-parieto-temporal cortical tissue thinning detected on VBM analysis as potential biomarkers of HAND progression and HIV related neurodegeneration.

6.4 Hospital Services Utilisation and Costs

Comparison of 100 HIV patients screening positive for cognitive impairment with 100 patients who screened negative for cognitive impairment found differences in hospital services utilisation and hospital care costs. While not all the differences were statistically significant, many were, as HIV patients screening positive for cognitive impairment accessed hospital services more frequently and had longer episodes of inpatient care. However, the clinical reasons for such discordance of

hospital services utilisation remain to be clarified. The CI+ group also accessed more of the GUIDe Medical Social Work services. The gathered data showed a statistically significant association between a positive screen for cognitive impairment and number of supports accessed in the area of housing / accommodation, and financial / social welfare / mortgages issues.

Comparison of people with HIV who screened positive for cognitive impairment with those who screened negative also revealed poorer health related outcomes for the CI+ group. These patients were more likely to be treatment non-compliant, hence more likely to have uncontrolled disease and a higher risk of death. As well as being non-compliant with treatment, this group of patients was also more likely to fail to attend their routine hospital outpatient appointments. They were also more likely to attend emergency department services. All of these factors contribute to making this group of patients more “resource intense” users of hospital services. Whether the poor treatment compliance and disengagement with services is caused by cognitive dysfunction (i.e. poor judgement and decision making, poor organisational skills and poor memory), or the poor socioeconomic status and other associated factors lead to cognitive impairment through poor control of disease remains to be further explored. Thaler et al. (2015) showed that poor socioeconomic factors affected treatment adherence in PLWH. Hinkin et al. (2002), in a cohort of HIV positive individuals with cognitive dysfunction, found that those who were more functionally impaired had more difficulties with adherence. High DNA rates and medication non-compliance could be used as surrogate markers of existing or emerging cognitive impairment in PLWH. These markers could be used in the GUIDe clinic to identify those who

need additional treatments / treatment adjustments or other types of support, to enable them to achieve a better disease control.

6.5 Seizures in HIV

A review of the hospital patient records of the 604 HIV+ individuals attending SJH GUIDe clinic who were screened for CI, also found a higher rate of seizures in this group of patients (2.4%), compared with the general population (0.8%). Interestingly, this was found to be higher in those who screened positive for cognitive impairment than in those who screened negative for CI (3.2% versus 1.7%, respectively). Seizure treatment in this group was not always effective, possibly because of drug interactions between antiretroviral therapies (ART) and anti-seizure drugs (ASDs). In addition, many of these patients disengaged from the hospital Neurology services and / or were reported to have poor adherence to ASD regimens. While careful consideration of the ASD prescription is important for HIV+ patients presenting with seizures, supplementary support services might help sustain patient engagement with the appropriate services.

6.6 Interventions That Could Treat or Slow Progression of HAND

There is a consensus in the field of HIV Medicine that HAART is currently the most important intervention that can allow people with HIV to live longer healthier lives and minimise its potential to cause neurocognitive dysfunction (499). In

addition to HAART, other interventions have been considered by researchers, but, to date, nothing approaching the impact of HAART has been found. In 2013, Weber et al. (2013) published a wide ranging review on pharmacological, and cognitive rehabilitation interventions that might benefit HIV+ individuals diagnosed with HAND (500). Once again, the review highlighted the paramount role played by HAART in the treatment of HAND.

In terms of non-ART pharmacotherapies, there are studies involving small numbers of patients that suggest some drugs such as lithium and serotonin reuptake inhibitors (SRIs) or certain antimicrobials / antimycotics such as minocycline improved patients' neurocognitive test performance. However, replication of these studies in larger cohorts of patients did not confirm such promising results. Sacktor et al. (2001) assessed efficacy of minocycline in 107 HIV infected individuals with CI and observed no improvement of cognitive status (501). Rumbaugh et al. (2008) agreed that, while pharmacotherapeutic approaches are some way from being a routine treatment, there are other potential therapies (502).

Some promising non-pharmacological interventions, including neurocognitive rehabilitation were found to be helpful. These were found to be effective in the domains of visual learning and speed of information processing. Some of these interventions, for example, cognitive stimulation (Becker et al., 2012) can be deployed via internet applications. Internet based cognitive stimulation was found to be a feasible intervention for PLWH in a trial on 60 subjects, and those who used the application consistently and frequently benefitted the most (503). Additionally, developing ways of compensating for the cognitive deficit (e.g.

making lists, taking notes when important information is given, keeping diaries, keeping objects in the same place) may help patients re-establish function.

Physical exercise is a low cost intervention that has been shown to have general health benefits and to be associated with higher levels of functioning and better neurocognitive test performance. Recent research by Fazeli et al. found that moderate amounts of physical activity in a group of 100 older adults with HIV (50 to 79 years) reduced the odds of neurocognitive impairment ($p=0.04$) and instrumental activities of daily living (IADLs) dependence (504). However, the feasibility study conducted at SJH by McDermott et al. (2017) in a small proportion of the currently described cohort, which explored the potential benefit of regular aerobic exercise on cognitive function, failed to show such benefit (505). The success of these programmes relies on regular participation and adherence to the exercise programme. Indeed, the study conducted in Dublin was limited by small participant numbers, high attrition rates, and poor adherence to the exercise programme.

In 2014, UNAIDS set its 90/90/90 goal (432). The third target focuses attention on treatment compliance. It is well known that patients who do not adhere to HAART treatment are more likely to develop drug resistance, become virally unsuppressed, and hence develop unstable HIV disease. Consequently, a key focus of HIV research is improving treatment adherence. Recent studies suggest that rates of treatment adherence might be improved if there were appropriate community based, social and other supports, such as supervised medication administration in place for people from more marginalised populations (506).

6.7 Study Strengths

This was a follow-up study of a well characterised cohort of HIV positive patients who access care in the largest HIV care service in Ireland. The retention rate was high in both the neuropsychology and neuroimaging studies. The neuropsychology tests used do not require specialist training and are easy to administer, therefore, they can be applied in clinical practice. A multimodal imaging data was acquired and analysed at both time points. Comparisons with age adjustments were made between the two time points and between HIV positive participants and healthy controls. For the hospital resource utilisation project, one third of the original cohort screened for cognitive impairment was followed for a relatively long interval of six years. There was a small differential attrition rate in the costs and hospital utilisation project, which permitted adequate comparisons between the groups.

6.8 Study Limitations

The study has a number of important limitations. It is possible that the interval between the baseline and follow-up neuropsychology assessments was too short to detect changes in a cohort with sustained disease control. Further follow-up assessments would be able to identify a more reliable trend in the progression of HAND, or otherwise, in this cohort. It is also possible that those who were more likely to decline were missed at the follow-up assessments. Therefore, following these patients clinically, where feasible, would be important and might yield more

information on the natural course of HAND in the context of widespread and early use of HAART.

The absence of a control group at baseline and at follow-up for the neuropsychology evaluation made it impossible to make comparisons with an adequately matched seronegative population or to control for the practice effects. In addition, application of some of these tests can be tester and environment dependent, and no cultural or language adjustments were made for patients of non-Irish origin or whose first language is not English. However, it was ascertained prior to the study enrolment, that all participants have a good command of English language. Importantly, the functional impairments were evaluated by applying the suggested questionnaire (Antinori et al., 2007). In most cases, these evaluations relied on self-reporting of impairments, which could be less objective than a validated quantitative functional assessment tool. Similarly, tester dependent variability could not be excluded when neurological assessments were performed. Therefore at future follow-up re-evaluation of this cohort, researchers would benefit from using validated quantitative tools and their published normative data for the functional assessment (The Bristol Activities of Daily Living Scale (BALDS) (507) or other validated questionnaires (508)). Similarly, for the eye movement abnormalities assessment, it would be beneficial to apply an eye tracker system (509). Using validated tools for the assessment of motor signs and skills (grip strength (510), grooved pegboard test (511), gait speed tests (512), tendon reflexes quantitative measure (513), tremor quantification technique (514)) would, perhaps, reduce the risk of tester specific biases and quantify reliably the impairments and their changes over time.

For the hospital utilisation and cost analysis, cost data was limited to only two years. Due to the limitations of the hospital costing system, the cost information collected only included the costs associated with inpatient, outpatient, and day case episodes of care and it was not possible to differentiate how much of the resources were spent on ART or other drug costs, blood tests, or other investigations. It was not possible to exclude additional healthcare costs incurred by other hospitals, primary care physicians, or costs incurred in the community. Another important limitation of the resource utilisation and cost analysis was lack of data pertaining the reasons for hospital admissions, as well as ED presentations, and reasons that led to more frequent OP attendances in the CI+ group.

For the “Seizures in HIV: The case for special consideration” study, apart from the small subject number, the lack of important clinical data precluded meaningful statistical analysis or indeed meaningful conclusions. Selection biases are not excluded in any of these projects, although the original prevalence study sub-cohort of 604 patients were judged to be representative of the St James’s Hospital 2,200 HIV+ cohort.

6.9 Future Recommendations

Patients, who are newly diagnosed with HIV, should be screened for cognitive impairment at baseline and every two years, or as clinically indicated, thereafter. Those with a positive screen for cognitive impairment should be formally referred

for detailed neurology and neuropsychology assessment and, where appropriate, for MRI brain, cerebrospinal fluid examination or other tests, and treated accordingly. In addition, patients who present with new onset of seizures or otherwise unexplained worsening seizure control (especially high risk group, i.e. IVDU) should be considered for HIV testing.

Current hospital utilisation and epilepsy in HIV studies show that, while PLWH may continue to engage with GUIDe clinic services, they tend to disengage from other services, including Neurology / Epilepsy clinics. For these reasons, there is a case for allocating Neurology resources to routine HIV care, with the goal of identifying those with cognitive impairment, i.e. HAND and prescribing service supports and treatments that would enable treatment compliance. Formalised and structured access to Neurology service should be available in HIV care programmes as an integral component of the multidisciplinary care for PLWH. This will facilitate timely specialised care and engagement with appropriate services. It is hoped that this would ensure better care for those who have impairment and improve engagement with the service through reduction of burden of appointments, thus improving treatment compliance, reducing the incidence and severity of cognitive impairment/HAND, and lowering the frequency of unplanned visits to the hospital ED that often lead to costly inpatient (IP and DC) episodes of care. Most importantly, it would offer PLWH a more appropriate structured programme of care.

The study also supports the call for providing patients and family / carers with education in the clinic, regarding possible early symptoms and signs of cognitive impairment. Patient information leaflets containing the simplified information

about recent research about HAND would help patients and their families to be more vigilant to the development of disease related cognitive dysfunction. Education regarding benefits of engagement and treatment adherence should be reinforced.

With respect to the future research, the current cohort should be followed further with neuropsychology and MRI assessments, at two to four year intervals, to evaluate the trajectory of neurocognitive and imaging changes. An appropriately matched seronegative control group (socioeconomically and educationally as well as age and gender matched) is mandatory for adequate comparisons and to facilitate control for practice effects. As most of the practice effects occur within the first year of re-testing, and tend to level off thereafter, an appropriately matched control group, followed at six weeks, six, twelve, eighteen and twenty-four months, to create a bank of normative data for future reference, would be important.

6.10 Conclusion

The main hypothesis of this work is that HAND is a progressive disorder due to neuro-degeneration resulting from ongoing neuro-inflammation triggered by HIV virus after entering the CNS despite the effective ART treatment. The main conclusion of this study is that, at a group level, no substantial decline was found between the two time points on neuropsychology assessments and this was reinforced by the follow-up MRI results, thus refuting the main hypothesis. This

suggests that HAART treatment stabilises the disease and possibly its related cognitive impairment in the majority of the treated patients. However, some indolent changes were noted on VBM and DTI analysis and a small proportion of patients showed progression of cognitive or functional impairment, suggesting that ongoing slowly progressive neurodegeneration cannot be excluded.

In addition, those PLWH who also have cognitive dysfunction were observed to have worse clinical outcomes, such as higher death rate, higher rate of other neurology conditions (epilepsy), and were observed to have poorer engagement with services. Poor adherence with treatment correlated with poor HIV disease control in both CI+ and CI- groups, however, this was higher in the cognitively impaired group. All of the above factors make them more hospital and resource intense, as shown by the hospital utilisation and cost data analysis.

Despite its limitations, this research further supports current clinical guidelines, advising early treatment initiation to achieve HIV disease stability for all PLWH. It also underpins the importance of promoting clinical, social and community supports that enable engagement with HIV services and facilitate treatment adherence.

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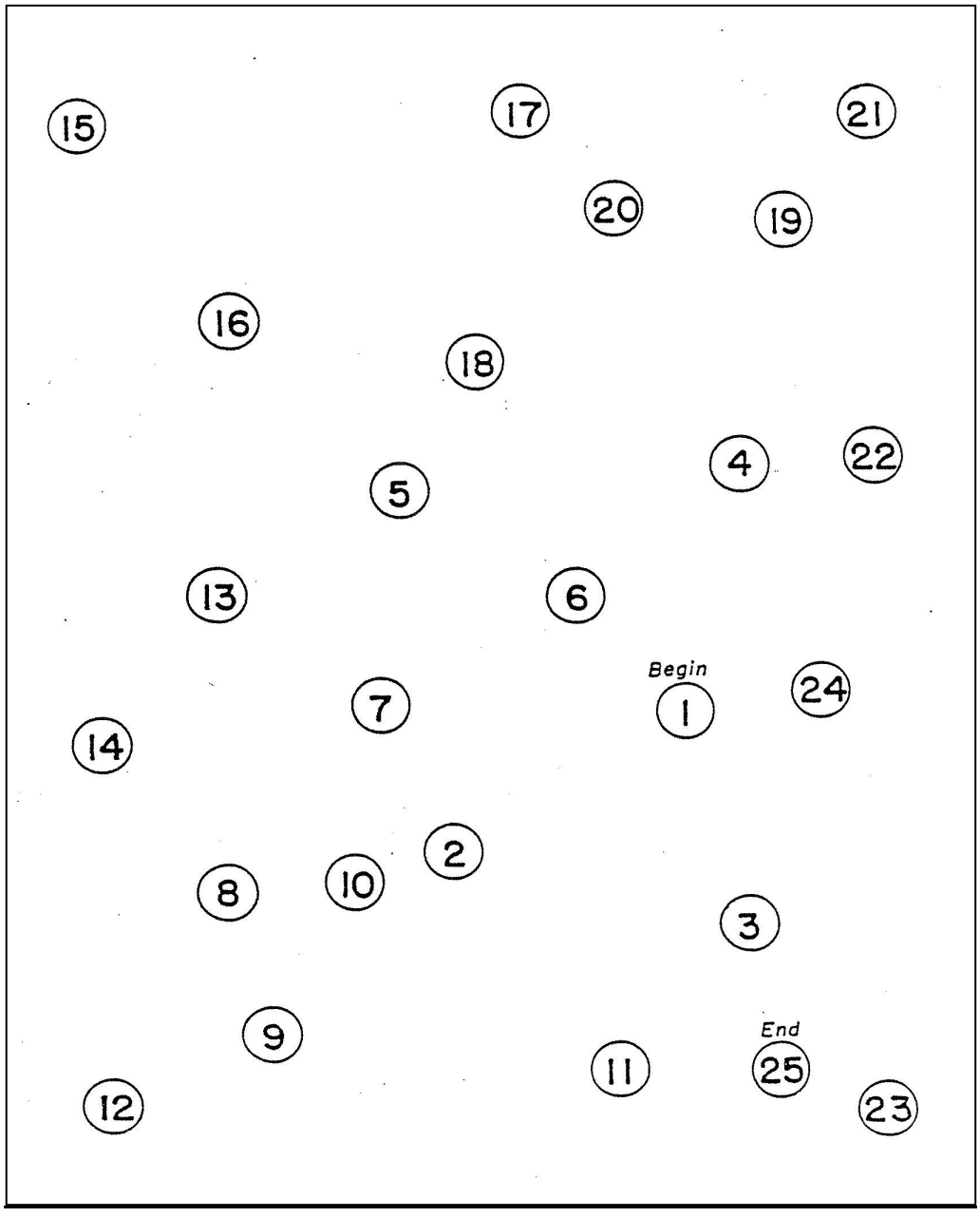
Appendices

Appendix 1: Brief NeuroCognitive Screen (BNCS)

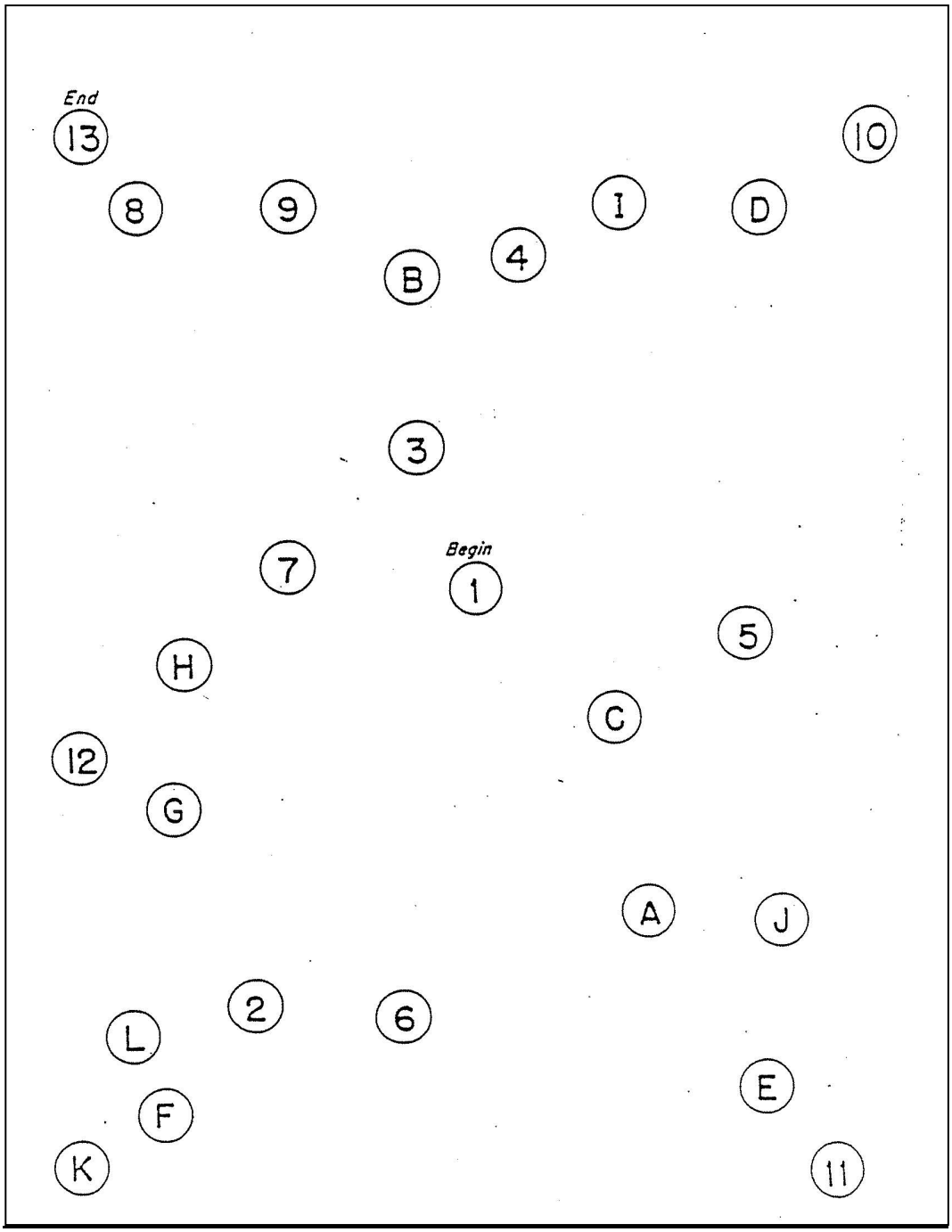
Brief NeuroCognitive Screen (BNCS) consists of three tests for a brief neuropsychology assessment. These tests include the Trail Making tests part A and part B, and Digit Symbol Test. For consistency the tests are administered in the following sequence:

1. Trail Making Part A
2. Trail Making Part B
3. Digit Symbol Test

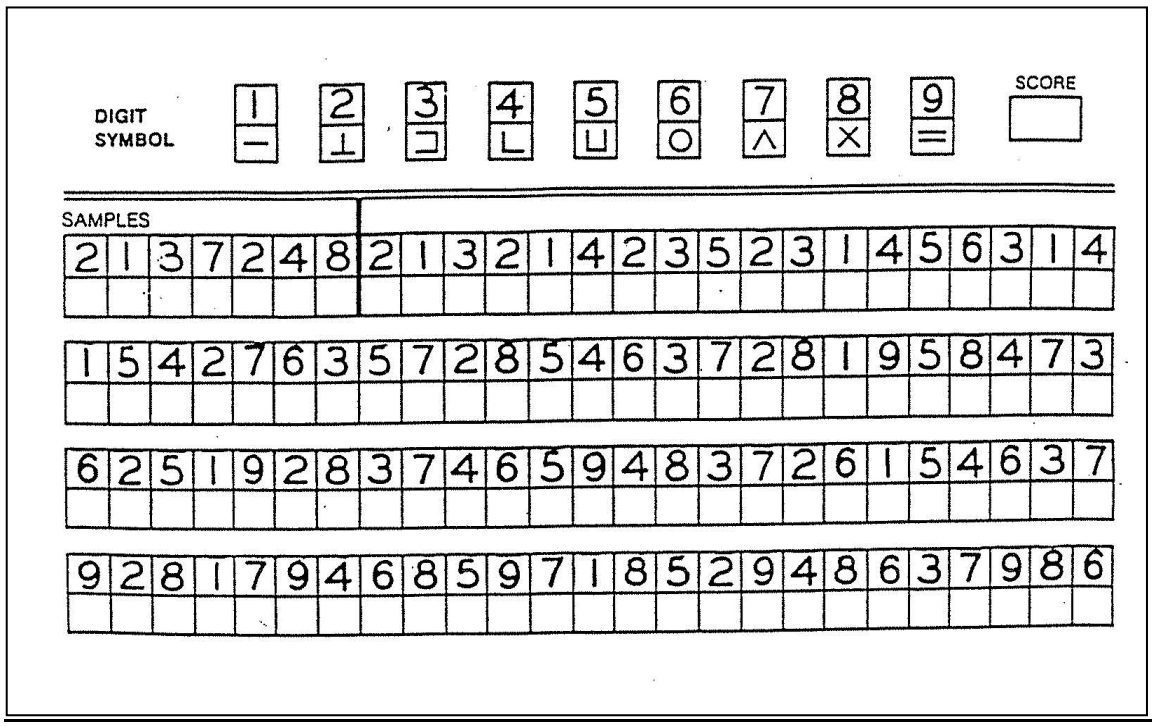
Trail Making Part A



Trail Making Part B



Digit Symbol Test



Appendix 2: Patient Information Leaflet and Consent Form for the Follow-up Neuropsychology Assessment.

SJH / AMNCH RESEARCH ETHICS COMMITTEE -Information an

Information and Consent for Patients

1. Title of study:

Cognitive impairment in HIV. Longitudinal follow-up of cognitive impairment in a well characterized cohort of HIV-positive patients.

2. Introduction:

One of the neurological complications of HIV is cognitive impairment. It occurs in 30-50% of people with HIV. People affected by this may experience symptoms such as forgetfulness, problems concentrating, problems with short term memory, language difficulties, clumsiness, unsteadiness, changes in personality, mood swings and inappropriate emotional responses. We hope that by carrying out this study we can contribute to the knowledge base about this condition and have a better understanding of it in order to help people who have it.

3. Procedures:

All patients previously recruited in the detailed neurocognitive study, attending the HIV clinic in St. James's Hospital will be offered follow-up tests at 12 and 18 months to look for symptoms or signs of cognitive dysfunction progression. These tests are called the Repeatable Battery for the Assessment of Neuropsychological Status; Addenbrooke's Cognitive Examination Revised; Montreal Cognitive Assessment; Frontal Assessment Battery and the Hospital Anxiety and Depression Scale. This will involve testing of your memory, thinking and concentration, mood and some simple tasks. It will take 60 minutes of your time. Patients will then have a neurological examination, which will involve testing the strength in your arms and legs, your co-ordination, your reflexes, your sensation (feeling) in your arms and legs. It will also involve testing the nerves in your face and your speech.

4. Benefits:

The information gathered from this study will help the medical community understand this condition better and aid diagnosis of future patients. This may help you and many others like you in the future.

5. Risks:

There are no risks associated with this study.

6. Exclusion from participation:

Anybody who does not wish to participate will not be included.

7. Alternative treatment:

You do not have to be a part of this study to be treated. Irrespective of whether you partake in the study or not, you will be followed up in the HIV clinic as before.

8. Confidentiality:

Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the hospital. All data gathered from this study will be kept strictly confidential with access only to responsible personnel involved in this project and no personal data will ever be divulged.

9. Compensation:

(Non-sponsored trial): Your doctors are covered by standard medical malpractice insurance. Nothing in this document restricts or curtails your rights.

10. Voluntary Participation:

You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits which you had before entering the study.

11. Stopping the study:

You understand that your doctor may stop your participation in the study at any time without your consent.

12. Permission:

This study has been approved by SJH/AMNCH ethics committee in its present form after careful scrutiny of the proposals submitted.

13. Further information:

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Dr. Lilia Zaporojan who can be emailed at lzaporojan@stjames.ie. If your doctor learns of important new information that might affect your desire to remain in the study, he or she will tell you.

SJH / AMNCH RESEARCH ETHICS COMMITTEE

CONSENT FORM

Consent for study Cognitive Impairment in HIV. Longitudinal follow-up of cognitive impairment in a well characterized cohort of HIV-positive patients.

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.

Name of sponsor:

PARTICIPANT'S NAME:

PARTICIPANT'S SIGNATURE:
Date

Date on which the participant was first furnished with this form:
Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained.

NAME OF CONSENTOR, PARENT or GUARDIAN:
SIGNATURE:
RELATION TO PARTICIPANT:

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him or her for the illness.

NAME OF FIRST WITNESS: SIGNATURE:
NAME OF SECOND WITNESS: SIGNATURE:

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any

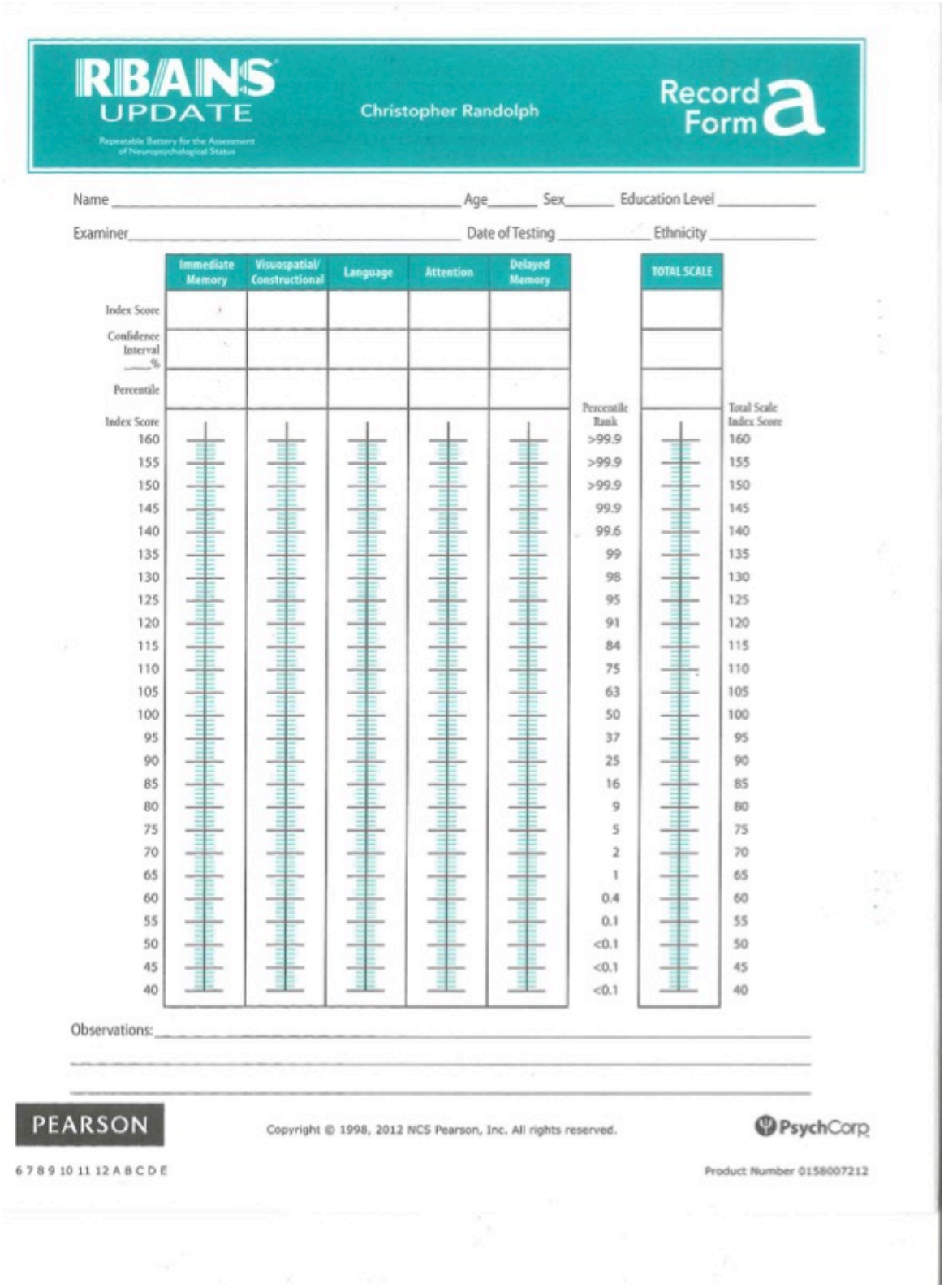
questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician’s signature:

Date:

(Keep the original of this form in the participant’s medical record, give one copy to the participant, keep one copy in the investigator’s records, and send one copy to the sponsor (if there is a sponsor).

Appendix 3: Battery of Neuropsychological Tests Used



RBANS UPDATE

Repeatable Battery for the Assessment of Neuropsychological Status

Christopher Randolph

Record Form a

Name _____ Age_____ Sex_____ Education Level _____

Examiner_____ Date of Testing _____ Ethnicity _____

	Immediate Memory	Visuospatial/ Constructional	Language	Attention	Delayed Memory	TOTAL SCALE
Index Score						
Confidence Interval ___%						
Percentile						

Index Score	Percentile Rank	Total Scale Index Score
160	>99.9	160
155	>99.9	155
150	>99.9	150
145	99.9	145
140	99.6	140
135	99	135
130	98	130
125	95	125
120	91	120
115	84	115
110	75	110
105	63	105
100	50	100
95	37	95
90	25	90
85	16	85
80	9	80
75	5	75
70	2	70
65	1	65
60	0.4	60
55	0.1	55
50	<0.1	50
45	<0.1	45
40	<0.1	40

Observations: _____

PEARSON

PsychCorp

6 7 8 9 10 11 12 A B C D E

Product Number 0158007212

1 List Learning

Trial 1

Say *I am going to read you a list of words. I want you to listen carefully and, when I finish, repeat back as many words as you can. You don't have to say them in the same order that I do—just repeat back as many words as you can remember, in any order. Okay?*

Trials 2–4

Say *I am going to read the list again. When I finish, repeat back as many words as you can, even if you have already said them before. Okay?*

Record responses in order.

Scoring: 1 point for each word correctly recalled on each trial.

List	Trial 1	Trial 2	Trial 3	Trial 4
Market				
Package				
Elbow				
Apple				
Story				
Carpet				
Bubble				
Highway				
Saddle				
Powder				

Number Correct		+		+		+		=	
	Total Trial 1		Total Trial 2		Total Trial 3		Total Trial 4		Total Score Range=0–40

PEARSON

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2 Story Memory

Trial 1

Say ***I am going to read you a short story. I'd like you to listen carefully and, when I finish, repeat back as much of the story as you can remember. Try and use the same wording, if you can. Okay?***

Read the story below, then say ***Now repeat back as much of that story as you can.***

Trial 2

Say ***I am going to read that same story again. When I finish, I want you to again repeat back as much of the story as you can remember. Try to repeat it as exactly as you can.***

Read the story below, then say ***Now repeat back as much of that story as you can.***

Scoring: 1 point for *verbatim* recall of bold, italic words or alternatives, shown below in color within parentheses. Record intrusions or variations in the Responses column.

Story	Trial 1 Responses	Trial 1 Score (0 or 1)	Trial 2 Responses	Trial 2 Score (0 or 1)	Item Score (0–2)
1. On ***Tuesday,***					
2. ***May***					
3. ***Fourth,***					
4. in ***Cleveland,*** Ohio,					
5. a ***3 alarm***					
6. ***fire*** broke out.					
7. ***Two***					
8. ***hotels***					
9. and a ***restaurant***					
10. were ***destroyed***					
11. before the ***firefighters*** ***(firemen)***					
12. were able to ***extinguish it*** ***(put it out).***					
				Total Score (Trial 1 + Trial 2) Range=0–24	

3 Figure Copy

Time Limit: 4 minutes

Fold this page back and present the Figure Copy Drawing Page along with the stimulus. Ask the examinee to make an exact copy of the figure. Tell the examinee that he or she is being timed, but that the score is based *only* on the exactness of his or her copy.

Scoring: 1 point for correctness and completeness (drawing), and 1 point for proper placement. See Appendix 1 in Stimulus Booklet A for complete scoring criteria and scoring examples.

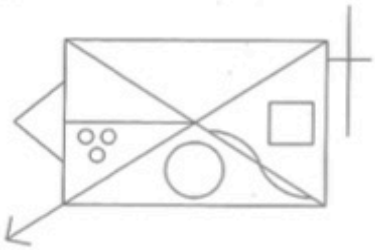


Figure Copy Criteria

(Fold back for use.)

Item	Drawing (0 or 1)	Placement (0 or 1)	Score (0, 1, or 2)	Scoring Criteria
1. rectangle				**Drawing:** lines are unbroken and straight; angles 90 degrees; top/bottom lines 25% longer than sides **Placement:** not rotated more than 15 degrees
2. diagonal cross				**Drawing:** lines are unbroken and straight and should approximately bisect each other **Placement:** ends of lines should meet corners of the rectangle without significant overlap or measurable distance between the ends of the lines and the corners
3. horizontal line				**Drawing:** line is unbroken and straight; should not exceed 1/2 the length of the rectangle **Placement:** should bisect left side of the rectangle at approximately a right angle and intersect the diagonal cross
4. circle				**Drawing:** round, unbroken and closed; diameter should be approximately 1/4–1/3 height of rectangle **Placement:** placed in appropriate segment; not touching any other part of figure
5. 3 small circles				**Drawing:** round, unbroken and closed; equal size; triangular arrangement; not touching each other **Placement:** in appropriate segment; not touching figure; triangle formed not rotated more than 15 degrees
6. square				**Drawing:** must be closed; 90 degree angles; lines straight and unbroken; height is 1/4–1/3 height of rectangle **Placement:** in appropriate segment; not touching any other part of figure; not rotated more than 15 degrees
7. curving line				**Drawing:** 2 curved segments are approximately equal in length and symmetrical; correct direction of curves **Placement:** ends of line touch diagonal; do not touch corner of rectangle or intersection of diagonal lines
8. outside cross				**Drawing:** vertical line of the outside cross is parallel to side of rectangle; >1/2 the height of rectangle; horizontal line crosses vertical at 90 degree angle and is between 20–50% of length of vertical line **Placement:** horizontal line of outside cross touches rectangle higher than 2/3 the height of rectangle, but below top; does not penetrate the rectangle
9. triangle				**Drawing:** angle formed by 2 sides of triangle is between 60–100 degrees; sides are straight, unbroken and meet in a point; distance on vertical side of rectangle subsumed by triangle is approximately 50% of the height of vertical side **Placement:** roughly centered on the left vertical side of the rectangle
10. arrow				**Drawing:** straight and unbroken; lines forming arrow are approximately equal in length and not more than 1/3 length of staff **Placement:** must protrude from appropriate corner of rectangle such that staff appears to be continuation of diagonal cross
	Total Score Range=0–20			

Figure Copy Drawing Page

(Fold back for use.)

4 Line Orientation

Time Limit: 20 seconds/item

Present the sample item, and say ***These two lines down here*** (indicate) ***match two of the lines on top. Can you tell me the numbers, or point to the lines that they match?*** Correct any errors and make sure the examinee understands the task. Continue with Items 1–10.

Scoring: 1 point for each line correctly identified.

Item	Responses	Correct Responses	Score (0, 1, or 2)
Sample		1, 7	
1.		10, 12	
2.		4, 11	
3.		6, 9	
4.		8, 13	
5.		2, 4	

Item	Responses	Correct Responses	Score (0, 1, or 2)
6.		1, 6	
7.		3, 10	
8.		5, 8	
9.		1, 3	
10.		11, 13	
		Total Score Range=0–20	

5 Picture Naming

Time Limit: 20 seconds/item

Ask the examinee to name each picture. Give the semantic cue only if the picture is obviously misperceived.

Scoring: 1 point for each item that is correctly named spontaneously or following semantic cue.

Item	Semantic Cue	Responses	Score (0 or 1)
1. chair	a piece of furniture		
2. pencil	used for writing		
3. well	you get water from it		
4. giraffe	an animal		
5. sailboat	used on the water (if "boat," query "what kind")		
6. cannon	a weapon, used in war		
7. pliers	a tool		
8. trumpet	a musical instrument ("cornet" okay)		
9. clothespin	used to hold laundry on a line		
10. kite	it's flown in the air		
		Total Score Range=0–10	

6 Semantic Fluency

Time Limit: 60 seconds

Say ***Now I'd like you to tell me the names of all of the different kinds of fruits and vegetables that you can think of. I'll give you one minute to come up with as many as you can. Ready?***

Scoring: 1 point for each correct response.

1. _____	11. _____	21. _____	31. _____
2. _____	12. _____	22. _____	32. _____
3. _____	13. _____	23. _____	33. _____
4. _____	14. _____	24. _____	34. _____
5. _____	15. _____	25. _____	35. _____
6. _____	16. _____	26. _____	36. _____
7. _____	17. _____	27. _____	37. _____
8. _____	18. _____	28. _____	38. _____
9. _____	19. _____	29. _____	39. _____
10. _____	20. _____	30. _____	40. _____

Total Score
Range=0–40 ☐

7 Digit Span

Say ***I am going to say some numbers, and I want you to repeat them after me. Okay?***
Read the numbers at the rate of 1 per second. Only read the second string in each set if the first string was failed. Discontinue after failure of both strings in any set.

Scoring: 2 points for the first string correct, 1 point for the second string correct, and 0 points for both strings failed.

Item	First String	String Score (0 or 2)	Second String	String Score (0 or 1)	Item Score (0–2)
1.	4—9		5—3		
2.	8—3—5		2—4—1		
3.	7—2—4—6		1—6—3—8		
4.	5—3—9—2—4		3—8—4—9—1		
5.	6—4—2—9—3—5		9—1—5—3—7—6		
6.	2—8—5—1—9—3—7		5—3—1—7—4—9—2		
7.	8—3—7—9—5—2—4—1		9—5—1—4—2—7—3—8		
8.	1—5—9—2—3—8—7—4—6		5—1—9—7—6—2—3—6—5		

Total Score
Range=0–16 ☐

8 Coding

Time Limit: 90 seconds

Say ***Look at these boxes*** (indicate key). ***For each one of these marks there is a number that goes with it. Down here there are marks, but no numbers. I want you to fill in the number that goes with each mark.***

Demonstrate the first three. Say ***Now I would like you to fill in the rest of these boxes up to the double lines*** (indicate) ***for practice.*** Correct any errors as they are made. Make sure that the examinee understands the task and has correctly completed the sample items before you begin timing.

Say ***Now I would like you to continue to fill in the numbers that match the marks. Go as quickly as you can without skipping any. When you reach the end of the line, go on to the next one. Ready? Go ahead.***

Redirect the examinee to the task if he or she becomes distracted. If the examinee is unable to comprehend the task, the subtest score is 0.

Scoring: 1 point for each item correctly coded within 90 seconds (*do not* score the sample items).

Note: Familiarize yourself with these instructions before administering this subtest.

Total Score
Range=0–89

⊂	∧	=	⌋	∨	⊃	+	⊥	⊢
1	2	3	4	5	6	7	8	9

SAMPLE_____

=	⊢	⊂	∧	+	⌋	⊥	⊃	∨	=	⊢	∧	⊃	+

⊥	⊃	∨	⊢	=	∧	⊂	+	⌋	∧	⊥	⊂	+	⌋

⊃	⊢	∧	=	∨	⊂	⌋	+	⊥	=	⊃	∧	⊢	⊂

+	⊂	⊢	⌋	=	⊢	+	∧	⊃	⊂	⌋	⊥	+	⊢

⊂	+	⊢	⊃	∧	=	⊥	⌋	⊂	=	+	∨	⊥	∧

∧	=	⌋	⊢	+	∨	⊥	⌋	∧	⊃	∨	⊥	⊂	⌋

+	⊂	⌋	⊃	∧	=	⊂	+	⊥	∨	⌋	∧	⊃	=

9 List Recall

Say ***Do you remember the list of words that I read to you in the beginning? Tell me as many of those words as you can remember now.***

Scoring: 1 point for each word correctly recalled.

List (Do not read.)	Response	Score (0 or 1)
Market		
Package		
Elbow		
Apple		
Story		
Carpet		
Bubble		
Highway		
Saddle		
Powder		
	Total Score Range=0–10	

10 List Recognition

Say ***I'm going to read you some words. Some of these words were on that list, and some of them weren't. I want you to tell me which words were on the list.*** For each word, ask ***Was _____ on the list?***

Scoring: 1 point for each word correctly identified. Circle the letter corresponding to examinee's response (y = yes, n = no); bold, capitalized (**Y, N**) letter indicates correct response.

List	Circle One		List	Circle One		List	Circle One		List	Circle One	
1. **Apple**	**Y**	n	6. sailor	y	**N**	11. **Bubble**	**Y**	n	16. **Saddle**	**Y**	n
2. honey	y	**N**	7. velvet	y	**N**	12. prairie	y	**N**	17. **Powder**	**Y**	n
3. **Market**	**Y**	n	8. **Carpet**	**Y**	n	13. **Highway**	**Y**	n	18. angel	y	**N**
4. **Story**	**Y**	n	9. valley	y	**N**	14. oyster	y	**N**	19. **Package**	**Y**	n
5. fabric	y	**N**	10. **Elbow**	**Y**	n	15. student	y	**N**	20. meadow	y	**N**
									Total Score Range=0–20		

11 Story Recall

Say ***Do you remember that story about a fire that I read to you earlier? Tell me as many details from the story as you can remember now.***

Scoring: 1 point for each *verbatim* recall of bold, italic words or alternatives, shown below in color within parentheses. Record intrusions or variations in the Responses column.

Story (Do not read.)	Responses	Item Score (0 or 1)
1. On ***Tuesday,***		
2. ***May***		
3. ***Fourth,***		
4. in ***Cleveland,*** Ohio,		
5. a ***3 alarm***		
6. ***fire*** broke out.		
7. ***Two***		
8. ***hotels***		
9. and a ***restaurant***		
10. were ***destroyed***		
11. before the ***firefighters*** *(firemen)*		
12. were able to ***extinguish it*** *(put it out)*.		
	Total Score Range=0–12	

12 Figure Recall

Say ***Do you remember that figure that I had you copy? I want you to draw as much of it as you can remember now. If you remember a part, but you're not sure where it goes, put it anywhere. Try to draw as much of it as you can.***

Now, present the Figure Recall Drawing Page.

Scoring: 1 point for correctness and completeness (drawing), and 1 point for proper placement. See Appendix 1 in Stimulus Booklet A for complete scoring criteria and scoring examples.

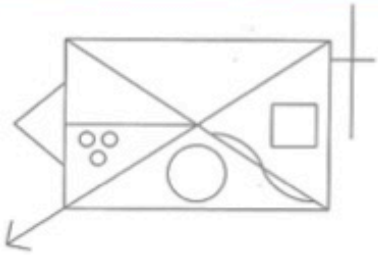


Figure Recall Criteria

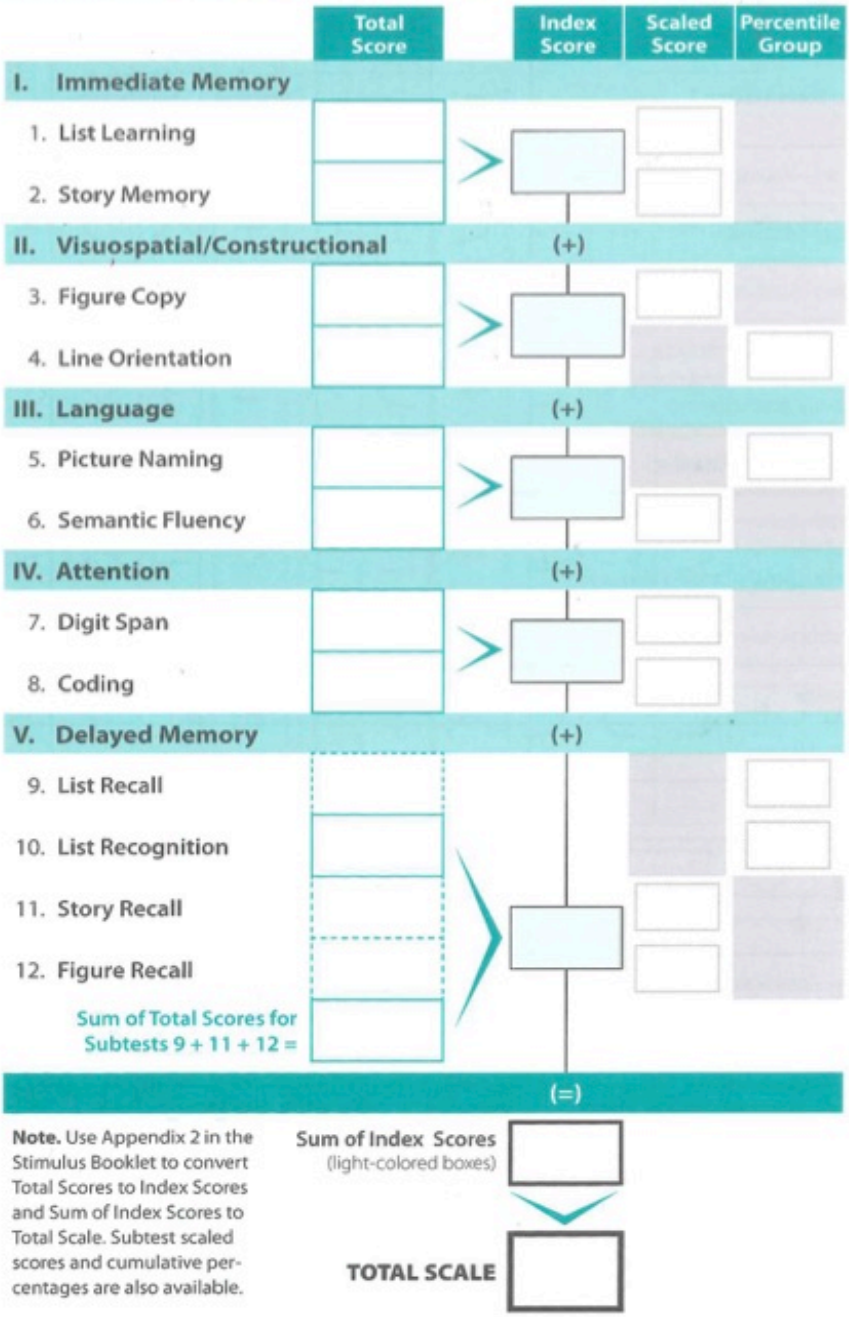
(Fold back for use.)

Item	Drawing (0 or 1)	Placement (0 or 1)	Score (0, 1, or 2)	Scoring Criteria
1. rectangle				**Drawing:** lines are unbroken and straight; angles 90 degrees; top/bottom lines 25% longer than sides **Placement:** not rotated more than 15 degrees
2. diagonal cross				**Drawing:** lines are unbroken and straight and should approximately bisect each other **Placement:** ends of lines should meet corners of the rectangle without significant overlap or measurable distance between the ends of the lines and the corners
3. horizontal line				**Drawing:** line is unbroken and straight; should not exceed 1/2 the length of the rectangle **Placement:** should bisect left side of the rectangle at approximately a right angle and intersect the diagonal cross
4. circle				**Drawing:** round, unbroken and closed; diameter should be approximately 1/4–1/3 height of rectangle **Placement:** placed in appropriate segment; not touching any other part of figure
5. 3 small circles				**Drawing:** round, unbroken and closed; equal size; triangular arrangement; not touching each other **Placement:** in appropriate segment; not touching figure; triangle formed not rotated more than 15 degrees
6. square				**Drawing:** must be closed; 90 degree angles; lines straight and unbroken; height is 1/4–1/3 height of rectangle **Placement:** in appropriate segment; not touching any other part of figure; not rotated more than 15 degrees
7. curving line				**Drawing:** 2 curved segments are approximately equal in length and symmetrical; correct direction of curves **Placement:** ends of line touch diagonal; do not touch corner of rectangle or intersection of diagonal lines
8. outside cross				**Drawing:** vertical line of the outside cross is parallel to side of rectangle; >1/2 the height of rectangle; horizontal line crosses vertical at 90 degree angle and is between 20–50% of length of vertical line **Placement:** horizontal line of outside cross touches rectangle higher than 2/3 the height of rectangle, but below top; does not penetrate the rectangle
9. triangle				**Drawing:** angle formed by 2 sides of triangle is between 60–100 degrees; sides are straight, unbroken and meet in a point; distance on vertical side of rectangle subsumed by triangle is approximately 50% of the height of vertical side **Placement:** roughly centered on the left vertical side of the rectangle
10. arrow				**Drawing:** straight and unbroken; lines forming arrow are approximately equal in length and not more than 1/3 length of staff **Placement:** must protrude from appropriate corner of rectangle such that staff appears to be continuation of diagonal cross
	Total Score Range=0–20			

Figure Recall Drawing Page

(Fold back for use.)

Score Conversion Page



Supplemental Discrepancy Analysis Page

Index Differences

Score 1–Score 2	Score 1	Score 2	Difference	Statistical Significance _____Level	Frequency of Difference in Standardization Sample
Immediate Memory—Visuospatial/Constructional					
Immediate Memory—Attention					
Immediate Memory—Language					
Immediate Memory—Delayed Memory					
Immediate Memory—Total Scale					
Visuospatial/Constructional—Attention					
Visuospatial/Constructional—Language					
Visuospatial/Constructional—Delayed Memory					
Visuospatial/Constructional—Total Scale					
Attention—Language					
Attention—Delayed Memory					
Attention—Total Scale					
Language—Delayed Memory					
Language—Total Scale					
Delayed Memory—Total Scale					

RBANS Adaptations

The above American version of the RBANS form was adapted for Irish patients as follows:

I. In the List Learning and List Recall subtests the word “highway” was replaced with “motorway”.

II. In the Story Memory and Story Recall subtests “…in Bangor, Wales, a serious fire…” was used instead of “…in Cleveland, Ohio, a 3 alarm fire…”.

III. In the Picture Naming subtest “peg” or “clothes peg” was used instead of “clothespin”.

IV. In the List Recognition subtest “grassland” was used instead of “prairie” and “motorway” was used instead of “highway”.

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R

Final Revised Version A (2005)

Name :
Date of birth :
Hospital no. :

Addressograph

Date of testing: ...../...../.....
Tester's name: .....
Age at leaving full-time education: .....
Occupation: .....
Handedness: .....

ORIENTATION

➢ Ask: What is the	Day	Date	Month	Year	Season	[Score 0-5]
➢ Ask: Which	Building	Floor	Town	County	Country	[Score 0-5]

REGISTRATION

➢ Tell: 'I'm going to give you three words and i'd like you to repeat after me: lemon, key and ball'. After subject repeats, say 'Try to remember them because i'm going to ask you later'. Score only the first trial (repeat 3 times if necessary).

Register number of trials .....

[Score 0-3]

ATTENTION & CONCENTRATION

➢ Ask the subject: ' could you take 7 away from a 100? After the subject responds, ask him or her to take away another 7 to a total of 5 subtractions. If subject make a mistake, carry on and check the subsequent answer (i.e. 93, 84, 77, 70, 63 -score 4)

Stop after five subtractions (93, 86, 79, 72, 65). ....

[Score 0-5]
(for the best performed task)

➢ Ask: 'could you please spell **WORLD** for me? Then ask him/her to spell it backwards:

....

MEMORY - Recall

➢ Ask: 'Which 3 words did I ask you to repeat and remember?'

....

[Score 0-3]

MEMORY - Anterograde Memory

➢ Tell: ' I'm going to give you a name and address and I'd like you to repeat after me. We'll be doing that 3 times, so you have a chance to learn it. I'll be asking you later'

Score only the third trial

[Score 0-7]

	1st Trial	2nd Trial	3rd Trial
Harry Barnes	....	....	....
73 Orchard Close	....	....	....
Kingsbridge	.....	.....	.....
Devon	.....	.....	.....

MEMORY - Retrograde Memory

[Score 0 -4]

➢ Name of current Prime Minister .....
➢ Name of the woman who was Prime Minister .....
➢ Name of the USA president .....
➢ Name of the USA president who was assassinated in the 1960's .....

ATTENTION&ORIENTATION MEMORY

VERBAL FLUENCY - Letter 'P' and animals

➢ **Letters**

Say: 'I'm going to give you a letter of the alphabet and I'd like you to generate as many words as you can beginning with that letter, but not names of people or places. Are you ready? You've got a minute and the letter is P'

[Score 0 - 7] ☐

>17	7
14-17	6
11-13	5
8-10	4
6-7	3
4-5	2
2-3	1
<2	0
total	correct

➢ **Animals**

Say: 'Now can you name as many animals as possible, beginning with any letter?

[Score 0 - 7] ☐

>21	7
17-21	6
14-16	5
11-13	4
9-10	3
7-8	2
5-6	1
<5	0
total	correct

FLUENCY

LANGUAGE - Comprehension

➢ Show written instruction: [Score 0-1] ☐ ☐

Close your eyes

➢ 3 stage command:
'Take the paper in your right hand. Fold the paper in half. Put the paper on the floor'

[Score 0-3] ☐ ☐

LANGUAGE - Writing

➢ Ask the subject to make up a sentence and write it in the space below:
Score 1 if sentence contains a subject and a verb (see guide for examples)

[Score 0-1] ☐ ☐

LANGUAGE

L A N G U A G E - Repetition

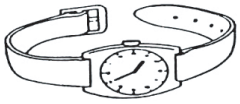
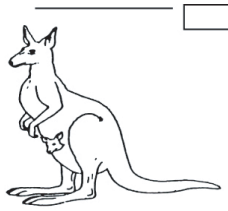
➢ Ask the subject to repeat:**' hippopotamus'; 'eccentricity; 'unintelligible'; 'statistician'**
Score 2 if all correct; 1 if 3 correct; 0 if 2 or less. [Score 0-2]

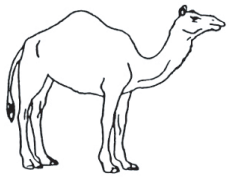
➢ Ask the subject to repeat: **'Above, beyond and below'** [Score 0-1]

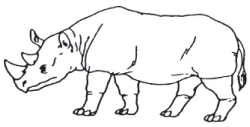
➢ Ask the subject to repeat: **'No ifs, ands or buts'** [Score 0-1]

L A N G U A G E - Naming

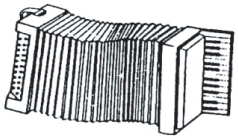
➢ Ask the subject to name the following pictures: [Score 0-2] pencil + watch







[Score 0-10]

L A N G U A G E - Comprehension

➢ Using the pictures above, ask the subject to: [Score 0-4]

- Point to the one which is associated with the monarchy _____
- Point to the one which is a marsupial _____
- Point to the one which is found in the Antarctic _____
- Point to the one which has a nautical connection _____

L A N G U A G E

LANGUAGE - Reading

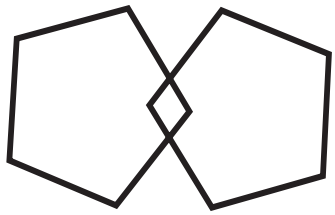
➢ Ask the subject to read the following words: [Score 1 only if all correct] [Score 0-1] ☐

sew
pint
soot
dough
height

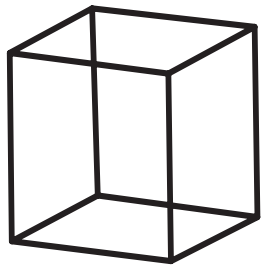
LANGUAGE

VISUOSPATIAL ABILITIES

➢ Overlapping pentagons: Ask the subject to copy this diagram: [Score 0-1] ☐☐



➢ Wire cube : Ask the subject to copy this drawing (for scoring, see instructions guide) [Score 0-2] ☐



➢ Clock: Ask the subject to draw a clock face with numbers and the hands at ten past five. (for scoring see instruction guide: circle = 1, numbers = 2, hands = 2 if all correct) [Score 0-5] ☐

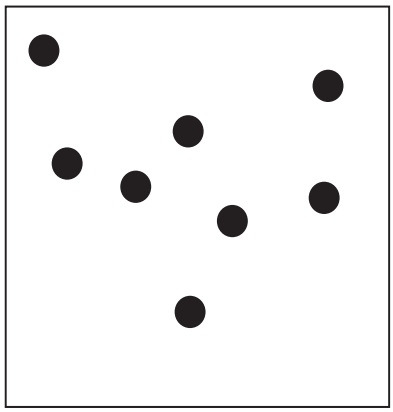
VISUOSPATIAL

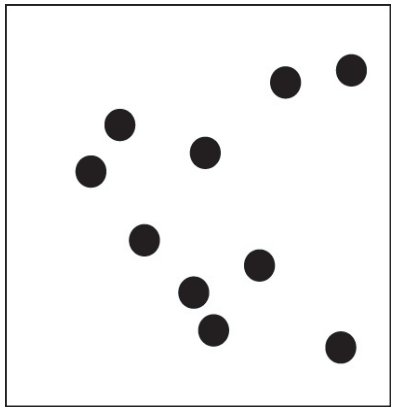
PERCEPTUAL ABILITIES

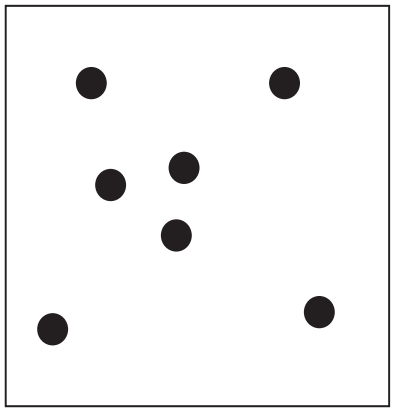
VISUOSPATIAL

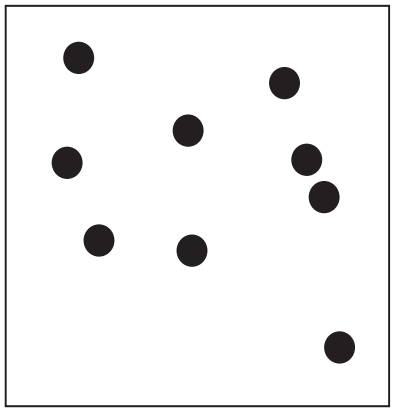
➢ Ask the subject to count the dots without pointing them

[Score 0-4]







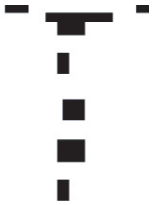


ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R

Final Revised Version A (2005)

PERCEPTUAL ABILITIES

➢ Ask the subject to identify the letters [Score 0-4]



VISUOSPATIAL

RECALL

➢ Ask "Now tell me what you remember of that name and address we were repeating at the beginning'"

[Score 0-7]

Harry Barnes	....
73 Orchard Close	....
Kingsbridge	.....
Devon	.....

RECOGNITION

➢ This test should be done if subject failed to recall one or more items. If all items were recalled, skip the test and score 5. If only part is recalled start by ticking items recalled in the shadowed column on the right hand side. Then test not recalled items by telling "ok, I'll give you some hints: was the name X, Y or Z?" and so on. Each recognised item scores one point which is added to the point gained by recalling.

[Score 0-5]

Jerry Barnes		Harry Barnes		Harry Bradford		recalled	
37		73		76		recalled	
Orchard Place		Oak Close		Orchard Close		recalled	
Oakhampton		Kingsbridge		Dartington		recalled	
Devon		Dorset		Somerset		recalled	

MEMORY

General Scores

SCORE

MMSE	/30
ACE-R	/100

Subscores

Attention and Orientation	/18
Memory	/26
Fluency	/14
Language	/26
Visuospatial	/16

Normative values based on 63 controls aged 52-75 and 142 dementia patients aged 46-86

Cut-off <88 gives 94% senstivity and 89% specificity for dementia
Cut-off <82 gives 84% sensitivity and 100% specificity for dementia

Frontal Assessment Battery

Purpose
The FAB is a brief tool that can be used at the bedside or in a clinic setting to assist in discriminating between dementias with a frontal dysexecutive phenotype and Dementia of Alzheimer's Type (DAT). The FAB has validity in distinguishing Fronto-temporal type dementia from DAT in mildly demented patients (MMSE > 24). Total score is from a maximum of 18, higher scores indicating better performance.

1. Similarities (conceptualization)
"In what way are they alike?"

- A banana and an orange

(In the event of total failure: "they are not alike" or partial failure: "both have peel," help the patient by saying: "both a banana and an orange are fruit"; but credit 0 for the item; do not help the patient for the two following items)

- A table and a chair
- A tulip, a rose and a daisy

Score (only category responses [fruits, furniture, flowers] are considered correct)

Three correct: 3 Two correct: 2 One correct: 1 None correct: 0

2. Lexical fluency (mental flexibility)
"Say as many words as you can beginning with the letter 'S,' any words except surnames or proper nouns."

If the patient gives no response during the first 5 seconds, say: "for instance, snake." If the patient pauses 10 seconds, stimulate him by saying: "any word beginning with the letter 'S.' The time allowed is 60 seconds.

Score (word repetitions or variations [shoe, shoemaker], surnames, or proper nouns are not counted as correct responses)

> 9 words: 3 6 -9 words: 2 3 -5 words: 1 < 3 words: 0

3. Motor series "Luria" test (programming)
"Look carefully at what I'm doing."

The examiner, seated in front of the patient, performs alone three times with his left hand the series of "fist–edge–palm."
"Now, with your right hand do the same series, first with me, then alone."
The examiner performs the series three times with the patient, then says to him/her:
"Now, do it on your own."

Score

Patient performs six correct consecutive series alone: 3
Patient performs at least three correct consecutive series alone: 2
Patient fails alone, but performs three correct consecutive series with the examiner: 1
Patient cannot perform three correct consecutive series even with the examiner: 0

4. Conflicting instructions (sensitivity to interference)
"Tap twice when I tap once."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 1-1-1.

"Tap once when I tap twice."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 2-2-2.

The examiner then performs the following series: 1-1-2-1-2-2-2-1-1-2.

Score No errors: 3 1 -2 errors: 2 > 2 errors: 1
Patient taps like the examiner at least four consecutive times: 0

5. Go–No Go (inhibitory control)
"Tap once when I tap once."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 1-1-1.

"Do not tap when I tap twice."
To ensure that the patient has understood the instruction, a series of 3 trials is run: 2-2-2.

The examiner then performs the following series: 1-1-2-1-2-2-2-1-1-2.

Score No errors: 3 1 -2 errors: 2 > 2 errors: 1
Patient taps like the examiner at least four consecutive times: 0

6. Prehension behaviour (environmental autonomy)

"Do not take my hands."

The examiner is seated in front of the patient. Place the patient's hands palm up on his knees. Without saying anything or looking at the patient, the examiner brings his own hands close to the patient's hands and touches the palms of both the patient's hands, to see if he will spontaneously take them. If the patient takes the examiner's hands, try again after asking the patient: "Now, do not take my hands."

Score

Patient does not take the examiner's hands: 3
Patient hesitates and asks what he/she has to do: 2
Patient takes the hands without hesitation: 1
Patient takes the examiner's hand even after he/she has been told not to do so: 0

Interpreting results
A cut off score of 12 on the FAB has a sensitivity of 77% and specificity of 87% in differentiating between frontal dysexecutive type dementias and DAT

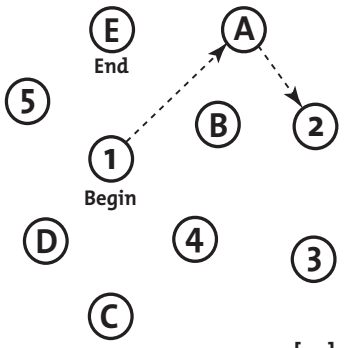
ReferenceS
Dubois, B. ; Litvan, I.; The FAB: A frontal assessment battery at bedside. Neurology. 55(11): 1621-1626, 2000.

Slachevsky, A; Dubois, B. Frontal Assessment Battery and Differential Diagnosis of Frontotemporal Dementia and Alzheimer Disease. Archives of Neurology. 61(7): 1104-1107, 2004.

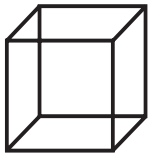
MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE



Copy cube



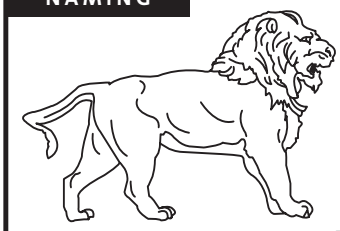
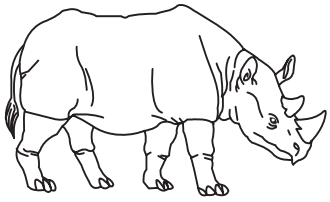
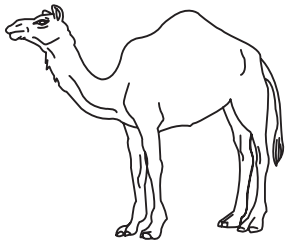
[ ] [ ]

Draw CLOCK (Ten past eleven)
(3 points)

[ ] Contour [ ] Numbers [ ] Hands

POINTS ___/5

NAMING

[ ] [ ] [ ]

___/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION

Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2 ___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B ___/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** ___/3

LANGUAGE

Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ] ___/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ____ (N ≥ 11 words) ___/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler ___/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

___/5

ORIENTATION

[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City ___/6

 Version November 7, 2004

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Normal ≥ 26 / 30

TOTAL ___/30

Add 1 point if ≤ 12 yr edu

Appendix 4: Functional Assessment Tool Used

Functional assessment in HAND

Mild Functional Decline (TWO or more of the following):			
1	**Increased assistance with at least TWO IALDs** (More IADLs could be considered as appropriate to the individual.)		
	Medication management		
	Financial management		
	Shopping		
	Meal preparation		
	Light housekeeping		
	Laundry		
	Driving		
	Use of public transportation		
	Maintaining personal schedules		
	Understanding media events		
	Child care		
2	**Unable to perform some aspects of a previous job, which is not due to medical symptoms**		
3	**Although maintains employment and/or full IALDs reports**		
	Less efficiency		
	Reduced productivity		
	More errors in performing tasks		
	More difficulty in meeting expectations		
	Greater effort expended performing the same activities		
4	**In the absence of significant depression (e.g. BDI > 17), which may bias reporting of symptoms, patient reports that he or she is experiencing difficulties with TWO or more aspects of cognition in daily life**		
	A. Meets HADS / other criteria for Depression		
	B. Meets HADS / other criteria for Anxiety		
	Memory for recent events (people, conversations, names, commitments, where things are placed)		
	Understanding conversations or reading materials		
	Word finding		
	Planning activities		
	Problem solving		
	Concentrating		
	Thinking clearly or logically		
	Finding his / her way about		
	Calculating		
	Following directions or instructions		
5	**Scores >1SD below an appropriate normative mean on at least ONE performance based standardised functional tasks**		

Major functional decline **(TWO or more of the following that are not readily attributable to medical or other comorbid conditions in the judgment of the examiner):**			
1	**Unable to maintain former employment not due to systemic illness or being dependent on disability status**		
2	**Requires substantially greater assistance or is dependent with more than > TWO IALDS**		
	Medication management		
	Financial management		
	Shopping		
	Meal preparation		
	Light housekeeping		
	Laundry		
	Driving		
	Use of public transportation		
	Maintaining personal schedules		
	Understanding media events		
	Child care		
3	**Greater difficulties with 4 or more aspects of cognition** However, self-report is not sufficient (would need confirmation by another informant) if patient is significantly depressed (e.g., BDI > 17).		
	A. Meets HADS / other Criteria for Anxiety		
	B. Meets HADS / other Criteria for Depression		
	Memory for recent events (people, conversations, names, commitments, where things are placed)		
	Understanding conversations or reading materials		
	Word finding		
	Planning activities		
	Problem solving		
	Concentrating		
	Thinking clearly or logically		
	Finding his / her way about		
	Calculating		
	Following directions or instructions		
4	**Scores >2SD below an appropriate normative mean on ONE performance based standardised functional task** **OR >1SD on at least TWO tasks**		

IADLs = instrumental activities of daily living, BDI = Beck Depression Inventory, HADS = Hospital Anxiety and Depression Scale, SD = standard deviation.

Adapted from Antinori et al. 2007

Appendix 5: Depression and Anxiety Screening Tool Used

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:
Total score: Depression (D) _____ Anxiety (A) _____
0-7 = Normal
8-10 = Borderline abnormal (borderline case)
11-21 = Abnormal (case)

References: Zigmond AS, & Snaith, R.P. The Hospital Anxiety And Depression Scale. Acta Psychiatrica Scandinavica. 1983;67(6):361-70

Appendix 6: Subtests Allocations for Z transformation by Domain

Sub Test	Z Score Domains						
	Attention & Processing Speed	**Working Memory**	**Executive Function**	**Memory**	**Visuos patial**	**Lang uage**	**Orien tation**
RBANS digit span forward	+						
RBANS digit coding	+						
RBANS list memory				+			
RBANS story memory				+			
RBANS list recall				+			
RBANS story recall				+			
RBANS figure copy					+		
RBANS line orientation					+		
RBANS categories						+	
RBANS phonemic fluency							
RBANS naming						+	
ACE-r anterograde memory				+			
ACE-r memory recall				+			
ACE-r word registration	+						
ACE-r backward tracking	+	+					
ACE-r phonemic fluency			+			+	
ACE-r pentagon copy					+		
ACE-r cube					+		
ACE-r clock drawing					+		
ACE-r perceptual abilities					+		
ACE-r categories						+	
ACE-r naming						+	

ACE-r language comprehension						+	
ACE-r writing						+	
ACE-r repetition						+	
ACE-r reading						+	
ACE-r orientation							+
ACE-r recall				+			
MoCA sustained attention	+		+				
MoCA backward tracking	+	+					
MoCA digit span forward	+						
MoCA backward span		+					
MoCA Abstraction			+				
MoCA phonemic fluency			+			+	
MoCA memory recall				+			
MoCA cube					+		
MoCA clock drawing					+		
MoCA categories							
MoCA naming						+	
MoCA repetition						+	
MoCA orientation							+
FAB similarities			+			+	
FAB Luria			+				
FAB conflicting instructions			+				
FAB go no go			+				
FAB prehension			+				
FAB phonemic fluency			+			+	

Appendix 7: Patient Information Leaflet and Consent Form for Follow-up MRI Data Acquisition

1. Title of study

The Inflammatory Neurodegenerative Continuum in HIV Related Cognitive Impairment – MRI and CSF characteristics. Follow-up MRI characteristics and correlation with clinical and neurocognitive assessment data.

2. Introduction

One of the neurological complications of HIV is cognitive impairment. It occurs in 20-50% of people with HIV. People affected by this complication of HIV infection may experience symptoms such as forgetfulness, problems concentrating, problems with short term memory, language difficulties, clumsiness, unsteadiness, changes in personality, mood swings and inappropriate emotional responses. We do not fully understand what causes memory problems in HIV and we hope that by carrying out this study we can contribute to the knowledge base about this condition and have a better understanding of it in order to help people who have it.

3. Procedures:

If you have been asked to participate in this study you have already had a MRI scan (Magnetic Resonance Imaging) in the past as part of your detailed assessment in "The Inflammatory Neurodegenerative Continuum in HIV Related Cognitive Impairment – MRI and CSF characteristics" study. The follow-up MRI brain scan will be carried out to look for new changes, if any, that HIV can cause in the brain that may be associated with the progression of memory difficulties. A new generation of MRI scanner called 3 Tesla MRI is available in St James's Hospital now. It is more advanced compared to conventional MRI in detecting changes in the structure of the brain. Those who have already underwent MRI scan as part of this clinical research will undergo a follow-up MRI scan of brain using the same 3T scanner. Patients are

placed on a moveable table in a large cylinder shaped scanner with openings on both ends. A microphone inside allows patients to talk to the person performing the scan. Once in the machine, a strong magnetic field is created around the patient while radio waves are directed toward the body. The radio signals are computer-processed and turned into images.

4. Benefits:

The information gathered from this study will help the medical community understand this condition better, learn about the causes of memory difficulties progression; aid diagnosis of future patients. This may help you and many others like you in the future.

5. Risks:

MRI scan does not involve any radiation. Scanning does not cause any pain.

The following conditions cause complications during the scan. If you have any of these conditions, the physician needs to be made aware prior to the scan. If you do not have any of the following, the procedure is risk free.

• Cerebral aneurysm clip (metal clip on a blood vessel in the brain)

• Cochlear (ear) implant for hearing impairment

• Metal in the eye or eye socket

• Implanted spine stabilization rods

• Heart pacemaker

• Implanted insulin pump/narcotics pump /implanted nerve stimulators

• Weight of more than 300 pounds

• Inability to lie on back for 30 to 60 minutes

• Pregnancy-***If you are a woman of childbearing age, you must not be pregnant or lactating and you must have a negative pregnancy test before the study begins. The effects of MRI on foetus or child are unknown and may be harmful. If you should become pregnant, please notify your doctor immediately.***

Rarely you may feel dizzy, have blurred vision or a metallic taste in your mouth for a few minutes after the scan. It is not a cause for concern and will resolve spontaneously.

6. Exclusion from participation:

Anybody who has not been part of the initial study will not be asked to partake in the follow-up study. Anybody who does not wish to participate will not be included.

7. Alternative treatment: ***You do not have to be a part of this study to be treated. Irrespective of whether you partake in the study or not, you will be followed up in the HIV clinic as before.***

8. Confidentiality:

Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the hospital. **All data gathered from this study will be kept strictly confidential with access only to responsible personnel involved in this project and no personal data will ever be divulged.**

9. Compensation:

(Non-sponsored trial): Your doctors are covered by standard medical malpractice insurance. Nothing in this document restricts or curtails your rights.

10. Voluntary Participation:

You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits which you had before entering the study.

11. Stopping the study:

You understand that your doctor may stop your participation in the study at any time without your consent.

12. Permission:

This study has been approved by SJH/AMNCH ethics committee in its present form after careful scrutiny of the proposals submitted.

13. Further information:

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Dr. Lilia Zaporojan who can be emailed at lzaporojan@stjames.ie. If your doctor learns of important new information that might affect your desire to remain in the study, he or she will tell you.

SJH / AMNCH RESEARCH ETHICS COMMITTEE

CONSENT FORM

Consent for study "The Inflammatory Neurodegenerative Continuum in HIV Related Cognitive Impairment – MRI and CSF Characteristics. Follow-up MRI characteristics and correlation with clinical and neurocognitive assessment data"

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.

Name of sponsor:

PARTICIPANT'S NAME:

PARTICIPANT'S SIGNATURE: **Date**

Date on which the participant was first furnished with this form:

Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the subject is a minor (under 18 years old) the signature of parent or guardian must be obtained:

NAME OF CONSENTOR, PARENT or GUARDIAN:

SIGNATURE:

RELATION TO PARTICIPANT:

Where the participant is capable of comprehending the nature, significance and scope of the consent required, but is physically unable to sign written consent, signatures of two witnesses present when consent was given by the participant to a registered medical practitioner treating him or her for the illness.

NAME OF FIRST WITNESS: SIGNATURE:

NAME OF SECOND WITNESS: SIGNATURE:

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician's signature:

Date:

Keep the original of this form in the participant's medical record, give one copy to the participant, keep one copy in the investigator's records, and send one copy to the sponsor (if there is a sponsor).

Appendix 8: MR Imaging Protocols

A. MRI protocol for T1-weighted Imaging

T1 3D TFE	
FOV FH (mm) =	256;
AP (mm) =	256;
RL (mm) =	160;
Voxel size FH (mm) =	1;
AP (mm) =	1;
RL (mm) =	1;
Recon voxel size (mm) =	1;
Reconstruction matrix =	256;
SENSE =	Yes;
P Reduction (AP) =	1;
P os factor =	1;
S reduction (RL) =	1.5;
Stacks =	1;
Slices =	160;
Slice orientation =	sagittal;
Fold-over direction =	AP;
Fat shift direction =	F;
Scan mode =	3D;
Technique =	FFE;
Contrast enhancement =	T1;
Acquisition mode =	cartesian;
Fast Imaging mode =	TFE;
shot mode =	multishot;
TFE factor =	240;
shot interval =	user defined;
(ms) =	3000;
TE =	user defined;
(ms) =	3.9
Flip angle (deg) =	8;
TR =	user defined;
(ms) =	8.5;
TFE prepulse =	invert;
slice selection =	no;
delay =	shortest;
NSA =	1;
Total scan duration =	07:29.;
ACQ matrix M x P =	256 x 240;
ACQ voxel MPS (mm) =	1.00 / 1.07 / 1.00;
REC voxel MPC (mm) =	1.00 / 1.00 / 1.00;
Act.WFS (pix) / BW (Hz) =	2.431 178.7;

B. MRI Protocol for DTI

DTI 32	
FOV RL (mm) =	245;
AP (mm) =	245;
FH (mm) =	150;
Voxel size RL (mm) =	2.5;
AP (mm) =	2.5;
Slice thickness (mm) =	2.5;
Recon voxel size (mm) =	2.45;
Fold-over suppression =	no;
Reconstruction matrix =	112;
SENSE =	yes;
P Reduction (AP) =	2;
P os factor =	1;
Stacks =	1;
Type =	Parallel;
Slices =	60;
Slice gap=	user defined;
gap (mm) =	0;
Slice orientation=	transverse;
Fold-over direction =	AP;
Fat shift direction =	P;
Scan mode =	MS;
Technique =	SE;
Fast imaging mode =	EPI;
Short mode =	Single-shot;
TE =	user defined;
(ms) =	59;
Flip angle (deg) =	90;
TR =	shortest;
(ms) =	8200
Halfscan =	yes;
Factor =	0.686;
Water-fat shift =	Minimum;
Shim =	PB-volume;
ShimAlign =	yes;
Fat suppression =	SPIR;
Strength =	strong;
Frequency offset (Hz) =	user defined;
offset (Hz) =	250;
Diffusion mode =	DTI;
Sequence =	SE;
Gradient duration =	Maximum;
Gradient overplus =	Yes;
Directional resolution =	High;
Nr of b-factors =	2;
b-factor order =	Ascending;
Max b-factor =	1100;
Total scan duration =	05:53.9.;
ACQ matrix M x P =	100 x 96;
ACQ voxel MPS (mm) =	2.45 / 2.55 / 2.50;

REC voxel MPC (mm) =	2.19 / 2.19 / 2.50;
EPI factor =	51;
WFS (pix) / BW (Hz) =	14.299 / 30.4;

Appendix 9: Seizure in HIV: The Case for Special Consideration. Article in Epilepsy and Behavior Case Reports Journal

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Case Report

Seizures in HIV: The case for special consideration

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ABSTRACT

Purpose: This study aimed to determine the rate, cause and management of seizures in the context of potential ART–ASD interactions in a cohort of HIV + individuals.
Methods: Records of 604 HIV + patients were reviewed and those reporting epilepsy/seizure diagnosis were further evaluated.
Results: This cohort exhibited a seizure rate of 2.4%. HIV + patients treated for epilepsy displayed low serum ASD levels and failed to achieve seizure control. They were more likely to disengage from Neurology follow-up.
Conclusion: For HIV + patients presenting with seizures/epilepsy the ASD prescription and the provision of supplementary support services needs to be carefully considered.

1. Introduction

In Ireland newly diagnosed cases of HIV have been reported at an annual rate that ranges from 7.0 to 7.5 per 100,000 [1]. Despite the introduction of highly active anti-retroviral therapy (HAART), 40–60% of HIV-infected individuals develop neurological complications [2–4]. The frequency of new seizures in the HIV positive (+) population is estimated to be between 4 and 11% in the populations studied [5]. To date the literature on the epidemiology of seizures and epilepsy in HIV has not generated reliable per patient year incidence estimates. Also no prevalence rates have been determined that can easily separate recurrent provoked seizures from recurrent unprovoked attacks (epilepsy). The data we have so far suggest a prevalence of all seizures of about 6% in a reasonably large HIV + cohort with approximately half of these identified as being unprovoked attacks [3].

These data compare with a point prevalence of 0.8% for epilepsy in Ireland in the general population [6], although there is an expected 10% life time risk of a seizure of any type in the general population [7]. Taking into account the wide variations in prevalence estimates for epilepsy due to differences in definitions and methodological approaches, a reasonable estimate of the prevalence of seizure disorders in people with HIV in Ireland is at least 3 times that of the general population.

Acute symptomatic seizures can be the presenting feature of HIV infection [8]. The main causes of seizures in HIV-infected individuals include the expected background genetic and environmental risk of epilepsy in that population, HIV infection itself or its CNS complications such as cerebral toxoplasmosis, tuberculoma, cryptococcal meningitis, PML, CNS lymphoma, syphilitic meningitis and HIV associated dementia [5,8–11]. HIV-related seizures may also be provoked by concurrently administered drugs [12]. Both HIV and seizures (including epilepsy and provoked seizures) may necessitate long term treatment with both antiretroviral therapy (ART) and anti-seizure drugs (ASD), which can lead to potentially serious ART–ASD interactions [11,13].The aim of this study was to determine the rate, type, cause and practiced treatment of new onset seizures (NOS) and epilepsy in a cohort of HIV-infected individuals attending St James's Hospital (SJH) in Dublin and to inform best practice seizure management in the context of potential ASD–ART interactions.

2. Methods

A dedicated HIV clinic in SJH is attended by a population of approximately 2200 HIV + patients. A subset of 604 HIV + individuals accessing this service previously participated in the Cognitive Impairment Prevalence Study, conducted between January 2011 and November 2013. Participants were screened for HIV-related cognitive impairment (CI) using the Brief Neurocognitive Assessment Screening (BNCS) tool [14]. In the current study, we further evaluated in detail the occurrence of seizure/epilepsy as another frequent neurological comorbidity in this population. For this purpose the Electronic Patient

Abbreviations: ART, antiretroviral therapy; ASD, anti seizure drug; NOS, new onset seizure; SJH, St. James's Hospital; EPR, electronic patient record; HAART, highly active antiretroviral therapy; CI, cognitive impairment.

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Records (EPR) of the 604 participants in the Cognitive Impairment Prevalence Study were reviewed for the diagnosis of "seizure", "seizure disorder" and "epilepsy"; as well as for neurology/epilepsy clinic attendances, EEG evaluations and ASD prescriptions. In those patients who met our search criteria, medical notes and EPR notes were further reviewed to obtain more detailed information about their history of seizures and epilepsy. This included recording the frequency of acute seizures and epilepsy in this cohort of patients, cause of seizures and their management.

Our assessment of patient adherence to prescribed treatment relied on the review of clinical notes. Adherence to ART was easier to ascertain as the ART therapy is observed in the HIV clinic. ASD therapy compliance on the other hand is usually interrogated with the patient (and with a family member or a caregiver where available) during the clinic appointment and is reconciled with the dispensing pharmacy. Checking serum ASD drug levels was also a helpful tool; however, the results were interpreted with caution in the context of possible drug–drug interactions.

3. Results

3.1. Demographic and clinical characteristics of HIV + patients with NOS

Out of 604 HIV + patients, a total of 15 (2.4%) had a history of epilepsy or a single provoked or unprovoked seizure at some stage in their life. The rate of epilepsy or seizures in those who screened positive for CI (311/604) was higher at 3.2% (10/311) than the 1.7% (5/293) for those who screened negative for CI. The male to female ratio amongst those who had seizures/epilepsy was 11:4. This ratio was consistent with the gender distribution in the Cognitive Impairment Prevalence Study cohort with the majority of participants being male (78.8%) [14]. In four of 15 patients, the history of epilepsy or seizures predated the diagnosis of HIV, of which two had established childhood onset epilepsy. Seizures re-occurred after the HIV diagnosis in one of the patients with childhood onset epilepsy and the other one experienced increased seizure frequency after the HIV diagnosis was established.

NOS, epilepsy diagnosis, or adulthood reoccurrence of seizures after or around the diagnosis of HIV was documented in a total of 13 patients with a mean latency of 69 months (5.8 years). A total of 14 out of 15 cases underwent further detailed analysis with one case excluded from the detailed analysis due to the lack of further information. (This participant had a single provoked event in childhood in the setting of a medication adverse event.) The mean age at NOS was 36 years, ranging from 13 to 57 years. Further clinical characteristics of HIV + patients with seizures are presented in Table 1.

Table 1
Demographic and clinical characteristics of HIV-positive patients with seizures.

Seizure onset relative to HIV Diagnosis (n = 15)	
Before HIV Diagnosis	Total 4
Acute Symptomatic Seizure in childhood	1
Childhood Epilepsy[a]	2[a]
One year before HIV Diagnosis	1
At/After HIV Diagnosis	Total 12
New Onset Seizure	11
Childhood epilepsy with seizure re-occurrence[a]	1[a]
Gender (n = 15)	M:F
	11:4
Way of infection (n = 15)	
Heterosexual	5
MSM	3
IVDU	7
Age at NOS/seizure re-occurrence (n = 14)[b]	
Mean	36
Range	13–57
Seizure onset latency after HIV Diagnosis (range; mean) (n = 14)[b]	0–168 months (14 years); 69 months (5.8 years)
0 (childhood with subsequent poor seizure control)	1
0 (12 months prior to HIV diagnosis)	1
At HIV diagnosis	2
1–6 months after HIV diagnosis	2
6–12 months after HIV diagnosis	1
36–168 months (3–15 years) after HIV diagnosis	7
CD4 + lymphocyte count at the time of NOS (n = 14)[b]	
<200 cell/mm^3	5
>200 cell/mm^3	5
NK	3
N/A	1
HAART treatment at NOS (n = 14)[b]	
No	8
Yes	3
NK	2
N/A	1

NOS – New Onset Seizure; NK - Not known due to presentation at an overseas healthcare center; N/A not applicable; IVDU – intravenous drug user; MSM – men having sex with men.

[a] In one case of known childhood epilepsy, seizures restarted in adulthood 5 years after the HIV diagnosis. The age at seizure re-occurrence was used for the purpose of this analysis.

[b] The childhood single seizure in acute settings was excluded from further analysis due to no available information.

3.2. Seizure semiology, etiology and evaluation

Repeated seizures led to the diagnosis of epilepsy in 6/14 (43%) patients necessitating ongoing ASD therapy. One of these had previously been diagnosed with Idiopathic Generalized Epilepsy (IGE) (or *Genetic Generalize Epilepsy (GGE)* according to the new ILAE classification of the epilepsies) [15]. Seizures were the presenting symptom of HIV in three cases (21%). This includes one case where the seizure occurrence was reported one year prior to the HIV diagnosis. In five patients (36%), seizures were the presenting feature of and were caused by a CNS opportunistic infection (one of them at the time of HIV diagnosis). Other causes included benzodiazepine withdrawal (4 patients). Alcohol withdrawal was documented during the first two presentations with seizure re-occurrence in adulthood in one of the patients with a previous history of childhood epilepsy. However, this patient developed further events while abstinent and was eventually diagnosed with epilepsy. No cause other than HIV was found in three patients, two of whom had focal gliosis shown by MRI (Table 2).

With regards to seizure type, one patient had *generalized tonic–clonic seizures*. *Focal seizures* (*aware* or *with impaired awareness*) were documented in four patients. In six patients seizure type was classified as probable *focal onset to bilateral tonic–clonic seizure*. Two patients had *unknown onset tonic–clonic seizures* and in one patient the seizure type was not possible to classify due to the lack of further information — *unclassified seizure* [16]. Status Epilepticus (SE) was documented in a total of three patients (21%) (one – non motor SE and two – motor SE).

EEG reports were available for nine of the patients. Only one of them had evidence of epileptiform discharges. A total of four patients showed focal dysfunction and one patient had generalized slow activity. Three patients showed no abnormality on EEG. Brain MRI or written reports were available for review for eleven patients (Table 2). Seven of them had focal brain lesions, and two had diffuse lesions shown by MRI (Table 2; Fig. 1).

A total of thirteen patients were reviewed by the Neurology service at the time of seizure presentation. Eight of them attended neurology/epilepsy services for follow-up with a subsequent high rate (5/8) of disengagement from these services.

3.3. ASD treatment, side effects and potential ASD–ART interactions

ASD treatment was begun in 10 patients: six of them were diagnosed with epilepsy and four presented with acute symptomatic seizures (Table 3).

The most commonly prescribed ASD was levetiracetam (prescribed 1st in 4 cases and 2nd or 3rd in 3 cases), which also had the highest

Table 2
Seizure semiology and etiology, diagnostic evaluation and Neurology specialist involvement. (n = 14).

Seizure type	
Focal onset seizure +/− to bilateral tonic–clonic	Total 4
- Focal aware	1
- Focal with impaired awareness	3
Focal to bilateral tonic–clonic[a]	6
Generalized Genetic Epilepsy (GGE)	1
Unknown onset tonic–clonic	2
Unclassified[b]	1
Seizure occurrence	
Acute symptomatic seizures	8
Recurrent seizures/epilepsy	6
EEG findings	
Generalized epileptic discharges	1
Focal dysfunction	4
Generalized slow activity	1
No abnormality	3
EEG not available	5
Brain MRI findings	
Focal brain lesions etiology	Total FBL 7
- CNS Toxoplasmosis	3
- Tuberculous meningitis	1
- Progressive multifocal leukoencephalopathy (PML)	1
- Focal gliosis (unspecified)	2
Diffuse brain lesions	2
Normal MRI	2
MRI not available/performed elsewhere[c]	3
Possible etiology of seizure	
CNS complication	Total CNS complications 5
- CNS Toxoplasmosis	3
- TB meningitis	1
- PML	1
Other:	Total other 9
Benzodiazepine withdrawal	4
Previous history of epilepsy	2
No other cause apart from HIV	3
Neurology Specialist Involvement	
Neurology review at NOS	13
Neurology/Epilepsy follow-up	8
Disengaged from Neurology/Epilepsy follow-up	5[d]

GGE – Generalized Genetic Epilepsy.
FBL - Focal brain lesions.
[a] Patients presented with witnessed episodes of tonic–clonic motor activity with impaired awareness and focal brain lesion on brain imaging or focal EEG changes.
[b] No further information was available to be able to classify seizure type for one participant.
[c] Two patients (one with GGE) initially presented elsewhere and information regarding imaging was not available; one patient had normal CT brain at seizure presentation.
[d] 2 of 5 patients who disengaged with epilepsy service still had documented seizures.

rate of adverse events (4/7), followed by lamotrigine (prescribed 1st in 2 cases, 2nd in one case and 3rd in 2 cases) and valproate (prescribed 1st in 4 cases, never as a second agent). Seizure freedom after introducing the first ASD was achieved in only three cases. In all three cases, patients presented with acute symptomatic seizures in the setting of a CNS opportunistic infection.

4. Discussion

Seizures and epilepsy are important CNS complications of HIV infection. Seizure prevalence in our HIV+ cohort is higher than in the general population (2.4% versus approximately 1.5% if we include symptomatic seizures and epilepsy). This difference is lower than in other reported studies [5,11]. Interestingly, we found a higher seizure prevalence rate (3.2%) in the HIV+ individuals who screened positive for cognitive impairment. Most of our patients (14/15) had at least one seizure after acquiring HIV infection. However, two of them had been previously diagnosed with epilepsy in childhood. After their diagnosis of HIV, one of these two patients had seizure reoccurrence and the other had increased seizure frequency. Notably, both had seizures refractory to ASD therapy, which could potentially be due to unfavorable ASD–ART interactions. Furthermore, eight of our patients had no other obvious risk factors for epilepsy apart from HIV and its CNS complications. This was further supported by lesional neuroimaging findings consistent with changes described in HIV encephalitis/encephalopathy or CNS opportunistic infection in seven of them.

HIV stage and immune status at NOS is important. Five of our patients had an AIDS defining CNS opportunistic infection at the time of seizure presentation. A further two patients had advanced HIV disease with CD4+ counts below 200 cells/mm^3. Slightly less than half (6/14) of those who underwent detailed analysis experienced the first seizure close to the time of HIV diagnosis (+/− 12 months) when infection is more likely to be uncontrolled (Table 1). Eight patients experienced only one seizure, half of these at the time of a concomitant CNS opportunistic infection. This is highly relevant as it highlights the importance of prompt diagnosis and treatment of opportunistic infection in those presenting with seizures in the context of a known or new HIV diagnosis. These patients may only require short term ASD therapy, thus avoiding potential long term ASD side effects and/or ASD–ART interactions.

Patients who were diagnosed with epilepsy (6/14) were less likely to have had CNS opportunistic infections. Only one patient with recurrent seizures was diagnosed with PML. Two patients had a preexisting epilepsy diagnosis. The remaining three had focal brain lesion reported on MRI and for two of these the focal brain lesion description was consistent with the MRI changes reported in HIV encephalitis/encephalopathy. These findings suggest that the direct effect of HIV on the CNS may also be responsible for the recurrent seizures in this category of patients. It was also found that these patients were more likely to present with seizures later in the course of their HIV disease, three years or more after their HIV diagnosis.

ART–ASD interactions can lead to increased serum drug levels in either drug class and increase the risk of toxicity [17]. ART–ASD interactions can also cause reduced drug serum levels with resulting consequences, such as reduced ASD levels and poor seizure control, or reduced ART levels with resulting poor virologic suppression and disease progression [18–20]. The latter is expected with the use of enzyme-inducing, older generation ASDs (phenobarbital, phenytoin and carbamazepine) [21–23]. The direct effects on HIV viral replication that could potentially lead to increased viral load by commonly used ASDs such as valproate have also been described [10,24].

In our study, of the three most prescribed ASDs LEV proved to be effective, provided the patient was compliant with the treatment and did not develop side effects. However, LEV had the highest incidence of side effects reported by patients (4/7) and had to be discontinued in two cases due to mood/personality disturbance. Levetiracetam has the least ASD–ART interactions reported in the literature but its propensity to cause intolerable neuropsychiatric side effects has been documented [10,25]. Therefore, careful consideration needs to be given to LEV prescription in patients as HIV-infected individuals may develop depression as part of their primary diagnosis. In this study, LEV was associated with viral control failure in two cases. However, this was not attributed to a possible ASD–ART interaction but to poor ART compliance.

LTG failed to demonstrate acceptable seizure control in our cohort. This can be explained by either the small dose prescribed or ASD–ART interactions not identified. Prescription of higher doses in these patients would have been appropriate but did not receive consideration. Overall, VPA showed poor seizure control in our cohort. However, VPA–ART interactions maybe possible and persistently low serum levels were recorded despite the high doses being prescribed. It was not possible to evaluate its effect on viral control due to the lack of data prior to 2012. Also, most patients who had been on VPA therapy had it discontinued by 2013.

Although the majority of patients were reviewed by neurology/epilepsy services in the acute settings and follow-up appointments were

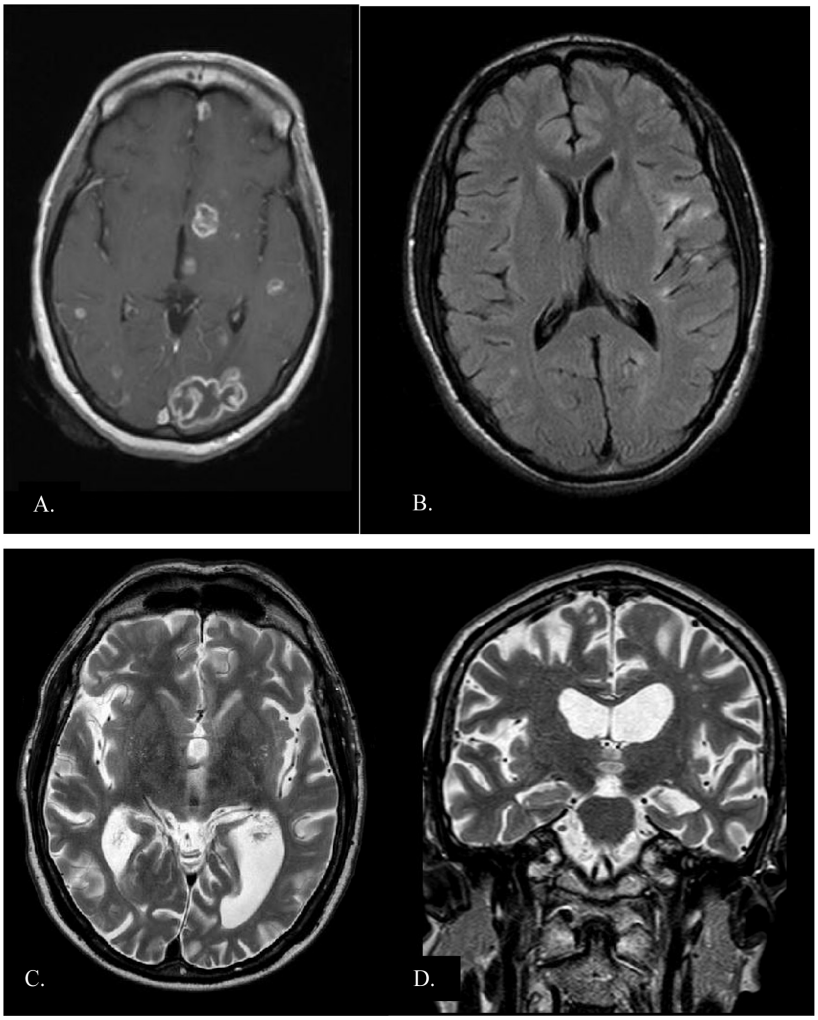

Fig. 1. MRI images: A. Case I - Axial T1 MRI image enhanced with contrast showing multiple ring enhancing lesions in a patient presenting with status epilepticus in the context of HIV and cerebral Toxoplasmosis; B. Case II - Axial T2 FLAIR image showing multiple high signal intensity foci in keeping with gliosis in the site of a previous infection in a patient treated for seizures on background of HIV and ongoing treatment for TB meningitis; C. and D. Case III - Axial and coronal T2 images showing focal atrophy of the left temporal lobe and hippocampus on background global atrophy in a patient with refractory epilepsy in the context of HIV and PML.

arranged, there was a high rate of disengagement with these services observed in our cohort (5/8). This is despite patients being contacted by one of the epilepsy nurse specialists to confirm the follow-up appointments. Additionally, an appointment letter is sent to the patients' home address, and a text message reminder is sent to the patients' mobile phone a week before any SJH follow-up appointment to ensure attendance. This experience suggests that this particular group of patients and especially those with concomitant CI may need supplementary counseling and support services to optimize their clinical care.

This study highlights the importance of both HIV and the neurologist's involvement in the evaluation and treatment of this category of patients and perhaps the need for integrated neurology/epilepsy service in the HIV clinic for relevant patients.

5. Study limitations

Our study has a number of important limitations. This is a small cohort evaluated by retrospective chart review. Although an initial group of 604 HIV+ patients were looked at, only a small number proved to have suffered from seizures/epilepsy. Selection bias is also possible. However, the 604 patients who consented to participate in the initial Cognitive Impairment Prevalence Study were judged to be fairly representative of the 2200 HIV+ patients attending the HIV services at SJH [14]. It was difficult to draw meaningful conclusions with regard to ASDs' effectiveness, adverse events and possible interactions for a number of reasons including: the small subject numbers, the retrospective nature of the study, the heterogeneity of anti-seizure drugs used in such a small cohort and the limitations of the clinical data available. Finally, many of the

Table 3
ASD treatment, side effects and potential ASD–ART Interactions (n = 10).

no	Engagement with neurology/epilepsy services	ASD/ Serum level	ASD Side effect	SZ free	Possible ART/ASD interactions	CSF penetration	Viral load while on ASD[a]
1	Disengaged	LTG-	–	NO	Abacavir – LTG ↓	High	–
					Efavirenz – no	High	
					Lamivudine – no	Low	
2	Disengaged	LTG-	–	NO	Efavirenz – no	High	–
					Tenofovir – no		
					Emtricitabine		
3	n/a	VPA	–	YES	↑ Lopinavir – VPA ↑	High	–
					Ritonavir – VPA	High	
					Tenofovir – no		
					Emtricitabine		
4	Disengaged	PHT↓		YES	Atazanavir – PHT		2200- < 50 (copies/ml)
		LEV	Mood		↓ Ritonavir – PHT	High	
					Tenofovir – PHT		
					Emtricitabine		
5	n/a	LEV		YES	Efavirenz – no	High	–
					Emtricitabine		
					Tenofovir – no		
6	Regular follow-up	VPA↔↓		NO	Tenofovir – no		ND
		LEV			Emtricitabine		
		LTG-			Ritonavir – LTG↓; VPA	High	
					Darunavir – VPA		
7	Regular follow-up	VPA↓↓		NO	Darunavir – VPA; ZOS		<40/ND
		LEV	Mood		Etravirine		
		LTG-			Ritonavir – LTG↓; ZOS; VPA	High	
		PG					
		ZOS↓↓	Cog				
8	Disengaged	LEV-	Mood	NO	Darunavir – no		<40/ND
					Raltegravir – no		
					Ritonavir – no	High	
					Tenofovir – no		
					Emtricitabine		
9	Regular follow-up	VPA		NO	Efavirenz – VPA; LAC↓	High	ND
		LTG↓↔			Abacavir – LTG↓	High	
		LEV↔	Pers		Lamivudine – no	Low	
		LAC↓			Dolutegravir – no		
10	n/a	LEV	–	–	Zidovudine – no	High	<40–4000 (copies/ml)
					Lamivudine – no	Low	
					Lopinavir – no	High	
					Ritonavir – no	High	

ASD - anti-seizure drug; ART – Antiretroviral Therapy; VL – Viral Load; ND – HIV RNA not detected; n/a - not applicable; no – no reported potential ARV-ASD interactions; n/k – not known. - information not available.
↔ ASD serum level within normal range.
↓ ASD serum level low/lowered by potential interaction.
↑ ASD serum level high/increased by potential interaction.
Cog – cognitive symptoms; Mood – mood disturbances; Pers – personality changes; SZ – seizure; LEV – Levetiracetam; LTG – Lamotrigine; LAC – Lacosamide; PHT – Phenytoin; PG – Pregabalin; VPA – Sodium Valproate; ZOS - Zonisamide.
[a] Viral Load results were not available prior to 2012.

patients had poor engagement with the neurology/epilepsy services, and adherence to ASD therapy was difficult to ascertain or manage.

6. Conclusion

While comparisons to the general population are difficult, it does appear that the prevalence of both symptomatic seizures and epilepsy (recurrent unprovoked attacks) is greater in HIV + individuals. However, the rates found in our cohort (2.4%) appear lower than other published estimates. An interesting finding is that the rate appears higher in those who screened positive for cognitive impairment (3.2%); a finding that requires further study. With respect to status epilepticus and seizure freedom rates, again, the HIV + population fairs worse than the general population. Finally, an important consideration is that HIV alone may not be the only cause of seizures/epilepsy in this study population. The majority of patients with seizures presented with focal or diffuse lesions shown by MRI and most of these were caused by opportunistic CNS infections.

It is in the area of treatment that more work needs to be done. ART–ASD interactions were not adequately evaluated in this cohort and might be responsible for ineffective treatment and potentially poor outcomes. Recently, evidence based recommendations on ASD treatment in the context of ART therapy have been jointly developed by the American Academy of Neurology and the International League Against Epilepsy [26]. These published guidelines should be carefully considered when it comes to making treatment decisions in patients with HIV.

Conflicts of interest

None.

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