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# **The Inflammatory Degenerative Continuum of HIV Related Cognitive Impairment**

A dissertation submitted to Trinity College for the Degree of Doctor of  
Philosophy (PhD) 2014

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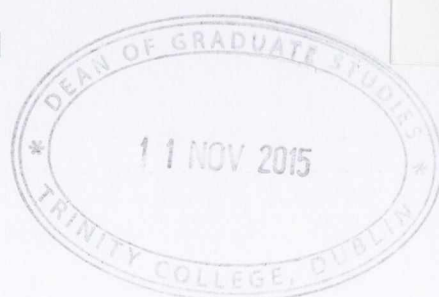
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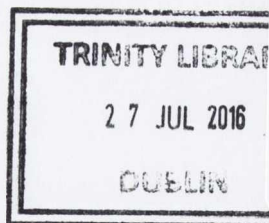
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**Dr Patricia Helena McNamara**



*Thesis 11057*

## **Dedication**

I dedicate this thesis to my parents, Patrick and Helen, and to my sister, Fiona, whose unconditional love and unwavering belief in me always sustains and nourishes me.



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## **Summary**

This thesis is the result of three years of research from January 2011 to December 2013 examining the role of cognition in HIV infection in an Irish specialist HIV service. This had not previously been examined in Ireland. Four studies were undertaken which were all linked. The first examined the prevalence of a positive screen for cognitive impairment. The second examined the cognitive profile of HAND in Ireland in the post highly active anti-retroviral therapy era. The third study investigated the magnetic resonance imaging (MRI) features of HAND with voxel based morphometry (VBM) and diffusion tensor imaging (DTI). The final study explored the immunological, inflammatory and degenerative biomarkers of HAND in cerebrospinal fluid. All of the studies were cross-sectional in nature.

The primary aim of the study was to explore the inflammatory degenerative continuum in HAND. A high prevalence rate was demonstrated of a positive screen for cognitive impairment. The cognitive profile was shown to be predominantly an amnesic, dysexecutive profile with cortical features. MRI showed both inflammatory and degenerative features on VBM and DTI with cortical atrophy and abnormalities across multiple DTI measures. CSF examination demonstrated that there was trafficking of activated T cells into the CSF of these patients with HAND despite viral suppression. All of these features highlight the roles that inflammation, immune activation and neurodegeneration play in this condition. The key findings of this study should hopefully add to the ever burgeoning literature on HAND and help to improve our understanding of this condition.

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# 1 Introduction

## 1.1 History of HIV Infection

Human immunodeficiency virus (HIV) first gained public notice when the United States Centres for Disease Control (CDC) and Prevention published a report, on five homosexual men in Los Angeles who had pneumocystis pneumonia, in their Morbidity and Mortality Report in June 1981. This was caused by a type of *Pneumocystis carinii* which is now recognised as a separate distinct species *Pneumocystis jiroveci*. All five patients also had evidence of current or previous infection with Cytomegalovirus (CMV) and candidal mucosal infection. Three of the patients who had been tested were noticed to have abnormal cellular-immune function. At the time the CDC suggested the possibility of a cellular-immune dysfunction which was related to a common exposure which then predisposed previously healthy individuals to unusual opportunistic infections (1).

In September 1981 in New York, a case series was published in the *Lancet* of eight homosexual men with Kaposi's sarcoma (KS) with unusual features of younger age at onset, poorer prognosis, previous history of sexually transmitted infections and positive antibodies for CMV and hepatitis B. Hymes et al postulated that sexually transmitted infections may have played a role in the pathogenesis of this unusual form of KS (2). Masur et al reported 11 cases of pneumocystis pneumonia in December 1981 in the *New England Journal of Medicine*. The affected patients were either drug users,



homosexual men or both. Absolute lymphocyte counts, T-cell counts and lymphocyte proliferation were all decreased suggesting a cellular immune dysfunction (3).

The CDC set up a special task force on Kaposi Sarcoma and Opportunistic Infections in 1981. The acronym AIDS for Acquired Immunodeficiency Syndrome was given to the condition in September 1982 by the CDC (4). At the end of 1981 and throughout 1982 more cases were reported throughout the USA of unusual opportunistic infections occurring and cases were also reported in the UK, Haiti and Europe (5-8). Infections were also noted amongst haemophiliacs (9). Cases were being described in Africa at this time which suggested that heterosexuals were also at risk of this disease (10, 11).

## **1.2 Isolation of the Causative Virus**

The virus itself was not isolated until 1983 when Dr. Luc Montagnier and his team at the Pasteur Institute in Paris isolated a T-lymphotropic retrovirus from the lymph node biopsy of a patient with signs and symptoms that often preceded acquired immune deficiency syndrome (AIDS), however they were uncertain if it caused AIDS or not. They named the virus lymphadenopathy-associated virus (LAV) (12). Robert Gallo and his team in the United States of America isolated the same virus and called it Human T-Lymphotropic Virus Type III (HTLV-III) but they also demonstrated a clear relationship between the virus and AIDS (13-16). The virus was renamed in 1986 by the International Committee on Taxonomy of Viruses to Human Immunodeficiency Virus (17).

There are two species of HIV; HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered by Dr. Luc Montagnier and Dr. Robert Gallo. It was previously termed both LAV and HTLV-III. HIV-1 is more virulent and more infective than HIV-2 and is the cause of the majority of HIV infections globally. HIV-2 has lower infectivity compared to HIV-1. It has remained largely confined to West Africa due to its lower capacity for transmission (18, 19).

### **1.3 Structure of HIV**

HIV is a lentivirus which is a member of the retroviridae family. It is a single-stranded, positive-sense, enveloped ribonucleic acid (RNA) virus. Its hallmark is the reverse transcription of viral RNA to deoxyribonucleic acid (DNA) by reverse transcriptase enzyme. This is a unique characteristic of retroviruses. The DNA is then integrated into the host's genome as a provirus. All the other non-retroviral viruses lead to the transcription of new RNA from a DNA strand.

HIV has a two layer phospholipid envelope derived from the host cell. Envelope (*env*) is a HIV protein that is embedded in the viral envelope. In retroviruses the expression of the *env* gene allows them to target and attach to specific cell types (20). HIV RNA codes for nine genes (21). Three of the genes, group specific antigen (*gag*), polymerase (*pol*), and *env*, encode structural proteins. HIV trans-activator (*tat*) and regulator of expression of virion proteins (*rev*) have an essential regulatory role. The remaining four genes are also involved in regulation but are not necessary for replication in all cells. *Tat* is a viral transcriptional transactivator (22). Glycoprotein 120 (gp120) and

glycoprotein 41 (gp41) are the primary glycoprotein envelope proteins contained in HIV. They are coded for by *env*. Gp120 is involved in determining viral tropism through its binding to target cell receptors. Gp41 is involved in the fusion between viral and cellular membranes (23).

#### **1.4 Genetic Variability**

HIV is very polymorphic and exists as a quasispecies, meaning that it consists of multiple genetically related variants. RNA viruses carry a much higher mutation rate than DNA viruses due to the high error rate in replication (21). The genetic diversity of HIV is due to this high viral replication rate along with its ability to recombine. This genetic diversity has been classified into subtypes called clades. The sequences within any one subtype are more similar to each other than to sequences from other subtypes throughout their genomes. They represent different lineages of HIV and there are some geographical associations. The use of clades or subtypes describes genetic clustering patterns and is a useful system for organizing viruses by genetic similarity. In Europe and North America the most common clade is subtype B while subtype C is the dominant form in Africa and Asia (24).

#### **1.5 Viral Tropism**

Viral tropism refers to the cell types that a virus can infect. When gp120 binds to cluster of differentiation (CD) 4 cells or macrophages it opens up a binding site allowing for attachment via two chemokines. Viruses which attach via the chemokine receptor CCR5 are called R5 tropic viruses and they primarily infect macrophages. X4



tropic viruses attach via the chemokine receptor CXCR4 and primarily infect primary CD4 T cells. Viruses which can use both chemokines are called dual or mixed tropic viruses (D/M tropic). After binding, gp41 then facilitates fusion of the host cell membrane and the viral membrane thus releasing the virus into the host cell cytoplasm and allowing the infective cycle to begin (25).

## **1.6 Epidemiology of HIV Infection Globally**

In 2013 an estimated 35 million people worldwide were living with HIV. In 2013 there were an estimated 2.1 million people who were newly infected with HIV and an estimated 1.5 million people died as a result of AIDS in the same time period (26). 70% of all new infections occurred in Sub-Saharan Africa in 2012 although the rate of new infections in adults had decreased by 34% since 2001. The rate of infection continues to rise in the Middle East, North Africa, Eastern Europe and Central Asia (27).

## **1.7 Epidemiology of HIV Infection in Ireland**

HIV became a notifiable disease in Ireland in September 2011. In 2012 there were 341 people newly diagnosed with HIV in Ireland. This gives a crude notification rate of 7.4 per 100,000 population. Since the early 1980's, 6,629 people have been newly diagnosed with HIV in Ireland (28). This number reflects solely patients who have been diagnosed. Tuite et al reviewed patients accessing HIV outpatient care in the Republic of Ireland over a 12 month time period from 2009 to 2010 and revealed that 3254 patients accessed HIV outpatient care in that time period (29).



Men who have sex with men (MSM) accounted for 49% of all new infections in 2012 and heterosexual contact was the route of infection for 38%. Intravenous drug users accounted for 3.8% of new infections in 2012. There were five cases recorded due to mother to child transmission. The country of infection was sub-Saharan Africa in all of the five cases. No children born in Ireland in 2012 were diagnosed with HIV. Probable route of transmission is not available for the other patients at this time (28).

Men who have sex with men have accounted for the highest number of new infections since 2009. 72% of new infections in 2012 were among men and women accounted for 28% of new infections. Based on the available geographic information, Irish born patients accounted for 36% of new infections. In 2012, 48% of patients newly diagnosed were born outside of Ireland (28).

15.5% of patients newly diagnosed in 2012 were co-infected with one or more sexually transmitted infections (STI) (syphilis/gonorrhoea/chlamydia). 7.3% of patients were co-infected with hepatitis C and 5% were co-infected with hepatitis B. At the time of their HIV diagnosis, 34 (10%) people were diagnosed with an AIDS defining illness. Pneumocystis pneumonia (PCP), Kaposi's sarcoma (KS), Pulmonary Tuberculosis (TB), extra-pulmonary TB and oesophageal candidiasis accounted for respectively, 32%, 24%, 12%, 12% and 12% of AIDS defining illnesses (28).

### **1.8 Mode of Transmission of HIV**

HIV is a blood borne virus. It can be transmitted sexually, through the use of contaminated needles in the intravenous drug using (IVDU) population, from mother to child and via infected blood products.

### **1.9 Mechanism of Infection**

HIV primarily infects CD4 positive T cells, macrophages and dendritic cells thereby suppressing the infected individual's immune system and predisposing them to opportunistic infections and some malignancies, most commonly Kaposi's sarcoma and non-Hodgkin's lymphoma.

Dendritic cells contain a receptor named DC-SIGN which is a HIV specific dendritic receptor. It binds HIV-1 at its gp-120 domain. The cell is not required to be directly infected. HIV-1 is then transported to lymphoid tissue. Replication of HIV occurs in regional lymph nodes. Dendritic cells act as antigen presenting cells. Naïve T cells are primed and rapid infection of T cells ensues. The entry of HIV-1 into host cells depends on binding at the chemokine sites CXCR4 or CCR5 (30). There is progressive depletion of CD4<sup>+</sup> T cells if the infection remains untreated.

T lymphocytes are part of the cellular immune response which involves the adaptive immune response. Cell mediated immunity involves the activation of macrophages and natural killer (NK) cells, the production of antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.

The activation, proliferation and function of helper T lymphocytes are crucial to an effective immune response (31). Dendritic cells act as antigen presenting cells. They are part of the innate immune response. After migration to local lymphoid tissue the dendritic cells mature and are distinguished by surface molecules. They are then involved in the activation of naïve T cells (32).

Antigens from pathogens that replicate in the cytoplasm are brought to the cell surface by major histocompatibility complex (MHC) class 1 molecules and are presented to CD8<sup>+</sup> T lymphocytes. This results in differentiation into cytotoxic CD8<sup>+</sup> that cause apoptosis of infected target cells. Cytotoxic CD8<sup>+</sup> control virally infected cells and tumour cells. Antigens from pathogens that replicate in intracellular vesicles are brought to the cell surface by MHC class 2 molecules and are presented to CD4<sup>+</sup> T lymphocytes (32).

There are two types of effector CD4<sup>+</sup> T cell, called T helper cell type 1 (TH1) and T helper cell type 2 (TH2). Intracellular antigens activate the TH1 pathway and extracellular antigens stimulate the TH2 response. TH1 cells induce B cells to produce immunoglobulin G (IgG) and induce phagocytosis. The humoral immune response is initiated by TH2 cells. Naïve antigen specific B cells then produce IgM antibodies (32).

HIV mediates its main effect on the immune system due to the infection and destruction of the TH lymphocytes. HIV infected CD4<sup>+</sup> and CD8<sup>+</sup> cells are more susceptible to apoptosis than TH lymphocytes in HIV negative individuals although HIV



endeavours to ensure productive infection and avoid apoptosis. The CD4<sup>+</sup> cell is more susceptible to apoptosis after binding of *env*. Uninfected cells also undergo apoptosis induced by HIV proteins gp120, *tat*, *nef* and *vpu* or activation induced cell death (31, 33).

### **1.10 Primary HIV Infection**

HIV commences as an acute, systemic infection. It is referred to as acute HIV, primary HIV or acute retroviral syndrome. It is often described as a “mononucleosis like” illness. 40% to 90% of patients develop symptoms attributable to acute infection with HIV (30). Symptom onset usually occurs ten days to four weeks after infection (34, 35). Patients may present with fever, myalgia, skin rash, night sweats, lethargy/malaise, arthralgia, pharyngitis, cervical adenopathy, oral ulcers, headache, thrush and cough (35). Diffuse lymphadenopathy may be present on physical examination. The rash associated with acute HIV infection may be diffuse and may affect the face, trunk, palms of the hands and soles of the feet. It occurs in 20% to 40% of patients (34).

Neurological findings may be present also at the time of acute infection. Neurological manifestations include aseptic meningitis, facial nerve palsies, encephalopathy, peripheral neuropathy and acute inflammatory demyelinating syndrome (AIDP) (30, 34).

Laboratory findings include anaemia, lymphopenia, thrombocytopenia and mild elevation of hepatic transaminases. The syndrome typically lasts up to two weeks but



may last several weeks. Seroconversion is terminated with the formation of HIV specific antibodies. They are usually present within three weeks of infection (30, 35).

During seroconversion HIV RNA levels are elevated, often to millions of copies per millilitre of blood. Levels of p24, a HIV glycoprotein antigen, are also elevated in acute HIV infection. These are present prior to the development of HIV specific antibodies and aid diagnosis in the early stages of infection. The level of RNA viraemia decreases to a nadir and this coincides temporally with the emergence of HIV specific CD8<sup>+</sup> T cells (30, 35).

### **1.11 Diagnosis of HIV Infection**

Diagnosis is made by the detection of antibodies to HIV-1 or HIV-2. This is first performed on a reactive enzyme immunoassay and then confirmed with a Western blot assay or an indirect immunofluorescence assay test. Detectable HIV nucleic acid (DNA or RNA) is also required and this is performed via polymerase chain reaction (PCR) testing. As stated above, in early infection HIV p24 antigen may be detected and used for diagnostic purposes (36).

### **1.12 Chronic HIV**

After seroconversion patients enter into a clinically latent phase of HIV. It is also referred to as chronic or asymptomatic HIV infection. There is still active replication of HIV throughout this phase. Cell mediated immunity responses are activated and cell death also occurs during this time period through the mechanisms described above.

There may be persistent generalised lymphadenopathy during this phase of the illness. During this phase quiescent CD4<sup>+</sup> memory T cells and macrophages act as reservoirs for latent infection (37). In the later stages of this phase, the viral load rises and the CD4<sup>+</sup> T cell count drops leading to symptomatic infection. Patients may develop constitutional symptoms of HIV and become susceptible to opportunistic infection. Disease progression is variable but without commencing highly active antiretroviral therapy (HAART) patients tend to develop AIDS defining illnesses within a mean time period of 10 to 11 years (38).

**1.13 Classification of HIV Infection**

The World Health Organisation (WHO) classifies HIV infection into four clinical stages based on the presence or absence of symptoms and the degree of symptoms if present (Table 1-1). The CDC also divides HIV infection into stages on the basis of CD4<sup>+</sup> T cell count and percentages and the presence or absence of AIDS defining illnesses (Table 1-2).

**Table 1-1 WHO Classification of HIV Infection**

|                                     |                                                                                                                                                                                       |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Clinical Stage 1<br>(Asymptomatic)  | Asymptomatic<br><br>Persistent generalized lymphadenopathy                                                                                                                            |
| Clinical Stage 2<br>(Mild Symptoms) | Moderate unexplained weight loss<br><br>(<10% of presumed or measured body weight)<br><br>Recurrent respiratory tract infections sinusitis, tonsillitis, otitis media and pharyngitis |

|                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                    | <p>Herpes zoster</p> <p>Angular cheilitis</p> <p>Recurrent oral ulceration</p> <p>Papular pruritic eruptions</p> <p>Seborrhoeic dermatitis</p> <p>Fungal nail infections</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <p>Clinical Stage 3</p> <p>(Advanced Symptoms)</p> | <p>Unexplained severe weight loss (&gt;10% of presumed or measured body weight)</p> <p>Unexplained chronic diarrhoea for longer than one month</p> <p>Unexplained persistent fever (above 37.6°C intermittent or constant, for longer than one month)</p> <p>Persistent oral candidiasis</p> <p>Oral hairy leukoplakia</p> <p>Pulmonary tuberculosis (current)</p> <p>Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia)</p> <p>Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis</p> <p>Unexplained anaemia (&lt;8 g/dl), neutropenia (&lt;0.5 × 10<sup>9</sup> per litre) or chronic thrombocytopenia (&lt;50 × 10<sup>9</sup> per litre)</p> |
| <p>Clinical Stage 4</p> <p>(Severe Symptoms)</p>   | <p>HIV wasting syndrome</p> <p>Pneumocystis pneumonia</p> <p>Recurrent severe bacterial pneumonia</p> <p>Chronic herpes simplex infection (orolabial, genital or anorectal)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

|  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <p>of more than one month's duration or visceral at any site)</p> <p>Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)</p> <p>Extra-pulmonary tuberculosis</p> <p>Kaposi's sarcoma</p> <p>Cytomegalovirus infection (retinitis or infection of other organs)</p> <p>Central nervous system toxoplasmosis</p> <p>HIV encephalopathy</p> <p>Extra-pulmonary cryptococcosis including meningitis</p> <p>Disseminated non-tuberculous mycobacterial infection</p> <p>Progressive multifocal leukoencephalopathy</p> <p>Chronic cryptosporidiosis (with diarrhoea)</p> <p>Chronic isosporiasis</p> <p>Disseminated mycosis (coccidiomycosis or histoplasmosis)</p> <p>Recurrent non-typhoidal Salmonella bacteraemia</p> <p>Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours</p> <p>Invasive cervical carcinoma</p> <p>Atypical disseminated leishmaniasis</p> <p>Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy</p> |
|--|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

WHO Classification of HIV Infection from WHO Report on HIV AIDS Programme: WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children 2007 (39).



Table 1-2 CDC Staging of HIV Infection

| Stage    | Laboratory Evidence                                                                                                                                             | Clinical Evidence                                                                                                          |
|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|
| 1        | Laboratory confirmation of HIV infection<br><br>and<br><br>CD4+ T-lymphocyte count of >500 cells/ $\mu$ L<br><br>or<br><br>CD4+ T-lymphocyte percentage of >29  | No AIDS defining illness                                                                                                   |
| 2        | Laboratory confirmation of HIV infection<br><br>and<br><br>CD4+ T-lymphocyte count of 200–499<br>cells/ $\mu$ L or<br><br>CD4+ T-lymphocyte percentage of 14–28 | No AIDS defining illness                                                                                                   |
| 3 (AIDS) | Laboratory confirmation of HIV infection<br><br>and<br><br>CD4+ T-lymphocyte count of <200 cells/ $\mu$ L<br><br>or<br><br>CD4+ T-lymphocyte percentage of <14  | or documentation of an<br><br>AIDS-defining condition<br><br>(with laboratory<br><br>confirmation of HIV<br><br>infection) |
| Unknown  | Laboratory confirmation of HIV infection<br><br>and<br><br>no information on CD4+ T-lymphocyte<br><br>count or percentage                                       | and no information on<br><br>presence of AIDS-defining<br><br>conditions                                                   |

CDC Staging of HIV Infection 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 (36).

#### **1.14 Treatment of HIV Infection**

The first clinically available medication for HIV was zidovudine. A trial was conducted in 1986 comparing zidovudine to placebo in HIV positive patients with AIDS, manifested by pneumocystis pneumonia or advanced AIDS related complex and stratified by CD4<sup>+</sup> T cell count. The results were published in 1987 and they showed that the use of zidovudine decreased mortality and the frequency of opportunistic infections in patients with AIDS (40). In subsequent years dual therapy, combination of zidovudine with didanosine or zalcitabine was shown to be superior to therapy with zidovudine alone. Dual therapy proved to be more effective at controlling viral replication and prolonging survival in HIV positive patients with and without AIDS (41-43), however there was still significant morbidity and mortality associated with HIV infection.

In January 1995 Ho et al published viral dynamics data in *Nature* and presented at the 11th International Conference on AIDS in Vancouver later that year. This data focussed on the rapid turnover and the genetic diversity of HIV by demonstrating that HIV positive patients produced up to 10 billion virions a day. It clearly highlighted the need for a better treatment strategy (44). A number of seminal papers were published soon after which established the superiority of combined antiretroviral therapy or HAART (45, 46). This marked the beginning of the era of combined antiretroviral therapy. Significant reductions were shown in morbidity and mortality with a 60 to 80%

reduction in the rate of AIDS, death and hospitalisation (47). Time to development of AIDS and time to death were prolonged with HAART (48). Life expectancies in developed countries for HIV positive patients are now approaching that of the general population thanks to successful treatment with HAART (49, 50).

Highly active antiretroviral therapy refers to the combination of drugs which have different viral targets to achieve virological suppression in patients with HIV. The list of antiretroviral therapies is shown in Table 1-3. There are a number of fixed dose combinations available also which improve adherence to therapy.

HAART drugs are classified according to their mechanism of action. Entry inhibitors (fusion inhibitors) interfere with binding, fusion and entry of HIV into the host cells. Maraviroc targets the chemokine receptor CCR5. Nucleoside reverse transcriptase inhibitors (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI) are nucleoside and nucleotide analogues which inhibit reverse transcription. They both act as competitive substrate inhibitors. Non-Nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase by binding to an allosteric site of the enzyme. NNRTIs act as non-competitive inhibitors of reverse transcriptase. Integrase inhibitors inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. Protease inhibitors (PIs) block the viral protease enzyme necessary to produce mature virions upon budding from the host membrane. PIs prevent the cleavage of *gag* and *gag/pol* precursor proteins.

**Table 1-3 List of Drugs Used in HAART**

|                                                                                                                                                                                                                                     |                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI)</b><br><br>Zidovudine<br><br>Didanosine<br><br>Zalcitabine<br><br>Stavudine<br><br>Lamivudine<br><br>Abacavir<br><br>Tenofovir disoproxil<br><br>Emtricitabine | <b>Non-nucleoside reverse transcriptase inhibitors (NNRTI)</b><br><br>Nevirapine<br><br>Delavirdine<br><br>Efavirenz<br><br>Etravirine<br><br>Rilvipirine |
| <b>Protease inhibitors (PI)</b><br><br>Saquinavir<br><br>Indinavir<br><br>Ritonavir<br><br>Nelfinavir<br><br>Amprenavir<br><br>Lopinavir<br><br>Fosamprenavir<br><br>Atazanavir<br><br>Tipranavir<br><br>Darunavir                  | <b>Integrase inhibitors (II)</b><br><br>Raltegravir<br><br><b>Entry or Fusion inhibitors (FI)</b><br><br>Enfuvirtide<br><br>Maraviroc (anti-CCR5)         |



1.15 Recommendations for Commencement of HAART

All patients with a CD4<sup>+</sup> T cell count less than 350 are recommended to initiate therapy. Recommendations for patients with higher CD4<sup>+</sup> T cell counts are shown in Table 1-4 (EACS Guidelines 2013). It is recommended to commence HAART in patients with cognitive impairment in the setting of HIV irrespective of CD4<sup>+</sup> count.

Table 1-4 Recommendations for Initiation of HAART

| Present Condition                                                                         | CD4 Count |      |
|-------------------------------------------------------------------------------------------|-----------|------|
|                                                                                           | 350-500   | >500 |
| Asymptomatic HIV infection                                                                | C         | C    |
| To reduce transmission of HIV                                                             | C         | C    |
| Symptomatic HIV disease<br>(CDC B or C conditions) incl. tuberculosis                     | R         | R    |
| Primary HIV infection                                                                     | C         | C    |
| Pregnancy (before third trimester)                                                        | R         | R    |
| Conditions (likely or possibly) associated with HIV, other than CDC stage B or C disease: | R         | R    |
| • HIV-associated kidney disease                                                           | R         | R    |
| • HIV-associated neurocognitive impairment                                                | R         | R    |
| • Hodgkin’s lymphoma                                                                      | R         | R    |
| • HPV-associated cancers                                                                  | R         | R    |
| • Other non-AIDS-defining cancers requiring chemo- and/or radiotherapy                    | C         | C    |
| • Autoimmune disease – otherwise unexplained                                              | C         | C    |
| • High risk for CVD (> 20% estimated 10-yr risk) or history of CVD                        | C         | C    |
| Chronic viral hepatitis:                                                                  |           |      |
| • HBV requiring anti-HBV treatment                                                        | R         | R    |
| • HBV not requiring anti-HBV treatment                                                    | R         | C    |
| • HCV for which anti-HCV treatment is being considered or given                           | R         | C    |
| • HCV for which anti-HCV treatment not feasible                                           | R         | C    |

Taken from European AIDS Clinical Guidelines 2013. R = use of HAART is recommended, C = use of HAART should be considered and actively discussed with the patient (51).

**1.16 Historical Nomenclature for HIV and Cognitive Impairment**

Many terms have been used to describe cognitive impairment or dementia secondary to HIV over the years. The term “sub-acute encephalitis” was first used in 1983 by Snider et al (52). In 1986 the term “AIDS dementia complex” was introduced (53, 54). “HIV encephalopathy” was another term used which was introduced in 1988 by Levy and Bredesen (55). Criteria were developed by the American Academy of Neurology AIDS Task Force for AIDS dementia in 1991 and the term HIV-1 associated cognitive/motor complex was introduced (56). The most recent term, HIV Associated Neurocognitive Disorders (HAND), was introduced by the American Academy of Neurology (AAN) in 2007 (Table 1-6) (57). "HIV encephalitis" (HIVE) should only be used to refer to the pathological features of HIV in the brain with the typical multinucleated giant cell encephalitis.

**Table 1-5 Clinical Staging of the AIDS Dementia Complex**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>Stage 0 (normal)</b></p> <ul style="list-style-type: none"><li>• Normal mental and motor function.</li></ul> <p><b>Stage 0.5 (equivocal and subclinical)</b></p> <ul style="list-style-type: none"><li>• Absent, minimal, or equivocal symptoms without impairment of work or capacity to perform activities of daily living. Mild signs (snout response, slowed ocular or extremity movements) may be present. Gait and strength are normal.</li></ul> |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Stage 1 (mild)**

- Able to perform all but the more demanding aspects of work or activities of daily living, but with unequivocal evidence (signs or 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 work 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 a rudimentary level. Nearly or absolutely mute. Paraparetic or paraplegic with urinary and faecal incontinence.

Taken from The AIDS Dementia Complex by Price and Brew in 1988 (58).



**Table 1-6 AAN Updated Research Nosology for HIV Associated Neurocognitive Disorders**

**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 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 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 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 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.

Taken from the 2007 American Academy Updated Research Nosology for HAND (56).



### 1.17 Neurological Complications of HIV

Snider et al in 1983 are credited with the first detailed pathological review of the neurological complications of AIDS in 50 patients (52). Neurological complications may occur as a direct result of HIV itself or secondary to immunosuppression. The timing of presentation and the CD4<sup>+</sup> T cell count are related to the type of neurological complication. 1-4% of patients develop neurological complications during seroconversion. Aseptic meningitis, meningoencephalitis, Bell's palsy, AIDP, brachial neuritis and transverse myelitis may develop during seroconversion or with a CD4<sup>+</sup> T cell count over 500. With CD4<sup>+</sup> T cell counts between 200 to 500, tuberculous (TB) meningitis, AIDP, distal sensory polyneuropathy (DPSN) and polymyositis may occur. With CD4<sup>+</sup> T cell counts of less than 200, opportunistic infections predominate. These include cryptococcal meningitis, toxoplasmosis, cytomegalovirus (CMV) radiculopathy and encephalitis and progressive multifocal leukoencephalopathy (PML). Other complications at this level of immunosuppression include primary central nervous system (CNS) lymphoma, vacuolar myelopathy and herpes encephalitis (59, 60). The incidence of neurological opportunistic infections has decreased in the HAART era from 13.1/1000 patient years in 1996-97 to 1/1000 patient years in 2006-07. There is also an increased risk of stroke (59, 61). Seizure disorders are also more common in the setting of HIV with reported prevalences as high as 11% (62). HIV associated neurocognitive disorders may occur at any CD4<sup>+</sup> T cell count but HIV associated dementia is associated with a nadir CD4<sup>+</sup> T cell count of 200 or less (60).

## **1.18 Prevalence of Cognitive Impairment in HIV**

### Prevalence in the Pre-HAART Era

In 1986 Navia et al described the clinical features and pathological findings of AIDS Dementia Complex. AIDS dementia complex was described in almost two thirds of autopsied patients (53, 54). Prior to the introduction of HAART up to 20% of patients with AIDS developed HIV dementia and it was associated with a high mortality rate with a mean survival of six months to one year after the development of dementia (63, 64). Prior to HAART mild neurocognitive impairment was described in 30% of patients with asymptomatic HIV disease and in up to 50% of patients with AIDS defining illnesses (65).

### Prevalence in the Post-HAART Era

The incidence of HIV associated dementia has declined in the post HAART era. This decline was evident even in the early years of the post HAART era with a 50% decline in the rates of HAD as Sacktor et al demonstrated by examining the incidence rates of HAD between 1992 to 1998 among homosexual men taking part in the Multicentre AIDS Cohort Study (66, 67). In a recent study published by Heaton et al only 2.4% of a tertiary referral clinic HIV positive population taking part in the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) Study had HIV associated dementia (68). Cognitive impairment in HIV positive patients however continues to be an ongoing clinical issue (69, 70) despite good virological control of HIV (65). HIV associated dementia remains an independent predictor of time to death (71).

Prevalence rates of 20% to 50% of HIV associated neurocognitive disorders have been demonstrated in large prospective studies in the United States, Europe and Australia (68, 70, 72-75). Chan et al demonstrated a prevalence rate of 22% in a South Asian population in 2012 (76). Wright et al found that 12% of patients attending ten centres in the Asia-Pacific region had moderate to severe cognitive impairment secondary to HIV (77). Patel et al found a prevalence rate of 14% for HIV associated dementia in an adult Malawian cohort (78). Lawler et al demonstrated a prevalence rate of 37% in a cohort of HIV positive patients in Botswana (79). 60.5% of a HIV positive Indian cohort had mild to moderate cognitive impairment with no functional impairment (80). Heaton et al demonstrated a prevalence rate of 34.2% in a HIV positive rural Chinese cohort and in the same study noted that 39.7% of those co-infected with HIV and hepatitis C were cognitively impaired (81). Pumpradit et al found a prevalence rate of 37.5% of HAND in a group of Thai patients on HAART (82). A study of South African patients commencing HAART showed a prevalence rate of 42.2% of mild neurocognitive disorder and 25.4% of the group had HIV dementia (83). The numbers studied in the developing countries are smaller compared to the larger trials in the US and Europe but still demonstrates a consistent pattern of cognitive impairment in the setting of HIV infection. This data illuminates the fact that HAND is a significant issue for patients worldwide and has not disappeared with successful viral suppression.

HIV clade or subtype has been speculated to influence the frequency of HAND. Clade B predominates in the USA and Europe while clades A, C and D are more common in sub Saharan Africa. Clade C is the predominant subtype in Asia. Results have been conflicting thus far. Sacktor et al reported a higher risk with clade D than clade A in



Uganda but the number of patients infected with clade D in the study was only 9; 8 of the 9 patients met criteria for HIV associated dementia (84). In a study of a paediatric population in Uganda, children infected with clade A subtype virus performed worse than children infected with clade D (85). An Ethiopian study found no significant differences between a HIV positive group and a HIV negative group (86). Clade was not determined in this particular study but clade C is the most common subtype found in Ethiopia. Other studies in countries where clade C is also common, such as Botswana and South Africa, have shown high prevalence rates of HAND although clade type was not determined (79, 83). Clade C predominates in India where rates of cognitive deficits of over 60% were reported (80). The reason for the conflicting evidence on the influence of subtype is unclear but may relate to multiple factors including methodology, ascertainment bias, lack of power due to small numbers, lack of control populations for some studies and lack of genetic subtyping (87).

## **1.19 Neuropathogenesis of HIV**

### **1.19.1 Pathological Features**

#### **Pre-HAART Era**

HIV is neuroinvasive (capable of entering the CNS), neurotropic (capable of infecting cells within the CNS) and neurovirulent (capable of causing neurological disease). HIV has been found in the cerebrospinal fluid (CSF) of patients during seroconversion, suggesting that HIV enters the CNS early in the course of infection (88-90). In an early post-mortem study of IV drug users who died prior to developing any symptoms related to HIV infection, PCR studies demonstrated low levels of HIV in brain tissue.



Pathological examination demonstrated a low grade lymphocytic meningitis, perivascular lymphocytic cuffing and perivascular macrophages in patients who had not developed AIDS while in those with AIDS, giant cell encephalitis was the most common finding (91). Oster et al demonstrated global neuronal loss of 37% in the brains of patients with AIDS (92).

#### Post-HAART Era

HIV enters the brain through infected monocytes and lymphocytes. They are used as a “Trojan horse” mechanism to cross the blood brain barrier (93). Neurons are not infected directly by HIV. Microglial cells and macrophages are the primary targets for productive HIV infection within the CNS as they both possess receptors for CD4 and CCR5. Microglia cells act as the brain’s native immune response cells. Perivascular macrophages and microglia are derived from bone-marrow cells that settle in the CNS during development and throughout adult life. It has been postulated that HIV may be spread within the CNS through cell-to-cell contact between infected macrophages and microglia (94). Astrocytes are also infected by HIV despite their lack of receptors for viral attachment but in a more restricted fashion (95). Astrocytes, oligodendrocytes and neurons are ectodermal derived cells unlike the mesodermal derived microglia and macrophages.

HIV can cause an inflammatory reaction upon entry to the CNS which may be manifested by a T cell reaction with vasculitis and leptomeningitis. There is also upregulation of MHC class II antigens, increased number of microglial cells found and increased production of cytokines (96). Perivascular microglia are likely involved early

in infection and the parenchymal microglia become involved in the later stages of infection (95). The chemokine receptor CCR5 is the dominant receptor used within the brain (97). There is proliferation of astrocytes and intense perivascular lymphocytic and macrophagic infiltration has been seen at autopsy which has been postulated to be related to immune reconstitution inflammatory syndrome (IRIS) (98).

The pathognomonic feature of HIV encephalitis (HIVE) on histological examination is the presence of multinucleated giant cells. These cells are the fusion of infected and uninfected microglia and macrophages and they stain positively for HIV antigens. Together they provide evidence of productive infection by HIV within the CNS. This pathological feature was present in 20-50% of patients who had autopsies performed in the first fifteen years of the HIV epidemic (99). HIVE has a predilection for the basal ganglia and white matter and affects the neocortical grey matter, cerebellum and brainstem to a lesser extent (100). Other pathological features of HIV include myelin pallor, axonal loss, microglial nodules and gliosis (99). There is also disruption of the blood brain barrier and apoptosis of astrocytes. This leads to dendritic and neuronal loss (101). Asare et al demonstrated a correlation between the reduction in pyramidal neuronal density and the severity of HAD (102). A correlation has also been demonstrated between the density of apoptotic astrocytes and rapid progression of dementia (103). Fischer et al further evaluated the neuronal loss seen in the pre-HAART era by demonstrating that large neurons were more susceptible than small neurons to the destruction caused by HIV and that the pattern of reduction in the number of large neocortical neurons was found in frontal, temporal, parietal as well as in occipital regions (104). CD8<sup>+</sup> positive lymphocytes were the primary cell type found

in the CNS infiltrates of patients without AIDS. CD20 positive B lymphocytes were also found in significant proportions (99).

#### 1.19.2 Role of Monocytes and Macrophages in the Post-HAART Era

Glass et al performed immunocytochemistry analysis on sections of frontal lobe and basal ganglia from the brains of a prospectively characterised cohort of 51 patients with AIDS who were stratified according to severity of dementia. They found a highly significant correlation between the degree of macrophage staining and the severity of dementia. They did not find a significant correlation between the presence of gp41 positive cells and the degree of dementia. They found that the presence of productive HIV infected cells did not correlate with HIV dementia and was in fact present in the majority of patients with AIDS therefore HIV is a pathological description and does not correlate with a clinical syndrome. They concluded that HIV is necessary but not sufficient to cause HIV dementia and that indirect mechanisms are likely involved which may be mediated through the actions of macrophages and microglia (105). Despite HAART the degree of macrophage and microglial activation remains similar in treated patients (99).

The contribution of activated perivascular macrophages to the development of HAND has many potential mechanisms. They release viral gene products and a significant number of proinflammatory cytokines which all have potential toxic effects on neuronal function. Chemokines and their respective receptors are essential components of neuronal physiology and they play a significant role in the balance



between neuroprotection and neurodegeneration within the CNS. Their roles involve migration, differentiation and activation of cells and also the proliferation of glia and neurons (106). Cytokines are soluble proteins that are involved in cell signalling and immunomodulation. Chemokines are a subset of cytokines which are capable of inducing chemotaxis in neighbouring cells. Their name derives from their function, chemotactic cytokines.

During HIV infection CD14<sup>+</sup>CD16<sup>+</sup> monocytes expand, especially during progression of chronic HIV infection to AIDS (107). They have been found to correlate with the presence of AIDS dementia along with CD14<sup>+</sup>CD69<sup>+</sup> monocytes (108). These CD16<sup>+</sup> monocytes act as a reservoir for HIV in *in vivo* studies and are also more susceptible to HIV infection. They may be capable of re-infecting the brain through continuous trafficking of infected monocytes across the blood brain barrier (109). Ancuta et al demonstrated that CD16<sup>+</sup> monocytes undergo transendothelial migration across brain microvascular endothelial cell monolayers under the effect of fractalkine (110). Fractalkine is a chemokine expressed in the brain, lymph nodes and intestinal cells (109). Stimulation with tumour necrosis factor (TNF) resulted in high expression of membrane bound fractalkine while stimulation with interferon gamma (IFN- $\gamma$ ) resulted in low expression of membrane bound fractalkine. Expression of vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1) followed the same pattern. Stimulation with TNF and IFN- $\gamma$  prevented transendothelial migration of CD16<sup>+</sup> monocytes in response to soluble fractalkine. They postulated that CD16<sup>+</sup> monocytes which are pro-inflammatory may be a contributing factor to the neuropathogenesis of HAD mediated via disruption of the integrity of the blood brain



barrier secondary to the accumulation onto brain microvascular endothelial cells which express fractalkine, VCAM-1 and ICAM-1 (110).

Soluble CD163 is another marker of activated macrophages. Brudo et al compared the level of soluble CD163 in the plasma and CSF of HIV positive patients across three groups: neuropsychologically normal, asymptomatic neurocognitive impairment and mild neurocognitive disorder. There was elevation of soluble CD163 in the plasma but not the CSF of patients with ANI and MND though the levels were higher in those patients with MND than those with ANI or who were neuropsychologically normal. It suggests persistent monocyte activation and may be useful as a marker for neurocognitive impairment (111). In HIV CD163<sup>+</sup>/CD16<sup>+</sup> macrophages have been found in brain parenchyma and also appear to represent the primary cell population involved in productive infection (109).

Clay et al examined the role of monocytes in an animal model of simian immunodeficiency virus (SIV) and demonstrated that trafficking of infected monocytes into the choroid plexus stromata and perivascular spaces of macaque monkeys occurred within 12 to 14 days post infection. These cells expressed the monocyte activation marker CD16 and the macrophage marker CD68. Neuroinvasion coincided with the appearance of proinflammatory chemokines CXCL9 and CCL2 (also known as monocyte chemoattractant protein 1 (MCP-1)). Prior to the establishment of neuroinvasion, peak viral loads peaked on day 11 and peripheral blood monocytes counts increased rapidly between days 4 and 8. These monocytes also demonstrated an increased ability to release CXCL9 and MCP-1. Clay et al demonstrated acute

monocytic infiltration in an animal model (112). Ragin et al found a positive correlation between levels of MCP-1 and subcortical brain injury in HIV (113).

### 1.19.3 Role of CD8<sup>+</sup> T cells in the Post-HAART Era

Immunological factors involved in the neuropathogenesis of HIV related cognitive impairment may include the changes that occur in T cells as people age and this effect may be further exaggerated in individuals who are HIV positive. Cytotoxic CD8<sup>+</sup> T cells play a central role in the control of intrathecal viral replication (114). Valcour et al hypothesised that the risk of cognitive impairment could be increased due to the loss of the specific cytotoxic CD8<sup>+</sup> immune response as people age (72). CD4<sup>+</sup> T cells are the main cell constituent of CSF in HIV negative individuals whereas in patients with HIV, CD8<sup>+</sup> T cells were the major cellular component of CSF (115). Miller et al described pathological features of two cases in 2004 of a syndrome that has since been termed CD8 encephalitis. Both patients had advanced HIV disease. One patient developed an acute onset rapidly progressive encephalopathy and the second patient developed worsening of a pre-existing encephalopathy after initiation of HAART with a resultant increase in CD4<sup>+</sup> T cell count and a marked reduction in HIV viral load. There was evidence of HIV DNA present in the brain tissue of both patients along with diffuse microglial hyperplasia. The most striking feature was the intense perivascular and intraparenchymal infiltration of CD8<sup>+</sup> T cells in the absence of CD4<sup>+</sup> T cells. They suggested that the recruitment of CD8<sup>+</sup> T cells into the CSF was due to the redistribution of lymphocytes in peripheral blood secondary to immune reconstitution (116). This suggests that while CD8<sup>+</sup> T cells may play a crucial role in the control of viral

replication, they may have a detrimental role in the setting of immune reconstitution. These findings have been supported by further studies since then (117, 118). There is a significant morbidity and mortality associated with this condition. Lescure et al described CD8 encephalitis in 14 patients; 5/14 died within 13 months of initiation of HAART. 5/14 patients had an excellent outcome with no sequelae and 4/14 had persistent cognitive deficits (118). While this is obviously a florid presentation, it may be possible that CD8<sup>+</sup> T cells in smaller numbers or with a less dramatic influx into the brain play a role in the development of HAND.

#### 1.19.4 Role of Microbial Translocation in the Post-HAART Era

The structural integrity of the gastrointestinal tract may be compromised in HIV infection which may result in bacteria entering the bloodstream. Elevated plasma lipopolysaccharide (LPS) is an indicator of microbial translocation from the gut. LPS induces monocyte activation and trafficking of monocytes into the CNS. Elevated levels of LPS were significantly associated with HAD. LPS was also more elevated in patients with AIDS who used intravenous heroin and/or ethanol and in patients who were co-infected with hepatitis C. Another potential mechanism for LPS induced transport of HIV-1 across the blood brain barrier is via a p38 mitogen activated protein kinase (MAPK). After HIV infected macrophages are stimulated with LPS, expression of E-selectin and VCAM-1 is induced (109, 119, 120).



#### 1.19.5 Viral Proteins in the Post-HAART Era

Extracellular viral products such as gp120 may cause toxicity of brain microvascular endothelial cells resulting in disruption of the blood brain barrier. The presence of gp120 induces toxicity and alters the expression of tight junction proteins in brain microvascular endothelial cells. Kanmogne et al explored the mechanism of toxicity to the blood brain barrier due to gp120. They demonstrated that exposure to gp120 resulted in decreased blood brain barrier tightness, increased permeability and increased migration across the blood brain barrier. The endothelial cells expressed HIV-1 co-receptors CCR5 and CXCR4. They were able to restore blood brain barrier integrity upon removal of gp120. Gp120 mediated monocyte migration and increased permeability of the blood brain barrier were blocked by antagonists of CCR5 and inhibitors of protein kinase C (PKC). Gp120 resulted in increased release of intracellular calcium and also activated three isoforms of protein kinase C. Specific PKC inhibitors were capable of blocking the gp120 mediated effects on the blood brain barrier. Gp120 mediated effects on the blood brain barrier are exerted through a CCR5/CXCR4 receptor mediated PKC activation mechanism (121). Khan et al also examined the mechanisms of gp120 induced cytotoxicity. They determined that the cytotoxicity required the presence of IFN- $\gamma$  and activation of p38 MAPK, however they were only able to demonstrate toxicity in the brain microvascular endothelial cells derived from children and not from adults (122). Another mechanism of gp120 induced disruption of tight junctions is mediated by proteasomal degradation of zonular occludens (ZO) 1 and 2. The protein 14-3-3 tau seems to counteract the down regulation of ZO-1 and ZO-2 but gp120 also increases expression of 14-3-3 tau (109). Gp120 reduces glial cell line derived neurotrophic factor expression resulting in dysfunction of the nigrostriatal



dopaminergic system (123). Bachis et al injected gp120 into rat striatum and hippocampus. The gp120 was then sequestered by neurons and underwent retrograde transport to distal neurons. Evidence of apoptosis was seen in neurons internalising and transporting gp120. The chemokine receptor CXCR4 mediated the retrograde transport of gp120 and apoptosis. They hypothesised that this could explain the widespread neurodegeneration seen in HIV despite the relatively low number of cells productively infected in the CNS and that the primary cell type infected consists of macrophages and microglia (124). Gp120 also alters glutamate signalling and calcium homeostasis. It can also result in dendritic alterations in cultures of human nervous tissue exposed to gp120. This mimics the pathology seen at autopsy of patients with cognitive impairment in the setting of HIV (125).

The viral protein transactivator of transcription (*tat*) also affects tight junction proteins and reduces expression of occludin, ZO-1 and ZO-2, in brain microvascular endothelial cells. *Tat* also induces adhesion molecules and chemokines in astrocytes and microglia which results in increased trafficking of monocytes into the CNS (109). The presence of *tat* results in neuronal cell death and excitotoxicity which is partially dependent on N-methyl-D-aspartate (NMDA) receptors. Neuronal apoptosis is enhanced by the combination of *tat* and TNF-alpha (123). Bruce-Keller et al genetically engineered rat glioma cells to produce *tat* and subsequently infused it into rat striatum or hippocampus. They found that *tat*, which was injected into the rat dentate gyrus, was transported to the CA3/4 region of the hippocampus and from the striatum to the substantia nigra. This resulted in behavioural abnormalities, neurotoxicity and reactive gliosis. The authors demonstrated that *tat* induced neuronal damage could occur at

sites distant to the site of infection and this demonstrated the role that *tat* plays in neuronal, glial and synaptic disruption in HAD (126). It has also been shown that *tat* can induce mitochondrial dysfunction and neuronal cell death at concentrations lower than that required for long terminal repeat (LTR) transactivation (127). There is also interest in differences in *tat* sequences across viruses and its possible role in variation of neurotropism in different clades. Mishra et al demonstrated that clade C *tat* is less neurotoxic when compared to clade B *tat* possibly due to differences within the dicysteine motif in the neurotoxic region of clade B *tat* at position 31 (128). Choi et al investigated *tat* sequences in the blood and CSF of 60 HIV positive individuals with clade B infection. They identified that a higher number of mixed bases in CSF *tat*, a measure of increased viral population diversity, was associated with HIV associated neurocognitive disorder (129). *Tat* appears to exert its clade-specific effect on HAND-related pathogenesis through its influences on the migration of mononuclear phagocytes into the CNS and also through direct neurotoxicity which is mediated through NMDA receptors (87). Nath et al demonstrated that *tat* did not require a continuous presence to exert its neurotoxic effects but was capable of causing neuronal damage and glial cell activation with a single exposure. Exposure to *tat* for a few minutes induced release of proinflammatory cytokines for several hours. They hypothesised that the mechanism behind this prolonged cytokine production was probably due to maintenance of a positive feedback loop by *tat* induced activation and cytokine production and that it was independent of extracellular calcium. They termed it a "hit and run" approach whereby a transient exposure resulted in an initial cascade of inflammation which was then able to continue to propagate even in the absence of

virus. They suggested that this may explain why measurement of viral load in the brain is not an accurate marker of viral damage within the CNS (130).

The viral protein *vpr* disrupts calcium homeostasis via downregulation of plasma membrane calcium ATPase. The calcium elevation induced by *vpr* impairs glutamate signalling in neuronal cells (131). Neuronal apoptosis has also been demonstrated due to *vpr* (132). *Nef* can induce apoptosis of brain microvascular endothelial cells (133). *Nef* has also been shown to cause neuronal damage through immune activation mediated by MCP-1 and result in spatial and recognition memory deficits in rats (134).

#### 1.19.6 Cytokines and Other Molecules in the Post-HAART Era

Factors released by HIV infected microglia and activated macrophages include TNF- $\alpha$ , interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), phospholipid activator platelet activating factor (PAF) and the free radical nitric oxide (NO). Insulin-like growth factor binding protein 2 (IGFBP-2) and monocyte chemoattractant protein 2 (MCP-2) are upregulated. IGFBP-2 blocks the neurotrophic effects of insulin growth factors 1 and 2 (IGF-1 and IGF-2) and therefore reduces neuronal survival. TNF- $\alpha$  and IL-1 $\beta$  release a neurotoxic cytokine L-cysteine and this interacts with the NMDA receptor. Gp120 also releases L-cysteine and is dependent on TNF- $\alpha$ . There is increased production of HIV in macrophages due to the presence of TNF-  $\alpha$ . When NMDA receptors are stimulated, neurons release PAF which increase glutamate release with resultant excitotoxicity (123). PAF increases the vulnerability of neurons to dendritic alterations (125).



Nitric oxide is released by macrophages and microglia. It is involved in blood-brain barrier breakdown in inflammatory conditions (135). Milstien et al did not demonstrate any increase in nitric oxide levels in CSF of patients with HIV in the pre HAART era (136). In the post HAART era Li et al measured metabolites of nitric oxide and found significant elevations of nitrite and nitrate in the CSF of patients with HIV but did not identify any correlation to cognitive status. They also examined the levels of 3-nitrotyrosine modified proteins, which are another measure of nitrosative stress, and these were significantly elevated in the CSF of patients with HIV and correlated to active dementia and a previous history of intravenous recreational drug use (137). Lisi et al demonstrated that antiretroviral agents, efavirenz, nevirapine, darunavir and atazanavir, increased nitric oxide production in microglial cells activated with Gp120 and IFN- $\gamma$ . This effect appeared to have been mediated by inhibition of arginase I activity (138).

Neopterin is a marker for monocytes, macrophages and microglia. Its primary stimulator is IFN- $\gamma$ . Levels of neopterin correlate with the release of reactive oxygen species by macrophages. The enzyme indoleamine 2, 3-dioxygenase is induced by IFN- $\gamma$ , *tat* and *nef* simultaneously with release of neopterin. This enzyme results in macrophages producing the neurotoxic factor quinolinic acid therefore neopterin is part of the neurotoxic cascade in HAND. Hagberg et al reviewed the experience of four centres with CSF neopterin. They found that CSF neopterin levels are usually elevated in patients with untreated HIV infection and that neopterin levels increase with worsening immunosuppression. High levels were particularly seen in patients with HIV dementia and CNS opportunistic infections. Neopterin levels decrease with HAART but



levels can remain mildly elevated even in those patients who are virally suppressed (139).

Quinolinic acid (QUIN) is a product of tryptophan metabolism and is produced by monocytes upon stimulation by IFN- $\gamma$  and HIV proteins. It is an excitotoxic agonist at NMDA receptors (140). There is also evidence that QUIN exerts its neurotoxic effects through more than just its effect on NMDA receptors. Free radical formation contributes significantly to the damage caused by QUIN (141). Heyes et al examined the relationship between CSF QUIN and cerebral atrophy on MRI and found that elevated levels of CSF QUIN correlated with worsening atrophy in regions sensitive to excitotoxic injury, i.e. striatum and limbic cortex, but not in regions more resistant to excitotoxicity. They also demonstrated a relationship between CSF QUIN levels and higher HIV-1 RNA levels in CSF (142). CSF QUIN levels decrease with antiretroviral therapy (143).

The expression of MCP-1 by astrocytes is induced by HIV and QUIN. There is upregulation of MCP-1 expression in patients with HIV. A correlation has also been shown between higher MCP-1 levels and poorer outcomes (140). "Elite" controllers are patients who maintain undetectable HIV RNA levels without HAART. Probasco et al demonstrated that elite controllers had similar levels of neopterin, MCP-1 and IFN- $\gamma$  induced protein 10 (IP-10) to HIV negative subjects and HIV positive subjects who were on HAART (144). Sevigny et al looked at markers of immune activation as predictors of time to death in advanced HIV infection and higher levels of MCP-1 trended towards statistical significance with a p value of 0.08 (71).

#### 1.19.7 Role of Astrocytes in the Post-HAART Era

Astrocytes are involved in the maintenance of the integrity of the blood brain barrier, maintaining low levels of excess excitatory neurotransmitter glutamate through re-uptake in order to avoid neuronal excitotoxicity. They are also involved in the genesis of neurotrophic factors. Astrocytes are infected with HIV despite lacking a CD4 receptor however they are not capable of de novo viral replication. Infected microglia release arachidonic acid, which is capable of impairing astrocytic mediated re-uptake of glutamate thereby leading to excitotoxicity. Astrocytosis also results due to the release of cytokines and is a pathological feature of HIV. When astrocytes are stimulated by pro-inflammatory cytokines they release chemokines which increase the entry of macrophages into the CNS across the blood brain barrier. Astrocyte apoptosis may propagate macrophage activation and also causes loss of neurotrophic support for neurons (100, 123).

#### 1.19.8 Synaptodendritic Injury in the Post-HAART Era

Synaptodendritic injury occurs in HIV and a number of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. The normal synaptodendritic network is complex and has a high degree of branching. The injured network is pruned and there is retraction of dendritic spines, dendritic beading and aberrant sprouting. This results in disruption of normal communication and impairment of directed transport of organelles and molecules along axons. While synaptodendritic injury occurs throughout the brain in HIV, there is a regional

vulnerability with the striatum and hippocampus being most affected. Dendritic beading results in neurons losing their capacity for long term potentiation (LTP). LTP is involved in synaptic plasticity and is essential for memory formation. Neurofilament protein (NFL) has been measured in CSF and is a marker of axonal injury. It is elevated in patients with HIV dementia (125). Brew et al also examined the role of NFL and demonstrated that elevated levels of NFL correlated with the presence of HIV dementia, increased with severity of dementia and decreased with commencement of HAART (145). Abdulle et al also demonstrated the value of NFL as a marker of axonal injury and as a useful marker of ongoing damage in the CNS (146).

#### 1.19.9 Compartmentalisation in the Post-HAART Era

The CNS may act as a sanctuary for HIV. There may be compartmentalisation of HIV within the CNS as the blood brain barrier may limit penetration of HAART into the CNS. Compartmentalisation is described as the development of discordance between plasma and CSF HIV viral loads. Viral suppression in the CSF has been found to correlate generally with plasma viral load. HAART induces viral suppression quickly, sometimes as quickly as within two weeks of commencement of HAART (147). Typical assays clinically available detect viral copies to less than 40 or 50 copies per millilitre. There are assays available that can detect single copies per millilitre. In one study greater than 80% of patients on HAART for 60 weeks had persistent low level viraemia of 1 copy/ml with a median number of 3.1 copies/ml (148). This low grade viral replication may be contributory to inflammation within the CNS and therefore play a role in the neuropathogenesis of HAND. Desplats et al demonstrated that HIV positive



patients classified as having latent infection in the CNS at the time of death (determined by high levels of HIV-1 DNA, no detectable HIV RNA or p24) had cognitive deficits and markers of neuroinflammation as evidenced by increased levels of chromatin modifiers (149). Despite evidence of low level viraemia within the CNS, HIV viral load no longer correlates as closely to HAND and significant evidence has not been produced to prove the hypothesis of CSF viral evolution in patients on HAART with successful viral suppression (150).

#### 1.19.10 CNS Penetration Effectiveness of HAART

There is significant interest in the therapeutic role that CNS penetrance of antiretroviral therapies may play in this disease process. The blood brain barrier limits entrance of HAART into the CNS and this is further compounded by the protein binding of HAART in plasma (61). Letendre et al developed a ranking system of antiretroviral therapies based on effectiveness of CNS penetration. They analysed data from over 400 patients and found that patients on regimes with lower CNS penetration effectiveness (CPE) scores were more likely to have detectable CSF viral loads. This study suggested that poorer penetration of HAART drugs into the CNS appeared to allow continued replication of HIV in the CNS as indicated by higher CSF HIV viral loads (151). In 2010 the ranking system was revised and the new system was more strongly correlated with CSF viral loads (152). A cross sectional study of 2636 participants on effective HAART showed better neurocognitive performance in those receiving higher CPE HAART (153). However other studies have not been quite so conclusive in their support and validation of the CPE ranking system.

Garvey et al examined data on over 22,000 HIV positive individuals and investigated the relationship between the development of CNS disease and CPE score. CNS disease included HIV encephalopathy, PML, toxoplasmosis and cryptococcal meningitis. CNS diseases were more common in patients on regimes with a score less than or equal to four and less frequently in those on a regime with a CPE score of ten or greater. These results were not statistically significant (154). Lanoy et al investigated the association between survival and CPE score.

At the beginning of the HAART era, the CPE score was significantly correlated with survival after severe neurological complications, while in the late HAART period, the additional effect of CPE score was negated by the more powerful antiretroviral regimens associated with plasma viral load control (155). McManus et al did not show any increased survival benefit for patients taking HAART with high CPE scores (156). In a prospective study of the validity of the CPE ranking system, 49 patients with mild to moderate HAND were randomly assigned to receive ART regimes with either high or low CPE scores. They did not find any statistically significant differences between the groups in term of their neuropsychometric performances (150). Vassallo et al followed up 256 patients for two years. They underwent neuropsychological testing and were categorised into one of five groups: cognitively normal, abnormalities within one cognitive domain, ANI, MND and HAD. Patients who cognitively deteriorated had a lower CPE score at the beginning of the study period and at the end of the two year follow up. The authors suggested that HAART with better CSF penetration may be protective against cognitive decline (157). There is unconvincing evidence currently to

support the ongoing use of the CPE ranking system to select HAART. While there may be inconclusive evidence to support the use of the CPE score to prevent cognitive impairment, the CPE ranking system may provide useful information about the possible neurotoxic side effects of HAART. Marra et al studied a cohort of 101 patients and they found that patients on regimes with higher CPE scores actually had poorer neurocognitive performance (158). It is possible that neurotoxicity secondary to HAART plays a role in the development of HAND.

#### 1.19.11 Neurotoxicity Due to HAART

NRTIs induce toxicity in peripheral tissues via disruption of mitochondrial function and PIs damage proteasome function (159). Robertson et al evaluated the effects of 15 different antiretrovirals on neurons in rat forebrain cultures. The highest neurotoxicities were seen with abacavir, efavirenz, etravirine, nevirapine and atazanavir, while the lowest neurotoxicities were found with darunavir, emtricitabine, tenofovir and maraviroc. They did not find any additive effects with combinations of drugs used clinically. They saw beading and pruning of dendrites with a corresponding decrease in microtubule associated protein-2 (MAP-2) staining on the neurons suggesting neuronal damage. The decrease in MAP-2+ processes was a sensitive index of neuronal damage. The antiretroviral compounds also caused destabilisation of neuronal calcium homeostasis and showed a reduced response to glutamate. The concentrations required for neurotoxicity were within therapeutic concentrations (160). In an earlier study Robertson et al found that neurocognitive performance improved for up to 96 weeks in patients who had discontinued HAART. These patients



were stable both virologically and immunologically. They accounted for practice effects. They postulated that neurotoxicity due to HAART could have been implicated in these results (161).

#### 1.19.12 HIV and Ageing in the Post-HAART Era

The interaction of HIV and the ageing brain is a growing area of interest. Neurodegenerative disorders such as Alzheimer's disease, Frontotemporal dementia and Parkinson's disease, are characterised by the deposition and accumulation of abnormal proteins within the brain including ubiquitin, amyloid-beta, tau and alpha synuclein. Amyloid-beta<sub>1-42</sub> is the main constituent of amyloid plaques. Tau and phosphorylated tau are markers of neuronal damage. Brew et al examined the CSF of 121 HIV positive patients, 20 patients with moderate to severe Alzheimer's Disease and 20 healthy controls. Amyloid-beta<sub>1-42</sub> levels were significantly lower in patients with AIDS dementia complex (ADC) compared with those without ADC and with the negative controls. Total tau levels were significantly increased in ADC Stage 2. There was a significant increase in phosphorylated tau levels in each of the ADC stages compared with the healthy controls and the patients with Alzheimer's Disease (162). Clifford et al measured amyloid-beta<sub>1-42</sub>, total and phosphorylated tau levels in patients with HIV and normal cognition, HIV positive patients with cognitive impairment and HIV negative patients with Alzheimer's Disease. They found that amyloid-beta levels in the CSF of patients with HAND were similar to those in patients with mild Alzheimer's dementia however total tau and phosphorylated tau levels were normal or slightly depressed in patients with HAND compared to patients with

Alzheimer's disease (163). The reason for the discrepancy in the phosphorylated tau levels is unclear but may be related to HAART, age of the participants or perhaps to a subcohort who had more neurodegenerative or Alzheimer's Disease like features.

Gisslen et al demonstrated reductions in soluble amyloid precursor proteins alpha and beta in patients with ADC and CNS opportunistic infections. They also demonstrated an elevation in total tau levels in some patients with ADC without the concomitant rise in phosphorylated tau. This suggests neural injury without accumulation of hyperphosphorylated tau. The authors postulated that these changes represented pathogenetic pathways to brain injury in ADC which differ to that in Alzheimer's Disease (164).

Khanlou et al examined tissue from the National NeuroAIDS Tissue Consortium in the United States of America for the presence of alpha synuclein and amyloid-beta in patients between the ages of 50 and 76. They found neuritic alpha-synuclein expression in the substantia nigra of 12/73 HIV positive patients and none in the older control cases. They found amyloid-beta deposition in the frontal and temporal cortical regions of 35/36 HIV positive patients examined (165). Achim et al examined tissue from the frontal cortex of 43 patients with HIV and HIV negative age matched controls. They found amyloid-beta staining in the pyramidal neurons and along axonal tracts. Patients with HIV had greater levels of intraneuronal amyloid-beta staining compared to HIV positive patients without evidence of HIV. The authors suggested that long-term survival with HIV might interfere with the clearance of amyloid-beta and worsen neuronal damage and cognitive impairment HIV positive individuals (166). Ances et al

performed imaging with the radioactive tracer Pittsburgh compound B (PiB), which binds to amyloid-beta<sub>1-42</sub>, on a group of 10 HIV positive individuals with normal cognition and 20 matched HIV negative controls. They did not demonstrate any increased uptake which would have been suggestive of amyloid deposition in the HIV positive patients (167). A further study was carried out on 16 HIV positive individuals, 11 of whom were cognitively normal and 5 of whom had HAND, and 19 HIV negative controls, 8 of whom were cognitively normal and 11 patients with symptomatic Alzheimer’s disease. Again there was no evidence of increased PiB uptake suggestive of amyloid deposition. The patient group were significantly younger than the control group (range of age in the patient group was 37-68 and range of age in the control group was 48-89) so this may have affected the outcome of the study and perhaps the amyloid deposition would be more obvious in an older HIV positive cohort (168).

**Table 1-7 Summary of Neuropathogenesis**

| Neuropathology                      |                                                                                                                                                                                                                                        |
|-------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Pathological Findings               | Lymphocytic meningitis<br>Multinucleated giant cells with encephalitis<br>Perivascular Lymphocytic and macrophagic infiltration<br>Myelin pallor<br>Axonal loss<br>Microglial nodules<br>Gliosis                                       |
| Inflammatory and Immune Activation  | Effects and Roles                                                                                                                                                                                                                      |
| Activated macrophages and monocytes | Release viral gene products and proinflammatory cytokines<br>CD163 is a marker of activated macrophages<br>Expansion of CD14 <sup>+</sup> /CD16 <sup>+</sup> monocytes<br>Migration of CD16 <sup>+</sup> cells across BBB increased by |





|                                            |                                                                                                                                                                                  |
|--------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                            | Increases vulnerability of neurons to dendritic alterations                                                                                                                      |
| Neopterin                                  | Elevated in CSF and remains mildly elevated even with viral suppression<br>Induces release of QUIN<br>Correlates with release of reactive oxygen species by macrophages          |
| QUIN                                       | Excitotoxic agonist at NMDA receptors<br>Forms free radicals<br>Correlates with cerebral atrophy                                                                                 |
| Monocyte chemoattractant protein 1 (MCP-1) | Upregulated in HIVE<br>Associated with poorer outcomes<br>Mediates neuronal death caused by <i>nef</i>                                                                           |
| Astrocytes                                 | Astrocytosis – feature of HIVE<br>Astrocyte apoptosis propagates macrophage activation and causes loss of neurotrophic support for neurons                                       |
| Synaptodendritic injury                    | Retraction of dendritic spines, dendritic beading and aberrant sprouting<br>Disrupts normal communication and impairs axonal transport<br>NFL is a marker of axonal injury       |
| HAART                                      | Mitochondrial dysfunction<br>Beading and pruning of dendrites<br>Destabilisation of neuronal $Ca^{++}$ homeostasis and a reduced response to glutamate<br>Increase NO production |
| Ageing                                     | Decreased levels of amyloid-beta<br>Normal or low levels of tau<br>Neuritic $\alpha$ -synuclein expression in substantia nigra                                                   |

The neuropsychological profile and the MRI characteristics of HAND will be discussed in detail in chapters 3 and 4 respectively.

## **1.20 Conclusion**

HIV has evolved into a chronic disease with the advent of HAART. Although the incidence rates of HIV associated dementia have decreased significantly in the post HAART era, it is evident that a milder spectrum of neurocognitive disorders is still quite prevalent. The exact aetiology of HAND remains to be fully elucidated despite many advances in our understanding of this disorder.

The role of on-going inflammation and immune activation in the aetiology of HIV related cognitive impairment is intriguing and may exist despite viral suppression in the primary blood compartment. Whether the on-going inflammation and immune activation are as a direct result of primary HIV infection in the CNS, a consequence of the CNS being a reservoir for on-going HIV replication despite adequate viral suppression in the primary blood compartment or as a consequence of HAART remains a subject of great interest and debate. It is also an area of ever burgeoning research. Whatever the mechanism of the initial inflammatory insult, there is evidence that low level inflammation continues even in those who are virally suppressed and does not require a continuous viral presence. It is most likely that the initial upregulation of inflammation and immune activation stems from a number of sources and pathways. This inflammatory cascade may then induce excitotoxicity resulting in neurotoxicity. This may be contributed to by HAART and also directly by HIV and its viral proteins. It is obvious that the neuropathogenesis of HIV is multifactorial and it is probably the result of the interaction of all these factors that contributes to the development of HAND.



Normal function of the brain requires a delicate balance between neuroprotective and neurodegenerative processes. These are tightly regulated to maintain normal neuronal function and it is likely that HIV disrupts this balance through many potential mechanisms as evidenced above. This also has implications for therapeutic interventions as it will be necessary for any successful intervention to be able to block or reverse the damaging effects of HIV, its resultant inflammatory cascade and neurodegenerative mechanisms without causing further disruption to this fine balance. This challenge is not unique to HAND but is also faced by researchers in the wider neurodegenerative field.

### **1.21 Hypothesis and Outline of the Thesis**

The literature review in this chapter highlighted the extensive research that has been carried out to date on HAND and the advances made thus far in our knowledge of the disorder and its neuropathogenesis. Despite the wonderful advances in HIV care in the last thirty years and the transformation of HIV from a devastating diagnosis without any treatment to a chronic disease manageable with HAART, there remains much to be learned about HIV and its impact on cognition. Gaps remain in our knowledge about HAND and it remains a condition worthy of further investigation. The primary objective of this study is to make a meaningful contribution to the knowledge base about HAND. To date no study has been carried out in Ireland assessing the prevalence or clinical features of HAND in Ireland.

The overarching hypothesis of the study is that HIV associated neurocognitive disorder has both inflammatory and degenerative features. It is likely that there is an inflammatory and immune mediated process early on in the disease process and that it becomes a neurodegenerative process later on. This hypothesis is based on the literature surrounding HAND that was discussed in this introduction. There is overwhelming evidence of both inflammatory and degenerative mechanisms involved in the neuropathogenesis of HAND. It is the hypothesis of the study that initially most of the inflammation is driven by immune activation and upregulation of inflammatory cytokines and that while HIV can have degenerative effects early on, it is later in the disease process that the degenerative phase becomes the predominant feature and is driven both by HIV and the excitotoxicity associated with the earlier inflammatory phase. Unfortunately it was beyond the scope of the study to fully elucidate this hypothesis as it would require considerable longitudinal follow-up and was not possible to complete within the time period of the study. My aims were to demonstrate that there were both inflammatory and degenerative features present in an Irish clinical cohort of HIV positive patients with cognitive impairment and to establish a well characterised cohort of patients who could be followed up longitudinally to further elaborate on the natural history of HAND in the post-HAART era and to establish the roles that inflammation and degeneration play over time.

The studies that were carried out for this PhD were all cross-sectional and were imperative to establish that there is a high rate of cognitive impairment in our clinical cohort, to examine the neuropsychological profile of patients in the post-HAART era and to examine the neuroradiological and CSF features of that cohort. Chapter 2 will

discuss a cross sectional study examining the rate of a positive screen for cognitive impairment in the HIV positive population attending our institution. Chapter 3 will discuss the neuropsychological profile and neurological features of patients with a positive screen for cognitive impairment who have had a formal neurological assessment. Chapter 4 will discuss the neuroimaging findings of patients with HAND. Chapter 5 will discuss the results of flow cytometry, cytokine analysis and tau and amyloid studies carried out on CSF of patients with HAND. Chapter 6 will discuss the implications of this research and future directions.

Throughout the thesis, the literature reviews contain studies that were published in the English language and were found through PUBMED and Google Scholar searches. The majority were published in the US, Europe and Australia however studies were included from Africa, Asia and South America as the clinical cohort studied were from a number of countries and therefore it was important to include studies that had examined cognition in their own cultural setting.



## **2 The Prevalence of a Positive Screen for Cognitive Impairment in a Cohort of HIV Positive Patients Attending an Irish Clinic**

### **2.1 Introduction**

#### **2.1.1 Outline of the chapter**

This chapter focuses on the cross sectional prevalence of a positive screen for cognitive impairment in a cohort of Irish HIV positive patients and the factors associated with it. The candidate expected to find a high prevalence rate based on international literature.

#### **2.1.2 Guidelines for Screening of HIV Associated Neurocognitive Disorders**

The European AIDS Clinical Society (EACS) recommends screening all patients without a highly confounding condition every two years for cognitive impairment. Highly confounding conditions include severe psychiatric conditions, abuse of psychotropic drugs, alcohol abuse, sequelae from previous central nervous system opportunistic infections (CNS-OIs) or other neurological diseases and current CNS-OIs or other neurological diseases. The 2013 guidelines from EACS recommend asking patients three questions every two years and if they reply “yes, definitely” to any one of the three questions then evaluation for cognitive impairment is suggested (51). The questions are outlined in Table 2-1. When this study commenced the 2009 EACS guidelines were in place and they suggested that HIV positive patients complaining of memory disturbance should be evaluated including neurological examination, cerebrospinal fluid examination and MR imaging of the brain. It suggested that

patients who were asymptomatic should be screened for cognitive impairment using the international HIV dementia scale if they had detectable plasma HIV RNA, had a nadir CD4 count less than 200, ongoing depression or were on HAART that had limited CNS penetration (169).

**Table 2-1 Screening Questions Suggested by EACS**

1. Do you experience frequent memory loss (e.g. do you forget the occurrence of special events even the more recent ones, appointments, etc.)?

2. Do you feel that you are slower when reasoning, planning activities, or solving problems?

3. Do you have difficulties paying attention (e.g. to a conversation, a book, or a movie)?
- For each question, patients can answer: a) never, b) hardly ever, or c) yes, definitely.

2.1.3 Screening Tests for HIV Associated Neurocognitive Disorders

The HIV dementia scale (HDS) was developed to screen for HIV associated dementia in the 1990s by Power et al. The sensitivity, specificity and positive predictive value of the HDS to detect HIV associated dementia were 80%, 91% and 78% respectively (170). The International HIV Dementia Scale (IHDS) was developed by Sacktor et al in 2005 for use in international settings. This was validated in patients in the United States and in Uganda and the sensitivity and specificity did not differ significantly between the two groups (80% and 57% in the US group and 80% and 55% in the Ugandan group) (171).

Zipursky et al carried out a systematic review of the literature on the evaluation of brief screening tools for HAND. They focused on studies that evaluated screening tools by comparing them with a reference standard or criterion (n=31). The majority of the studies were conducted in the USA but were also conducted in Australia, South Africa, the UK, Germany, Belgium, Netherlands, Switzerland, Uganda, Kenya and India. They performed a meta-analysis for the HDS and IHDS. They concluded that these tools are not ideal for detecting a range of neurocognitive impairment and are best used to detect HIV associated dementia (172). Kamminga et al also reviewed screening tests in HAND and restricted their review to studies in the post HAART era. The screen that was the most valid was the demographically adjusted HIV Dementia Scale but only in the setting of HAD. They concluded that it was inappropriate for use in the post HAART era where ANI is the most common form of HAND. The CogState computerised battery was found to be similar to the neuropsychological battery used in the study but it was only performed in patients with advanced HIV and 11/60 participants were assessed as AIDS dementia complex stage 1 or 2 so this is likely to have inflated the study findings (173, 174).

The Brief NeuroCognitive Screen was developed by the AIDS Clinical Trial Group to identify people with HAND. It was validated by Ellis et al and had a sensitivity of 65% and a specificity of 72% to detect HAND (175). The trail making tests A and B assess visual conceptual and visuomotor tracking, psychomotor speed, mental flexibility, attention, visual perceptual abilities and executive speed (176). The digit symbol test assesses processing speed, visual scanning, sustained attention, associative learning and sequencing (177, 178).



#### 2.1.4 Risk Factors for HIV Associated Neurocognitive Disorders

Risk factors for HAND that have been demonstrated to date include a low nadir CD4<sup>+</sup> T cell count (68, 179-181), older age (180, 182) substance abuse (183-187), lower educational level (181), co-infection with hepatitis C (185, 187, 188), CCR2 polymorphisms (186, 189), the 2578G variant of CCL2 (MCP-1) (186, 190), TNF- $\alpha$  308A allele (186, 191) and systemic features such as low haemoglobin, constitutional symptoms and a low body mass index (64, 101). Apolipoprotein E status, particularly in older patients (186, 192), has also been associated with HAND in some studies, although a recent study from the CHARTER group did not demonstrate any increased risk associated with apolipoprotein E4 carrier status (193).

#### 2.1.5 Functional Consequences of HAND

HIV associated dementia remains an independent predictor of time to death. Sevigny et al followed a cohort of 329 HIV positive patients with CD4 T cell counts less than 200 at time of recruitment and HIV dementia was statistically associated with time to death with a hazard ratio of 6.1 (71). These patients were followed up between 1998 and 2002. In a Canadian cohort study of 1651 HIV positive patients followed up from 1998 to 2008, HAND was associated with a higher mortality rate with a hazard ratio of 3.1 (194). Heaton et al examined a cohort of 267 patients and found that patients who were neuropsychologically impaired demonstrated a high rate of functional impairment in the areas of work assessment, finances, medication management, cooking and shopping. Within this cohort there was also a statistically higher rate of unemployment in the patients who were neuropsychologically impaired (195).

Woods et al demonstrated that patients with prospective memory (“remembering to remember”) impairment had poorer medication management (196). Prospective memory refers to the ability to carry out a future task or intention. Impairment in prospective memory is associated with dependence in activities of daily living (197). Hinkin et al also demonstrated that HAND was associated with poor medication adherence (198). Ettenhofer et al demonstrated that older HIV positive patients with neurocognitive impairment or substance abuse problems were more likely to be less adherent to their antiretroviral regimes (199). Albert et al demonstrated that patients with HAND who had low scores on executive and psychomotor tests were more likely to rely on fixed medication schedules in order to avoid forgetting to take their medications (200).

Blalock et al demonstrated that employment status in patients with HIV tended to fluctuate due to the severity of their medical condition (201). Van Gorp et al examined predictors of the ability to return to work in HIV positive patients who were out of work. Memory function was the most important predictor of return to employment (202). Van Gorp et al in an earlier study examined the relationship between neurocognitive impairment and employment in 130 HIV positive patients. Patients who were unemployed performed poorer on neuropsychological tests of memory, cognitive set shifting and psychomotor speed (203). Rabkin et al examined predictors of employment in 141 HIV positive men and found that lifetime depressive disorder, physical limitations and cognitive impairment all represented significant barriers to employment. The patients who were unemployed performed worse on

neuropsychological tests of memory, executive function and psychomotor speed when age, CD4 T cell count and physical limitations were accounted for (204).

Marcotte et al completed neuropsychological assessment and a driving assessment of 40 HIV positive patients and 20 HIV negative control subjects. Driving was assessed with two driving simulations, a useful field of view test and an on-road assessment. HIV positive patients who were neuropsychologically impaired were at an increased risk for impairment of on-road driving but HIV positive patients with normal cognition were similar to HIV negative controls. Executive dysfunction was the strongest neuropsychological predictor of failing the on-road driving assessment (205).

#### 2.1.6 Psychiatric Disorders in HIV

Depression is the most common psychiatric disorder associated with HIV infection and is more prevalent in HIV positive people compared to the general population (206-209). Women are at increased risk for depression from early adolescence to their mid-50s. Women have an increased lifetime risk of a major depressive order 1.7 to 2.7 that of men (210). Symptoms of depression include depressed mood, loss of interest and enjoyment, reduced energy leading to increased fatiguability and diminished activity, reduced concentration and attention, reduced self-esteem and self-confidence, ideas of guilt and unworthiness, bleak and pessimistic views of the future, ideas or acts of self-harm or suicide, disturbed sleep and diminished appetite. In order to meet the diagnostic criteria of a depressive episode at least two of the following symptoms should be present for two weeks or more: depressed mood, anhedonia or increased



fatigability along with two of the remaining symptoms described above (211).  
Diagnostic criteria for anxiety are described in Table 2-2 (211).

**Table 2-2 Diagnostic Criteria for Anxiety from the ICD-10 Classification**

|                                                                                                                                     |
|-------------------------------------------------------------------------------------------------------------------------------------|
| <b>Generalised Anxiety Disorder</b>                                                                                                 |
| A. A period of at least six months with prominent tension, worry and feelings of apprehension, about every-day events and problems. |
| B. At least four symptoms out of the following list of items must be present, of which at least one from items (1) to (4).          |
| Autonomic arousal symptoms                                                                                                          |
| (1) Palpitations or pounding heart, or accelerated heart rate.                                                                      |
| (2) Sweating.                                                                                                                       |
| (3) Trembling or shaking.                                                                                                           |
| (4) Dry mouth (not due to medication or dehydration).                                                                               |
| Symptoms concerning chest and abdomen                                                                                               |
| (5) Difficulty breathing.                                                                                                           |
| (6) Feeling of choking.                                                                                                             |
| (7) Chest pain or discomfort.                                                                                                       |
| (8) Nausea or abdominal distress (e.g. churning in stomach).                                                                        |
| Symptoms concerning brain and mind                                                                                                  |
| (9) Feeling dizzy, unsteady, faint or light-headed.                                                                                 |
| (10) Feelings that objects are unreal (derealisation), or that one's self is distant or "not really here" (depersonalization).      |
| (11) Fear of losing control, going crazy, or passing out.                                                                           |
| (12) Fear of dying.                                                                                                                 |
| General symptoms                                                                                                                    |
| (13) Hot flushes or cold chills.                                                                                                    |

(14) Numbness or tingling sensations.

Symptoms of tension

(15) Muscle tension or aches and pains.

(16) Restlessness and inability to relax.

(17) Feeling keyed up, or on edge, or of mental tension.

(18) A sensation of a lump in the throat, or difficulty with swallowing.

Other non-specific symptoms

(19) Exaggerated response to minor surprises or being startled.

(20) Difficulty in concentrating, or mind going blank, because of worrying or anxiety.

(21) Persistent irritability.

(22) Difficulty getting to sleep because of worrying.

C. The disorder does not meet the criteria for panic disorder, phobic anxiety disorders, obsessive-compulsive disorder or hypochondriacal disorder.

D. Most commonly used exclusion criteria: not sustained by a physical disorder, such as hyperthyroidism, an organic mental disorder or psychoactive substance-related disorder, such as excess consumption of amphetamine-like substances, or withdrawal from benzodiazepines.

Significant cognitive deficits in psychomotor speed, attention, visual learning, memory and executive function have been shown in the first episode of a major depressive disorder (212). In the pre-HAART era Hinkin et al did not find any significant difference on neuropsychological test performance between HIV positive patients with high and low scores on the Beck Depression Inventory (213). In the post-HAART era Castellon et al examined the components of depression and their relationship to neurocognitive performance in HIV. They divided the Beck Depression Inventory into three factors: self-reproach, mood-motivation disturbance and somatic disturbance. Mood-

motivation disturbance was associated with poorer performance on tests of verbal memory, executive function and motor speed (214). Kalechstein et al demonstrated that depression predicted procedural memory deficits in HIV (215). Cysique et al conducted a prospective longitudinal study on 227 HIV positive patients and did not find any association between depression and neuropsychological performance (216). The conflicting evidence on the link between depression and performance on neuropsychological tests may be due to differing methodology in relation to depression scales and clinical interviews.

Morrison et al demonstrated that there was a higher rate of a major depressive episode and more symptoms of depression and anxiety in HIV positive women than in HIV negative women (217). Sewell et al found that there was no significant difference in the rates of anxiety between HIV positive and HIV negative homosexual men but they found that compared with the general population both HIV positive and HIV negative homosexual men reported more symptoms of anxiety and stress (218).

Depression and anxiety have been associated with non-adherence to HAART (219, 220). Treatment with anti-depressants improves adherence to HAART (221, 222). Ickovics et al examined the impact of depressive symptoms on mortality and HIV disease progression on HIV positive women. Depressive symptoms were associated with HIV progression (223) however Rabkin et al demonstrated stability of mood in HIV positive homosexual men despite HIV progression (224).



Cell-mediated activation and inflammation contribute to depressive symptoms and are mediated by interleukin-1, interleukin-2, interleukin-6, tumour necrosis factor alpha and interferon-gamma. Serotonin depletion may occur secondary to the upregulation of pro-inflammatory cytokines and there is an increase in oxidative and nitrosative stress pathways with resultant neuronal apoptosis and neurodegeneration (225). HIV also results in upregulation of pro-inflammatory cytokines and it is possible that the increase in depressive symptoms and episodes in HIV may be secondary to the neuroinflammation of HIV infection. HIV also results in enhanced degradation of the serotonin precursor tryptophan (226). Schroecksnadel et al found that among 152 HIV positive patients, those who were not depressed had lower plasma levels of neopterin, lower rates of degradation of tryptophan, higher CD4 T cell counts, higher haemoglobin levels and better quality of life scores than patients with depression (226). Warriner et al also examined the relationship between depression and markers of immune activation in HIV infection. Neopterin levels were higher in those who were depressed compared to those who were not depressed but only for patients taking anti-depressant medications (227).

#### 2.1.7 Description of the Study Site

St. James's Hospital is the largest university teaching hospital in Dublin with 950 beds. The Genitourinary and Infectious Diseases (GUIDe) Clinic provides the largest outpatient service in Ireland for patients living with HIV. It also has outpatient clinics for patients with sexually transmitted infections (STI) and other infectious diseases. It has a dedicated inpatient service for patients with HIV and other infectious diseases.

The service is provided by consultants in infectious diseases and genitourinary medicine, a medical team composed of specialist registrars and senior house officers, clinical nurse specialists, health advisors, medical social workers, a clinical nutritionist and a dedicated pharmacy team. Three HIV clinics are held weekly, including one for patients co-infected with hepatitis C. There are four STI clinics a week, two of which provide a walk-in service. There is a weekly infectious diseases clinic.

Competing needs in a busy outpatient HIV clinic setting affect ability to screen all patients for cognitive impairment. Prior to the initiation of this study there was no formal screening programme for cognitive impairment in place at this institution. A retrospective study carried out over a six week period by the PhD candidate from August 2010 to September 2010 reviewed 500 consecutive patients attending HIV clinics in St. James's Hospital to examine if the use of surrogate markers could help to identify patients at risk of cognitive impairment. Only 7/500 charts reviewed had documentation of patients being asked about cognitive symptoms and all seven patients had cognitive symptoms. Surrogate markers only identified a small percentage of patients (13.8%, 69/500) as being at risk of cognitive impairment, considerably lower than international prevalence rates therefore the need for a prospective screening programme was highlighted (228).

## **2.2 Study Aims**

**Aim 1:** To determine the prevalence of a positive screen for cognitive impairment in the HIV positive population who attend St. James's Hospital.

**Aim 2:** To assess risk factors for the development of cognitive impairment in the HIV positive population who attend St. James's Hospital.

**Aim 3:** To determine the prevalence of a positive screen for anxiety in the HIV positive population who attend St. James's Hospital and the risk factors for a positive screen.

**Aim 4:** To determine the prevalence of a positive screen for depression in the HIV positive population who attend St. James's Hospital and the risk factors for a positive screen.

## **2.3 Methodology**

### **2.3.1 Study Design and Recruitment**

This was a cross-sectional observational study. Patients were recruited from the outpatient HIV clinics at St. James's Hospital, Dublin. Recruitment commenced on 22 December 2010 and was completed on 11 February 2013. The PhD candidate attended three weekly HIV clinics to recruit patients and all cognitive testing was carried out by the candidate in order to limit any potential bias in the methodology of testing. Patients were recruited during their routine outpatient appointment. They were given a patient information leaflet (see Appendix 1), signed a consent form (see Appendix 2) and completed testing on the same day. Unfortunately, ethical approval was not granted to include a HIV negative cohort in this study or any of the subsequent studies and therefore limits our interpretation of the results. A HIV negative group would have been preferable in order to control for sociodemographic features and also substance abuse.



### 2.3.2 Determination of Sample Size

2200 HIV positive patients attend St. James's Hospital. The prevalence of cognitive impairment ranges from 20% to 50% according to international literature reviewed in chapter 1. A prevalence rate of 35% was chosen for the purposes of calculating the sample size. A Raosoft sample size calculator was used to calculate the sample size. With a population of 2200, a margin of error of 5%, a confidence interval of 95% and a prevalence rate of 35% a sample size of 302 HIV positive patients would be required.

It was anticipated that a minimum of 302 patients would be recruited into the study but in order to ensure that we had adequate numbers in each group, patients with a positive screen for cognitive impairment and patients with a negative screen for cognitive impairment, to carry out multivariate analysis; our plan was to recruit 600 patients into the study. A total number of 600 patients recruited should yield approximately 200 patients with cognitive impairment. This then allowed for at least 10 patients per variable in the multivariate analysis and also allowed for possible losses due to incomplete or missing data.

### 2.3.3 Inclusion and Exclusion Criteria

Patients were included in the study if they were HIV positive, attending the outpatient clinic in St. James's Hospital, over the age of 18, capable of giving informed consent, had sufficient use of the English language to be able to participate and were willing to voluntarily participate in the study.

Patients were excluded if they were HIV negative, under the age of 18, had insufficient English to partake, had a physical disability or limitation which affected their ability to complete the cognitive testing or were unwilling to participate voluntarily in the study.

#### 2.3.4 Screening Tools Used

Patients were screened for cognitive impairment using the Brief NeuroCognitive Screen (BNCS). This test has been validated for use in HIV positive patients (175). There is no one ideal test for screening for cognitive impairment in the setting of HIV however the BNCS was chosen as it was validated in the setting of HIV infection, it was less likely to miss patients who were only mildly impaired compared to the HIV dementia scale which tests for patients who are more significantly impaired and there was normative data available in a wide age range for the three subtests within the BNCS. The components of the BNCS were Trail Making Test Part A (TMT-A), Trail Making Test Part B (TMT-B) and the Digit Symbol Test. A copy of the BNCS is in Appendix 3. All three subsections of the test were timed. The candidate ensured that each participant understood the tests involved in the screening process by asking patients to explain back the tasks they were asked to perform and patients completed a sample of each test prior to undertaking them. The participants were aware that the tests were being timed.

For Trails A and B patients were told to connect the dots in sequence: numbers 1 to 25 for TMT-A and alternate between letters and numbers starting at number 1 and then letter A and so forth for TMT-B. Patients completed a sample of TMT-A (numbers 1 to

5) and a sample of TMT-B (numbers 1 to 5 and letters A to E) prior to completing the tests. If the patient made any error it was corrected immediately and the patient was directed back to the last correct target. If the patient was unable to locate the next correct target assistance was provided after ten seconds and they were pointed to the next correct target. If the patient was unable to complete TMT-A within 180 seconds or TMT-B within 240 seconds, testing was stopped and the scores recorded as 180 or 240 seconds. For the digit symbol test, patients completed a sample consisting of 7 boxes with the numbers entered and they were required to enter the matching symbols. The key with numbers 1 to 9 and their respective symbols remained at the top of the page throughout testing. Patients were then required to insert the matching symbols for as many of the printed 93 numbers that they could in 90 seconds. All tests were timed using the stopwatch function of an iPhone. Three scores were recorded for the BNCS: time taken to complete Trails A, time taken to complete Trails B and the number of correctly matched symbols in the digit symbol test completed in 90 seconds.

Anxiety and depression were screened for using the Hospital Anxiety and Depression (HAD) Scale (see Appendix 4). This is a 14 item questionnaire consisting of 7 items relating to depression and 7 items relating to anxiety. This was completed by the patients but the candidate remained present to assist or answer any questions that they may have had.



### 2.3.5 Determination of a Positive Screen for Cognitive Impairment

A positive screen for cognitive impairment was defined as one standard deviation below the population mean on two tests or two standard deviations below the population mean on one test based on the BNCS validated screening tool (175). The BNCS has been used in other studies with HIV positive patients also (153). The normative data for the Trail Making Test (TMT) were taken from a meta-analysis published in *The Handbook of Normative Data for Neuropsychological Assessment* (176). For the meta-analysis 28 studies were included for TMT-A and 29 studies were included for TMT-B. The studies included were published between 1980 and 2004. The countries in which the studies were carried out included England, Canada, United States of America and Australia. Participants ranged in age from 16 to 91 years of age. Education ranged from 8 to 16 years. Over 6300 participants were included in the meta-analysis. Scores for TMT-A were adjusted for education by adding 1.31 for every year of education below 14 and subtracting 1.31 for every year of education over 14 between 12 and 17 years of education. A similar adjustment was made for TMT-B by adding or subtracting 6.45 for every year below or above 14 years of education. The normative data for the digit symbol test were taken from the *Weschler Adult Intelligence Scale Revised (WAIS-R)* normative data (229).

A positive screen for anxiety or depression was a score of 11 or greater on the respective sub-sections of the HAD Scale (230). The HAD scale has been validated for use in patients with HIV and has been used in a number of international settings (231-234).

### 2.3.6 Ethical Approval and Data Protection

The study had full ethical approval from the combined ethics committee of the Adelaide and Meath Hospitals Incorporating the National Children's Hospital (AMNCH) and St. James's Hospital (SJH). All patients who participated in the study were given patient information leaflets to read and were given the opportunity to ask any questions that they had prior to agreeing to participate in the study. The voluntary nature of participation was emphasised and that refusal to participate would not affect their ongoing medical care in any way. Written informed consent was obtained from all participants. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

Data was initially collected using a paper case report form in the clinic setting (see Appendix 5). Data was subsequently transferred to a password protected computerised database using Microsoft Access 2010 (R). All identifying information was treated in a strictly confidential manner. A copy of the data dictionary is attached in appendix 6.

Data was pseudo-anonymised. Each participant was assigned a unique study identifying number. This study number was entered on the paper case report form which did not contain the patient's name or medical record number. An excel worksheet was created to link the unique study ID number and the participants medical record number. This document was encrypted and password protected and only accessed by the PhD candidate. It was necessary to pseudo-anonymise the data to ensure that there was not duplication of data and to be able to give patients the

results of their cognitive screening at any point in the future also if they requested it again.

Hard copies of the case report forms were stored in a locked office in the Department of Neurology in St. James's Hospital. The consent forms were also stored in this office but did not contain any reference to the unique study number. These documents were only accessed by the candidate. The pseudo-anonymised database was encrypted and password protected and could only be accessed by the candidate.

### 2.3.7 Data Collection and Data Recorded

All demographic data was collected at the time of review in the HIV outpatient clinic and clinical data that was not available at that time (e.g. nadir CD4 count, antiretroviral history, results from T cell lymphocytes and HIV viral load tests that were taken on the same day) were recorded subsequently when the data was available.

Demographic data collected included gender, nationality, handedness, age at the time of the study, highest level of education reached and number of years spent in education, employment history, smoking history, alcohol history and history of use of recreational/illicit substances.

Clinical and disease related data collected included year of diagnosis, mode of transmission of HIV, endorsement of cognitive symptoms, nadir CD4<sup>+</sup> T cell count, current CD4<sup>+</sup> T cell count and current HIV viral load. Medical history was documented



specifically for the presence of co-infection with hepatitis B or C, hypertension, hypercholesterolaemia, diabetes and history of infection with syphilis. If patients were co-infected with hepatitis C, their hepatitis C PCR status was also documented. Neurological disorders and neurological complications of HIV were recorded (cryptococcal meningitis, CNS TB, toxoplasmosis, encephalitis, PML, IRIS, epilepsy, neuropathy and stroke). Psychiatric history was also recorded (depression, BPAD, anxiety, schizophrenia and psychosis). Medication history was also recorded (lipid lowering medications, anti-hypertensive medications, anti-epileptic medications, methadone, benzodiazepines, anti-depressants and anti-psychotics). Antiretroviral history was recorded. It was documented if patients were naïve to HAART, currently on HAART, had only been on HAART to prevent mother to child transmission and if they had been on HAART previously but were not currently on HAART. Date of initiation of HAART and date of most recent regime were documented. The current regime was documented separately and all previous regimes were documented together.

Clinical and disease related data were gathered at the time of screening the patient for cognitive impairment, from interrogation of their paper medical chart and electronic notes used by the Genitourinary and Infectious Diseases (GUIDE) clinic and review of the cards used by the GUIDE Pharmacy department. Blood results were taken from the electronic patient record (EPR) at our institution. EPR was initiated in 2006. Blood results required from time periods prior to this were taken from the older electronic systems (St. James's Patient Administration System (PAS) and the old St. James's Laboratory system). All efforts were made to ensure full data collection where possible.

Viral suppression was entered as a separate variable and was defined as a HIV viral load less than 50 copies per ml. The CPE score was calculated for each individual patient also based on their current regime of HAART.

#### 2.3.8 Missing Data

The only missing data points in this database are four nadir CD4<sup>+</sup> T cell counts from patients who were diagnosed in countries other than Ireland, date of commencement of HAART for one patient who commenced HAART in a different hospital and current CD4<sup>+</sup> T cell count and viral load from one patient who also attends services in a different country and had bloods taken there. Missing data was excluded from individual analysis involving those variables and only patients with data on all variables entered into the multivariate model were used in the multivariate analysis.

#### 2.3.9 Role of the PhD Candidate

The candidate recruited and consented all of the study participants. The candidate performed all of the neurocognitive screening and statistical analysis. The candidate created and maintained the study database.

### 2.4 Statistical Analysis

Statistical analysis was carried out using SPSS Version 21 (SPSS Inc., Chicago IL). Qualitative (categorical) variables were presented as absolute number (N) and relative frequencies (percentages). Continuous variables were presented as absolute number,

means with standards deviations (SD) and medians. Descriptive analysis was carried out on qualitative variables using Chi square ( $\chi^2$ ) testing to examine intergroup comparisons. The Bonferroni correction method was used to adjust for multiple comparisons. Fishers exact test was used where cells contained less than ten data points. Continuous variables were examined using the independent samples t test. Where data failed to meet criteria for parametric tests, the Mann Whitney U test was performed for continuous variables. All tests were two tailed and statistical significance was set at  $p < 0.05$ .

Multivariate analysis was performed to identify predictors of the presence of a positive screen for cognitive impairment. Binary logistic regression was carried out and the method used was Enter. Only variables that were statistically significant on univariate analysis were entered into the model.

## **2.5 Results**

### **2.5.1 Results of Entire Cohort**

There were 604 patients recruited to the study. Their characteristics are described in Table 2-3. 51.5% of the study cohort had a positive screen for cognitive impairment based on the predefined criteria outlined in the methodology. Of note, the first 96 patients recruited to this study were also recruited to the CRANlum study, which was a cross-sectional epidemiology study in Western Europe and Canada and was conducted to describe and compare the prevalence of a positive screen for neurocognitive impairment, depressive symptoms, and anxiety in an HIV-positive population either



naïve to HAART or on HAART. They found a positive screen for cognitive impairment in 41.5% of patients and a greater percentage of women compared with men had a positive screen (51.78% vs. 35.1%;  $p < 0.0001$ ) (235).

The majority of the study cohort were male (78.8%), mean age was 40.9 (SD 10.2) years and 70.9% were Irish. Mode of transmission was heterosexual in 32.8%, men who have sex with men in 49.3% and due to IV drug use in 15.4%. Just over 83% were on HAART and 88.7% were virally suppressed. A study was carried out nationally looking at HIV positive patients accessing care and from July 2009 to June 2010 in St James's Hospital 1745 patients accessed outpatient care. The majority were male (68.2%) and Irish (62.6%). Their mean age was 40.2 (SD 9.5) years. Mode of transmission was heterosexual in 42.9%, men who have sex with men in 32.4% and IV drug use in 22.2%. The majority were on HAART (80%) and virally suppressed (88%) (236). The study cohort is fairly representative of the overall cohort attending St James's Hospital.

Table 2-3 Characteristics of Study Group

| <u>Characteristics</u>                |               | <u>(n = 604)</u> |            |
|---------------------------------------|---------------|------------------|------------|
|                                       |               | <u>n</u>         | <u>(%)</u> |
| <b>Handedness</b>                     | Right         | 555              | (91.9)     |
|                                       | Left          | 49               | (8.1)      |
| <b>Gender</b>                         | Male          | 476              | (78.8)     |
|                                       | Female        | 128              | (21.2)     |
| <b>Symptoms</b>                       | Yes           | 246              | (40.7)     |
|                                       | No            | 358              | (59.3)     |
| <b>Country of Birth</b>               | Ireland       | 428              | (70.9)     |
|                                       | Europe        | 52               | (8.6)      |
|                                       | Africa        | 100              | (16.6)     |
|                                       | Asia          | 8                | (1.3)      |
|                                       | South America | 12               | (2.0)      |
|                                       | Other         | 4                | (0.7)      |
| <b>English as a first Language</b>    | Yes           | 468              | (77.5)     |
|                                       | No            | 136              | (22.5)     |
| <b>Mode of Transmission</b>           | Heterosexual  | 198              | (32.8)     |
|                                       | MSM           | 298              | (49.3)     |
|                                       | IVDU          | 93               | (15.4)     |
|                                       | Vertical      | 3                | (0.5)      |
|                                       | Other         | 12               | (2.0)      |
| <b>Virally Suppressed<br/>(n=504)</b> | Yes           | 447              | (88.7)     |
|                                       | No            | 57               | (11.3)     |
| <b>Hepatitis B</b>                    | Yes           | 47               | (7.8)      |
|                                       | No            | 557              | (92.2)     |
| <b>Hepatitis C</b>                    | Yes           | 118              | (19.5)     |
|                                       | No            | 486              | (80.5)     |
| <b>Hep C PCR Positive<br/>(n=118)</b> | Yes           | 73               | (61.9)     |
|                                       | No            | 45               | (38.1)     |
| <b>Hypertension</b>                   | Yes           | 67               | (11.1)     |
|                                       | No            | 537              | (88.9)     |
| <b>Hypercholesterolaemia</b>          | Yes           | 100              | (16.6)     |
|                                       | No            | 504              | (83.4)     |
| <b>Diabetes</b>                       | Yes           | 15               | (2.5)      |
|                                       | No            | 589              | (97.5)     |
| <b>Syphilis</b>                       | Yes           | 140              | (23.2)     |
|                                       | No            | 464              | (76.8)     |
| <b>Cryptococcal Meningitis</b>        | Yes           | 2                | (0.3)      |
|                                       | No            | 602              | (99.7)     |
| <b>CNS TB</b>                         | Yes           | 5                | (0.8)      |
|                                       | No            | 599              | (99.2)     |
| <b>Toxoplasmosis</b>                  | Yes           | 12               | (2)        |
|                                       | No            | 592              | (98)       |

|                               |            |     |        |
|-------------------------------|------------|-----|--------|
| <b>Encephalitis</b>           | Yes        | 5   | (0.8)  |
|                               | No         | 599 | (99.2) |
| <b>PML</b>                    | Yes        | 4   | (0.7)  |
|                               | No         | 600 | (99.3) |
| <b>IRIS</b>                   | Yes        | 1   | (0.2)  |
|                               | No         | 603 | (99.8) |
| <b>Epilepsy</b>               | Yes        | 15  | (2.5)  |
|                               | No         | 589 | (97.5) |
| <b>Neuropathy</b>             | Yes        | 25  | (4.1)  |
|                               | No         | 579 | (95.9) |
| <b>Stroke</b>                 | Yes        | 5   | (0.8)  |
|                               | No         | 599 | (99.2) |
| <b>Depression</b>             | Yes        | 145 | (24)   |
|                               | No         | 459 | (76)   |
| <b>Bipolar Disorder</b>       | Yes        | 7   | (1.2)  |
|                               | No         | 597 | (98.8) |
| <b>Anxiety</b>                | Yes        | 22  | (3.6)  |
|                               | No         | 582 | (96.4) |
| <b>Schizophrenia</b>          | Yes        | 2   | (0.3)  |
|                               | No         | 602 | (99.7) |
| <b>Psychosis</b>              | Yes        | 7   | (1.2)  |
|                               | No         | 597 | (98.8) |
| <b>Lipid Lowering Therapy</b> | Yes        | 66  | (10.9) |
|                               | No         | 538 | (89.1) |
| <b>Anti-hypertensives</b>     | Yes        | 63  | (10.4) |
|                               | No         | 541 | (89.6) |
| <b>Anti-epileptics</b>        | Yes        | 16  | (2.6)  |
|                               | No         | 588 | (97.4) |
| <b>Methadone</b>              | Yes        | 71  | (11.8) |
|                               | No         | 533 | (88.2) |
| <b>Benzodiazepines</b>        | Yes        | 79  | (13.1) |
|                               | No         | 525 | (86.9) |
| <b>Anti-depressants</b>       | Yes        | 66  | (10.9) |
|                               | No         | 538 | (89.1) |
| <b>Anti-psychotics</b>        | Yes        | 19  | (3.1)  |
|                               | No         | 585 | (96.9) |
| <b>Employment History</b>     | Employed   | 323 | (53.5) |
|                               | Student    | 49  | (8.1)  |
|                               | Unemployed | 211 | (34.9) |
|                               | Retired    | 21  | (3.5)  |
| <b>Smoking History</b>        | Smoker     | 278 | (46)   |



|                                                 |                    |                 |        |
|-------------------------------------------------|--------------------|-----------------|--------|
|                                                 | Non smoker         | 214             | (35.4) |
|                                                 | Ex-smoker          | 112             | (18.5) |
| <b>Alcohol History</b>                          | None               | 119             | (19.7) |
| <b>Units per week</b>                           | 1-10 units         | 289             | (47.8) |
|                                                 | 11-20 units        | 100             | (16.6) |
|                                                 | 21-30 units        | 40              | (6.6)  |
|                                                 | 31-40 units        | 12              | (2.0)  |
|                                                 | > 40 units         | 21              | (3.5)  |
|                                                 | Hx of ADS          | 23              | (3.8)  |
| <b>Illicit Substance Use</b>                    | Never              | 385             | (63.7) |
|                                                 | Current Non IV Use | 94              | (15.6) |
|                                                 | Current IV Use     | 9               | (1.2)  |
|                                                 | Previous Hx        | 118             | (19.5) |
| <b>Antiretroviral Therapy</b>                   | Naïve              | 95              | (15.7) |
|                                                 | On HAART           | 504             | (83.4) |
|                                                 | MTCT               | 3               | (0.5)  |
|                                                 | Previous exposure  | 2               | (0.3)  |
| <b>Positive Screen for Anxiety</b>              | Yes                | 148             | (24.5) |
|                                                 | No                 | 456             | (75.5) |
| <b>Positive Screen for Depression</b>           | Yes                | 55              | (9.1)  |
|                                                 | No                 | 549             | (90.9) |
| <b>Positive Screen for Cognitive Impairment</b> | Yes                | 311             | (51.5) |
|                                                 | No                 | 293             | (48.5) |
| <b>Age (in years)</b>                           | Mean (SD)          | 40.9 (10.2)     |        |
|                                                 | Median (range)     | 41 (18-77)      |        |
| <b>Time since Diagnosis (years)</b>             | Mean (SD)          | 8.2 (6.9)       |        |
|                                                 | Median (range)     | 6.5 (0.03-28.9) |        |
| <b>Nadir CD4 Count</b>                          | Mean (SD)          | 235.6 (102.3)   |        |
|                                                 | Median (range)     | 222 (1-907)     |        |
| <b>Current CD4 Count</b>                        | Mean (SD)          | 563.3 (252.7)   |        |
|                                                 | Median (range)     | 541 (19-1525)   |        |
| <b>Years of Education</b>                       | Mean (SD)          | 14.3 (3.9)      |        |
|                                                 | Median (range)     | 14 (0-26)       |        |
| <b>Exposure to HAART (total) (years)</b>        | Mean (SD)          | 6.1 (4.75)      |        |
|                                                 | Median (range)     | 4.9 (0.01-18.7) |        |
| <b>Exposure to HAART (current) (years)</b>      | Mean (SD)          | 2.9 (2.06)      |        |
|                                                 | Median (range)     | 2.6 (0.01-12.2) |        |
| <b>CPE Score</b>                                | Mean (SD)          | 7.081 (0.8711)  |        |
|                                                 | Median (range)     | 7 (5-13)        |        |
| <b>HADS - Anxiety Score</b>                     | Mean (SD)          | 7.576 (4.401)   |        |
|                                                 | Median (range)     | 7 (0-21)        |        |
| <b>HADS - Depression Score</b>                  | Mean (SD)          | 4.568 (4.0323)  |        |
|                                                 | Median (range)     | 4 (0-20)        |        |

There were 311/604 patients (51.5%) who had a positive screen for cognitive impairment. They were more likely to be female ( $p=0.000011$ ), endorse cognitive symptoms ( $p=0.041$ ), to have been born in Africa ( $p=0.000001$ ), speak English as a second language ( $p<0.000001$ ), to have acquired HIV through heterosexual transmission ( $p=0.000012$ ), to not be virally suppressed ( $p=0.005$ ), to be co-infected with hepatitis C ( $p=0.012$ ), to be PCR positive for hepatitis C ( $p=0.004$ ), to be on methadone replacement therapy ( $p=0.000032$ ), to be on benzodiazepines ( $p<0.000001$ ), to be on anti-depressants ( $p=0.036$ ), to be on anti-psychotic medications ( $p=0.004$ ), to be unemployed ( $p=0.000001$ ), to consume no alcohol per week ( $p=0.03$ ), to have a positive screen for anxiety on the HAD scale ( $p=0.041$ ), to have a positive screen for depression on the HAD scale ( $p=0.003$ ), to be younger ( $p=0.004$ ), to have a lower nadir CD4<sup>+</sup> T cell count ( $p=0.023$ ), to have a lower current CD4<sup>+</sup> T cell count ( $p=0.011$ ), to have fewer years of education ( $p=0.012$ ) and to have higher scores on both the anxiety ( $p=0.027$ ) and depression ( $p=0.000033$ ) subsections of the HAD scale. The presence of neuropathy ( $p=0.007$ ), syphilis infection ( $p=0.007$ ), hypercholesterolaemia ( $p=0.002$ ) and being on lipid lowering therapy ( $p=0.002$ ) were all associated with a reduced likelihood of a positive screen for cognitive impairment.

Table 2-4 Characteristics of Patients with a Positive Screen and Patients with a Negative Screen for Cognitive Impairment – Nominal and Categorical Variables Analysed using Chi Square Tests or Fisher Exact Tests.

| Characteristics               | (n = 604)    |            | Positive Screen<br>(n=311) |        | Negative Screen<br>(n=293) |        | P Value                                                 |
|-------------------------------|--------------|------------|----------------------------|--------|----------------------------|--------|---------------------------------------------------------|
|                               | n            | (%)        | n                          | (%)    | n                          | (%)    |                                                         |
| Handedness                    | Right        | 555 (91.9) | 289                        | (52.1) | 266                        | (47.9) | 0.335                                                   |
|                               | Left         | 49 (8.1)   | 22 (44.9)                  |        | 27 (55.1)                  |        |                                                         |
| Gender                        | Male         | 476 (78.8) | 223                        | (46.8) | 253                        | (53.2) | 0.000011                                                |
|                               | Female       | 128 (21.2) | 88 (68.8)                  |        | 40 (31.3)                  |        |                                                         |
| Symptoms                      | Yes          | 246 (40.7) | 139                        | (56.5) | 107                        | (43.5) | 0.041                                                   |
|                               | No           | 358 (59.3) | 172 (48)                   |        | 186 (52)                   |        |                                                         |
| Country of Birth              | Ireland      | 428 (70.9) | 194                        | (45.3) | 274                        | (54.7) | <0.000001                                               |
|                               | Europe       | 52 (8.6)   | 21 (40.4)                  |        | 31 (59.6)                  |        |                                                         |
|                               | Africa       | 100 (16.6) | 86 (86)                    |        | 14 (14)                    |        |                                                         |
|                               | Asia         | 8 (1.3)    | 3 (37.5)                   |        | 5 (62.5)                   |        |                                                         |
|                               | S. America   | 12 (2.0)   | 7 (58.3)                   |        | 5 (41.7)                   |        |                                                         |
|                               | Other        | 4 (0.7)    | 0 (0)                      |        | 4 (100)                    |        |                                                         |
| English as a first Language   | Yes          | 468 (77.5) | 210                        | (44.9) | 258                        | (74.3) | <0.000001                                               |
|                               | No           | 136 (22.5) | 101 (55.1)                 |        | 35 (25.7)                  |        |                                                         |
| Mode of Transmission          | Heterosexual | 198 (32.8) | 124                        | (62.6) | 74                         | (37.4) | 0.000012<br>Std Residual<br>for Heterosexual<br>was 2.2 |
|                               | MSM          | 298 (49.3) | 121                        | (40.6) | 177                        | (59.4) |                                                         |
|                               | IV/DU        | 93 (15.4)  | 57                         | (61.3) | 36                         | (38.7) |                                                         |
|                               | Vertical     | 3 (0.5)    | 2 (66.7)                   |        | 1 (33.3)                   |        |                                                         |
|                               | Other        | 12 (2.0)   | 7 (58.3)                   |        | 5 (41.7)                   |        |                                                         |
| Virally Suppressed<br>(n=504) | Yes          | 447 (88.7) | 221                        | (49.4) | 226                        | (50.6) | 0.005                                                   |
|                               | No           | 57 (11.3)  | 41 (71.9)                  |        | 16 (28.1)                  |        |                                                         |
| Hepatitis B                   | Yes          | 47 (7.8)   | 28                         | (59.6) | 19                         | (40.4) | 0.248                                                   |
|                               | No           | 557 (92.2) | 283 (50.8)                 |        | 274 (49.2)                 |        |                                                         |



| Characteristics               |     | (n = 604) |        | Positive Screen<br>(n=311) |         | Negative Screen<br>(n=293) |        | P Value |
|-------------------------------|-----|-----------|--------|----------------------------|---------|----------------------------|--------|---------|
|                               |     | n         | (%)    | n                          | (%)     | n                          | (%)    |         |
| Hepatitis C                   | Yes | 118       | (19.5) | 73                         | (61.9)  | 45                         | (38.1) | 0.012   |
|                               | No  | 486       | (80.5) | 238                        | (49)    | 248                        | (51)   |         |
| Hep C PCR Positive<br>(n=118) | Yes | 73        | (61.9) | 51                         | (69.9)  | 22                         | (30.1) | 0.04    |
|                               | No  | 45        | (38.1) | 22                         | (48.9)  | 23                         | (51.1) |         |
| Hypertension                  | Yes | 67        | (11.1) | 30                         | (44.8)  | 37                         | (55.2) | 0.244   |
|                               | No  | 537       | (88.9) | 281                        | (52.3)  | 256                        | (47.7) |         |
| Hypercholesterolaemia         | Yes | 100       | (16.6) | 37                         | (37)    | 63                         | (63)   | 0.002   |
|                               | No  | 504       | (83.4) | 274                        | (54.4)  | 230                        | (45.6) |         |
| Diabetes                      | Yes | 15        | (2.5)  | 9                          | (60)    | 6                          | (40)   | 0.504   |
|                               | No  | 589       | (97.5) | 302                        | (51.3)  | 287                        | (48.7) |         |
| Syphilis                      | Yes | 140       | (23.2) | 58                         | (41.4)  | 82                         | (58.6) | 0.007   |
|                               | No  | 464       | (76.8) | 253                        | (54.5)  | 211                        | (45.5) |         |
| Cryptococcal Meningitis       | Yes | 2         | (0.3)  | 2                          | (100)   | 0                          | (0)    | 0.169   |
|                               | No  | 602       | (99.7) | 309                        | (51.30) | 293                        | (48.7) |         |
| CNS TB                        | Yes | 5         | (0.8)  | 5                          | (100)   | 0                          | (0)    | 0.062   |
|                               | No  | 599       | (99.2) | 306                        | (51.1)  | 293                        | (48.9) |         |
| Toxoplasmosis                 | Yes | 12        | (2)    | 7                          | (58.3)  | 5                          | (41.7) | 0.632   |
|                               | No  | 592       | (98)   | 304                        | (51.4)  | 288                        | (48.6) |         |
| Encephalitis                  | Yes | 5         | (0.8)  | 4                          | (80)    | 1                          | (20)   | 0.2     |
|                               | No  | 599       | (99.2) | 307                        | (51.3)  | 292                        | (48.7) |         |
| PML                           | Yes | 4         | (0.7)  | 4                          | (100)   | 0                          | (0)    | 0.124   |
|                               | No  | 600       | (99.3) | 307                        | (51.2)  | 293                        | (48.8) |         |

| Characteristics        |     | (n = 604) |        | Positive Screen<br>(n=311) |        | Negative Screen<br>(n=293) |        | P Value |
|------------------------|-----|-----------|--------|----------------------------|--------|----------------------------|--------|---------|
|                        |     | n         | (%)    | n                          | (%)    | n                          | (%)    |         |
| IRIS                   | Yes | 1         | (0.2)  | 1                          | (100)  | 0                          | (0)    | 1       |
|                        | No  | 603       | (99.8) | 310                        | (51.4) | 293                        | (48.6) |         |
| Epilepsy               | Yes | 15        | (2.5)  | 10                         | (66.7) | 5                          | (33.3) | 0.299   |
|                        | No  | 589       | (97.5) | 301                        | (51.1) | 288                        | (48.9) |         |
| Neuropathy             | Yes | 25        | (4.1)  | 6                          | (24)   | 19                         | (76)   | 0.007   |
|                        | No  | 579       | (95.9) | 305                        | (52.7) | 274                        | (47.3) |         |
| Stroke                 | Yes | 5         | (0.8)  | 5                          | (100)  | 0                          | (0)    | 0.062   |
|                        | No  | 599       | (99.2) | 306                        | (51.1) | 293                        | (48.9) |         |
| Depression             | Yes | 145       | (24)   | 79                         | (54.5) | 66                         | (45.5) | 0.408   |
|                        | No  | 459       | (76)   | 232                        | (50.5) | 227                        | (49.5) |         |
| Bipolar Disorder       | Yes | 7         | (1.2)  | 4                          | (57.1) | 3                          | (42.9) | 1       |
|                        | No  | 597       | (98.8) | 307                        | (51.4) | 290                        | (48.6) |         |
| Anxiety                | Yes | 22        | (3.6)  | 12                         | (54.5) | 10                         | (45.5) | 0.77    |
|                        | No  | 582       | (96.4) | 299                        | (51.4) | 283                        | (48.6) |         |
| Schizophrenia          | Yes | 2         | (0.3)  | 2                          | (100)  | 0                          | (0)    | 0.5     |
|                        | No  | 602       | (99.7) | 309                        | (51.3) | 293                        | (48.7) |         |
| Psychosis              | Yes | 7         | (1.2)  | 6                          | (85.7) | 1                          | (14.3) | 0.124   |
|                        | No  | 597       | (98.8) | 305                        | (51.5) | 292                        | (48.9) |         |
| Lipid Lowering Therapy | Yes | 66        | (10.9) | 22                         | (33.3) | 44                         | (66.7) | 0.002   |
|                        | No  | 538       | (89.1) | 289                        | (53.7) | 249                        | (46.3) |         |
| Anti-hypertensives     | Yes | 63        | (10.4) | 31                         | (49.2) | 32                         | (50.8) | 0.702   |
|                        | No  | 541       | (89.6) | 280                        | (51.8) | 261                        | (48.2) |         |

| Characteristics                   | (n = 604)          |            | Positive Screen<br>(n=311) |            | Negative Screen<br>(n=293) |           | P Value                               |
|-----------------------------------|--------------------|------------|----------------------------|------------|----------------------------|-----------|---------------------------------------|
|                                   | n                  | (%)        | n                          | (%)        | n                          | (%)       |                                       |
| Anti-epileptics                   | Yes                | 16 (2.6)   | 11 (68.8)                  | 5 (31.3)   | 288 (49)                   | 0.161     |                                       |
|                                   | No                 | 588 (97.4) | 300 (51)                   |            |                            |           |                                       |
| Methadone                         | Yes                | 71 (11.8)  | 53 (74.6)                  | 18 (25.4)  | 275 (53.1)                 | 0.000032  |                                       |
|                                   | No                 | 533 (88.2) | 258 (48.4)                 |            |                            |           |                                       |
| Benzodiazepines                   | Yes                | 79 (13.1)  | 65 (82.3)                  | 14 (17.7)  | 279 (51.6)                 | <0.000001 |                                       |
|                                   | No                 | 525 (86.9) | 246 (46.9)                 |            |                            |           |                                       |
| Anti-depressants                  | Yes                | 66 (10.9)  | 42 (63.6)                  | 24 (36.4)  | 269 (50)                   | 0.036     |                                       |
|                                   | No                 | 538 (89.1) | 269 (50)                   |            |                            |           |                                       |
| Anti-psychotics                   | Yes                | 19 (3.1)   | 16 (84.2)                  | 3 (15.8)   | 290 (49.6)                 | 0.004     |                                       |
|                                   | No                 | 585 (96.9) | 295 (50.4)                 |            |                            |           |                                       |
| Employment History                | Employed           | 323 (53.5) | 135 (41.8)                 | 188 (58.2) | 12 (57.1)                  | 0.000001  |                                       |
|                                   | Student            | 49 (8.1)   | 29 (59.2)                  | 20 (40.8)  |                            |           |                                       |
|                                   | Unemployed         | 211 (34.9) | 138 (65.4)                 | 73 (34.6)  |                            |           |                                       |
|                                   | Retired            | 21 (3.5)   | 9 (42.9)                   |            |                            |           |                                       |
| Smoking History                   | Smoker             | 278 (46)   | 153 (55)                   | 125 (45)   | 106 (49.5)                 | 0.166     |                                       |
|                                   | Non smoker         | 214 (35.4) | 108 (50.5)                 |            |                            |           |                                       |
|                                   | Ex-smoker          | 112 (18.5) | 50 (44.6)                  | 62 (55.4)  |                            |           |                                       |
| Alcohol History<br>Units per week | None               | 119 (19.7) | 75 (63)                    | 44 (37)    | 8 (34.8)                   | 0.03      | Std Residual for<br>No alcohol is 1.8 |
|                                   | 1-20 units         | 389 (64.4) | 18.5 (47.6)                | 204 (52.4) |                            |           |                                       |
|                                   | 21-40 units        | 52 (8.6)   | 25 (48.1)                  | 27 (51.9)  |                            |           |                                       |
|                                   | > 40 units         | 21 (3.5)   | 11 (52.4)                  | 10 (47.6)  |                            |           |                                       |
| Illicit Substance Use             | Hx of ADS          | 23 (3.8)   | 15 (65.2)                  |            | 189 (49.1)                 | 0.352     |                                       |
|                                   | Never              | 385 (63.7) | 196 (50.9)                 |            |                            |           |                                       |
|                                   | Current Non IV Use | 94 (15.6)  | 54 (57.4)                  | 40 (42.6)  |                            |           |                                       |
|                                   | Current IV Use     | 9 (1.2)    | 5 (71.4)                   | 2 (28.6)   |                            |           |                                       |



| <u>Characteristics</u>         | (n = 604)         |            | Positive Screen<br>(n=311) |            | Negative Screen<br>(n=293) |     | P Value |
|--------------------------------|-------------------|------------|----------------------------|------------|----------------------------|-----|---------|
|                                | n                 | (%)        | n                          | (%)        | n                          | (%) |         |
| Antiretroviral Therapy         | Previous Hx       | 118 (19.5) | 56 (47.5)                  | 62 (52.5)  |                            |     |         |
|                                | Naïve             | 95 (15.7)  | 46 (48.4)                  | 49 (51.6)  |                            |     | 0.877   |
|                                | On HAART          | 504 (83.4) | 262 (52)                   | 242 (48)   |                            |     |         |
|                                | MTCT              | 3 (0.5)    | 2 (66.7)                   | 1 (33.3)   |                            |     |         |
|                                | Previous exposure | 2 (0.3)    | 1 (50)                     | 1 (50)     |                            |     |         |
| Positive Screen for Anxiety    | Yes               | 148 (24.5) | 87 (58.8)                  | 61 (41.2)  |                            |     | 0.041   |
|                                | No                | 456 (75.5) | 224 (49.1)                 | 232 (50.9) |                            |     |         |
| Positive Screen for Depression | Yes               | 55 (9.1)   | 39 (70.9)                  | 16 (29.1)  |                            |     | 0.003   |
|                                | No                | 549 (90.9) | 272 (49.5)                 | 277 (50.5) |                            |     |         |

IV = intravenous, Std = Standardised, Hx = history, ADS = Alcohol Dependence Syndrome and MTCT = Maternal to child transmission.

Table 2-5 Demographics of Patients with a Positive Screen and a Negative Screen for Cognitive Impairment – Linear Variables Analysed using Independent T Test or Mann Whitney U Test.

|                                       |           | Positive Screen<br>(n=311) | Negative Screen<br>(n=293) | P Value | 95% CI                  |
|---------------------------------------|-----------|----------------------------|----------------------------|---------|-------------------------|
| Age (in years)                        | Mean (SD) | 39.733 (9.946)             | 42.126 (10.377)            | 0.004   | (-4.0173) - (-0.7691)   |
|                                       | Median    | 39                         | 42                         |         |                         |
| Time since Diagnosis<br>(days)        | Mean (SD) | 2817.675 (2302.8863)       | 3162.27 (2699.4141)        | 0.338*  |                         |
|                                       | Median    | 2365                       | 2471                       |         |                         |
| Nadir CD4 Count                       | Mean (SD) | 221 (157.409)              | 251.038 (166.2)            | 0.023   | (-55.98) - (-4.0915)    |
|                                       | Median    | 207                        | 233                        |         |                         |
| Current CD4 Count                     | Mean (SD) | 538.116 (259.18)           | 590.161 (243.177)          | 0.011   | (-92.3022) - (-11.7882) |
|                                       | Median    | 505                        | 570.5                      |         |                         |
| Years of Education                    | Mean (SD) | 13.865 (4.1076)            | 14.724 (3.66)              | 0.012*  |                         |
|                                       | Median    | 14                         | 14                         |         |                         |
| Exposure to HAART (total)<br>(days)   | Mean (SD) | 2053.487 (1589.9178)       | 2410.078 (1861.0586)       | 0.088*  |                         |
|                                       | Median    | 1760                       | 1885                       |         |                         |
| Exposure to HAART (current)<br>(days) | Mean (SD) | 1021.172 (729.0108)        | 1082.012 (778.7706)        | 0.366   | (-193.06) - (-71.38)    |
|                                       | Median    | 890                        | 1003                       |         |                         |
| CPE Score                             | Mean (SD) | 7.069 (0.9197)             | 7.095 (4.1029)             | 0.735   | (-0.1791) - (0.1264)    |
|                                       | Median    | 7                          | 7                          |         |                         |

|                                |           | Positive Screen<br>(n=311) | Negative Screen<br>(n=293) | P Value  | 95% CI          |
|--------------------------------|-----------|----------------------------|----------------------------|----------|-----------------|
| <b>HADS - Anxiety Score</b>    | Mean (SD) | 7.977 (4.6354)             | 7.15 (4.1029)              | 0.027*   |                 |
|                                | Median    | 8                          | 7                          |          |                 |
| <b>HADS - Depression Score</b> | Mean (SD) | 5.225 (4.2715)             | 3.87 (3.641)               | 0.000033 | 0.7187 - 1.9909 |
|                                | Median    | 4                          | 3                          |          |                 |

\* Performed using Mann Whitney U Tests and no confidence intervals available for the difference. SD = Standard Deviation. CI = Confidence Interval.



**Table 2-6 Results of Binary Logistic Regression of Study Cohort.**

| Variable                       | Wald   | P Value   | Odds Ratio | Confidence Interval |
|--------------------------------|--------|-----------|------------|---------------------|
| Symptoms                       | 5.098  | 0.024     | 1.7        | 1.073 - 2.696       |
| African country of birth       | 34.051 | <0.000001 | 11.075     | 4.938 - 24.839      |
| Neuropathy                     | 5.474  | 0.019     | 0.229      | 0.066 - 0.787       |
| Use of benzodiazepines         | 12.828 | 0.000341  | 6.746      | 2.373 - 19.176      |
| Unemployed                     | 6.969  | 0.008     | 2.163      | 1.2 - 3.835         |
| Alcohol consumption > 40 units | 4.443  | 0.035     | 0.228      | 0.058 - 0.902       |

Logistic regression analysis showed that the main factors predictive of a positive screen for cognitive impairment were the endorsement of cognitive symptoms, being born in Africa, the use of benzodiazepines, being unemployed and consumption of more than 40 units of alcohol weekly. The presence of neuropathy predicted a reduced likelihood of a positive screen. Being born in Africa contributed the most to the model followed by the use of benzodiazepines.

There were 79/604 (13.1%) patients taking benzodiazepines. Of the 79 patients taking benzodiazepines 53 (67.1%) were male; 73 were Irish (92.4%); 74 were on antiretroviral therapy (93.7%) and 59 of the 74 patients on HAART were virally suppressed (79.7%); 64/79 were unemployed (81%); 56 were on methadone replacement therapy (70.9%); 52 had acquired HIV through intravenous drug use (65.8%); 3 (3.8%) and 24 (30.4%) patients respectively were currently using illicit substances intravenously and non-intravenously. These are all potential confounding factors that may contribute to the association between the use of benzodiazepines and the presence of a positive screen for cognitive impairment.

There were 100 patients in the study cohort who were born in Africa; 86/100 had a positive screen for cognitive impairment; 36/100 endorsed cognitive symptoms; 45/100 were male; 9/100 spoke English as their first language; 88/100 acquired HIV through heterosexual transmission; 89/100 were on HAART and 77/89 (86.5%) were virally suppressed. Univariate analysis showed that the only significant variable among the African patients contributing to a positive screen for cognitive impairment was younger age ( $p=0.05$ ). Mean age in the African patients with a positive screen for cognitive impairment was 37.36 years and in those with a negative screen it was 42 years.

When comparing the African patients to the rest of the study cohort, the African patients were more likely to be female ( $p<0.000001$ ), to be younger ( $p=0.003$ ), to have acquired HIV through heterosexual transmission or vertical transmission ( $p<0.000001$ ), to have had CNS TB ( $p=0.000127$ ), to be students ( $p=0.000052$ ), to not drink alcohol ( $p<0.000001$ ), to never have used illicit substances ( $p<0.000001$ ) and to have been on HAART to prevent maternal to child transmission ( $p=0.021$ ). They were less likely to speak English as their first language ( $p<0.000001$ ), to be co-infected with hepatitis C ( $p=0.000018$ ) or syphilis ( $p=0.008$ ), to be on methadone replacement therapy ( $p=0.000257$ ), benzodiazepines ( $p=0.001$ ) or antidepressants ( $p=0.005$ ), to be smokers ( $p<0.000001$ ) and had a shorter duration of HAART therapy ( $p=0.007$ ). None of these factors definitively explain the increased prevalence of a positive screen for cognitive impairment amongst this patient group but the lack of English as a first language and cultural factors that cannot be accounted for may be contributory however all patients had sufficient proficiency in English to participate in the study. Clade or other unknown

host factors such as genetic predispositions may contribute to the increased prevalence of a positive screen for cognitive impairment within the African patients.



Table 2-7 Demographics of the African Patients Compared to the Rest of the Cohort (Categorical Variables)

| Characteristics           | African Patients<br>(n=100) |     |              | Rest of the Cohort<br>(n=504) |      | P Value    | Std Residual for<br>African Patients |
|---------------------------|-----------------------------|-----|--------------|-------------------------------|------|------------|--------------------------------------|
|                           | n                           | (%) |              | n                             | (%)  |            |                                      |
| Symptoms                  |                             |     | Yes          | 36                            | (36) | 210 (41.7) | 0.317                                |
| Gender                    |                             |     | Male         | 45                            | (45) | 431 (85.5) | <0.000001                            |
|                           |                             |     | Female       | 55                            | (55) | 73 (14.5)  | 7.3                                  |
| English as a 1st language |                             |     | Yes          | 9                             | (9)  | 459 (91.1) | <0.000001                            |
| Mode of Transmission      |                             |     | Heterosexual | 88                            | (88) | 110 (21.8) | 9.6                                  |
|                           |                             |     | MSM          | 7                             | (7)  | 291 (57.7) | -6                                   |
|                           |                             |     | IVDU         | 3                             | (3)  | 40 (17.9)  | -3.2                                 |
|                           |                             |     | Vertical     | 2                             | (2)  | 1 (0.2)    | 2.1                                  |
|                           |                             |     | Other        | 0                             | (0)  | 12 (2.4)   | -1.4                                 |
| Virally Suppressed        |                             |     | Yes          | 77                            | (77) | 370 (73.4) | 0.2                                  |
| Hepatitis C co-infection  |                             |     | Yes          | 4                             | (4)  | 114 (22.6) | 0.000018                             |
| Syphilis                  |                             |     | Yes          | 13                            | (13) | 127 (25.2) | 0.008                                |
| CNS TB                    |                             |     | Yes          | 4                             | (4)  | 1 (0.2)    | 0.000127                             |
| Depression                |                             |     | Yes          | 13                            | (13) | 132 (26.2) | 0.005                                |
|                           |                             |     |              |                               |      |            | -2.2                                 |

| <u>Characteristics</u>       | African Patients<br>(n=100) |      | Rest of the Cohort<br>(n=504) |        | P Value   | Std Residual for<br>African Patients |
|------------------------------|-----------------------------|------|-------------------------------|--------|-----------|--------------------------------------|
|                              | n                           | (%)  | n                             | (%)    |           |                                      |
| <b>Methadone</b>             |                             |      |                               |        |           |                                      |
| Yes                          | 1                           | (1)  | 70                            | (13.9) | 0.000257  | -3.1                                 |
| <b>Benzodiazepine Use</b>    |                             |      |                               |        |           |                                      |
| Yes                          | 3                           | (3)  | 76                            | (15.1) | 0.001     | -2.8                                 |
| <b>Antidepressants</b>       |                             |      |                               |        |           |                                      |
| Yes                          | 3                           | (3)  | 63                            | (12.5) | 0.005     | -2.4                                 |
| <b>Employment History</b>    |                             |      |                               |        |           |                                      |
| Employed                     | 50                          | (50) | 273                           | (54.2) | 0.000052  | -0.5                                 |
| Student                      | 19                          | (19) | 30                            | (6)    |           | 3.8                                  |
| Unemployed                   | 31                          | (31) | 180                           | (35.7) |           | -0.7                                 |
| Retired                      | 0                           | (0)  | 21                            | (4.2)  |           | -1.9                                 |
| <b>Smoking History</b>       |                             |      |                               |        |           |                                      |
| Smoker                       | 18                          | (18) | 260                           | (51.6) | <0.000001 | -4.1                                 |
| <b>Alcohol History</b>       |                             |      |                               |        |           |                                      |
| None                         | 40                          | (40) | 79                            | (15.7) | <0.000001 | 4.6                                  |
| 1-20 units                   | 55                          | (55) | 334                           | (66.3) |           | -1.2                                 |
| 21-40 units                  | 4                           | (4)  | 48                            | (9.5)  |           | -1.6                                 |
| > 40 units                   | 0                           | (0)  | 21                            | (4.2)  |           | -1.9                                 |
| Hx of ADS                    | 1                           | (1)  | 22                            | (4.4)  |           | -1.4                                 |
| <b>Illicit Substance Use</b> |                             |      |                               |        |           |                                      |
| Never                        | 93                          | (93) | 292                           | (57.9) | <0.000001 | 3.7                                  |
| Current Non IV Use           | 2                           | (2)  | 92                            | (18.3) |           | -3.4                                 |
| Current IV Use               | 0                           | (0)  | 7                             | (1.4)  |           | -1.1                                 |
| Previous                     | 5                           | (5)  | 113                           | (22.4) |           | -3.3                                 |

| <u>Characteristics</u>                   | African Patients<br>(n=100)  |            | Rest of the Cohort<br>(n=504) |     | P Value   | Std Residual for<br>African Patients |
|------------------------------------------|------------------------------|------------|-------------------------------|-----|-----------|--------------------------------------|
|                                          | n                            | (%)        | n                             | (%) |           |                                      |
| Antiretroviral Therapy                   | Naïve                        | 9<br>(9)   | 86<br>(17.1)                  |     | 0.021     | -1.7                                 |
|                                          | On HAART                     | 89<br>(89) | 415<br>(82.5)                 |     |           | 0.6                                  |
|                                          | MTCT<br>Previous<br>exposure | 2<br>(2)   | 1<br>(0.2)                    |     |           | 2.1                                  |
|                                          |                              | 0<br>(0)   | 2<br>(0.4)                    |     |           | -0.6                                 |
| Positive Screen for Cognitive Impairment | Yes                          | 86<br>(86) | 225<br>(44.6)                 |     | <0.000001 | 4.8                                  |

IV = intravenous, Std = Standardised, Hx = history, ADS = Alcohol Dependence Syndrome and MTCT = Maternal to child transmission.



Table 2-8 Demographics of African Patients Compared to the Rest of the Cohort (Linear Variables)

|                                | African Patients<br>(n=104) |                      | Rest of the Cohort<br>(n=504) | P Value |
|--------------------------------|-----------------------------|----------------------|-------------------------------|---------|
| Age                            | Mean (SD)                   | 38.01 (8.2382)       | 41.47 (10.4814)               | 0.003*  |
|                                | Median                      | 37                   | 236                           |         |
| Time since Diagnosis<br>(days) | Mean (SD)                   | 2239.45 (1285.15)    | 3132.732 (2660.21)            | 0.058   |
|                                | Median                      | 2192.5               | 2468.5                        |         |
| Nadir CD4 Count                | Mean (SD)                   | 217.8 (164.05)       | 239.124 (161.87)              | 0.23    |
|                                | Median                      | 218.5                | 224                           |         |
| Current CD4 Count              | Mean (SD)                   | 533.55 (234.09)      | 569.24 (256.03)               | 0.197   |
|                                | Median                      | 495                  | 542                           |         |
| Years of Education             | Mean (SD)                   | 14.83 (3.78)         | 14.17 (3.94)                  | 0.05    |
|                                | Median                      | 15                   | 14                            |         |
| Duration of ART (total)        | Mean (SD)                   | 1638.182 (1122.5456) | 2349.637 (1813.6171)          | 0.007   |
|                                | Median                      | 1414                 | 1879.5                        |         |
| CPE Score                      | Mean (SD)                   | 7.114 (0.82)         | 7.075 (0.88)                  | 0.702   |
|                                | Median                      | 7                    | 7                             |         |
| HADS - Anxiety Score           | Mean (SD)                   | 6.98 (4.89)          | 7.69 (4.29)                   | 0.138   |
|                                | Median                      | 6                    | 7                             |         |

| HADS - Depression Score | African Patients |             | Rest of the Cohort |             | P Value |
|-------------------------|------------------|-------------|--------------------|-------------|---------|
|                         | Mean (SD)        | (n=104)     | Mean (SD)          | (n=504)     |         |
| Median                  |                  | 3           |                    | 4           |         |
|                         |                  | 4.47 (4.03) |                    | 4.59 (4.03) | 0.791   |

\*Performed using Mann Whitney U Tests and no confidence intervals available for the difference. SD = Standard Deviation. CI = Confidence Interval.

There were 211 unemployed patients in the study; 70.6% (149/211) were male; 75.8% (145/211) were Irish; 33.6% (71/211) had acquired HIV through intravenous drug use; 29.4% (62/211) were on methadone replacement therapy; mean age was 41.18 years; mean nadir CD4 T cell count was 198.828; mean current CD4 T cell count was 527.63; mean length of education was 12.1 years and mean duration of HIV diagnosis was 9.33 years.

Patients who were unemployed were more likely to endorse cognitive symptoms ( $p=0.000001$ ); to be male ( $p=0.000307$ ); to have acquired HIV through IV drug use ( $p<0.000001$ ); to be co-infected with hepatitis C ( $p<0.000001$ ); to have epilepsy ( $p=0.013$ ); to have a previous history of depression ( $p=0.000247$ ); to be on anti-epileptic medications ( $p=0.006$ ), methadone replacement therapy ( $p<0.000001$ ), benzodiazepines ( $p<0.000001$ ), antidepressants ( $p=0.000044$ ) or antipsychotics ( $p=0.013$ ); to be smokers ( $p<0.000001$ ); to have a positive screen for anxiety ( $p=0.008$ ) and/or depression ( $p=0.000001$ ); to consume greater than 40 20 units of alcohol per week or to have a history of ADS ( $p<0.000001$ ); to have been diagnosed for longer with HIV ( $p=0.002$ ); to have a lower nadir CD4 T cell count ( $p=0.000046$ ); to have a lower current CD4 T cell count ( $p=0.001$ ); to have fewer years of education ( $p<0.000001$ ) and were less likely to be virally suppressed ( $p=0.000214$ ) or have syphilis ( $p=0.001$ ). All of these characteristics are likely to influence cognition in this group and contribute to the higher prevalence rate amongst patients who were unemployed.



Table 2-9 Demographics of the Unemployed Patients Compared to the Rest of the Study Cohort (Categorical Variables).

| Characteristics          | Unemployed<br>(n=211) |        | Rest of the Cohort<br>(n=393) |        | P Value   | Std Residual for<br>Unemployed |
|--------------------------|-----------------------|--------|-------------------------------|--------|-----------|--------------------------------|
|                          | n                     | (%)    | n                             | (%)    |           |                                |
| Symptoms                 |                       |        |                               |        |           |                                |
| Yes                      | 114                   | (54)   | 132                           | (33.6) | 0.000001  |                                |
| Gender                   |                       |        |                               |        |           |                                |
| Male                     | 149                   | (70.6) | 327                           | (83.2) | 0.000307  |                                |
| Female                   | 62                    | (29.4) | 66                            | (16.8) |           |                                |
| Country of Birth         |                       |        |                               |        |           |                                |
| Ireland                  | 160                   | (75.8) | 268                           | (68.2) | 0.206     |                                |
| Europe                   | 16                    | (7.6)  | 36                            | (9.2)  |           |                                |
| Africa                   | 31                    | (14.7) | 69                            | (17.6) |           |                                |
| Asia                     | 0                     | (0)    | 8                             | (2)    |           |                                |
| South Africa             | 3                     | (1.4)  | 9                             | (2.3)  |           |                                |
| Other                    | 1                     | (0.5)  | 3                             | (0.8)  |           |                                |
| Mode of Transmission     |                       |        |                               |        |           |                                |
| Heterosexual             | 75                    | (35.5) | 123                           | (31.3) | <0.000001 |                                |
| MSM                      | 61                    | (28.9) | 237                           | (60.3) |           |                                |
| IVDU                     | 71                    | (33.6) | 22                            | (5.6)  |           | 6.8                            |
| Vertical                 | 1                     | (0.5)  | 2                             | (0.5)  |           |                                |
| Other                    | 3                     | (1.4)  | 9                             | (2.3)  |           |                                |
| Virally Suppressed       |                       |        |                               |        |           |                                |
| Yes                      | 152                   | (82.2) | 295                           | (92.5) | 0.000214  |                                |
| Hepatitis C co-infection |                       |        |                               |        |           |                                |
| Yes                      | 83                    | (70.3) | 35                            | (8.9)  | <0.000001 |                                |
| Syphilis                 |                       |        |                               |        |           |                                |
| Yes                      | 33                    | (15.6) | 107                           | (27.2) | 0.001     |                                |

| <u>Characteristics</u>            | <u>Unemployed</u><br>(n=211) |            | <u>Rest of the Cohort</u><br>(n=393) |            | <u>P Value</u> | <u>Std Residual for</u><br><u>Unemployed</u> |
|-----------------------------------|------------------------------|------------|--------------------------------------|------------|----------------|----------------------------------------------|
|                                   | <u>n</u>                     | <u>(%)</u> | <u>n</u>                             | <u>(%)</u> |                |                                              |
| <b>Epilepsy</b>                   |                              |            |                                      |            |                |                                              |
| Yes                               | 10                           | (4.7)      | 5                                    | (1.3)      | 0.013          |                                              |
| <b>Depression</b>                 |                              |            |                                      |            |                |                                              |
| Yes                               | 69                           | (32.7)     | 76                                   | (19.3)     | 0.000247       |                                              |
| <b>Anti-epileptic medications</b> |                              |            |                                      |            |                |                                              |
| Yes                               | 11                           | (5.2)      | 5                                    | (1.3)      | 0.006          |                                              |
| <b>Methadone</b>                  |                              |            |                                      |            |                |                                              |
| Yes                               | 62                           | (29.6)     | 9                                    | (2.3)      | <0.000001      |                                              |
| <b>Benzodiazepine Use</b>         |                              |            |                                      |            |                |                                              |
| Yes                               | 64                           | (30.3)     | 15                                   | (3.8)      | <0.000001      |                                              |
| <b>Antidepressants</b>            |                              |            |                                      |            |                |                                              |
| Yes                               | 38                           | (18)       | 28                                   | (7.1)      | 0.000044       |                                              |
| <b>Antipsychotic medications</b>  |                              |            |                                      |            |                |                                              |
| Yes                               | 12                           | (5.7)      | 7                                    | (1.8)      | 0.013          |                                              |
| <b>Smoking History</b>            |                              |            |                                      |            |                |                                              |
| Smoker                            | 134                          | (63.5)     | 144                                  | (36.6)     | <0.000001      |                                              |
| <b>Alcohol History</b>            |                              |            |                                      |            |                |                                              |
| None                              | 50                           | (23.7)     | 69                                   | (17.6)     | <0.000001      | 1.3                                          |
| <b>Units per week</b>             |                              |            |                                      |            |                |                                              |
| 1-20 units                        | 111                          | (52.6)     | 278                                  | (70.7)     |                | -2.1                                         |
| 21-40 units                       | 18                           | (8.5)      | 34                                   | (8.7)      |                | 0                                            |
| > 40 units                        | 14                           | (6.6)      | 7                                    | (1.8)      |                | 2.5                                          |
| Hx of ADS                         | 18                           | (8.5)      | 5                                    | (1.3)      |                | 3.5                                          |
| <b>Illicit Substance Use</b>      |                              |            |                                      |            |                |                                              |
| Never                             | 95                           | (45)       | 290                                  | (73.8)     | <0.000001      | -3.4                                         |
| Current Non IV Use                | 48                           | (22.7)     | 46                                   | (11.7)     |                | 2.6                                          |

| <u>Characteristics</u>                   | Unemployed<br>(n=211) |               | Rest of the Cohort<br>(n=393) |     | P Value  | Std Residual for<br>Unemployed |
|------------------------------------------|-----------------------|---------------|-------------------------------|-----|----------|--------------------------------|
|                                          | n                     | (%)           | n                             | (%) |          |                                |
|                                          | Current IV Use        | 6<br>(2.8)    | 1<br>(0.3)                    |     |          | 2.3                            |
|                                          | Previous              | 62<br>(29.5)  | 56<br>(14.2)                  |     |          | 3.2                            |
| Antiretroviral Therapy                   | Naïve                 | 23<br>(10.9)  | 72<br>(18.3)                  |     | 0.026    |                                |
|                                          | On HAART              | 185<br>(87.7) | 319<br>(81.2)                 |     |          |                                |
|                                          | MTCT                  | 1<br>(0.5)    | 2<br>(0.5)                    |     |          |                                |
|                                          | Previous exposure     | 2<br>(0.9)    | 0<br>(0)                      |     |          |                                |
| Positive Screen for Depression           | Yes                   | 36<br>(17.1)  | 19<br>(4.8)                   |     | 0.000001 |                                |
| Positive Screen for Anxiety              | Yes                   | 65<br>(30.8)  | 83<br>(21.1)                  |     | 0.008    |                                |
| Positive Screen for Cognitive Impairment | Yes                   | 138<br>(65.4) | 173<br>(44.0)                 |     | 0.000001 |                                |

IV = intravenous, Std = Standardised, Hx = history, ADS = Alcohol Dependence Syndrome, MTCT = Maternal to child transmission

Table 2-10 Characteristics of Unemployed Patients Compared to the Rest of the Cohort (Linear Variables)

|                                     |           | Unemployed<br>(n=211) | Rest of the Cohort<br>(n=393) | P Value   | 95% CI                 |
|-------------------------------------|-----------|-----------------------|-------------------------------|-----------|------------------------|
| Age (in years)                      | Mean (SD) | 41.18 (9.2808)        | 40.774 (10.6989)              | 0.68      | (-2.05) - (1.37)       |
|                                     | Median    | 41                    | 40                            |           |                        |
| Time since Diagnosis<br>(days)      | Mean (SD) | 3406.265 (2640.7879)  | 2758.575 (2405.1423)          | 0.002     | (-1065.03) - (-230.36) |
|                                     | Median    | 2933                  | 2186                          |           |                        |
| Nadir CD4 Count                     | Mean (SD) | 198.828 (161.8907)    | 255.207 (159.2693)            | 0.000046  | 29.42 - 83.34          |
|                                     | Median    | 178                   | 248                           |           |                        |
| Current CD4 Count                   | Mean (SD) | 527.63 (267.2708)     | 582.528 (242.6641)            | 0.011     | 12.72 - 97.08          |
|                                     | Median    | 506                   | 566                           |           |                        |
| Years of Education                  | Mean (SD) | 12.109 (3.6177)       | 15.448 (3.5626)               | <0.000001 | 2.74 - 3.94            |
|                                     | Median    | 12                    | 16                            |           |                        |
| Exposure to HAART (total)<br>(days) | Mean (SD) | 2291.71 (1748.871)    | 2186.638 (1725.9282)          | 0.512     | (-420.95) - (209.48)   |
|                                     | Median    | 1792.5                | 1807                          |           |                        |
| CPE Score                           | Mean (SD) | 6.946 (0.9683)        | 7.16 (0.7999)                 | 0.008     | 0.06 - 0.37            |
|                                     | Median    | 7                     | 7                             |           |                        |
| HADS - Anxiety Score                | Mean (SD) | 8.45 (4.622)          | 7.107 (4.2093)                | 0.000329  | (-2.07) - (-0.61)      |
|                                     | Median    | 8                     | 6                             |           |                        |



| HADS - Depression Score | Mean (SD) | Unemployed<br>(n=211) | Rest of the Cohort<br>(n=393) | P Value | 95% CI |
|-------------------------|-----------|-----------------------|-------------------------------|---------|--------|
|                         | Median    | 6.294 (4.4763)        | 3.641 (3.4369)                | <0.001* |        |

\*Performed using Mann Whitney U Tests and no confidence intervals available for the difference. SD = Standard Deviation. CI = Confidence Interval.

On univariate analysis the presence of neuropathy was associated with a reduced likelihood of a positive screen for cognitive impairment and on multivariate analysis the presence of neuropathy was also less likely to predict a positive screen. The numbers of patients with neuropathy in our cohort were small ( $n=25$ ) and the type of neuropathy was not classified further as to whether it was due to antiretroviral therapy or directly related to HIV. Due to the small number of patients with neuropathy in the study group, it is difficult to draw any significant conclusions about the relationship between neuropathy and cognitive impairment.

#### 2.5.2 Results of Irish Patients

Analysis was also carried out on the predictors of a positive screen for cognitive impairment in the patients who were all born in Ireland. All of these patients spoke English as their first language and would not have had any cultural issues with the cognitive screening tests as they would all have been accustomed to the concepts used in the screening tests through the Irish education system. The characteristics of the Irish patients compared to the rest of the study cohort are described in Table 2-11 and Table 2-12. The Irish patients were older ( $p<0.001$ ), had been diagnosed for longer ( $p<0.0001$ ), had fewer years of education ( $p=0.000003$ ), had been on HAART for longer ( $p<0.0001$ ), were more likely to be male ( $p<0.000001$ ), to have acquired HIV through intravenous drug use ( $p<0.000001$ ), to be co-infected with hepatitis C ( $p<0.000001$ ), to have a history of depression ( $p=0.000022$ ), to be on methadone replacement therapy ( $p=0.0000003$ ), benzodiazepines ( $p=0.000006$ ) or antidepressants ( $p=0.000003$ ) and were more likely to be smokers ( $p<0.000001$ ).

Table 2-11 Characteristics of Irish Patients Compared to the Rest of the Study Cohort (Categorical Variables)

| Characteristics          | Irish Patients<br>(n=428) |        |              | Rest of the Cohort<br>(n=176) |        |  | P Value   | Std Residual for<br>Irish Patients |
|--------------------------|---------------------------|--------|--------------|-------------------------------|--------|--|-----------|------------------------------------|
| Gender                   | n                         | (%)    |              | n                             | (%)    |  | <0.000001 |                                    |
|                          | 363                       | (84.8) | Male         | 113                           | (64.2) |  |           |                                    |
|                          | 65                        | (15.2) | Female       | 63                            | (35.8) |  |           |                                    |
| Mode of Transmission     | 94                        | (22)   | Heterosexual | 104                           | (59.1) |  | <0.000001 | -3.9                               |
|                          | 236                       | (55.1) | MSM          | 62                            | (35.2) |  |           |                                    |
|                          | 85                        | (19.9) | IVDU         | 8                             | (4.5)  |  |           |                                    |
|                          | 1                         | (0.2)  | Vertical     | 2                             | (1.1)  |  |           |                                    |
|                          | 12                        | (2.8)  | Other        | 0                             | (0)    |  |           |                                    |
| Virally Suppressed       | 320                       | (89.4) | Yes          | 127                           | (86.9) |  | 0.728     |                                    |
| Hepatitis C co-infection | 106                       | (24.8) | Yes          | 12                            | (6.8)  |  | <0.000001 | 2.4                                |
| Hepatitis B co-infection | 30                        | (7)    | Yes          | 17                            | (9.7)  |  | 0.269     |                                    |
| Depression               | 123                       | (28.7) | Yes          | 22                            | (12.5) |  | 0.000022  | 2                                  |
| Methadone                | 67                        | (15.7) | Yes          | 4                             | (2.3)  |  | 0.000003  | 2.4                                |
| Benzodiazepine Use       | 73                        | (17.1) | Yes          | 6                             | (3.4)  |  | 0.000006  | 2.3                                |
| Antidepressants          | 63                        | (14.7) | Yes          | 3                             | (1.7)  |  | 0.000003  | 2.4                                |

| <u>Characteristics</u>                   | Irish Patients<br>(n=428) |        | Rest of the Cohort<br>(n=176) |        | P Value   | Std Residual for<br>Irish Patients |
|------------------------------------------|---------------------------|--------|-------------------------------|--------|-----------|------------------------------------|
|                                          | n                         | (%)    | n                             | (%)    |           |                                    |
| Smoking History                          |                           |        |                               |        |           |                                    |
| Smoker                                   | 227                       | (53)   | 51                            | (29)   | <0.000001 | 2.1                                |
| Illicit Substance Use                    |                           |        |                               |        |           |                                    |
| Never Current Non IV Use                 | 240                       | (56.1) | 145                           | (82.4) | <0.000001 | -2                                 |
| Current IV Use                           | 81                        | (18.9) | 13                            | (7.4)  |           | 1.8                                |
| Previous Use                             | 7                         | (1.6)  | 0                             | (0)    |           | 0.9                                |
|                                          | 100                       | (23.4) | 18                            | (10.2) |           | 1.8                                |
| Antiretroviral Therapy                   |                           |        |                               |        |           |                                    |
| Naïve                                    | 68                        | (15.9) | 27                            | (15.3) | 0.476     |                                    |
| On HAAART                                | 358                       | (83.6) | 146                           | (83)   |           |                                    |
| MTCT Previous exposure                   | 1                         | (0.2)  | 2                             | (1.1)  |           |                                    |
|                                          | 1                         | (0.2)  | 1                             | (0.6)  |           |                                    |
| Positive Screen for Cognitive Impairment |                           |        |                               |        |           |                                    |
| Yes                                      | 194                       | (45.3) | 117                           | (66.5) | 0.000002  | -1.8                               |

IV = intravenous, Std = Standardised, Hx = history, MTCT = Maternal to child transmission.



Table 2-12 Characteristics of Irish Patients Compared to the Rest of the Study Cohort (Linear Variables)

|                                     |           | Irish Patients<br>(n=428) | Rest of the Cohort<br>(n=176) | P Value  | 95% CI           |
|-------------------------------------|-----------|---------------------------|-------------------------------|----------|------------------|
| Age (in years)                      | Mean (SD) | 42.194 (10.5)             | 37.733 (8.6)                  | <0.001*  |                  |
|                                     | Median    | 42                        | 37                            |          |                  |
| Time since Diagnosis<br>(days)      | Mean (SD) | 3318.306 (2730.7)         | 2173.903 (1589.833)           | <0.001*  |                  |
|                                     | Median    | 2637                      | 1957                          |          |                  |
| Nadir CD4 Count                     | Mean (SD) | 238.631 (162.85)          | 228.131 (161.15)              | 0.472    |                  |
|                                     | Median    | 225                       | 218                           |          |                  |
| Current CD4 Count                   | Mean (SD) | 581.534 (259.2)           | 519.125 (230.9)               | 0.006    | 18.201 - 106.62  |
|                                     | Median    | 556                       | 486                           |          |                  |
| Years of Education                  | Mean (SD) | 13.811 (3.835)            | 15.426 (3.89)                 | 0.000003 | (-2.3) - (-0.94) |
|                                     | Median    | 13.5                      | 16                            |          |                  |
| Exposure to HAART (total)<br>(days) | Mean (SD) | 2425.344 (1829.15)        | 1735.178 (1359.43)            | <0.001   |                  |
|                                     | Median    | 1934.5                    | 1465.5                        |          |                  |
| CPE Score                           | Mean (SD) | 7.072 (0.9)               | 7.103 (0.797)                 | 0.718    |                  |
|                                     | Median    | 7                         | 7                             |          |                  |
| HADS - Anxiety Score                | Mean (SD) | 7.916 (4.35)              | 6.75 (4.433)                  | 0.003    | 0.4 - 1.93       |
|                                     | Median    | 7                         | 6                             |          |                  |

| HADS - Depression Score | Irish Patients |         | Rest of the Cohort | P Value | 95% CI |
|-------------------------|----------------|---------|--------------------|---------|--------|
|                         | Mean (SD)      | (n=428) |                    |         |        |
|                         | 4.792 (4.198)  |         | 4.023 (3.55)       | 0.074   |        |
|                         | Median         | 4       | 3                  |         |        |

\*Performed using Mann Whitney U Tests and no confidence intervals available for the difference. SD = Standard Deviation. CI = Confidence Interval.

Table 2-13 Predictors of a Positive Screen for Cognitive Impairment in Patients Born in Ireland – Binary and Categorical Variables.

| <u>Characteristics</u>               |              | <u>Positive Screen</u><br>(n=194) |            | <u>Negative Screen</u><br>(n=234) |            | <u>P Value</u>                                  |
|--------------------------------------|--------------|-----------------------------------|------------|-----------------------------------|------------|-------------------------------------------------|
|                                      |              | <u>n</u>                          | <u>(%)</u> | <u>n</u>                          | <u>(%)</u> |                                                 |
| <b>Gender</b>                        | Male         | 157                               | (43.3)     | 206                               | (56.7)     | 0.041                                           |
|                                      | Female       | 37                                | (56.9)     | 28                                | (43.1)     |                                                 |
| <b>Mode of Transmission</b>          | Heterosexual | 43                                | (45.7)     | 51                                | (54.3)     | 0.000486<br>Std Residual<br>for IVDU<br>was 2.7 |
|                                      | MSM          | 89                                | (37.7)     | 147                               | (62.3)     |                                                 |
|                                      | IVDU         | 55                                | (64.7)     | 30                                | (35.3)     |                                                 |
|                                      | Vertical     | 0                                 | (0)        | 1                                 | (100)      |                                                 |
|                                      | Other        | 7                                 | (58.3)     | 5                                 | (41.7)     |                                                 |
| <b>Virally Suppressed</b><br>(n=504) | Yes          | 134                               | (41.9)     | 186                               | (58.1)     | 0.00029                                         |
|                                      | No           | 29                                | (76.3)     | 9                                 | (23.7)     |                                                 |
| <b>Hepatitis C</b>                   | Yes          | 68                                | (64.2)     | 38                                | (35.8)     | 0.000007                                        |
|                                      | No           | 126                               | (39.1)     | 196                               | (60.9)     |                                                 |
| <b>Hep C PCR Positive</b><br>(n=118) | Yes          | 48                                | (72.7)     | 18                                | (27.3)     | 0.000003                                        |
|                                      | No           | 20                                | (50)       | 20                                | (50)       |                                                 |
| <b>Hypercholesterolaemia</b>         | Yes          | 23                                | (30.3)     | 53                                | (69.7)     | 0.004                                           |
|                                      | No           | 171                               | (48.6)     | 181                               | (51.4)     |                                                 |
| <b>Neuropathy</b>                    | Yes          | 4                                 | (21.1)     | 15                                | (78.9)     | 0.034                                           |
|                                      | No           | 190                               | (46.5)     | 219                               | (53.5)     |                                                 |

| <u>Characteristics</u>        | <u>Positive Screen</u><br>(n=194) |            | <u>Negative Screen</u><br>(n=234) |            | <u>P Value</u> |
|-------------------------------|-----------------------------------|------------|-----------------------------------|------------|----------------|
|                               | <u>n</u>                          | <u>(%)</u> | <u>n</u>                          | <u>(%)</u> |                |
| <b>Stroke</b>                 | Yes                               | 4 (100)    | 0 (0)                             | (0)        | 0.041          |
|                               | No                                | 190 (44.8) | 234 (55.2)                        | (55.2)     |                |
| <b>Depression</b>             | Yes                               | 67 (54.5)  | 56 (45.5)                         | (45.5)     | 0.016          |
|                               | No                                | 127 (41.6) | 178 (58.4)                        | (58.4)     |                |
| <b>Lipid Lowering Therapy</b> | Yes                               | 17 (29.8)  | 40 (70.2)                         | (70.2)     | 0.015          |
|                               | No                                | 177 (47.7) | 194 (52.3)                        | (52.3)     |                |
| <b>Methadone</b>              | Yes                               | 52 (77.6)  | 15 (22.4)                         | (22.4)     | <0.000001      |
|                               | No                                | 142 (39.3) | 219 (60.7)                        | (60.7)     |                |
| <b>Benzodiazepines</b>        | Yes                               | 62 (84.9)  | 11 (15.1)                         | (15.1)     | <0.000001      |
|                               | No                                | 132 (37.2) | 223 (62.8)                        | (62.8)     |                |
| <b>Anti-depressants</b>       | Yes                               | 39 (61.9)  | 24 (38.1)                         | (38.1)     | 0.006          |
|                               | No                                | 155 (42.5) | 210 (57.5)                        | (57.5)     |                |
| <b>Anti-psychotics</b>        | Yes                               | 14 (82.4)  | 3 (17.6)                          | (17.6)     | 0.002          |
|                               | No                                | 180 (43.8) | 231 (56.2)                        | (56.2)     |                |
| <b>Employment History</b>     | Employed                          | 73 (32.6)  | 151 (67.4)                        | (67.4)     | <0.000001      |
|                               | Student                           | 7 (30.4)   | 16 (69.6)                         | (69.6)     |                |
|                               | Unemployed                        | 105 (65.6) | 55 (34.4)                         | (34.4)     |                |



| <u>Characteristics</u>         | Positive Screen<br>(n=194) |     | Negative Screen<br>(n=234) |     | P Value |
|--------------------------------|----------------------------|-----|----------------------------|-----|---------|
|                                | n                          | (%) | n                          | (%) |         |
| Smoking History                | Retired                    | 9   | (42.9)                     | 12  | (57.1)  |
|                                | Smoker                     | 125 | (55.1)                     | 102 | (44.9)  |
|                                | Non smoker                 | 35  | (30.2)                     | 81  | (69.8)  |
|                                | Ex-smoker                  | 34  | (40)                       | 51  | (60)    |
| Illicit Substance Use          | Never                      | 92  | (38.3)                     | 148 | (61.7)  |
|                                | Current Non IV Use         | 49  | (60.5)                     | 32  | (39.5)  |
|                                | Current IV Use             | 5   | (71.4)                     | 2   | (28.6)  |
|                                | Previous Hx                | 48  | (48)                       | 52  | (52)    |
| Positive Screen for Anxiety    | Yes                        | 62  | (53.4)                     | 54  | (46.6)  |
|                                | No                         | 132 | (42.3)                     | 180 | (57.7)  |
| Positive Screen for Depression | Yes                        | 31  | (67.4)                     | 15  | (32.6)  |
|                                | No                         | 163 | (42.7)                     | 219 | (57.3)  |

Std = Standardised, Hx = History, IV = Intravenous.

Table 2-14 Predictors of a Positive Screen for Cognitive Impairment in Irish Patients – Linear Variables Analysed by Independent T Test or Mann Whitney U Test.

|                      |           | Positive Screen<br>(n=194) | Negative Screen<br>(n=234) | P Value | 95% CI                |
|----------------------|-----------|----------------------------|----------------------------|---------|-----------------------|
| Nadir CD4 Count      | Mean (SD) | 221.375 (158.8195)         | 252.85 (165.0894)          | 0.047   | (-62.56) - (-0.383)   |
|                      | Median    | 207.5                      | 236                        |         |                       |
| Current CD4 Count    | Mean (SD) | 554.887 (281.9671)         | 603.721 (236.9321)         | 0.007   | (-98.1909) - (-0.522) |
|                      | Median    | 521                        | 587                        |         |                       |
| Years of Education   | Mean (SD) | 13.041 (4.0679)            | 14.449 (3.5132)            | <0.001* |                       |
|                      | Median    | 12                         | 14                         |         |                       |
| HADS - Anxiety Score | Mean (SD) | 8.619 (4.5055)             | 7.333 (4.1319)             | 0.002*  |                       |
|                      | Median    | 8.5                        | 7                          |         |                       |

\* Performed using Mann Whitney U Tests and no confidence intervals available for the difference. SD = Standard Deviation. CI = Confidence Interval.

**Table 2-15 Results of Logistic Regression Analysis of Irish Born Patients – R<sup>2</sup> was 22.7.**

| Variable                    | Wald  | P Value | Odds Ratio | Confidence Interval |
|-----------------------------|-------|---------|------------|---------------------|
| Mode of transmission - IVDU | 3.835 | 0.05    | 0.398      | 0.158 - 1.001       |
| Absence of neuropathy       | 5.45  | 0.02    | 0.282      | 0.098 - 0.816       |
| Use of benzodiazepines      | 11.94 | 0.001   | 5.411      | 2.077 - 14.099      |
| Unemployed                  | 0.011 | 6.463   | 1.803      | 1.145 - 2.84        |

IVDU – intravenous drug user

On univariate analysis female gender (p=0.041), endorsement of cognitive symptoms (p=0.01), acquisition of HIV through intravenous drug use (p=0.000486), not being virally suppressed (p=0.00029), co-infection with hepatitis C (p=0.000007), previous history of stroke (p=0.041) or depression (p=0.016), being on methadone replacement therapy (p<0.000001), benzodiazepines (p<0.000001), antidepressants (p=0.006) or antipsychotics (p=0.002), being unemployed (p<0.000001), smoking (p=0.000037), current non-intravenous use of illicit substances (p=0.002), positive screen for anxiety (p=0.04) or depression (p=0.001), lower nadir CD4 T cell count (p=0.047), lower current CD4 T cell count (p=0.007), and fewer years of education (p<0.001) were all associated with a positive screen for cognitive impairment. Presence of neuropathy (p=0.034) was associated with a reduced risk of a positive screen for cognitive impairment. IV drug use as a mode of acquisition of HIV, being a smoker and current non-intravenous drug use were all statistically significantly associated with a positive screen for cognitive impairment in the Irish group but not for the entire study cohort. This is likely a reflection of the demographics of the Irish patients recruited to the study. On multivariate analysis the factors associated with a positive screen for cognitive impairment were IV drug use as a mode of transmission, use of benzodiazepines and being unemployed.

### 2.5.3 Results of Patients Co-infected with Hepatitis C

Hepatitis C is a known risk factor for HAND (185, 187, 188). Sub-group analysis was carried out on patients co-infected with hepatitis C. Patients co-infected with hepatitis C were predominantly male (86/118, 72.9%) and Irish (106/118, 89.8%). Over half (66/118, 55.9%) endorsed cognitive symptoms. The majority were on HAART (111/118, 94.1%) and 85.6% (95/111) were virally suppressed. Just over half were on methadone replacement therapy (64/118, 54.2%). The majority were unemployed (83/118, 70.3%). Three quarters were smokers (90/118, 76.3%). Ex-IVDUs comprised 44.9% (53/118) of patients co-infected with hepatitis C but 6/118 (5.1%) were still currently injecting drugs and 38/118 (32.2%) were using illicit substances either orally or via inhalation. Almost two thirds of patients co-infected with hepatitis C (73/118, 61.9%) had a positive screen for cognitive impairment.



**Table 2-16 Predictors of a Positive Screen for Cognitive Impairment in Patients co-infected with Hepatitis C – Univariate Analysis using Chi Square Analysis or Fisher Exact Test.**

|                               |              | Positive Screen |            | Negative Screen |            | P Value  |
|-------------------------------|--------------|-----------------|------------|-----------------|------------|----------|
| <u>Characteristics</u>        |              | <u>(n=73)</u>   |            | <u>(n=45)</u>   |            |          |
|                               |              | <u>n</u>        | <u>(%)</u> | <u>n</u>        | <u>(%)</u> |          |
| Symptoms                      | Yes          | 49              | (74.2)     | 17              | (25.8)     | 0.002    |
|                               | No           | 24              | (46.2)     | 28              | (53.8)     |          |
| Gender                        | Male         | 50              | (58.1)     | 36              | (41.9)     | 0.172    |
|                               | Female       | 23              | (71.9)     | 9               | (28.1)     |          |
| Mode of Transmission          | Heterosexual | 17              | (73.9)     | 6               | (26.1)     | 0.369    |
|                               | MSM          | 5               | (45.5)     | 6               | (54.5)     |          |
|                               | IVDU         | 47              | (61.8)     | 29              | (38.2)     |          |
|                               | Other        | 4               | (50)       | 4               | (50)       |          |
| Virally suppressed            | Yes          | 55              | (57.8)     | 40              | (42.4)     | 0.178    |
|                               | No           | 13              | (81.2)     | 3               | (18.8)     |          |
| Hep C PCR Positive<br>(n=118) | Yes          | 51              | (69.9)     | 22              | (30.1)     | 0.04     |
|                               | No           | 22              | (48.9)     | 23              | (51.1)     |          |
| Lipid Lowering Therapy        | Yes          | 0               | (0)        | 4               | (100)      | 0.019    |
|                               | No           | 73              | (64)       | 41              | (46)       |          |
| Methadone                     | Yes          | 48              | (75)       | 16              | (25)       | 0.002    |
|                               | No           | 25              | (46.3)     | 29              | (53.7)     |          |
| Benzodiazepines               | Yes          | 46              | (82.1)     | 10              | (17.9)     | 0.000016 |
|                               | No           | 27              | (43.5)     | 35              | (56.5)     |          |
| Smoking History               | Smoker       | 61              | (67.8)     | 29              | (32.2)     | 0.044    |
|                               | Non smoker   | 7               | (500       | 7               | (50)       |          |
|                               | Ex-smoker    | 5               | (35.7)     | 9               | (64.3)     |          |

Table 2-17 Predictors of a Positive Screen for Cognitive Impairment in Patients Co-infected with Hepatitis C – Analysis of Linear Variables Using Independent T Test.

|                      |           | Positive Screen<br>(n=73) | Negative Screen<br>(n=45) | P Value | 95% CI                |
|----------------------|-----------|---------------------------|---------------------------|---------|-----------------------|
| Years of Education   | Mean (SD) | 10.712 (3.1157)           | 12.178 (3.6387)           | 0.022   | (-2.7132) - (10.2172) |
|                      | Median    | 10                        | 12                        |         |                       |
| HADS - Anxiety Score | Mean (SD) | 9.616 (4.3064)            | 7.533 (4.4803)            | 0.013   | 0.4415 - 3.7247       |
|                      | Median    | 10                        | 7                         |         |                       |

\* Performed using Mann Whitney U Tests and no confidence intervals available for the difference. SD = Standard Deviation. CI = Confidence Interval.

Table 2-18 Logistic Regression of Patients Co-infected with Hepatitis C – R<sup>2</sup> 33.5.

| Variable           | Wald  | P Value | Odds Ratio | Confidence Interval |
|--------------------|-------|---------|------------|---------------------|
| Symptoms           | 5.136 | 0.023   | 2.913      | 1.155 - 7.342       |
| Hep C PCR positive | 3.857 | 0.05    | 2.503      | 1.002 - 6.255       |
| Unemployed         | 0.011 | 6.463   | 1.803      | 1.145 - 2.84        |

Predictors of a positive screen for cognitive impairment in the group of patients co-infected with hepatitis C were the endorsement of cognitive symptoms ( $p=0.002$ ), being PCR positive for hepatitis C RNA ( $p=0.032$ ), being on methadone replacement therapy ( $p=0.002$ ), benzodiazepines use ( $p=0.000016$ ), smoking ( $p=0.044$ ) and fewer years of education ( $p=0.022$ ). Being on lipid lowering therapy was associated with a reduced likelihood of a positive screen for cognitive impairment in this group ( $p=0.019$ ) but only four patients within this group were on lipid lowering therapy so these results have to be interpreted with caution. On logistic regression analysis the factors associated with a positive screen for cognitive impairment within this group were the endorsement of cognitive symptoms, being PCR positive for hepatitis C RNA and the use of benzodiazepines.

#### 2.5.4 Results of Female Patients

Female gender was associated with a higher rate of a positive screen for cognitive impairment in this study group and subgroup analysis was carried out on the 128 female participants. Demographics of the female patients in this study are outlined in Table 2-19. They were more likely to endorse cognitive symptoms ( $p=0.028$ ), to be African ( $p<0.000001$ ), to have acquired HIV through heterosexual transmission ( $p<0.000001$ ), to be on benzodiazepines ( $p=0.006$ ), to be students or unemployed ( $p=0.000005$ ), to be non-smokers ( $p=0.01$ ), to not drink alcohol ( $p<0.000001$ ), to have a positive screen for anxiety ( $p=0.007$ ) and depression ( $p=0.000019$ ) and to have a lower nadir CD4 T cell count ( $p=0.01$ ).

Table 2-19 Demographics of Female Patients (Categorical Variables).

| Characteristics             | Women<br>(n=128) |        | Men<br>(n=476) |        | P Value   | Std Residual for<br>Female Patients |
|-----------------------------|------------------|--------|----------------|--------|-----------|-------------------------------------|
| Symptoms                    | n                | (%)    | n              | (%)    |           |                                     |
| Yes                         | 63               | (49.2) | 183            | (38.4) | 0.028     |                                     |
| English as a first language |                  |        |                |        |           |                                     |
| Yes                         | 73               | (57)   | 395            | (83)   | <0.000001 |                                     |
| Mode of Transmission        |                  |        |                |        |           |                                     |
| Heterosexual                | 101              | (78.9) | 97             | (20.4) | <0.000001 | 9.1                                 |
| MSM                         | NA               |        | 298            | (62.6) |           |                                     |
| IVDU                        | 25               | (19.5) | 68             | (14.3) |           | 1.2                                 |
| Vertical                    | 1                | (0.8)  | 2              | (0.4)  |           | 0.5                                 |
| Other                       | 1                | (0.8)  | 11             | (2.3)  |           | -1                                  |
| Country of Birth            |                  |        |                |        |           |                                     |
| Ireland                     | 65               | (50.8) | 363            | (76.3) | <0.000001 | -2.7                                |
| Europe                      | 6                | (4.7)  | 46             | (9.7)  |           | -1.5                                |
| Africa                      | 55               | (43)   | 45             | (9.5)  |           | 7.3                                 |
| Asia                        | 1                | (0.75) | 7              | (1.5)  |           | -0.5                                |
| South Africa                | 0                | (0)    | 12             | (2.5)  |           | -1.6                                |
| Other                       | 1                | (0.75) | 3              | (0.5)  |           | 0.2                                 |
| Virally Suppressed          |                  |        |                |        |           |                                     |
| Yes                         | 98               | (86)   | 349            | (89.4) | 0.088     |                                     |
| Hypercholesterolaemia       |                  |        |                |        |           |                                     |
| Yes                         | 11               | (8.6)  | 89             | (18.7) | 0.005     |                                     |
| Syphilis                    |                  |        |                |        |           |                                     |
| Yes                         | 7                | (5.5)  | 133            | (27.9) | <0.000001 |                                     |



| Characteristics                  | Women<br>(n=128) |        | Men<br>(n=476) |        | P Value   | Std Residual for<br>Female Patients |
|----------------------------------|------------------|--------|----------------|--------|-----------|-------------------------------------|
|                                  | n                | (%)    | n              | (%)    |           |                                     |
| Depression                       |                  |        |                |        |           |                                     |
| Yes                              | 32               | (28.1) | 109            | (22.9) | 0.219     |                                     |
| Anxiety                          |                  |        |                |        |           |                                     |
| Yes                              | 6                | (4.7)  | 16             | (3.4)  | 0.477     |                                     |
| Methadone                        |                  |        |                |        |           |                                     |
| Yes                              | 19               | (14.8) | 52             | (10.9) | 0.222     |                                     |
| Benzodiazepine Use               |                  |        |                |        |           |                                     |
| Yes                              | 26               | (20.3) | 53             | (11.1) | 0.006     |                                     |
| Smoking History                  |                  |        |                |        |           |                                     |
| Smoker                           | 53               | (41.4) | 225            | (47.3) | 0.01      | -0.8                                |
| Ex-smoker                        | 16               | (12.5) | 96             | (20.2) |           | -1.6                                |
| Non smoker                       | 59               | (46.1) | 155            | (32.6) |           | 2                                   |
| Illicit Substance Use            |                  |        |                |        |           |                                     |
| Never                            | 94               | (72.4) | 291            | (61.1) | 0.017     | 1.4                                 |
| Current Non IV Use               | 9                | (7)    | 85             | (17.9) |           | -2.4                                |
| Current IV Use                   | 1                | (0.8)  | 6              | (1.3)  |           | -0.4                                |
| Previous Use                     | 24               | (18.8) | 94             | (19.7) |           | -0.2                                |
| Alcohol History (units per week) |                  |        |                |        |           |                                     |
| None                             | 52               | (40.6) | 67             | (14.1) | <0.000001 | 5.3                                 |
| 1 - 20 units                     | 64               | (50)   | 325            | (68.3) |           | -2                                  |
| 21 - 40 units                    | 3                | (2.4)  | 49             | (10.3) |           | -2.4                                |
| >40 units                        | 4                | (3.1)  | 17             | (3.6)  |           | -0.2                                |
| Hx of ADS                        | 5                | (3.9)  | 18             | (3.8)  |           | 0.1                                 |

| <u>Characteristics</u>                   | Women<br>(n=128) |               | Men<br>(n=476) |        | P Value  | Std Residual for<br>Female Patients |
|------------------------------------------|------------------|---------------|----------------|--------|----------|-------------------------------------|
|                                          | n                | (%)           | n              | (%)    |          |                                     |
| Employment History                       | Employed         | 47<br>(36.7)  | 276<br>(58)    | (58)   | 0.000005 | -2.6                                |
|                                          | Student          | 18<br>(14.1)  | 31<br>(6.5)    | (6.5)  |          | 2.4                                 |
|                                          | Unemployed       | 62<br>(48.4)  | 149<br>(31.3)  | (31.3) |          | 2.6                                 |
|                                          | Retired          | 1<br>(0.8)    | 20<br>(4.2)    | (4.2)  |          | -1.6                                |
| Positive Screen for Anxiety              | Yes              | 43<br>(33.6)  | 105<br>(22.1)  | (22.1) | 0.007    |                                     |
| Positive Screen for Depression           | Yes              | 24<br>(18.8)  | 31<br>(6.5)    | (6.5)  | 0.000019 |                                     |
| Positive Screen for Cognitive Impairment | Yes              | 88<br>(68.80) | 223<br>(46.8)  | (46.8) | 0.000011 |                                     |

Std = Standardised, ADS = Alcohol Dependence Syndrome.

Table 2-20 Demographics of Female Patients (Linear Variables).

|                                     |           | Women<br>(n=128)   | Men<br>(n=476)     | P Value |
|-------------------------------------|-----------|--------------------|--------------------|---------|
| Age (in years)                      | Mean (SD) | 39.313 (8.85)      | 41.319 (10.5)      | 0.054   |
|                                     | Median    | 37.5               | 41                 |         |
| Time since Diagnosis<br>(days)      | Mean (SD) | 3148.5 (2237.35)   | 2940.826 (2575.03) | 0.063   |
|                                     | Median    | 2637               | 2243.5             |         |
| Nadir CD4 Count                     | Mean (SD) | 203.82 (144.65)    | 244.178 (165.85)   | 0.01    |
|                                     | Median    | 198.5              | 227                |         |
| Current CD4 Count                   | Mean (SD) | 539.055 (255.21)   | 569.857 (251.88)   | 0.221   |
|                                     | Median    | 526.5              | 542                |         |
| Years of Education                  | Mean (SD) | 13.81 (3.94)       | 14.41 (3.9)        | 0.127   |
|                                     | Median    | 14                 | 14                 |         |
| Exposure to HAART (total)<br>(days) | Mean (SD) | 2267.417 (1681.88) | 2212.997 (1750.32) | 0.768   |
|                                     | Median    | 1886               | 1768               |         |
| CPE Score                           | Mean (SD) | 7.07 (0.896)       | 7.09 (0.865)       | 0.869   |
|                                     | Median    | 7                  | 7                  |         |
| HADS - Anxiety Score                | Mean (SD) | 8.49 (5.04)        | 7.33 (4.19)        | 0.026   |
|                                     | Median    | 8                  | 7                  |         |
| HADS - Depression Score             | Mean (SD) | 5.688 (4.85)       | 4.267 (3.73)       | 0.008   |
|                                     | Median    | 4                  | 3                  |         |

Table 2-21 Predictors of a Positive Screen for Cognitive Impairment in Female Patients – Univariate Analysis using Chi Square Analysis or Fisher Exact Test.

| <u>Characteristics</u> | Positive Screen<br>(n=88) |              | Negative Screen<br>(n=40) |     | P Value  |
|------------------------|---------------------------|--------------|---------------------------|-----|----------|
|                        | n                         | (%)          | n                         | (%) |          |
| Country of Birth       | Ireland                   | 37<br>(56.9) | 28<br>(43.1)              |     | 0.000144 |
|                        | Europe                    | 2<br>(33.3)  | 4<br>(66.7)               |     |          |
|                        | Africa                    | 49<br>(89.1) | 6<br>(10.9)               |     |          |
|                        | Asia                      | 0<br>(0)     | 1<br>(100)                |     |          |
|                        | Other                     | 0<br>(0)     | 1<br>(100)                |     |          |
| Use of Benzodiazepines | Yes                       | 24<br>(92.3) | 2<br>(7.7)                |     | 0.004    |
|                        | No                        | 64<br>(62.7) | 38<br>(37.3)              |     |          |

Table 2-22 Predictors of a Positive Screen for Cognitive Impairment in Female Patients – Analysis of Linear Variables Using Independent T Test.

|                                   | Positive Screen<br>(n=88) |                      | Negative Screen<br>(n=40) |  | P Value | 95% CI                |
|-----------------------------------|---------------------------|----------------------|---------------------------|--|---------|-----------------------|
| Age in years                      | Mean (SD)                 | 37.898 (8.5689)      | 42.425 (8.7468)           |  | 0.007   | (-7.7819) - (-1.2727) |
|                                   | Median                    | 36                   | 43                        |  |         |                       |
| Duration of ART (total)<br>(days) | Mean (SD)                 | 1934.101 (1488.0496) | 2998.861 (1864.8847)      |  | 0.005*  |                       |
|                                   | Median                    | 1521                 | 3013                      |  |         |                       |

\* Performed using Mann Whitney U Tests and no confidence intervals available for the difference. SD = Standard Deviation. CI = Confidence Interval.



The predictors of a positive screen for cognitive impairment in female patients were younger age ( $p=0.007$ ), African country of birth ( $p=0.000144$ ), use of benzodiazepines ( $p=0.004$ ) and shorter duration of antiretroviral therapy ( $p=0.005$ ). On logistic regression analysis of the female patients the only variable that was significant was the use of benzodiazepines ( $R^2$  was 47.4, WALD 11.388,  $p=0.001$ , OR 16.196, 95% CI 3.214 – 81.627).

#### 2.5.5 Results of Screening for Depression

There was a positive screen for depression in 9.1% (55/604) of the study group. 56.4% (31/55) were male; 83.6% (46/55) were Irish; 38.2% (21/55) acquired HIV through heterosexual transmission, 27.3% (15/55) acquired HIV through homosexual transmission, 30.9% (17/55) acquired HIV through IV drug use, one person (1.8%) acquired HIV through vertical transmission and one person (1.8%) through occupational risks; 92.7% (51/55) were on HAART and 39/51 (76.5%) were virally suppressed; 65.5% (36/55) were unemployed and 61.8% (34/55) were smokers. Median age was 40, median duration of diagnosis was 7.3 years, median nadir CD4 count was 227, median current CD4 count was 545, median years of education was 12 and median duration of HAART was 6.2 years. There was a history of depression in 37/55 (67.3%) of those with a positive screen for depression. Of the group with a positive screen for depression, 6/55 (10.9%) had a history of an anxiety disorder.

On univariate analysis a positive screen for depression was associated with female gender ( $p=0.000019$ ), acquisition of HIV through IV drug use ( $p=0.001$ ), lack of

virological suppression ( $p=0.001$ ), co-infection with hepatitis C ( $p=0.000059$ ), a history of depression ( $p<0.000001$ ), a history of anxiety ( $p=0.01$ ), being on anti-epileptic medications ( $p=0.025$ ), methadone replacement therapy ( $p<0.000001$ ), benzodiazepines ( $p<0.000001$ ), antipsychotic medications ( $p=0.00002$ ), being unemployed ( $p=0.000012$ ), smoking ( $p=0.048$ ), consuming more than 40 units of alcohol weekly or a history of alcohol dependence syndrome ( $p=0.003$ ), a previous history of illicit substance abuse ( $p=0.007$ ), a positive screen for cognitive impairment ( $p=0.003$ ) and fewer years of education ( $p=0.001$ ). Logistic regression analysis showed that a history of depression and the use of anti-psychotics were independent predictors of a positive screen for depression. These factors likely reflect patients who had a diagnosis of depression and were on treatment for a psychiatric disorder.

**Table 2-23 Characteristics of Patients with a Positive Screen for Depression – Statistically Significant Variables.**

| Characteristics               |              | Positive Screen |        | Negative Screen |        | P Value                                      |
|-------------------------------|--------------|-----------------|--------|-----------------|--------|----------------------------------------------|
|                               |              | (n=55)          |        | (n=549)         |        |                                              |
|                               |              | n               | (%)    | n               | (%)    |                                              |
| Gender                        | Male         | 31              | (6.5)  | 445             | (93.5) | 0.000019                                     |
|                               | Female       | 24              | (18.8) | 104             | (81.2) |                                              |
| Mode of Transmission          | Heterosexual | 21              | (10.6) | 177             | (89.4) | 0.001<br>Std Residual<br>for IVDU was<br>2.9 |
|                               | MSM          | 15              | (5)    | 283             | (95)   |                                              |
|                               | IVDU         | 17              | (18.3) | 76              | (81.7) |                                              |
|                               | Vertical     | 1               | (33.3) | 2               | (66.7) |                                              |
|                               | Other        | 1               | (8.3)  | 11              | (91.7) |                                              |
| Virally Suppressed<br>(n=504) | Yes          | 39              | (8.7)  | 408             | (91.3) | 0.001                                        |
|                               | No           | 12              | (21.1) | 45              | (78.9) |                                              |
| Hepatitis C                   | Yes          | 22              | (18.6) | 96              | (81.4) | 0.000059                                     |
|                               | No           | 33              | (6.8)  | 453             | (93.2) |                                              |
| History of Depression         | Yes          | 37              | (25.5) | 108             | (74.5) | <0.000001                                    |
|                               | No           | 18              | (3.9)  | 441             | (96.1) |                                              |
| History of Anxiety            | Yes          | 6               | (27.3) | 16              | (72.7) | 0.01                                         |
|                               | No           | 49              | (8.4)  | 533             | (91.6) |                                              |
| Methadone                     | Yes          | 19              | (26.8) | 52              | (73.2) | <0.000001                                    |
|                               | No           | 36              | (6.8)  | 497             | (93.2) |                                              |
| Benzodiazepines               | Yes          | 24              | (30.4) | 55              | (69.6) | <0.000001                                    |
|                               | No           | 31              | (5.9)  | 494             | (94.1) |                                              |
| Anti-epileptic medications    | Yes          | 4               | (25)   | 12              | (75)   | 0.025                                        |
|                               | No           | 51              | (8.7)  | 537             | (91.3) |                                              |
| Anti-psychotics               | Yes          | 7               | (36.8) | 12              | (63.2) | 0.00002                                      |
|                               | No           | 48              | (8.2)  | 537             | (91.8) |                                              |
| Employment History            | Employed     | 16              | (5)    | 307             | (95)   | 0.000012                                     |
|                               | Student      | 3               | (6.1)  | 46              | (93.9) |                                              |
|                               | Unemployed   | 36              | (17.1) | 175             | (82.9) |                                              |
|                               | Retired      | 0               | (0)    | 21              | (100)  |                                              |
| Smoking History               | Smoker       | 34              | (12.2) | 244             | (87.8) | 0.048                                        |
|                               | Non smoker   | 14              | (6.5)  | 200             | (93.5) |                                              |

|                                             |                    | Positive Screen |            | Negative Screen |            | P Value                     |
|---------------------------------------------|--------------------|-----------------|------------|-----------------|------------|-----------------------------|
| <u>Characteristics</u>                      |                    | <u>(n=55)</u>   |            | <u>(n=549)</u>  |            |                             |
|                                             |                    | <u>n</u>        | <u>(%)</u> | <u>n</u>        | <u>(%)</u> |                             |
|                                             | Ex-smoker          | 7               | (6.3)      | 105             | (93.7)     |                             |
| Alcohol Consumption<br>(Units per week)     | None               | 17              | (14.3)     | 102             | (85.7)     | 0.003                       |
|                                             | 1 - 10 units       | 17              | (5.9)      | 272             | (94.1)     |                             |
|                                             | 11 - 20 units      | 7               | (7)        | 93              | (93)       |                             |
|                                             | 21 - 30 units      | 2               | (5)        | 38              | (95)       |                             |
|                                             | 31 - 40 units      | 2               | (16.7)     | 10              | (83.3)     |                             |
|                                             | > 40 units         | 5               | (23.8)     | 16              | (76.2)     |                             |
|                                             | Hx of ADS          | 5               | (21.7)     | 18              | (78.3)     |                             |
| Illicit Substance Use                       | Never              | 25              | (6.5)      | 360             | (93.5)     | 0.007                       |
|                                             | Current Non IV Use | 9               | (9.6)      | 85              | (90.4)     |                             |
|                                             | Current IV Use     | 1               | (14.3)     | 6               | (85.7)     |                             |
|                                             | Previous Hx        | 26              | (16.9)     | 98              | (83.1)     |                             |
| Positive Screen for<br>Cognitive Impairment | Yes                | 39              | (12.5)     | 272             | (87.5)     | 0.003                       |
|                                             | No                 | 16              | (5.5)      | 277             | (94.5)     |                             |
| Years of Education                          | Mean (SD)          | 12.6 (3.5984)   |            | 14.45 (3.9114)  |            | 0.001                       |
|                                             | Median             | 12              |            | 14              |            | 95% CI<br>(-2.93) - (-0.77) |

Std = Standardised, Hx = History, IV = Intravenous.

**Table 2-24 Binary Logistic Regression Results of Predictors of a Positive Screen for Depression.**

| <b>Variable</b>    | <b>Wald</b> | <b>P Value</b> | <b>Odds Ratio</b> | <b>Confidence Interval</b> |
|--------------------|-------------|----------------|-------------------|----------------------------|
| Hx of Depression   | 27.38       | <0.001         | 6.717             | 3.291 - 13.71              |
| Antipsychotic Meds | 5.119       | 0.024          | 4.437             | 1.221 - 16.131             |

Hx = History. Meds = medications.

## 2.5.6 Results of Screening for Anxiety

There was a positive screen for anxiety in 24.5% (148/604) of the study group. Of the 148 patients with a positive screen for anxiety 70.9% (105/148) were male; 78.4% (116/148) were Irish; 33.1% (49/148) had acquired HIV through heterosexual



transmission, 45.9% (68/148) through homosexual transmission, 19.6% (29/148) through IV drug use, one person (0.7%) acquired HIV vertically and one person through occupational risks; 84.5% (125/148) were on HAART and 84.8% (106/125) were virally suppressed; 54.7% (81/148) were smokers; 46.6% (69/148) were employed, 7.4% were students (11/148), 44% (65/148) were unemployed and 2% (3/148) were retired. Median age was 39, median duration of diagnosis was 6.35 years, median nadir CD4 count was 234.5, median current CD4 count was 543, median number of years was 13 and median duration of HAART was 5.2 years. There was a history of an anxiety disorder in 14/148 (9.5%) of those with a positive screen for anxiety. There was a history of depression in 72/148 (48.6%) of the group with a positive screen for anxiety.

A positive screen for anxiety was associated with female gender ( $p=0.007$ ), co-infection with hepatitis C ( $p=0.016$ ), neuropathy ( $p=0.021$ ), a history of depression ( $p<0.000001$ ), a history of anxiety ( $p=0.000014$ ), methadone replacement therapy ( $p=0.005$ ), use of benzodiazepines ( $p<0.000001$ ), anti-depressants ( $p=0.000007$ ), anti-psychotics ( $p=0.019$ ), smoking ( $p=0.05$ ), fewer years of education ( $p=0.012$ ) and a positive screen for cognitive impairment ( $p=0.041$ ). Logistic regression analysis showed that female gender, a history of depression, a history of anxiety and neuropathy were all independent predictors of a positive screen for anxiety.

Table 2-25 Characteristics of Patients with a Positive Screen for Anxiety – Statistically Significant Variables.

| Characteristics                          |            | Positive Screen |        | Negative Screen |        | P Value          |
|------------------------------------------|------------|-----------------|--------|-----------------|--------|------------------|
|                                          |            | (n=148)         |        | (n=456)         |        |                  |
|                                          |            | n               | (%)    | n               | (%)    |                  |
| Gender                                   | Male       | 105             | (22.1) | 371             | (77.9) | 0.007            |
|                                          | Female     | 43              | (33.6) | 85              | (66.4) |                  |
| English as 1st Language                  | Yes        | 124             | (26.5) | 344             | (73.5) | 0.035            |
|                                          | No         | 24              | (17.6) | 112             | (82.4) |                  |
| Hepatitis C                              | Yes        | 39              | (33.1) | 79              | (66.8) | 0.016            |
|                                          | No         | 109             | (22.4) | 377             | (77.6) |                  |
| Neuropathy                               | Yes        | 11              | (44)   | 14              | (56)   | 0.021            |
|                                          | No         | 137             | (23.7) | 442             | (76.3) |                  |
| History of Depression                    | Yes        | 72              | (49.7) | 73              | (50.3) | <0.000001        |
|                                          | No         | 76              | (16.6) | 383             | (83.4) |                  |
| History of Anxiety                       | Yes        | 14              | (63.6) | 8               | (36.4) | 0.000014         |
|                                          | No         | 134             | (23)   | 448             | (77)   |                  |
| Methadone                                | Yes        | 27              | (38)   | 44              | (62)   | 0.005            |
|                                          | No         | 121             | (22.7) | 412             | (77.3) |                  |
| Benzodiazepines                          | Yes        | 38              | (48.1) | 41              | (51.9) | <0.000001        |
|                                          | No         | 110             | (21)   | 415             | (79)   |                  |
| Anti-depressants                         | Yes        | 31              | (47)   | 35              | (53)   | 0.000007         |
|                                          | No         | 117             | (21.7) | 421             | (78.3) |                  |
| Anti-psychotics                          | Yes        | 9               | (47.4) | 10              | (52.6) | 0.019            |
|                                          | No         | 139             | (23.8) | 446             | (76.2) |                  |
| Smoking History                          | Smoker     | 81              | (29.1) | 197             | (70.9) | 0.05             |
|                                          | Non smoker | 23              | (20.5) | 89              | (79.5) |                  |
|                                          | Ex-smoker  | 44              | (20.6) | 170             | (79.4) |                  |
| Positive Screen for Cognitive Impairment | Yes        | 87              | (28)   | 224             | (72)   | 0.041            |
|                                          | No         | 61              | (20.8) | 232             | (79.2) |                  |
| Years of Education                       | Mean (SD)  | 13.581 (3.6122) |        | 14.509 (3.989)  |        | 0.012            |
|                                          | Median     | 13              |        | 15              |        |                  |
|                                          |            |                 |        |                 |        | (-1.65) - (-0.2) |

**Table 2-26 Binary Logistic Regression Results of Predictors of a Positive Screen for Anxiety.**

| Variable         | Wald   | P Value | Odds Ratio | Confidence Interval |
|------------------|--------|---------|------------|---------------------|
| Female Gender    | 4.433  | 0.035   | 1.719      | 1.038 - 2.847       |
| Hx of Depression | 38.248 | <0.001  | 4.655      | 2.859 - 7.578       |
| Hx of Anxiety    | 9.178  | 0.002   | 4.68       | 1.724 - 12.703      |
| Hx of Neuropathy | 4.747  | 0.029   | 2.766      | 1.108 - 6.909       |

Hx = history.

**2.6 Discussion**

**2.6.1 Discussion of Results of Screening for Cognitive Impairment**

Just over 50% of the study group had a positive screen for cognitive impairment. The statistically significant factors associated with a positive screen for cognitive impairment included female gender, younger age, an African country of birth, endorsement of cognitive symptoms, co-infection with hepatitis C, lack of virological suppression, being on medications with CNS effects (methadone, benzodiazepines, anti-depressants, anti-psychotics), being unemployed, lower nadir and current CD4 counts, fewer years of education and positive screens for anxiety and depression.

Carter et al demonstrated that cognitive complaints predicted poorer neuropsychological performance (237). Rourke et al found that cognitive complaints were correlated significantly with depressive symptoms and with neuropsychological measures of attention, working memory, psychomotor skills and learning efficiency. They found that depressive symptoms accounted for the majority of variance explained in cognitive complaints while psychomotor efficiency predicted the remaining variance (238). Bassel et al studied the relationship between working



memory and subjective cognitive complaints in 36 HIV positive adults. Working memory was the strongest predictor of cognitive complaints and depressive symptoms also independently predicted cognitive complaints (239).

The effect of country of birth may be due to the lack of culturally appropriate normative data for the screening tests. Cultural differences and acculturation have been shown to affect the results of neuropsychological testing in African-Americans compared to Caucasian Americans (240). Acculturation explains the process of cultural and psychological change that results from the meeting of different cultures. It also refers to the degree to which an ethnic group adopts the behaviours, language and values of the dominant culture.

Cultural and racial influences on neuropsychological testing have been documented previously. Manly et al examined English speaking African-Americans and Caucasian Americans in Manhattan and demonstrated that after accounting for occupational attainment and medical conditions, African Americans performed worse on measures of categorical fluency, figure memory, verbal abstraction and visuospatial skills (241). Jacobs et al performed neuropsychological testing on English speaking and Spanish speaking older people. There were significant differences between the English speakers and the Spanish speakers on non-verbal tests as well as category fluency (242). Other factors influence the results of neuropsychological testing in different ethnic groups such as reading level and educational quality (243, 244). There is significant heterogeneity in cultural, educational and linguistic exposures within ethnic



groups so even creating separate normative data for different ethnic groups is challenging and not without potential disadvantages (245).

A recent study from Uganda showed that 68.6% of a cohort of HIV positive patients had evidence of cognitive impairment compared to 16% of a HIV negative control group suggesting that there is a significant effect of HIV on cognition independent of African race and that it is not just due to a lack of culturally appropriate tests and normative data (246). It is also possible that the higher rate of a positive screen for cognitive impairment in the African patients in this study may be due to a clade effect however clade status was not recorded as part of this study.

Female gender, endorsement of cognitive symptoms, lack of virological suppression, co-infection with hepatitis C, use of methadone, benzodiazepines, anti-depressants or anti-psychotics, unemployment, positive screens for anxiety and/or depression, lower nadir and/or current CD4 T cell counts and fewer years of education were all predictive of a positive screen for cognitive impairment both in the entire study cohort and in just the Irish patients. All of the Irish patients spoke English as their first language and hence should have fewer or no cultural issues with the screening tests used therefore it suggests that these factors are an accurate assessment of the predictors of a positive screen for cognitive impairment. It should be noted that the Irish patients formed the largest group within the study cohort so are likely to be driving some of the statistically significant results found in the entire cohort analysis. The younger age is being driven primarily by the African patients for whom it was the only predictor of a positive screen.

Women have been under-represented in studies on cognition in HIV as a lot of studies focused on men due to the higher incidence of HIV among men than women, particularly in the United States, due to the predominance of HIV among men who have sex with men and the higher number of men who are IV drug users compared to women (247). Of 236 studies conducted on the neurological and neuropsychological consequences of HIV between 1988 and 1997, only 31% of studies included women with approximate representational parity (248).

In the early years of the epidemic it seemed that women might be more vulnerable to the development of the neurological consequences of AIDS. A retrospective review of 6548 patients with AIDS in Europe found that the risk of AIDS dementia complex was twice as high in women compared to men (249). A prospective study of 146 patients with HIV in the United States did not find any gender difference in the neurocognitive complications of HIV (250). A review of 6293 patients who seroconverted to HIV did not find any gender differences in the pre-HAART era but from 1997 onwards they found that women had a lower risk of AIDS, death and development of AIDS dementia complex (251). A prospective study of 71 patients with HIV associated dementia did not find any gender difference in progression of HAD (252). Wojna et al demonstrated a rate of cognitive impairment of 77.6% in Hispanic women in Puerto Rica (253).

HIV positive women performed worse on tests of motor skill and probabilistic learning than HIV positive men. These are tests of nondeclarative or procedural learning and rely on the integrity of the fronto-striatal tracts (254). There was a higher prevalence rate of cognitive impairment among HIV positive women who were not on HAART in a

multi-centre study of women living with HIV (255). Richardson et al examined the role of hepatitis C co-infection on cognition in women with HIV. HIV positive women who were co-infected with hepatitis C and were not on HAART were more likely to be cognitively impaired than those who were HIV and hepatitis C negative (256).

Substance abuse, including excess alcohol consumption, is a known risk factor for HAND (183). Alcohol abuse and HIV have additive and interactive effects on cognition (257). In an observational study of British civil servants excessive alcohol consumption among men was associated with a faster cognitive decline compared to those who consumed light to moderate amounts of alcohol (258). A meta-analysis of fifteen studies did not show an increased risk of dementia amongst those who were classified as "heavy drinkers". This may reflect a selection bias within the studies used (259). Some protective effect of moderate alcohol consumption has been demonstrated. Older people without the APOE epsilon 4 allele who consumed up to three units of wine daily had a lower risk of developing Alzheimer's Disease (260). Other meta-analyses also demonstrated a protective relationship between moderate consumption of alcohol and the development of Alzheimer's Disease but not for vascular dementia or cognitive decline (261-263). Therefore it is not surprising that patients with a history of excess alcohol consumption were more likely to have a positive screen for cognitive impairment and that it was an independent predictor of a positive screen for cognitive impairment on multivariate analysis.

People on methadone replacement therapy have demonstrated impairment in psychomotor speed, working memory, decision making and inhibitory mechanisms



(264). A study in 2005 examined the effects of long-term opioid abuse from methadone maintenance therapy. The performance of abstinent abusers on neuropsychological tests fell between that of the control group and the methadone maintenance group, suggesting that methadone maintenance may be associated with additional impairment over and above that associated with long-term abuse of opioids (265). In a further study 60% of people currently abusing opiates showed significant impairment on neuropsychological tests. The drug free group of addicts fell between the controls and the group actively abusing opioids without any significant differences. The authors suggested that recovery of cognitive function may occur during abstinence (266).

In a pilot study of 30 HIV positive and 30 HIV negative substance abusers, the HIV positive substance abusers displayed significantly more motor impairment which was specifically related to impaired cognition in this group and independent of degree of immunocompromise. HIV status was a significant predictor of learning and speeded processing after controlling for reading level, methadone replacement therapy and positive urine toxicology (267). Margolin et al examined the factors affecting cognition in HIV positive drug users. Independent predictors of neuropsychological test performance were HIV viral load, educational level and premorbid medical and psychiatric conditions (268).

Long term use of benzodiazepines has been shown to affect cognition particularly the domains of visuospatial ability, speed of processing, sustained attention and verbal learning (269-271). There is evidence of improvement of cognitive functioning after



withdrawal of benzodiazepine use but patients may still remain impaired compared to healthy controls or normative data at least six months post withdrawal of benzodiazepines (269, 272). In a French study of long-term benzodiazepine use on cognitive functioning in young adults, benzodiazepine use was associated with impairment in long-term memory only in women (273).

Unemployment has been shown to be a functional consequence of HAND (201-204). Due to the limitations of cross-sectional studies it is not possible to establish a causative relationship between unemployment and cognitive impairment. It was statistically significantly related to the presence of a positive screen for cognitive impairment in this study. Patients with cognitive impairment are more likely to have difficulties maintaining employment but it is also possible that patients who are unemployed may be more likely to perform poorer on cognitive testing due to sociodemographic reasons or the co-existence of a substance abuse disorder.

A lower nadir CD4 T cell count (68, 179-181) and a lower educational level (181) have both been shown to be risk factors for HAND in international studies. It has been hypothesised that a lower nadir CD4 T cell count represents a “legacy event” that contributes to the development of HAND (68). A lower nadir CD4 T cell count also indicates that patients were more immunocompromised prior to initiation of HAART and this may relate to late presentation, poor engagement in care and sociodemographic factors such as poor social circumstances or a chaotic lifestyle.

Within this cohort we found that 9.1% had a positive screen for depression and that 24.5% had a positive screen for anxiety. In a European prevalence study the rate of depression in adults was 8.56% (274). A Swedish study found prevalence rates of 17.2% and 14.7% for depression and anxiety respectively however it must be noted that data was obtained through a postal survey with a response rate of 44.3% and there was likely to have been a selection bias in those who chose to respond which may account for the higher prevalence rates in this study (275). Mackenzie et al examined the prevalence rate of generalised anxiety disorder in 12,312 adults over the age of 55 and found a prevalence rate of 2.8% (276).

Both of these screens were associated with a positive screen for cognitive impairment. Female gender, co-infection with hepatitis C, a history of depression or anxiety, methadone replacement therapy, anti-psychotic medications, smoking and fewer years of education were all associated with positive screens for depression and anxiety. These factors were also significantly related to a positive screen for cognitive impairment suggesting that there is a complex interplay amongst these factors in relation to cognitive impairment, depression and anxiety.

#### 2.6.2 Strengths of the Study

The major strength of this study is the large number of participants from a single centre who were unselected and were a representative sample of a clinical population living with HIV in Ireland. All of the cognitive screens were conducted by the PhD candidate to avoid bias or differences in the administration of the test.

### 2.6.3 Limitations of the Study

The BNCS is a screening tool and while it was designed to be sensitive to HIV related cognitive impairment, it may also detect other causes of cognitive impairment. It was not designed to draw conclusions about the severity of the impairment in those who had a positive screen. Due to its higher rate of false positives and negatives, it may be more suited to large cohort studies as opposed to individual patients (175). The lack of available normative data for all the cultural and racial groups within our study is another limitation and therefore local, cultural or racial differences may account for some of the statistically significant results found in this study however most of these statistically significant relationships were also seen within the Irish sub-group analysis, all of whom spoke English as their first language and for whom the normative data should be applicable. This suggests that the statistically significant relationships found in this study are relevant and not just a bias of the lack of culturally appropriate normative data. Another limitation of the study is the cross-sectional nature of the study as it does not allow definitive conclusions with regard to causality to be drawn and only allows conclusions with regard to associations between the dependent variable and independent variables.

## 2.7 Conclusions

This was the first large scale cross-sectional screening programme in Ireland for cognitive impairment in the setting of HIV infection. A prevalence rate of 51.5% of a positive screen for cognitive impairment was demonstrated in this study and highlighted the necessity of a structured prospective screening programme for

cognitive impairment at our institution. Multiple risk factors exist for HAND. HAND may be a heterogeneous disease with differing risk factors contributing to the development of HAND in susceptible individuals, possibly with synergistic effects of individual risk factors. HAND and affective symptoms or disorders are likely to be inter-related and not entirely separate entities co-occurring.



### **3 Neuropsychological Assessment of Patients with a Positive Screen for Cognitive Impairment**

#### **3.1 Introduction**

##### **3.1.1 Outline of the chapter**

This chapter focuses on the patients who underwent detailed neuropsychological assessment in order to determine the cognitive profile of a cohort of HIV positive Irish patients with a positive screen for cognitive impairment. The underlying hypothesis of the study is that there are both inflammatory/immune mediated and degenerative changes in HAND. The candidate expected to find that HIV positive patients would exhibit features consistent with other neurodegenerative conditions and that there would be evidence of more cortical features on neuropsychological assessment in the post HAART era as opposed to the predominant subcortical features of the pre-HAART era.

##### **3.1.2 Clinical features and Neurocognitive Profile of HIV Associated Neurocognitive Disorders**

###### ***3.1.2.1 Clinical Features in the 1980s***

Navia et al described the clinical features of AIDS dementia complex in a series of 46 patients in 1986 on whom they also had autopsy findings. There were 24 patients who were not demented in the study also. The cognitive impairment developed after the diagnosis of AIDS in most of their patients but in one third of their patients the

patients were cognitively impaired either at the time of diagnosis of AIDS or prior to the diagnosis of AIDS. Patients exhibited disturbance in cognitive, behavioural and motor function. Patients complained of forgetfulness, loss of concentration, difficulty recalling recent events and losing their train of thought. Motor symptoms included lower limb weakness, loss of balance and abnormal co-ordination. Apathy and social withdrawal were the most common behavioural symptoms (53).

On testing of cognition, patients demonstrated inattention and recent memory loss with difficulty performing serial 7s and recalling a series of objects at 5 minutes. The most common finding on neurological examination was ataxia of gait while one third of patients had hyperreflexia. In the later stages of the disease patients demonstrated global cognitive dysfunction and the majority had psychomotor retardation. They also exhibited worsening ataxia, hypertonia, urinary or faecal incontinence and in some cases lower limb weakness requiring the use of a wheelchair. Grasp reflexes and tremor were also commonly seen. Navia et al coined the term AIDS dementia complex in this paper and suggested that it was consistent with a subcortical dementia given the prominent psychomotor slowing and the absence of cortical signs such as aphasia and apraxia (53). The neuropathology findings of the 70 patients supported this hypothesis given the predominance of white matter and subcortical nuclei pathology (54). Grant et al also found impairments in abstraction, learning and speed of information processing in patients with AIDS in the 1980s (277). Heaton et al found increased rates of neuropsychological impairment at each successive stage of HIV infection in 389 HIV positive men. The domains most often affected were attention, speed of information processing and learning efficiency and they suggested that this

pattern was consistent with earliest involvement of subcortical or fronto-striatal brain systems (278). Power et al also described the clinical triad of progressive cognitive decline, motor dysfunction and behavioural abnormalities associated with HIV associated dementia and agreed with the subcortical nature of the pathology (279).

#### ***3.1.2.2 Changes in HAND Before and During the HAART Era***

Heaton et al reviewed the changes in HAND before and during the HAART era. In the post HAART era more cortical findings were demonstrated. Impairment in learning and memory and executive function were more predominant than the subcortical impairments in motor skills, cognitive speed and verbal fluency that were common in the pre-HAART era. Recall, working memory and attention were stable across the two time periods in the study by Heaton et al (280). Cysique et al also examined the changing pattern of HAND across the two eras and demonstrated an improvement in attention, verbal fluency and visuospatial skills, but there was a deterioration in learning efficiency and complex attention (281).

#### ***3.1.2.3 Motor Skills and Speed of Information Processing***

Impairment in motor skills and information processing has been evident in HAND since the early 1980s. Motor slowing was a required feature of HIV associated dementia in the 1991 diagnostic criteria (56). Bradykinesia and bradyphrenia are prominent neurological features of cognitive impairment in the setting of HIV infection (282). Older HIV positive adults were more impaired on the motor scale of the Unified Parkinson's Disease Rating Scale compared to HIV negative age, gender and ethnicity



matched controls, especially on measures of bradykinesia, hypomimia, action/postural tremor and hand agility (283). Motor slowing in HIV infection has also been assessed using timed gait test (284), finger tapping (278, 285) and manual dexterity (286, 287). Woods et al suggested that impaired performance on tests of information processing speed could arise from a variety of deficits including attention, visuoperception, working memory, praxis and motor skills (65).

#### **3.1.2.4 Episodic Memory**

Episodic memory impairment is a sensitive indicator of HAND (286). Peavy et al administered the California Verbal Learning Test to 31 symptomatic HIV positive, 94 asymptomatic HIV positive and 40 HIV negative subjects to examine the prevalence and nature of verbal memory deficits in HIV. Symptomatic HIV positive subjects were significantly impaired compared to HIV negative control subjects on measures of acquisition and retention. They were significantly less likely than control subjects to use a semantic clustering strategy to support recall. They concluded that the profile of verbal memory deficits exhibited by the subgroup of impaired HIV positive subjects was similar to that of patients with Huntington's Disease (a typical subcortical dementia) and different from that of patients with Alzheimer's disease (a cortical dementia) and this finding was consistent with the known predominance of subcortical neuropathological changes associated with HIV infection (288). Meyer et al examined the effects of HIV and recent illicit drug use on verbal memory in 952 HIV positive and 443 HIV negative women. HIV infection and recent illicit drug use were both associated with statistically significantly worse verbal learning and memory independently and there was an additional interaction between recent illicit drug use and HIV serostatus



which negatively impacted verbal learning and memory only in HIV positive women (289).

Episodic memory deficits are most consistent with a mixed encoding and retrieval profile (65) however there is considerable heterogeneity in the memory profile of HIV infection but most support a frontal-striatal conceptualization of verbal memory performance in the setting of HIV infection (290). This pattern of mixed encoding and retrieval issues is characterised by impaired immediate and delayed free recall but relatively better performance on tasks of recognition (65, 291). Rapid forgetting is rarely displayed in HIV infection and when it does occur has been attributed to shallow encoding (65). Scott et al demonstrated that patients with HAD relied disproportionately on recency regions of the list when assessing recall using the Hopkins Verbal Learning Test Revised. This indicated a passive recall style of echoing only the words within their auditory attention span. The patients with HAD did not show significant improvement on measures of recognition and Scott et al concluded that this was most consistent with a primary encoding deficit (292). Woods et al have shown that HIV positive patients have deficits in strategic aspects of memory encoding and retrieval with a computerised visuospatial temporal order memory task further supporting the hypothesis of a mixed encoding and retrieval problem in HAND (293). Castelo et al have demonstrated impairment of the hippocampal system as well as the prefrontal system during episodic encoding in HIV positive patients during a functional MRI study. The HIV positive patients demonstrated significantly reduced signal intensity changes in the right posterior hippocampus, right inferior frontal gyrus, and left lingual gyrus. They also exhibited more activity within the lateral frontal and

posterior parietal regions (294). This suggests that there is hippocampal involvement in addition to the role of the fronto-striatal circuits in the memory profile of HIV.

#### **3.1.2.5 Prospective Memory**

Prospective memory (“remembering to remember”) involves the execution of future intentions and is postulated to be largely dependent on prefrontal-striatal circuits (295). It is also affected in HIV and particularly affects daily functioning that requires self-initiated cues and retrieval (296). Prospective memory performance was correlated with validated clinical measures of executive functions, episodic memory (free recall), and verbal working memory, but not with tests of semantic memory, retention, or recognition discrimination in a study of 42 HIV positive and 29 HIV negative subjects. Carey et al suggested that these findings indicated that prospective memory impairment in the setting of HIV infection is primarily driven by a breakdown in the strategic/executive aspects of retrieving future intentions and this is consistent with a prefronto-striatal circuit pathogenesis (295).

Gonzalez et al demonstrated deficits in complex motor skills without any evidence of impairment of procedural learning (297). In an earlier study (1993) Martin et al had suggested that there may be impairment of motor skill learning in HIV (298). A further study in 2011 found that HIV positive women performed poorly on tests of motor skill learning and probabilistic learning but there were no significant differences between HIV positive and negative men (254).

### **3.1.2.6 Attention and Concentration**

Reger et al conducted a meta-analysis of the neuropsychological sequelae of HIV infection and found that the deficits with the largest effect sizes in the group of patients with AIDS were attention and concentration with a moderate effect size and a large effect size in motor functioning (299). Attentional deficits have been shown in the areas of response inhibition (300), divided attention as assessed by a dual task condition of both auditory and visual choice paradigms (301) and visual attention (302). Visual attentional deficits were associated with driving accidents in HIV positive subjects with cognitive impairment (302). Hardy et al reviewed reaction time in HIV infection and using Brinkley plots demonstrated that patients with HIV were on average 22% slower than HIV negative controls (282).

Chang et al conducted a functional MRI study of 11 HIV positive patients and suitably matched HIV negative controls in order to evaluate the neural correlates of attention and working memory deficits. They performed tasks that required varying levels of attention for working memory. Patients with HIV showed greater activation in the parietal regions for the simpler tasks and showed greater activation additionally in the frontal lobes when performing the more difficult tasks. They postulated that the task-dependent increased frontal activation in patients with HIV suggested that the neural correlate of attentional deficits may be excessive attentional modulation as a result of fronto-striatal brain injury (303). In a further study by Chang et al they performed fMRI while subjects underwent a set of visual attention tasks with increasing levels of attentional load. Patients showed decreased activation in the normal visual attention network (dorsal parietal, bilateral prefrontal and cerebellar regions) and increased



activation in adjacent or contralateral brain regions. They concluded that HIV-associated brain injury led to reduced efficiency in the normal attention network, thus requiring reorganisation and increased usage of neural reserves to maintain performance during attention-requiring tasks (304). Ernst et al performed magnetic resonance spectroscopy and functional MR imaging in 14 HIV positive patients. They found positive correlations between the blood oxygenation level dependent (BOLD) signal strength in the working memory network (posterior parietal cortex and lateral prefrontal cortex) and the concentrations of frontal white matter and basal ganglia metabolites that are predominant in glial cells. There was no correlation with the concentration of N-acetyl compounds, which are markers of neuronal viability, or with metabolite concentrations in the frontal grey matter. Ernst et al proposed that the inflammatory glial abnormalities reduced the efficiency of neural processing necessitating compensatory increases in attention in patients, and associated BOLD signals, to perform a given task (305).

#### **3.1.2.7 Executive Function**

Executive functions include reasoning, task flexibility, problem solving, planning, complex problem solving abilities, self-directed behaviours and set shifting functions. Executive function may be assessed by abstraction, problem solving, set shifting and reasoning. Executive function depends on the frontal cortex, basal ganglia and posterior parietal lobe (65). HIV positive patients usually score lower on executive tasks such as the Stroop Colour Word Test (289, 300, 306, 307), Trail Making Test Part B (278, 299, 308) and the Wisconsin Card Sorting Test (237). These three tests assess cognitive shifting and response inhibition. Kupprat et al examined a cohort of HIV



positive men who have sex with men and found that executive impairment was present regardless of age and educational level (309).

### **3.1.2.8 Visuospatial Skills**

Visuospatial/visuoconstructional skills are the ability to detect, understand, manipulate and integrate visual stimuli in the context of its environment (65). Visuospatial deficits were not seen prominently in early studies of HAND however deficits were noted in spatial abilities that relied on the integrity of the fronto-striato-parietal networks such as egocentric spatial tasks (65). Abnormalities were noted in spatial attentional tasks (310, 311). Hardy et al found perceptual span deficits in patients with HIV and did not find any differences in response strategy due to disinhibition or psychomotor speed (312). Olesen et al demonstrated deficits on tasks of mental rotation and hierarchical pattern perception in HIV and these are tasks which depend highly on parietal function (313). Weber et al explored mental rotation further and examined mental rotation of hands in HIV infection and found that HIV positive patients made more errors on the task than did HIV negative controls. The errors were associated with poorer performance on tasks of executive function and working memory suggesting that the fronto-striatal-parietal networks were disrupted (314). Bogdanova et al demonstrated impairment of numerical and spatial cognition in HIV infection through tasks of mental number line bisection, physical line bisection and physical number line orientation. This again provided evidence for disruption of fronto-striato-parietal networks in HAND (315).

### **3.1.2.9 *Speech and Language***

Speech and language were long thought to be normal in HAND and for deficits, such as aphasia, to occur more commonly in the setting of opportunistic infection or other intra-cerebral pathology (65). There is however evidence of impairment of language in HIV infection. Lopez et al reviewed six HIV positive patients with speech motor control disorders after HIV infection. The patients exhibited an ataxic dysarthria along with other neurological signs including action-intention tremors, ataxic gait and release signs. The authors attributed the speech disorder to cerebellar dysfunction. The patients were followed up for one year and did not develop HIV associated dementia within that time frame but the authors commented in the paper that the patients were slow in timed decision-making tasks and had impaired procedural learning. This may suggest that although the patients did not develop a frank dementia within the follow-up time period they may still have been cognitively impaired (316). McCabe et al examined pragmatic communication skills in five HIV positive men and found abnormalities with fluency, turn taking quantity, turn taking interruption/overlap and vocal quality. They concluded that these behaviours were associated with subcortical impairment (317).

White et al studied verbal fluency in patients with HAD and found that they were significantly impaired on letter fluency with relatively intact category fluency (318). Woods et al examined the cognitive underpinnings of verbal fluency deficits in 21 patients with HAD, 51 HIV positive non demented patients and 30 HIV negative healthy controls. The HAD group displayed fewer switches between phonemic clusters and a higher proportion of response errors, predominantly intrusion errors, compared to the

healthy control group and the non-demented HIV positive group but there were no significant differences between the groups on average phonemic cluster size. They concluded that deficits occurred as a result of disruption of rule-guided lexical-semantic search strategies in HAD rather than depleted lexical-semantic memory stores. They postulated that these deficits could be mediated by prefrontal-striatal circuit dysfunction (319). A further study evaluated action and noun fluency in HIV infection. Action fluency has been shown to be more sensitive to frontal-basal ganglia disruption than noun fluency. HIV positive patients generated fewer actions (verbs) than control subjects (320). Iudicello conducted a meta-analysis of verbal fluency in HIV and concluded that mild word generation deficits were evident in HIV infection with similar impairments in both letter and category fluency. Effect size increased with advancing HIV disease severity (321). A subsequent study by Iudicello et al found that HIV infection was associated with greater impairments in switching, but not semantic clustering within an animal fluency task. When the same group were administered an alternating category fluency task the deficits in switching were further exacerbated (322). A further study by Iudicello et al demonstrated that HIV positive patients benefitted from cueing in verbal fluency tasks (323).

### 3.1.3 History of the Neuropsychological Tests Used

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was initially developed in 1998 and examines five cognitive domains (immediate recall, visuospatial and constructional skills, attention, language and delayed recall). It was developed to identify and characterise abnormal cognitive decline in older adults and as a screening test for younger adults (324). It has been widely used in a variety of



diseases including depression (325), mild cognitive impairment (326), Parkinson's disease (327, 328) and Huntington's disease (329). It has not been validated for use in HIV positive populations to date.

The Addenbrooke's Cognitive Exam Revised (ACE-R) is a 100-point test that assesses five cognitive domains (attention/orientation, memory, verbal fluency, language and visuospatial abilities). It has been validated in patients with dementia (Alzheimer's disease and Frontotemporal dementia), mild cognitive impairment and healthy controls (330, 331). It has not been validated for use in the setting of HIV however the ACE-R has been shown to be sensitive to early cognitive impairment (330) and to distinguish between patients with progressive degenerative disorders and those with affective disorders (332). It is easy to use and does not require specialist training. Larner evaluated its utility in a routine cognitive clinical setting and a wide age range (24 – 85) and confirmed its diagnostic utility (333).

The Montreal Cognitive Assessment (MoCA) is a 30-point test that encompasses eight cognitive domains (334). The domains tested include executive, visuospatial, language, attention, and concentration, working memory, memory and orientation. It is freely available, has been translated into over thirty languages and is easy to use and score (335). It examines both cortical and subcortical processes (336). The MoCA was initially validated for the detection of mild cognitive impairment and early Alzheimer's disease and a cut-off score of less than 26 was utilised (334). This test has been validated in the setting of HIV (337, 338). Overton et al evaluated 200 HIV positive patients on HAART and the MoCA was compared to a standardised neuropsychological battery and using a



cut-off score of less than 26 yielded a sensitivity of 63%, a specificity of 71%, a positive predictive value of 79% and a negative predictive value of 53% (337). Hasbun et al evaluated 100 HIV positive patients naïve to HAART and found that the MoCA had good screening accuracy with a cut-off score of less than 26 (sensitivity 85%, specificity 40%, positive predictive value 81% and negative predictive value 48%) (338). Milanini et al evaluated the utility of the MoCA to screen for HAND in patients over the age of 60 and found a sensitivity of 72% and specificity of 67% with a cut-off of less than 26 (339). These three studies were published after the initiation of the candidate's study.

The Frontal Assessment Battery (FAB) is an 18-point test that focuses on executive function. It was devised by Dubois et al in order to assess frontal lobe function easily and quickly in a clinical setting. Dubois et al demonstrated that the FAB was sensitive to executive function and differentiated between healthy controls and patients with neurodegenerative diseases with prominent executive involvement (Parkinson's disease, multiple system atrophy, corticobasal degeneration and Frontotemporal dementia) (340). It can be used to discriminate between dementias with a frontal dysexecutive syndrome or phenotype and those with an amnesic presentation such as Alzheimer's disease (341, 342).

#### 3.1.4 Use of Retention Scores

Retention scores are the relative amount of material retained on delayed recall in relation to the amount initially acquired. They have been recommended as an index of rapid forgetting which is considered a hallmark of hippocampal dysfunction. Alzheimer's disease is the prototypical example of a disorder with characteristic rapid

forgetting or a densely amnesic picture on neuropsychological testing and loss of hippocampal volume on neuroimaging. Retention scores of word and story memory have been used to differentiate between Alzheimer's disease and Frontotemporal dementia (343). Auditory verbal learning test percent retention was used to differentiate between Alzheimer's disease and dementia with Lewy bodies (344). Percent retention scores have also been used to predict progression to Alzheimer's disease from amnesic mild cognitive impairment (MCI). Griffith et al successfully classified 85.7% of their study group (49 patients with MCI) as either converters to Alzheimer's disease or MCI non-converters, with 76.9% sensitivity and 88.9% specificity using Dementia Rating Scale Initiation/Perseveration and Wechsler Memory Scale Visual Reproduction percent retention scores (345). Albert et al also supported the use of retention scores in the assessment of episodic memory impairment to distinguish patients with MCI due to Alzheimer's disease (346).

Clark et al examined the diagnostic accuracy of percent retention scores on the verbal memory subtests of the RBANS for the diagnosis of Alzheimer's disease and mild cognitive impairment. They examined 73 patients with Alzheimer's disease, 44 patients with amnesic MCI and 20 healthy controls. They found that in order to distinguish between healthy controls and patients with Alzheimer's disease the optimal cut off scores were less than 60% on list retention and less than 70% on story retention. These cut off points yielded a correct classification in 94.6% of healthy controls and 91.4% of patients with Alzheimer's disease. With the use of likelihood ratios patients with a score of less than 60% on list retention were ten times more likely to have Alzheimer's disease and patients with a score greater than 60% were twenty-two times more likely

to not have Alzheimer's disease. Scores of less than 70% on list retention and less than 80% on story retention yielded the most optimal accuracy for distinguishing between healthy controls and patient with amnesic MCI. A score of less than 70% on list retention indicated that patients were six times more likely to have amnesic MCI when compared with healthy controls and scores of less than 80% on story retention indicated that patients were five times more likely to have amnesic MCI (347).

### **3.2 Study Aims**

**Aim 1:** To determine the detailed cognitive profile of a group of Irish patients with HIV in the post HAART era who have had a positive screen for cognitive impairment.

### **3.3 Methodology**

#### **3.3.1 Study Design and Recruitment**

This was a cross-sectional observational study. Patients with a positive screen for cognitive impairment were recruited. Recruitment commenced on 4 August 2011 and was completed on 28 November 2013. All assessments were carried out by the candidate in order to limit any potential bias in the methodology of testing. They were given a patient information leaflet (see Appendix 7) and signed a consent form (see Appendix 8).

#### **3.3.2 Inclusion and Exclusion Criteria**

Patients were included in the study if they were HIV positive, attending the outpatient clinic in St. James's Hospital, had a positive screen for cognitive impairment, over the



age of 18, capable of giving informed consent, had sufficient use of the English language to be able to participate and were willing to voluntarily participate in the study.

Patients were excluded if they were HIV negative, under the age of 18, had insufficient English to partake, had a physical disability or limitation which affected their ability to complete the cognitive testing, were unwilling to participate voluntarily in the study or were actively injecting illicit substances.

### 3.3.3 Neuropsychological Tests Used

All patients underwent a full neurological history and examination. Neuropsychological assessment was performed using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), the Addenbrooke Cognitive Examination Revised (ACE-R), the Montreal Cognitive Assessment (MoCA) and the Frontal Assessment Battery (FAB). A copy of these tests can be found in Appendix 9.

The ACE-R assesses five cognitive domains. No adjustment was made for the age or educational level of the participant. The total score possible was 100 and consisted of the sum of the scores of each of the five cognitive domains. A score of less than 88 was considered abnormal.

The MoCA assesses eight domains. The total score possible was 30 and consisted of the sum of the scores of each of the eight domains. One point was added for



participants who had 12 or less years of education (334, 335). An abnormal score was less than 26.

The FAB assesses executive function only. The total score possible was 18. The normative data used was taken from that published by Appollonio et al (348). It was adjusted for age (from 20 up to 95) and educational attainment of the patient (from 1-3 years up to >13 years). Although the normative data is taken from an Italian paper, there is a paucity of normative data published for this test and it allowed for adjustment for age and education which was important for this study given the wide age range and educational level of the study participants. Slachevsky et al found that a cut-off score of 12 had the highest sensitivity and specificity to differentiate patients with Alzheimer's disease and Frontotemporal dementia however no adjustment was suggested for age or education and the populations studied were older so the Italian normative data was deemed to be the most appropriate to use for this study instead of a standard cut-off score.

All tests were administered and scored according to published instructions.

#### 3.3.4 Determination of a Diagnosis of HIV Associated Neurocognitive Disorders

The RBANS was used for the determination of a diagnosis of HAND as there were standard deviations and normative data available for it for a wide age range. Asymptomatic neurocognitive impairment (ANI) was defined as one standard deviation below the mean on two domains with no functional impairment. Mild neurocognitive disorder (MND) was defined as one standard deviation below the mean on two

domains with mild functional impairment. HIV associated dementia (HAD) was defined as two standard deviations below the mean on two domains with significant functional impairment. Full diagnostic criteria are outlined in Table 1-6 (57). Functional impairment was assessed during neurological history taking and was based on self-report from the study participants and by collateral history where available (see Table 3-1). Contributing and confounding conditions were classified according to the guidelines set by the expert working group of the National Institute of Mental Health and the National Institute of Neurological Diseases and Stroke (57).

**Table 3-1 Assessment of Function in HAND**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>Mild Functional Decline (2 or more of the following)</b></p> <p>1) Self report or other report of some increased assistance with at least two IADLs such as medication management, financial management, shopping, meal preparation, light housekeeping, laundry, driving, use of public transportation, maintaining personal schedules, understanding media events, and child care. (More IADLs could be considered as appropriate to the individual.)</p> <p>2) Patient is unable to perform some aspects of a previous job. This is not due to medical symptoms.</p> <p>3) Although patient may maintain employment and/or full IADL independence, he or she reports less efficiency, reduced productivity, more errors in performing tasks, more difficulty meeting expectations, or greater effort expended performing the same activities.</p> <p>4) In the absence of significant depression (e.g. Beck Depression Inventory &gt; 17), which may bias reporting of symptoms, patient reports that he or she is experiencing increased difficulty with two or more aspects of cognition in daily life. These may include difficulties with 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 or her way about, calculating, or following directions or instructions. Reports of these difficulties also may be obtained from a knowledgeable informant. (Instruments to assess</p> |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

depression that allow one to separate out items concerning somatic symptoms from those describing depressed mood are preferred for this purpose, as somatic symptoms associated with depression may also be caused by HIV itself.)

5) If performance-based, standardized functional tests are administered, patient scores  $>1$  SD below an appropriate normative mean on at least one such task.

**Major functional decline requires two or more of the following that are not readily attributable to medical or other comorbid conditions in the judgment of the examiner:**

1) Patient is unable to maintain former employment and this is not due to systemic illness or other factors not related to cognitive impairment (e.g., healthcare coverage being dependent upon disability status).

2) Patient requires substantially greater assistance (or is dependent) with more than two IADLs, as listed above.

3) Patient or a knowledgeable informant reports that he or she experiences/shows significantly greater difficulty with 4 or more aspects of cognition, as listed above. However, self-report is not sufficient (would need confirmation by another informant) if patient is significantly depressed (e.g., BDI  $> 17$ ).

4) If performance-based, standardized functional tasks are administered, patient scores  $>2$  SD below an appropriate normative mean on at least one such task, or  $>1$  SD below the mean on at least two tasks.

IADLs = instrumental activities of daily living, BDI = Beck Depression Inventory, SD = standard deviation.

Taken from Antinori et al (57).

### 3.3.5 Ethical Approval and Data Protection

The study had full ethical approval from the combined ethics committee of the Adelaide and Meath Hospitals Incorporating the National Children's Hospital (AMNCH) and St. James's Hospital (SJH). All patients who participated in the study were given patient information leaflets to read and were given the opportunity to ask any questions that they had prior to agreeing to participate in the study. The voluntary



nature of participation was emphasised and that refusal to participate would not affect their ongoing medical care in any way. Written informed consent was obtained from all participants. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

Neurological history and examination were recorded on paper and neuropsychological data was recorded on the individual neuropsychological test sheets. Data was subsequently transferred to a computerised password protected database using Microsoft Access 2010 (R). All identifying information was treated in a strictly confidential manner.

Data was pseudo-anonymised. Each participant maintained the unique study identifying number assigned to them in the prevalence study. Hard copies of the assessments were stored in a locked office in the Department of Neurology in St. James's Hospital. The consent forms were also stored in this office but did not contain any reference to the unique study number. These documents were only accessed by the candidate.

#### 3.3.6 Data Collection and Data Recorded

Demographic data collected in the prevalence study was included for the respective participants who agreed to take part in the detailed neuropsychological assessment and entered into the study database. CD4 T cell count, HIV viral load and antiretroviral therapy history were updated if any changes had occurred in the intervening period. Additional clinical data recorded included details of the neurological history: word



finding difficulties, difficulties remembering names or faces, repeating themselves, being forgetful, misplacing objects, difficulties with directions or navigation, difficulties learning new information, making errors at work, safety concerns, symptoms of dyspraxia, behavioural changes, personality changes, being slower completing tasks, poor concentration and a family history of neurodegenerative disorders. The neurological examination was also recorded: cranial nerve findings, tone, power, reflexes, co-ordination, sensation, gait, apraxia and rapid alternating movements. The scores from the respective sections of each of the neuropsychological tests used were also entered into the database. A copy of the data dictionary is attached in Appendix 10.

### 3.3.7 Missing Data

Nadir CD4<sup>+</sup> T cell counts were not available for two patients who were diagnosed outside of Ireland. One patient commenced neuropsychological testing but stopped after completing the RBANS and the FAB and therefore there is no data on this patient for the ACE-R or MoCA. Missing data was excluded from individual analysis involving those variables.

### 3.3.8 Calculation of Retention Scores

Retention scores were only calculated based on the RBANS list and story memory subtests and were deemed abnormal if patients recalled less than 53% of the list items that they had acquired and less than 61% of the story items that they had acquired. The healthy controls in Clark et al's paper had mean percent retention scores of 77.11

(standard deviation of 12.01) on list memory and 86.32 (standard deviation of 12.51) on story memory (347). For the purposes of this study an abnormal score was taken as the mean score minus two standard deviations. These percentages were slightly lower than the optimal cut-off scores used in the validation paper to differentiate between healthy controls and patients with Alzheimer’s disease but it was felt that using the mean score minus two standard deviations ensured that patients were scoring significantly lower than healthy controls. There was no adjustment made for age and 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 this cohort was 42 years so the majority of study subjects were significantly younger than the controls used in the normative data and should score at least similarly to them and most likely higher therefore an abnormal retention score in this study cohort should be of significance and suggest abnormality.

Retention scores were calculated as follows on the list and story memory subtests of the RBANS:

$$\text{List Recall Score} \div \text{List Learning Score on Final Trial} \times 100$$
$$\text{Story Recall Score} \div \text{Story Memory Score on Final Trial} \times 100$$

3.3.9 Calculation of Abnormal List Recognition Scores

The normative data in the RBANS supplement provide mean scores and standard deviations based on age groups for list recognition and these were used to determine if a patient had abnormal list recognition or not (349). List recognition was deemed to be abnormal if the individual patient’s score was more than two standard deviations away from the mean score for that age group.

### 3.3.10 Domain Based Analysis

A domain based analysis was carried out whereby all the subsections of the neuropsychological tests used were grouped according to the domain that they tested. Attention and processing speed consisted of digit span forward from the RBANS and MoCA, digit coding from the RBANS, word registration and backward tracking from the ACE-R, sustained attention task and backward tracking from the MoCA. Working memory consisted of backward span on the MoCA and backward tracking from the MoCA and the ACE-R. Executive function consisted of abstraction from the MoCA and FAB, phonemic fluency from the MoCA and ACE-R and similarities, LURIA sequence, conflicting instructions, go-no-go and prehension from the FAB. Memory domain consisted of list memory, story memory, list recall and story recall from the RBANS, anterograde memory and memory recall from the ACE-R and memory recall from the MoCA. Visuospatial domain consisted of figure copy and line orientation from the RBANS, pentagon copying, cube and clock drawing and perceptual abilities from the ACE-R and cube and clock drawing from the MoCA. Language domain consisted of categorical and phonemic fluency tests from RBANS, ACE-R, MoCA and FAB, naming tests from the RBANS, ACE-R and MoCA, language comprehension, writing, repetition and reading from the ACE-R and repetition from the MoCA. Temporal orientation consisted of orientation from the MoCA and ACE-R. Each score was z transformed and then averaged to give an average z score for each domain for each patient. In order to perform the z transformation, the mean and standard deviation was calculated for the patients with normal neuropsychological testing. The calculation for the z transformation is shown below.



$$z = \frac{x - \mu}{\sigma}$$

z : z score, x = individual score,  $\mu$  = mean,  $\sigma$  = standard deviation.

### 3.3.11 The Role of the PhD Candidate

The candidate recruited and consented all of the study participants. The candidate performed all of the detailed neuropsychological testing and statistical analysis. The candidate created and maintained the study database.

## 3.4 Statistical Analysis

Statistical analysis was carried out using SPSS Version 21 (SPSS Inc., Chicago IL). Qualitative (categorical) variables were presented as absolute number (N) and relative frequencies (percentages). Continuous variables were presented as absolute number, means with standards deviations (SD) and medians. Descriptive analysis was carried out on qualitative variables using Chi square ( $X^2$ ) testing to examine intergroup comparisons. The Bonferroni correction method was used to adjust for multiple comparisons. Fishers exact test was used where cells contained less than ten data points. Continuous variables were examined using the independent samples t test or one way analysis of variance (ANOVA). Where data failed to meet criteria for parametric tests, the Mann Whitney U test or the Kruskal Wallis one way analysis of variance by ranks were performed for continuous variables. All tests were two tailed and statistical significance was set at  $p < 0.05$ . Tukey's honestly significant difference (HSD) test was performed for post hoc analysis of any significant differences found on



ANOVA as it accounts for performing multiple comparisons and is a more conservative approach also thereby reducing the likelihood of a Type I error.

### **3.5 Results**

#### **3.5.1 Characteristics of Study Cohort**

There were 104 patients recruited. Their characteristics are described in Table 3-2. The majority were male (80.8%), Irish (79.8%) and spoke English as their first language (86.5%). A quarter of the group were co-infected with hepatitis C. Just over a half were employed (51.9%) and 49% were smokers. There were 95 patients on HAART (91.3%) and 90/95 (94.7%) were virally suppressed. There was a positive screen for anxiety in 28.8% (30/104) and a positive screen for depression in 8.7% (9/104) of the study cohort.

Table 3-2 Patient Characteristics

| <b>Characteristics</b>               |               | <b>(n = 104)</b> |                   |
|--------------------------------------|---------------|------------------|-------------------|
|                                      |               | <b><u>n</u></b>  | <b><u>(%)</u></b> |
| <b>Handedness</b>                    | Right         | 92               | (88.5)            |
|                                      | Left          | 12               | (11.5)            |
| <b>Gender</b>                        | Male          | 84               | (80.8)            |
|                                      | Female        | 20               | (19.2)            |
| <b>Country of Birth</b>              | Ireland       | 83               | (79.8)            |
|                                      | Europe        | 6                | (5.8)             |
|                                      | Africa        | 14               | (13.4)            |
|                                      | South America | 1                | (1.0)             |
| <b>English as a First Language</b>   | Yes           | 90               | (86.5)            |
| <b>Mode of Transmission</b>          | Heterosexual  | 34               | (32.7)            |
|                                      | MSM           | 48               | (46.2)            |
|                                      | IVDU          | 19               | (18.3)            |
|                                      | Other         | 3                | (2.9)             |
| <b>Virally Suppressed</b><br>(n=95)  | Yes           | 90               | (94.7)            |
|                                      | No            | 5                | (5.3)             |
| <b>Hx of Hepatitis B</b>             | Yes           | 8                | (7.8)             |
| <b>Hx of Hepatitis C</b>             | Yes           | 26               | (25)              |
| <b>Hepatitis C PCR Positive</b>      | Yes           | 17               | (16.3)            |
| <b>Hypertension</b>                  | Yes           | 10               | (9.6)             |
| <b>Hypercholesterolaemia</b>         | Yes           | 13               | (12.5)            |
| <b>Diabetes</b>                      | Yes           | 4                | (3.8)             |
| <b>Hx of Syphilis</b>                | Yes           | 15               | (4.4)             |
| <b>Hx of Cryptococcal Meningitis</b> | Yes           | 1                | (1)               |
| <b>Hx of CNS TB</b>                  | Yes           | 2                | (1.9)             |
| <b>Hx of Toxoplasmosis</b>           | Yes           | 2                | (1.9)             |
| <b>Hx of Encephalitis</b>            | Yes           | 1                | (1)               |

|                                      |             |    |        |
|--------------------------------------|-------------|----|--------|
| <b>Hx of PML</b>                     | Yes         | 1  | (1)    |
| <b>Epilepsy</b>                      | Yes         | 5  | (4.8)  |
| <b>Neuropathy</b>                    | Yes         | 2  | (1.9)  |
| <b>Stroke</b>                        | Yes         | 2  | (1.9)  |
| <b>Hx of Depression</b>              | Yes         | 27 | (26)   |
| <b>Hx of Bipolar Disorder</b>        | Yes         | 1  | (1)    |
| <b>Hx of Anxiety</b>                 | Yes         | 3  | (2.9)  |
| <b>Hx of Schizophrenia</b>           | Yes         | 1  | (1)    |
| <b>Hx of Psychosis</b>               | Yes         | 2  | (1.9)  |
| <b>Lipid Lowering Therapy</b>        | Yes         | 10 | (9.6)  |
| <b>Anti-hypertensives</b>            | Yes         | 11 | (10.6) |
| <b>Anti-epileptics</b>               | Yes         | 6  | (5.8)  |
| <b>Methadone replacement therapy</b> | Yes         | 16 | (15.4) |
| <b>Benzodiazepines</b>               | Yes         | 22 | (21.2) |
| <b>Anti-depressants</b>              | Yes         | 14 | (13.5) |
| <b>Anti-psychotics</b>               | Yes         | 3  | (2.9)  |
| <b>Employment History</b>            | Employed    | 54 | (51.9) |
|                                      | Student     | 5  | (4.8)  |
|                                      | Unemployed  | 41 | (39.5) |
|                                      | Retired     | 4  | (3.8)  |
| <b>Smoking History</b>               | Smoker      | 51 | (49)   |
|                                      | Non smoker  | 33 | (31.7) |
|                                      | Ex-smoker   | 20 | (19.2) |
| <b>Alcohol History</b>               | None        | 19 | (18.3) |
| <b>Units per week</b>                | 1-10 units  | 51 | (49)   |
|                                      | 11-20 units | 15 | (14.4) |
|                                      | 21-30 units | 11 | (10.6) |

|                                          |                    |        |                  |
|------------------------------------------|--------------------|--------|------------------|
|                                          | 31-40 units        | 0      | (0)              |
|                                          | > 40 units         | 3      | (2.9)            |
|                                          | Hx of ADS          | 5      | (4.8)            |
| <b>Illicit Substance Use</b>             | Never              | 68     | (65.4)           |
|                                          | Current Non IV Use | 18     | (17.3)           |
|                                          | Current IV Use     | 0      | (0)              |
|                                          | Previous Hx        | 18     | (17.3)           |
| <b>Antiretroviral Therapy</b>            | Naïve              | 7      | (6.7)            |
|                                          | On HAART           | 95     | (91.3)           |
|                                          | MTCT               | 2      | (1.9)            |
| <b>Positive Screen for Anxiety</b>       | Yes                | 30     | (28.8)           |
| <b>Positive Screen for Depression</b>    | Yes                | 9      | (8.7)            |
| <b>Age (in years)</b>                    | Mean (sd)          | 42.2   | (10.12)          |
|                                          | Median (range)     | 41.5   | (21-69)          |
| <b>Time since Diagnosis (years)</b>      | Mean (sd)          | 9.432  | (7.29)           |
|                                          | Median (range)     | 7.45   | (0.12 - 28.27)   |
| <b>Nadir CD4 Count</b>                   | Mean (sd)          | 246.64 | (169.583)        |
|                                          | Median (range)     | 241    | (5 - 848)        |
| <b>Current CD4 Count</b>                 | Mean (sd)          | 603.87 | (289.912)        |
|                                          | Median (range)     | 585.5  | (59 - 1485)      |
| <b>Years of Education</b>                | Mean (sd)          | 13.56  | (4.065)          |
|                                          | Median (range)     | 14     | (6 - 23)         |
| <b>Exposure to HAART (total) (years)</b> | Mean (sd)          | 6.51   | (5.06)           |
|                                          | Median (range)     | 6.01   | (0.008 - 16.496) |
| <b>HADS - Anxiety Score</b>              | Mean (sd)          | 7.93   | (4.481)          |
|                                          | Median (range)     | 7      | (0-21)           |
| <b>HADS - Depression Score</b>           | Mean (sd)          | 5.41   | (4.216)          |
|                                          | Median (range)     | 5      | (0 - 20)         |

ADS=Alcohol dependence syndrome, IV=intravenous, HAART=highly active antiretroviral therapy,

MTCT=maternal to child transmission, sd=standard deviation, HADS=Hospital Anxiety and Depression Scale.

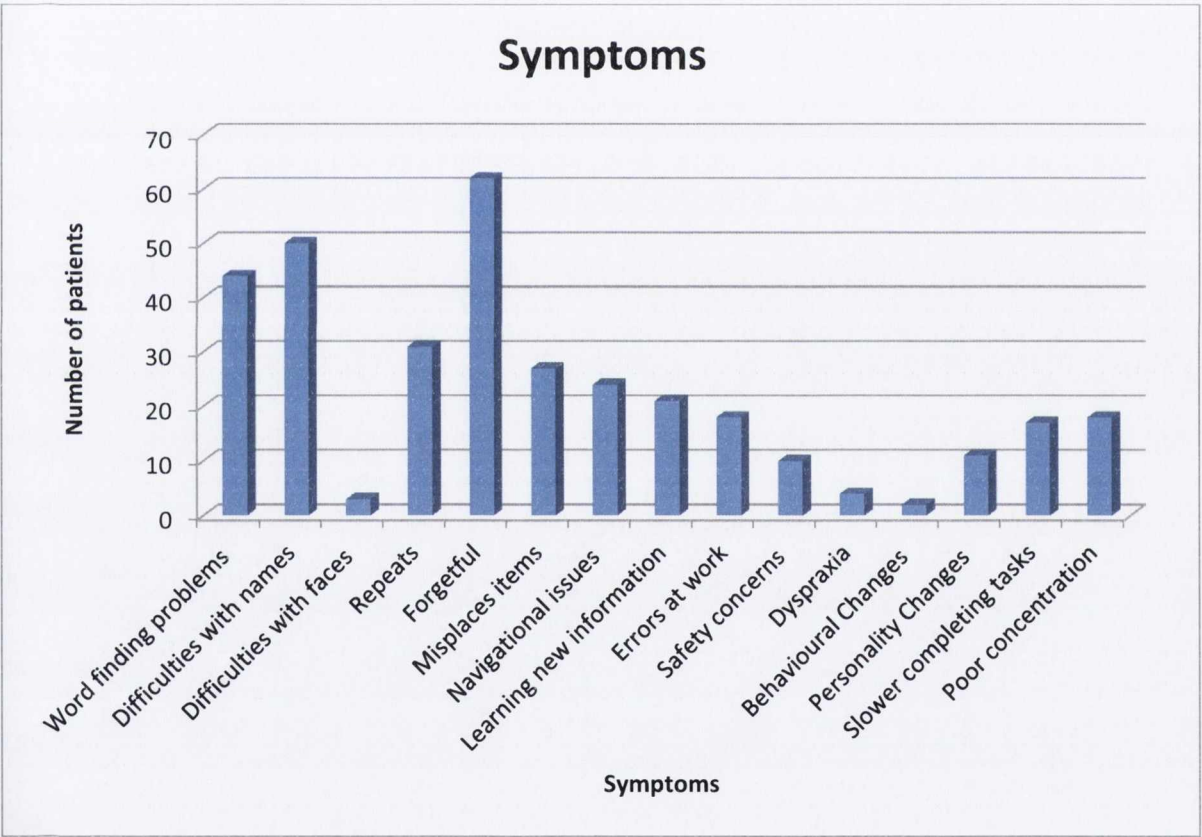


### 3.5.2 Symptoms

The most common cognitive symptoms patients complained of were forgetfulness (62/104, 59.6%), difficulties remembering names of friends and family members (50/104, 48.1%) and word finding difficulties (44/104, 42.3%). The symptoms included under forgetfulness included difficulties following conversations, following the plotlines on television shows and in books, forgetting appointments, increasing reliance on memory aides and forgetting important dates and events. Almost 30% of the study group stated that their family members complained that the patients frequently repeated the same questions and told them the same stories. Patients also complained that they frequently misplaced items and objects such as keys, wallets or purses and mobile phones and often spent considerable amounts of time daily searching for them (27/104, 26%). Navigational difficulties included new difficulties following directions, using a map and getting lost easily in familiar environments (24/104, 23.7%). It did not include people who had lifelong difficulties following directions or using a map. Patients stated that they had difficulties learning new information, both at work and in further education, in terms of learning new processes, pathways and procedures at work and learning and retaining new information from college or postgraduate courses (21/104, 20.2%). A number of patients (18/104, 17.3%) stated that they frequently made errors at work and had experienced difficulties at work due to this. A similar percentage of patients complained of decreased concentration when performing tasks, reading, watching television or learning new information (18/104, 17.3%) and 16.3% (17/104) complained of being slower completing tasks. Safety concerns encompassed difficulties remembering to close windows and doors when leaving their home, difficulties remembering to set burglar alarms, frequently forgetting about food cooking and burning the food and/or setting off smoke alarms as a consequence and getting lost and being

unable to find their way home (10/104, 9.6%). Personality changes (11/104, 10.6%) and changes in behaviour (2/104, 1.9%) were also noted in the study group and included increased aggression and anger and decreased interest and empathy. Symptoms of dyspraxia only occurred in 3.9% of the study group and included difficulties using technology and household appliances. The least frequent symptom was difficulty recognising faces (3/104, 2.9%).

Figure 3-1 Symptoms of Study Cohort



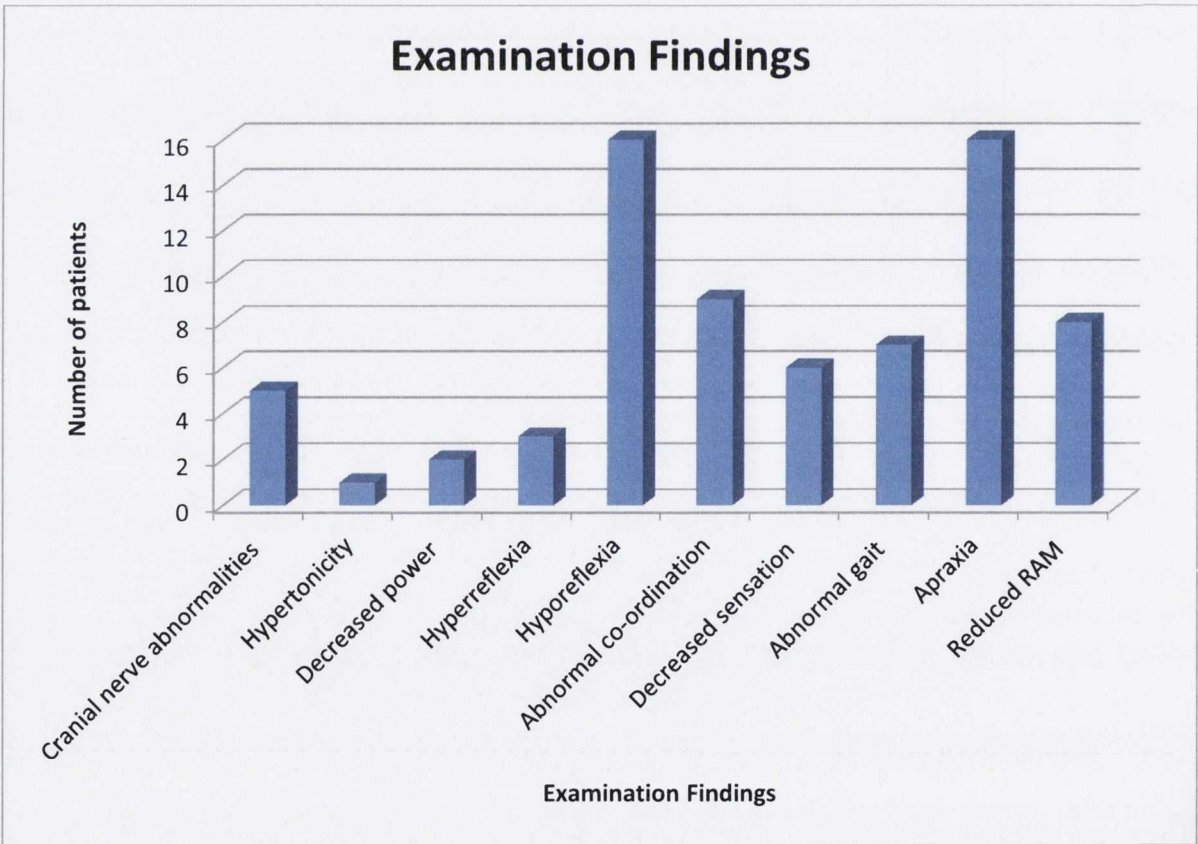
Number of patients who experienced these symptoms.

3.5.3 Examination Findings

The most common findings on neurological examination were hyporeflexia (16/104, 15.4%) and apraxia (16/104, 15.4%). Patients demonstrated features of ideomotor apraxia and also some features of limb-kinetic apraxia. Apraxia was examined by asking the patient to copy

movements of the hands, mime using a toothbrush or a hairbrush, wave goodbye, perform a salute, whistle and mime blowing out a candle. Patients had most difficulties miming using a toothbrush or a hairbrush, often using their finger as the object. They also had difficulties copying movements of the hands such as a “thumbs-up” position, opposition of thumb to index finger and holding up various fingers. They were slower and more imprecise completing these tasks. It was usually present bilaterally. There were no features of orobuccal apraxia. The cranial nerve findings were nystagmus in two patients, a visual field deficit in one patient secondary to a stroke, visual loss and hearing loss on the left in another patient and slow saccadic eye movements.

Figure 3-2 Abnormal Examination Findings of Study Cohort (Number of Patients)



Number of patients who had abnormal examination findings. RAM=Rapid alternating movements.



### 3.5.4 Breakdown of HAND Diagnosis

There were 62/104 patients who met neuropsychological criteria for HAND, of whom, 40/104 (38.5%) met criteria for asymptomatic neurocognitive disorder (ANI), 20/104 (19.2%) mild neurocognitive disorder (MND) and 2/104 (1.9%) HIV associated dementia (HAD). There were ten patients who had entirely normal testing on all four neuropsychological batteries. There were 32 patients who had abnormalities on testing that did not meet diagnostic criteria for HAND: abnormalities within one domain on RBANS or scoring below the cut-off mark for the ACE-R, MOCA or FAB or a combination thereof.

One patient who met criteria for ANI had a previous history of a stroke and had cognitive symptoms as a consequence therefore it was a confounding condition and it was not possible to attribute the abnormalities to HIV. Another patient who met criteria for MND had a previous history of progressive multifocal leukoencephalopathy with improvement of cognitive symptoms for a few years after initiation of HAART but with subsequent decline in the setting of stable neuroimaging and no evidence of further opportunistic infection. It was deemed to be a contributing condition which was compatible with a diagnosis of HIV related cognitive impairment. There were 18 patients who reported non intravenous use of recreational substances of whom four met criteria for ANI and five met criteria for MND. All patients gave a clear history of deterioration of cognitive functioning in the presence of stable substance use. They were neither intoxicated nor in withdrawal at the time of cognitive testing. Two of these patients were in full time employment. They were classified as contributing conditions. There were three patients who consumed more than 40 units of alcohol weekly but none of them met diagnostic criteria for HAND.



On comparison between patients with normal neuropsychological testing, patients who had abnormalities on testing that were not diagnostic of HAND and patients with HAND, the only statistically significant difference was in years of education on ANOVA:  $F(2, 101) = 4.77$ ,  $p=0.01$ . Post hoc comparisons using the Tukey HSD test indicated that the mean score for patients with HAND (mean = 12.76, SD = 4.08) was significantly different from those with normal neuropsychological testing (mean = 16.55, SD = 4.824) ( $p=0.010914$ ). Patients who had abnormalities which did not meet diagnostic criteria for HAND (mean = 14.1, SD = 3.197) did not differ significantly from patients with normal neuropsychological testing or HAND.

Table 3-3 Comparison of Baseline Characteristics Between Groups.

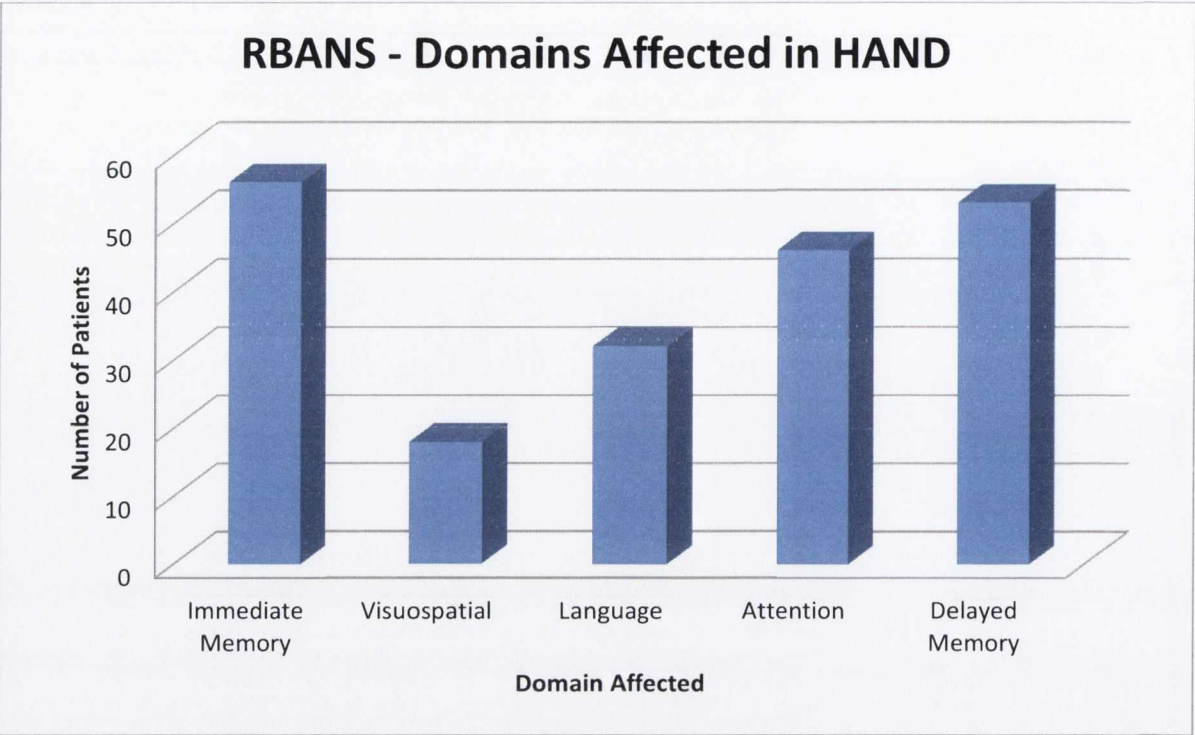
| Characteristics             | Normal<br>(n = 10) |              | Non Diagnostic Abnormalities<br>(n=32) |              | HAND<br>(n=62) |     | P Value |
|-----------------------------|--------------------|--------------|----------------------------------------|--------------|----------------|-----|---------|
|                             | n                  | (%)          | n                                      | (%)          | n              | (%) |         |
| Gender                      | Male               | 11<br>(100)  | 26<br>(63.9)                           | 47<br>(75.8) |                |     | 0.15    |
|                             | Female             | 0<br>(0)     | 5<br>(16.1)                            | 15<br>(24.2) |                |     |         |
| Country of Birth            | Ireland            | 11<br>(100)  | 26<br>(83.9)                           | 46<br>(74.2) |                |     | 0.293   |
|                             | Europe             | 0<br>(0)     | 3<br>(9.7)                             | 3<br>(4.8)   |                |     |         |
|                             | Africa             | 0<br>(0)     | 2<br>(6.5)                             | 12<br>(19.4) |                |     |         |
|                             | S. America         | 0<br>(0)     | 0<br>(0)                               | 1<br>(1.6)   |                |     |         |
| English as a first language | Yes                | 11<br>(100)  | 29<br>(93.5)                           | 50<br>(80.6) |                |     | 0.088   |
| Mode of Transmission        | Heterosexual       | 1<br>(9.1)   | 11<br>(35.5)                           | 22<br>(35.5) |                |     | 0.092   |
|                             | MSM                | 9<br>(81.8)  | 15<br>(48.4)                           | 24<br>(38.7) |                |     |         |
|                             | IVDU               | 1<br>(9.1)   | 3<br>(9.7)                             | 15<br>(24.2) |                |     |         |
|                             | Other              | 0<br>(0)     | 2<br>(6.5)                             | 1<br>(1.6)   |                |     |         |
| Virally Suppressed (n=95)   | Yes                | 10<br>(90.9) | 26<br>(83.9)                           | 54<br>(87.1) |                |     | 0.166   |
| Hx of Hepatitis C           | Yes                | 3<br>(27.3)  | 4<br>(12.9)                            | 19<br>(30.6) |                |     | 0.173   |
| Hx of Depression            | Yes                | 4<br>(36.4)  | 5<br>(16.1)                            | 18<br>(29)   |                |     | 0.289   |
| Methadone                   | Yes                | 1<br>(9)     | 3<br>(9.7)                             | 12<br>(19.4) |                |     | 0.394   |
| Smoking History             | Smokers            | 4<br>(36.4)  | 16<br>(51.6)                           | 31<br>(50)   |                |     | 0.832   |

| <u>Characteristics</u>                | Normal<br>(n = 11) | Non Diagnostic Abnormalities<br>(n=31) | HAND<br>(n=62)    | P Value |
|---------------------------------------|--------------------|----------------------------------------|-------------------|---------|
| <b>Alcohol History</b>                |                    |                                        |                   |         |
| Units per week                        |                    |                                        |                   |         |
| None                                  | 2<br>(18.2)        | 3<br>(9.7)                             | 14<br>(22.5)      | 0.17    |
| 1-10 units                            | 3<br>(27.3)        | 17<br>(54.8)                           | 31<br>(50)        |         |
| 11-20 units                           | 3<br>(27.3)        | 5<br>(16.1)                            | 7<br>(11.3)       |         |
| 21-30 units                           | 2<br>(18.2)        | 4<br>(12.9)                            | 5<br>(8.1)        |         |
| 31-40 units                           | 0<br>(0)           | 0<br>(0)                               | 0<br>(0)          |         |
| > 40 units                            | 1<br>(9.1)         | 2<br>(6.5)                             | 0<br>(0)          |         |
| Hx of ADS                             | 0<br>(0)           | 0<br>(0)                               | 5<br>(5.1)        |         |
| <b>Illicit Substance Use</b>          |                    |                                        |                   |         |
| Never                                 | 7<br>(63.6)        | 21<br>(67.7)                           | 40<br>(64.5)      | 0.455   |
| Current Non IV Use                    | 1<br>(9.1)         | 8<br>(25.8)                            | 9<br>(14.5)       |         |
| Current IV Use                        | 0<br>(0)           | 0<br>(0)                               | 0<br>(0)          |         |
| Previous Hx                           | 3<br>(27.3)        | 2<br>(6.5)                             | 13<br>(21)        |         |
| <b>Antiretroviral therapy</b>         |                    |                                        |                   |         |
| On HAART                              | 10<br>(90.9)       | 27<br>(87.1)                           | 58<br>(93.5)      | 0.351   |
| <b>Positive Screen for Anxiety</b>    |                    |                                        |                   |         |
| Yes                                   | 0<br>(0)           | 11<br>(35.5)                           | 19<br>(30.6)      | 0.073   |
| <b>Positive Screen for Depression</b> |                    |                                        |                   |         |
| Yes                                   | 1<br>(9.1)         | 2<br>(6.5)                             | 6<br>(9.7)        | 0.872   |
| <b>Age</b>                            |                    |                                        |                   |         |
| Mean (SD)                             | 39.55 (6.3)        | 39.97 (10.6)                           | 43.82 (10.2)      | 0.145   |
| Median (Range)                        | 39 (30 - 51)       | 40 (21 - 50)                           | 43 (26 - 69)      |         |
| <b>Time Since Diagnosis (days)</b>    |                    |                                        |                   |         |
| Mean (SD)                             | 3105.91 (1885.96)  | 3187.48 (2607.23)                      | 3630.08 (2819.68) | 0.685   |
| Median (Range)                        | 2707 (645 - 7239)  | 2484 (341 - 10189)                     | 3032 (44 - 10318) |         |

| Characteristics          | Non Diagnostic Abnormalities |                   | P Value |
|--------------------------|------------------------------|-------------------|---------|
|                          | Normal<br>(n = 11)           | HAND<br>(n=62)    |         |
| Nadir CD4 T Cell Count   | Mean (SD)                    | 294.81 (201.5)    | 0.053   |
|                          | Median (Range)               | 284 (9 - 790)     |         |
| Current CD4 T Cell Count | Mean (SD)                    | 650.68 (242.22)   | 0.139   |
|                          | Median (Range)               | 618 (273 - 1485)  |         |
| Years of Education       | Mean (SD)                    | 14.1 (3.197)      | 0.01    |
|                          | Median (Range)               | 14 (8.2)          |         |
| Exposure to HAART (days) | Mean (SD)                    | 2522.46 (1804.28) | 0.895   |
|                          | Median (Range)               | 2515 (185 - 6021) |         |



Figure 3-3 Breakdown of Domains Affected in the RBANS Battery for Patients with HAND



Breakdown of domains affected in HAND by number of patients.

For the five domains within the RBANS test, the raw subtest scores are converted to a domain based index score adjusted for age. List learning and story memory total raw scores are converted into an index score for immediate memory. Figure copy and line orientation total scores are converted into an index score for a visuospatial and constructional domain. Picture naming and semantic fluency total scores are converted into an index score for language. Digit span and coding total scores are converted into an index score for attention. List recall, list recognition, story recall and figure recall total scores are converted into an index score for delayed memory. Immediate memory (90.3%) and delayed memory (85.5%) are the most affected domains within patients who met criteria for HAND. Attention was abnormal in 74.2%, language in 51.6% and visuospatial skills in 29.03% of patients with HAND.

Table 3-4 Breakdown of RBANS Index Scores

| RBANS Index Scores  | Normal<br>n=10 | Non Diagnostic Abnormalities<br>n=32 | HAND<br>n=61    | P value |
|---------------------|----------------|--------------------------------------|-----------------|---------|
| Immediate Memory    |                |                                      |                 |         |
| Mean (SD)           | 93 (8.093)     | 86.28 (10.929)                       | 70.89 (13.013)  | <0.001  |
| Median              | 90             | 85                                   | 73              |         |
| Visuoconstructional |                |                                      |                 |         |
| Mean (SD)           | 105.1 (8.621)  | 104.72 (13.182)                      | 94.19 (17.4)    | 0.008   |
| Median              | 105            | 103.5                                | 96              |         |
| Language            |                |                                      |                 |         |
| Mean (SD)           | 97.3 (5.908)   | 94.16 (7.932)                        | 81.32 (15.959)  | <0.001  |
| Median              | 98.5           | 95.5                                 | 84              |         |
| Attention           |                |                                      |                 |         |
| Mean (SD)           | 96.1 (10.104)  | 94.97 (9.62)                         | 75.27 (15.428)  | <0.001  |
| Median              | 95.5           | 94                                   | 79              |         |
| Delayed Memory      |                |                                      |                 |         |
| Mean (SD)           | 94.1 (6.367)   | 88.41 (15.368)                       | 66.53 (116.014) | <0.001  |
| Median              | 93             | 93                                   | 64              |         |

There were statistically significant differences on the index scores between the three groups and the pairwise comparisons showed differences between the patients with HAND and those with normal neuropsychological testing and also those with non-diagnostic abnormalities on all domains except visuoconstructional on which there were only differences between the group with HAND and the group with non-diagnostic abnormalities.

The RBANS was used to derive a cortical-subcortical deviation score by subtracting the mean of the delayed memory index and the language index from the mean of the attention index and visuospatial constructional index and scores above zero are considered cortical and scores below zero are considered subcortical (324, 350). This method has been validated using patients with Alzheimer's disease (a prototypical cortical dementia) and patients with prototypical subcortical dementias (Huntington's disease and Parkinson's disease) (351). Of the 62 patients with HAND, 48 had scores greater than zero suggesting that their underlying pathology was more likely to be cortical, 13 had scores below zero suggesting subcortical pathology and one patient had a score of zero.

#### 3.5.5 Retention scores

In the entire cohort there were 43 patients who had abnormal retention of the list items, 24 patients who had abnormal retention of the story items and 16 patients who had abnormal retention of both. There was only one patient from the group with normal neuropsychological testing who had an abnormal retention score. That patient only remembered 50% of the list items that they had learned which was just below the normal limit. None of the other patients in the group with normal neuropsychological testing had

abnormal retention scores. In the group with HAND, there were 32 (51.6%) patients with abnormal retention of the list items, 20 (32.3%) patients with abnormal retention of the story items and 14 (22.6%) patients with abnormal retention of both list and story items. Within the group of patients with abnormalities on neuropsychological testing that were not diagnostic of HAND, there were ten patients (31.25%) who had abnormal retention of the list items, four patients (12.5%) who had abnormal retention of the story items and two patients (6.25%) with abnormal retention of both list and story items. The retention scores of patients with HAND are shown in Table 3-5 and those with non-diagnostic abnormalities are shown in Table 3-6.



Table 3-5 Summary of Retention Scores in Patients with HAND who had Abnormal Retention

| Abnormalities in Either List or Story |                     | Abnormalities in Both List and Story |                     |
|---------------------------------------|---------------------|--------------------------------------|---------------------|
| % of List Recalled                    | % of Story Recalled | % of List Recalled                   | % of Story Recalled |
| 28                                    | 50                  | 0                                    | 37.5                |
| 0                                     | 37.5                | 0                                    | 40                  |
| 42.8                                  | 37.5                | 28.5                                 | 60                  |
| 0                                     | 40                  | 0                                    | 25                  |
| 0                                     | 40                  | 0                                    | 37.5                |
| 28.5                                  | 60                  | 14.5                                 | 45.5                |
| 42.8                                  | 25                  | 0                                    | 25                  |
| 0                                     | 37.5                | 20                                   | 50                  |
| 0                                     | 55                  | 0                                    | 50                  |
| 0                                     | 45.5                | 33.3                                 | 27.3                |
| 0                                     | 50                  | 42.9                                 | 54.5                |
| 14.5                                  | 25                  | 50                                   | 33.3                |
| 33                                    | 50                  | 33                                   | 37.5                |
| 0                                     | 50                  | 0                                    | 0                   |
| 20                                    | 58.3                |                                      |                     |
| 0                                     | 27.3                |                                      |                     |
| 0                                     | 54.5                |                                      |                     |
| 20                                    | 33.3                |                                      |                     |
| 42                                    | 37.5                |                                      |                     |
| 0                                     |                     |                                      |                     |
| 42.9                                  |                     |                                      |                     |
| 33                                    |                     |                                      |                     |
| 0                                     |                     |                                      |                     |
| 33.3                                  |                     |                                      |                     |
| 42.9                                  |                     |                                      |                     |
| 42.8                                  |                     |                                      |                     |
| 0                                     |                     |                                      |                     |
| 50                                    |                     |                                      |                     |
| 33.3                                  |                     |                                      |                     |
| 40                                    |                     |                                      |                     |
| 50                                    |                     |                                      |                     |
| 0                                     |                     |                                      |                     |

**Table 3-6 Summary of Retention Scores in Patients with Non-Diagnostic Abnormalities on Neuropsychological Testing who had Abnormal Retention**

| Abnormalities in Either List or Story |                     | Abnormalities in Both List and Story |                     |
|---------------------------------------|---------------------|--------------------------------------|---------------------|
| % of List Recalled                    | % of Story Recalled | % of List Recalled                   | % of Story Recalled |
| 50                                    | 55.6                | 0                                    | 50                  |
| 50                                    | 50                  | 50                                   | 58.3                |
| 50                                    | 54.5                |                                      |                     |
| 0                                     | 58.3                |                                      |                     |
| 0                                     |                     |                                      |                     |
| 0                                     |                     |                                      |                     |
| 37.5                                  |                     |                                      |                     |
| 50                                    |                     |                                      |                     |
| 42.8                                  |                     |                                      |                     |
| 50                                    |                     |                                      |                     |

3.5.6 List Recognition on RBANS

There were abnormalities within list recognition in 48 patients in the cohort; one with normal testing (n=10), seven (21.9%) in the group with non-diagnostic abnormalities (n=32) and 40/62 (64.5%) patients with HAND (p=0.000001). Of the patients with abnormal retention of list items, 32/43 (74.4%) had an abnormal list recognition score. Within the group of patients with HAND, 26/32 (81.3%) patients with abnormal retention score on list memory had abnormal list recognition (p=0.007). In the group of patients with non-diagnostic abnormalities, five (50%) with abnormal retention score on list memory had poor list recognition (p=0.019) and one person who had normal neuropsychological testing had an abnormal retention score on list memory and also had poor list recognition (p=0.1).

3.5.7 Results of Addenbrooke’s Cognitive Exam – Revised (ACE-R)

A score of less than 88 on the ACE-R was considered abnormal. There were 63 patients who had an abnormal total score on the ACE-R, 48 of whom met criteria for HAND based on the RBANS and the remaining 15 did not. A domain was deemed to be abnormal if the patient’s

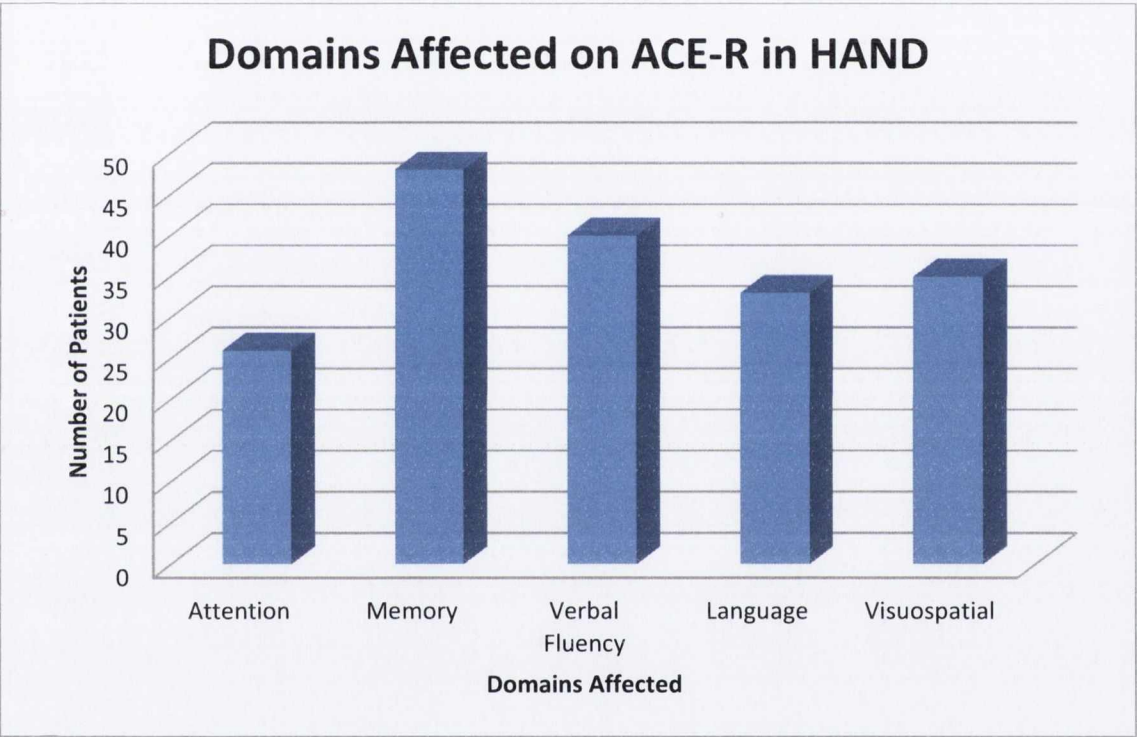
score was more than one standard deviation away from the mean score in that domain in the control group in Mioshi's published validation of the ACE-R (330). One standard deviation was chosen as the diagnostic criteria for HAND use scores of one standard deviation or more away from the mean score in ANI and MND (57).

Figure 3-4 demonstrates the domains affected in the 48 patients with HAND who had an abnormal ACE-R score. The domains most affected were memory (100%) and verbal fluency (83.3%). Visuospatial skills were affected in 76.8%, language in 68.8% and attention in 54.2% of patients with HAND. Table 3-7 shows the total and domain based scores obtained for patients who had an abnormal total ACE-R score. There were statistically significant differences between the mean scores of the two groups with an abnormal ACE-R test in the domains of memory and verbal fluency and also the total ACE-R score. There were statistically significant differences between the mean scores of the three groups (normal neuropsychological testing, patients with abnormal neuropsychological testing who did not meet diagnostic criteria for HAND and patients with HAND) in the domains of memory, verbal fluency, language and visuospatial skills. These were performed using Kruskal-Wallis tests as the data was non-parametric. Pairwise comparisons showed differences between patients with HAND and those with non-diagnostic abnormalities in memory ( $p=0.003$ ), verbal fluency ( $p=0.05$ ) and language ( $p=0.002$ ). Pairwise comparisons showed statistically significant differences between patients with HAND and those with normal testing in the domains of memory ( $p<0.001$ ), verbal fluency ( $p<0.001$ ), language ( $p=0.001$ ), and visuospatial skills ( $p=0.039$ ).



Patients with an abnormal ACE-R score were more likely to be unemployed ( $p=0.009$ ), to be older (44 years of age compared to 39.6 years) ( $p=0.031$ ), to have fewer years of education (12.3 years compared to 15.65 years) ( $p<0.001$ ) and to have hyper-reflexia ( $p=0.004$ ), abnormal co-ordination ( $p=0.011$ ), gait abnormalities ( $p=0.041$ ), apraxia ( $p=0.004$ ) and reduced rapid alternating movements ( $p=0.022$ ) on examination.

Figure 3-4 Domains Affected on ACE-R in Patients with HAND



Breakdown of domains affected in HAND by number of patients.



**Table 3-7 Scores on Domains within ACE-R in Patients who had an Abnormal Total ACE-R Score**

|              |        | <b>HAND<br/>(n=48)</b> | <b>Non Diagnostic Abnormalities<br/>(n=15)</b> | <b>Total Score<br/>Possible</b> | <b>P Value</b> |
|--------------|--------|------------------------|------------------------------------------------|---------------------------------|----------------|
| Attention    |        |                        |                                                | 18                              |                |
|              | Mean   | 16.79                  | 17.33                                          |                                 | 0.23           |
|              | Median | 17                     | 18                                             |                                 |                |
|              | Range  | 10 - 18                | 15 - 18                                        |                                 |                |
| Memory       |        |                        |                                                | 26                              |                |
|              | Mean   | 15.06                  | 18.6                                           |                                 | 0.001          |
|              | Median | 15.5                   | 18                                             |                                 |                |
|              | Range  | 8 - 20                 | 13 - 25                                        |                                 |                |
| Fluency      |        |                        |                                                | 14                              |                |
|              | Mean   | 8.13                   | 9.4                                            |                                 | 0.041          |
|              | Median | 8                      | 9                                              |                                 |                |
|              | Range  | 3 - 12                 | 7 - 12                                         |                                 |                |
| Language     |        |                        |                                                | 26                              |                |
|              | Mean   | 22.21                  | 23.73                                          |                                 | 0.06           |
|              | Median | 23                     | 24                                             |                                 |                |
|              | Range  | 14 - 16                | 19 - 26                                        |                                 |                |
| Visuospatial |        |                        |                                                | 16                              |                |
|              | Mean   | 13.98                  | 14.73                                          |                                 | 0.252          |
|              | Median | 15                     | 15                                             |                                 |                |
|              | Range  | 4 - 16                 | 12 - 16                                        |                                 |                |
| Total        |        |                        |                                                | 100                             |                |
|              | Mean   | 75.75                  | 83.8                                           |                                 | 0.001          |
|              | Median | 77.5                   | 85                                             |                                 |                |
|              | Range  | 42 - 87                | 70 - 87                                        |                                 |                |

Table 3-8 Domain Based Scores on the ACE-R for the Whole Cohort

|              |               | Normal<br>(n=10) | Non Diagnostic Abnormalities<br>(n=32) | HAND<br>(n=61) | P Value |
|--------------|---------------|------------------|----------------------------------------|----------------|---------|
| Attention    | Mean          | 17.3             | 17.53                                  | 16.75          | 0.193   |
|              | Median        | 17.5             | 18                                     | 18             |         |
|              | Std Deviation | 0.823            | 0.803                                  | 2.35           |         |
| Memory       | Mean          | 23.1             | 19.91                                  | 16.64          | <0.001  |
|              | Median        | 23.5             | 20.5                                   | 16             |         |
|              | Std Deviation | 1.595            | 3.246                                  | 4.431          |         |
| Fluency      | Mean          | 10.6             | 10.75                                  | 8.67           | <0.001  |
|              | Median        | 10.6             | 11                                     | 9              |         |
|              | Std Deviation | 1.35             | 1.867                                  | 2.372          |         |
| Language     | Mean          | 25.4             | 24.53                                  | 22.74          | <0.001  |
|              | Median        | 26               | 25                                     | 23             |         |
|              | Std Deviation | 0.843            | 1.759                                  | 2.786          |         |
| Visuospatial | Mean          | 15.6             | 15.03                                  | 14.23          | 0.018   |
|              | Median        | 16               | 16                                     | 15             |         |
|              | Std Deviation | 0.699            | 1.231                                  | 2.217          |         |

Std = Standard

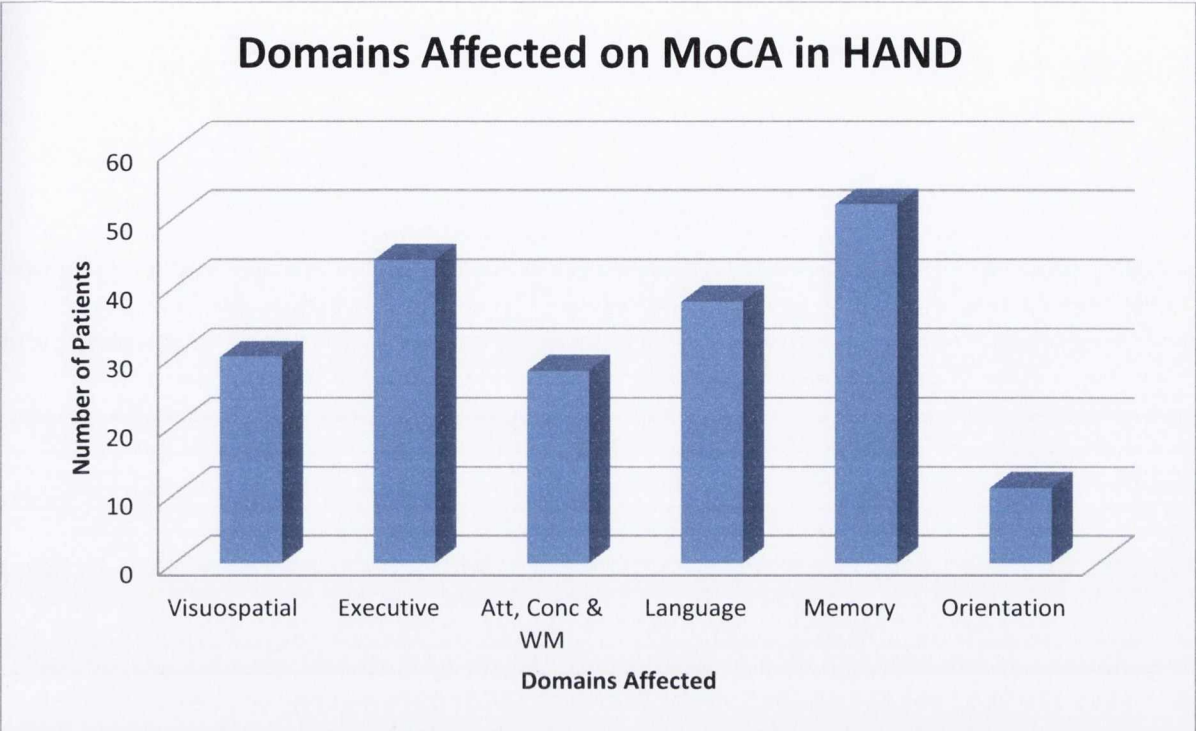
3.5.8 Results of Montreal Cognitive Assessment (MoCA)

An abnormal MoCA was a score less than 26. In total, 73 patients had an abnormal score, 52 of whom met criteria for HAND. As there are no published data available for the mean scores and standard deviations of each domain within the MoCA, for the purposes of this study a domain was considered abnormal if the domain score was one point or more away from the total possible score. This is less than ideal for an analysis of domains affected as a total score of 26 or less is abnormal, thereby allowing participants to lose up to 4 points on one or any domain before their total score is deemed abnormal, but given the lack of published domain based means and standard deviations for the MoCA it was deemed to be the best approach possible.

The domains most affected in the 52 patients with HAND who had an abnormal total MoCA score were memory (100%), executive function (84.6%) and language (73.1%). Visuospatial skills were affected in 57.7% of patients with HAND, attention, concentration and working memory in 53.8% and orientation in 21.2%. In patients who had abnormalities on neuropsychological testing that failed to meet criteria for HAND (n=21) the domains most affected were delayed recall (100%) and executive function (66.7%). Language and visuospatial skills were equally affected in 52.4%, attention, concentration and working memory in 19% and orientation in 9.5% of patients with abnormalities on neuropsychological testing that failed to meet criteria for HAND.

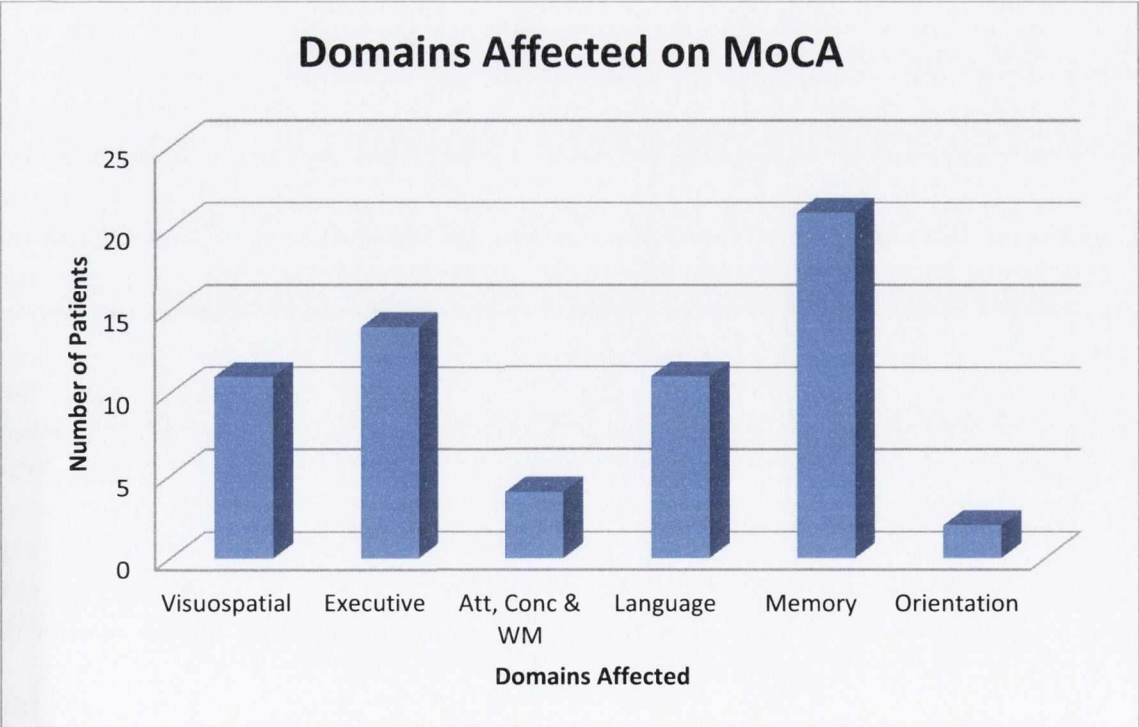
Patients with an abnormal MoCA score were more likely to be unemployed ( $p=0.46$ ), to have a positive screen for anxiety ( $p=0.008$ ), to be older (43.64 years compared to 39 years of age) ( $p=0.034$ ), to have a lower nadir CD4 T cell count (221.56 compared to 304.63) ( $p=0.024$ ), to have a lower current CD4 T cell count (564.36 compared to 704.7) ( $p=0.025$ ), to have fewer years of education (12.66 years compared to 15.9) ( $p<0.001$ ), to have a lower score on both the anxiety (8.45 compared to 6.47) ( $p=0.04$ ) and depression (5.73 compared to 3.73) ( $p=0.029$ ) subsections of the hospital anxiety and depression scale.

Figure 3-5 Domains Affected on MoCA in Patients with HAND



Breakdown of domains affected in HAND by number of patients. Att, Conc & WM = Attention, Concentration and Working Memory.

Figure 3-6 Domains Affected on MoCA in Patients with Non-Diagnostic Abnormalities on Neuropsychological Testing



Breakdown of domains affected in patients with non-diagnostic abnormalities by number of patients.



**Table 3-9 Recall Scores on the MoCA by Patient Group**

|        | Normal<br>(n = 10) | Non Diagnostic Abnormalities<br>(n=32) | HAND<br>(n=62) | P Value |
|--------|--------------------|----------------------------------------|----------------|---------|
| Mean   | 3.1                | 1.47                                   | 1.21           | 0.001   |
| Median | 3                  | 1                                      | 1              |         |

On comparison between patients with normal neuropsychological testing, patients who had abnormalities on testing that were not diagnostic of HAND and patients with HAND, the recall score on the MoCA was statistically significantly different on ANOVA:  $F(2, 100) = 7.315$ ,  $p=0.001$ . Post hoc comparisons using the Tukey HSD test indicated that the mean score for patients with HAND was significantly different from those with normal neuropsychological testing ( $p=0.001$ ). Post hoc analysis also demonstrated that the mean score for patients with non-diagnostic abnormalities was significantly different from those with normal neuropsychological testing ( $p=0.007$ ). There were no significant differences between patients with non-diagnostic abnormalities and HAND ( $p=0.698$ ).

**3.5.9 Results of Frontal Assessment Battery Test (FAB)**

There were 36 patients with abnormal frontal assessment battery scores; eight patients (8/32, 25%) with abnormalities on neuropsychological testing who did not meet diagnostic criteria for HAND and 28 (28/62, 45.2%) patients who met diagnostic criteria for HAND. Within the group of patients with HAND, 15/40 (37.5%) patients with ANI had abnormal scores, 11/20 (55%) patients with MND had abnormal FAB scores and both patients with HAD scored abnormally. Patients with an abnormal score on the frontal assessment battery were more likely to complain of forgetfulness ( $p=0.039$ ), misplacing items ( $p=0.04$ ), difficulties learning new information ( $p=0.009$ ) and have experienced safety concerns ( $p=0.032$ ). There were also statistically significant relationships to the presence of hyper-

reflexia ( $p=0.04$ ), gait abnormalities ( $p=0.008$ ) and apraxia ( $p=0.001$ ) on examination. Subjective complaints of personality change ( $p=0.051$ ) and a positive screen for depression ( $p=0.065$ ) approached statistical significance.

**Table 3-10 Scores on FAB Test**

|           | Normal<br>(n=10) | Non Diagnostic Abnormalities<br>(n=32) | HAND<br>(n=61) |
|-----------|------------------|----------------------------------------|----------------|
| Mean (SD) | 16.6 (1.174)     | 16.47 (1.502)                          | 14.73 (2.53)   |
| Median    | 16.5             | 17                                     | 15             |
| Range     | 15 - 18          | 13 - 18                                | 4 - 18         |

### 3.5.10 Domain Based Analysis

Table 3-11 and Table 3-12 show the domain based score for all the neuropsychological tests combined for the entire cohort and patients with HAND respectively. As the data was non parametric analysis was carried out using Kruskal-Wallis tests. There were statistically significant differences between groups (normal, non-diagnostic abnormalities and HAND) in all domains except the temporal and orientation domain. Pairwise comparisons demonstrated statistically significant differences between patients with HAND and those with normal testing in attention and processing speed ( $p=0.007$ ), executive function ( $p=0.005$ ), memory ( $p<0.001$ ) and visuospatial domain ( $p=0.008$ ). Pairwise comparisons also demonstrated statistically significant differences between patients with HAND and those with non-diagnostic abnormalities in attention and processing speed ( $p<0.001$ ), working memory ( $p=0.022$ ), executive function ( $p=0.001$ ), memory ( $p<0.001$ ) and visuospatial domain ( $p=0.012$ ).

Table 3-11 Mean and Median Z scores of each Domain for Entire Cohort

|                          | Normal<br>(n=10)  | Non Diagnostic<br>Abnormalities<br>(n=32) | HAND<br>(n=62)     | P Value |
|--------------------------|-------------------|-------------------------------------------|--------------------|---------|
| Attention and Processing |                   |                                           |                    |         |
| Speed                    |                   |                                           |                    |         |
| Mean                     | 0                 | 0.086                                     | -0.538             | <0.001  |
| Median                   | -0.007            | 0.103                                     | -0.401             |         |
| Range                    | (-0.62) - (0.48)  | (-0.49) - (0.53)                          | (-2.69) - (0.36)   |         |
| Working Memory           |                   |                                           |                    |         |
| Mean                     | 0                 | 0.241                                     | -0.391             | 0.025   |
| Median                   | 0.5               | 0.5                                       | 0.105              |         |
| Range                    | (-1.08) - (0.5)   | (-0.685) - (0.5)                          | (-4.9) - (0.5)     |         |
| Executive Function       |                   |                                           |                    |         |
| Mean                     | 0                 | -0.132                                    | -0.662             | <0.001  |
| Median                   | 0.0005            | -0.135                                    | -0.645             |         |
| Range                    | (-0.642) - (0.51) | (-1.24) - (0.824)                         | (-3.31) - (0.98)   |         |
| Memory                   |                   |                                           |                    |         |
| Mean                     | -0.008            | -1.009                                    | -2.18              | <0.001  |
| Median                   | 0.088             | -0.977                                    | -2.13              |         |
| Range                    | (-1.05) - (1.12)  | (-2.33) - (-0.45)                         | (-4.36) - (-0.081) |         |
| Visuospatial Skills      |                   |                                           |                    |         |
| Mean                     | 0                 | -0.246                                    | -0.844             | 0.001   |
| Median                   | 0.0339            | -0.98                                     | -0.4277            |         |
| Range                    | (-0.43) - (0.295) | (-1.538) - (0.36)                         | (-7.25) - (0.295)  |         |
| Language Skills          |                   |                                           |                    |         |
| Mean                     | 0                 | -0.128                                    | -0.729             | <0.001  |
| Median                   | 0.0285            | -0.053                                    | -0.498             |         |
| Range                    | (-0.32) - (0.31)  | (-2.12) - (0.59)                          | (-3.27) - (0.54)   |         |

|                          |        | Normal<br>(n=10)  | Non Diagnostic<br>Abnormalities<br>(n=32) | HAND<br>(n=62)    | P Value |
|--------------------------|--------|-------------------|-------------------------------------------|-------------------|---------|
| Temporal and Orientation | Mean   | 0                 | -0.351                                    | -0.459            | 0.393   |
|                          | Median | 0.158             | 0.158                                     | 0.158             |         |
|                          | Range  | (-1.42) - (0.158) | (-4.01) - (0.158)                         | (-5.09) - (0.158) |         |



Table 3-12 Mean and Median Z Scores of each Domain for Patients with HAND

|                                | ANI<br>(n=40)      | MND<br>(n=20)      | HAD<br>(n=2)       | P Value  |
|--------------------------------|--------------------|--------------------|--------------------|----------|
| Attention and Processing Speed |                    |                    |                    | 0.45     |
| Mean                           | -0.476             | -0.568             | -1.489             |          |
| Median                         | -0.353             | -0.565             | -1.489             |          |
| Range                          | (-2.1) - (0.35)    | (-1.44) - (0.232)  | (-2.68) - (-0.289) |          |
| Working Memory                 |                    |                    |                    | 0.737    |
| Mean                           | -0.329             | -0.331             | -2.2               |          |
| Median                         | 0.303              | 0.105              | -2.2               |          |
| Range                          | (-3.32) - (0.5)    | (-2.13) - (0.5)    | (-4.29) - (0.5)    |          |
| Executive Function             |                    |                    |                    | 0.000379 |
| Mean                           | -0.603             | -0.589             | -2.54              |          |
| Median                         | -0.553             | -0.749             | -2.54              |          |
| Range                          | (-2.34) - (0.989)  | (-1.42) - (0.329)  | (-3.31) - (-1.7)   |          |
| Memory                         |                    |                    |                    | 0.224    |
| Mean                           | -2.077             | -2.28              | -3.192             |          |
| Median                         | -2.096             | -2.2               | -3.192             |          |
| Range                          | (-3.93) - (-0.082) | (-3.866) - (-0.75) | (-4.36) - (-2.3)   |          |
| Visuospatial Skills            |                    |                    |                    | 0.185    |
| Mean                           | -0.719             | -0.757             | -4.16              |          |
| Median                         | -0.518             | -0.359             | -4.16              |          |
| Range                          | (-2.85) - (0.29)   | (-5.59) - (0.23)   | (-7.25) - (-1.08)  |          |
| Language Skills                |                    |                    |                    | 0.153    |
| Mean                           | -0.646             | -0.79              | -1.82              |          |
| Median                         | -0.407             | -0.713             | -1.82              |          |
| Range                          | (-3.27) - (0.39)   | (-2.98) - (0.537)  | (-2.04) - (-1.6)   |          |

|                          |        | ANI<br>(n=40)    | MND<br>(n=20)    | HAD<br>(n=2)       | P Value |
|--------------------------|--------|------------------|------------------|--------------------|---------|
| Temporal and Orientation | Mean   | -0.221           | -0.639           | -3.5               | 0.008   |
|                          | Median | 0.158            | 0.158            | -3.5               |         |
|                          | Range  | (-3.5) - (0.158) | (-3.5) - (0.158) | (-5.09) - (-1.923) |         |
|                          |        |                  |                  |                    |         |

### 3.6 Discussion

#### 3.6.1 Discussion of the Results

In the study cohort 62/104 (59.6%) of patients who had a positive screen for cognitive impairment met diagnostic criteria for HAND on further neuropsychological evaluation while 32/104 (30.8%) had abnormalities on only one domain and therefore failed to meet diagnostic criteria for HAND but did not have normal neuropsychological testing. The remaining ten patients (9.6%) had entirely normal cognitive testing. The sensitivity of the screening tool that identified patients for recruitment into this study was 65% in the validation study by Ellis et al (175) so the figure found in this study would be consistent with the published sensitivity.

The predominant clinical neurocognitive profile of patients within this cohort was that of a dysexecutive, amnesic presentation. Immediate and delayed memory were the domains that were most abnormal on the RBANS. While only the RBANS was used for diagnosis of HAND, the predominant domains that were abnormal on the ACE-R and MoCA were also memory, verbal fluency/language and executive. The FAB which predominantly tests executive function was also abnormal in 45% of patients with HAND.

While many of the memory deficits may be due to poor encoding and retrieval issues, there were a significant proportion of the group with HAND who had poor retention scores demonstrating evidence of rapid forgetting on assessment of the list and story memory percent retention. Just over half of the patients with HAND had abnormal

retention of list, one third had abnormal story retention and 22.6% had abnormal retention of both list and story memory. This demonstrates that a subgroup of the patients had densely amnesic features on neuropsychological testing and that it was more common within the group who met criteria for HAND. Significantly, within the group who had abnormal neuropsychological testing but who did not meet criteria for HAND, densely amnesic features were also demonstrated. While this was less common than in the patients with HAND, only one patient who had normal neuropsychological testing had an abnormal list retention score and none of them had abnormal story retention scores suggesting that this is a significant finding both within the group of patients with HAND and those who had abnormalities that were not diagnostic of HAND. It is possible that the memory deficits in our patients with HIV are due to shallow encoding but within this subgroup of patients with features of rapid forgetting as suggested by abnormal retention scores it was demonstrated that they were statistically more likely to also have poor list recognition. The combination of poor retention and poor list recognition suggest that these patients exhibit rapid forgetting, which is a hallmark of hippocampal dysfunction. It is likely that the patients have a mixed picture with deficits in encoding and retrieval and a subgroup of patients with rapid forgetting. This suggests that there is disruption of both fronto-striatal networks and hippocampal networks in memory deficits in HIV infection. It may be possible to use retention scores to discriminate patients with less significant abnormalities or abnormalities that are not diagnostic of HAND who are likely to progress to HAND however longitudinal follow up would be required to determine its utility. It may also be possible to use this in future trials to select patients who may



benefit from medications that are aimed at hippocampal dysfunction such as donepezil.

Patients who failed to meet diagnostic criteria for HAND as they only had impairment on either one domain on the RBANS and/or abnormalities within the other cognitive batteries that were performed, may in fact represent an earlier form of HAND as they also had deficits within the executive, memory and visuospatial domains similar to the patients with HAND. There were no statistically significant differences between the three groups on analysis of demographic data and disease characteristics other than in years of education with those patients who had HAND having fewer years of education compared to those with normal neuropsychological testing. Although there were no statistically significant differences between their global domain scores and those of the ten patients with entirely normal neuropsychological testing we believe that over time these patients are highly likely to meet diagnostic criteria for HAND in the future and longitudinal follow up is warranted.

Interestingly apraxia was the most common finding on neurological examination along with hyporeflexia. Apraxia is typically deemed to be a cortical finding. It is thought to result secondary to disruption of the fronto-parietal-basal ganglia networks. It is more commonly associated with left hemisphere lesions/disruption (352). Hanna-Pladdy et al examined the cortical and subcortical contributions to apraxia and found qualitative differences between the anatomical locations. The patients with cortical abnormalities had abnormal production of transitive and intransitive gestures to verbal command and imitation and also impaired gesture discrimination whereas the patients with

subcortical pathology had mild deficits in production and execution of transitive pantomimes but with normal imitation and discrimination (353). Apraxia is commonly seen in many neurodegenerative conditions including Alzheimer's disease, corticobasal degeneration, Parkinson's disease and posterior cortical atrophy (352, 354-356).

Apraxia has been described in HIV infection in the setting of opportunistic infections (357) and immune reconstitution syndrome (358) but is considered to be rare in the setting of HAND (359-361). There is a case report of Balint's syndrome, which consists of simultanagnosia, optic ataxia and oculomotor apraxia, secondary to HIV encephalitis (362). Eye-lid opening apraxia was described in a 44 year old woman with a severe blepharospasm-omandibular dystonia syndrome secondary to HIV encephalopathy which improved with HAART (363). The 16 patients in this study who had apraxia had mild apraxia with predominantly ideomotor apraxia, often using their hand as the object or performing the task clumsily and less often limb-kinetic apraxia, again having difficulties copying hand movements with less precision and increased clumsiness. There was no evidence of oro-buccal apraxia in this group. These findings suggest cortical dysfunction, most likely of fronto-parietal origin. Apraxia has not been described often in HAND previously. This group of patients may be different than other groups previously described which would be unusual or apraxia may not necessarily have been examined that often in previous studies as the focus may have been more on neuropsychological testing rather than neurological examination findings.

Using the RBANS cortical-subcortical deviation score 48 patients with HAND had scores greater than zero suggesting underlying cortical pathology. Duff et al showed that 37% of a cohort of older adults attending primary care scored ten or more points on this

score and cautioned about clinical interpretation of this score (364) however it has been shown to reliably differentiate typical cortical dementias from typical subcortical dementias (324, 351). The combination of an amnesic, dysexecutive syndrome on neurocognitive testing with some densely amnesic findings, cortical scores on the cortical-subcortical deviation score on the RBANS and the presence of apraxia suggest that there are prominent cortical features to HAND in this cohort of patients. Alzheimer's disease also has an amnesic, dysexecutive neurocognitive profile (365, 366) and prominent apraxia (354). It is a prototypical cortical dementia and a neurodegenerative disorder. While HAND does not share the same pathophysiology as Alzheimer's disease, the overlap in clinical features suggests that there may be similar network disruption in both diseases and that in addition to fronto-striatal network disruption in HIV there is also neocortical and hippocampal involvement. These findings also support the hypothesis that there are degenerative features to HAND given their predominance and their presence in other neurodegenerative disorders like Alzheimer's disease.

### 3.6.2 Strengths of the study

This is a well characterised cohort of HIV positive patients who had a positive screen for cognitive impairment. The neuropsychological tests used are easy to use in a clinical setting and could be easily applied to other English speaking clinical populations. All of the assessments were carried out by the candidate and therefore eliminate inter-rater bias and differences.



### 3.6.3 Limitations of the study

The cohort studied are similar to the overall population who attend the clinic at St James's Hospital however as with all cross-sectional studies there may be bias within those who volunteer to participate in studies. It is possible that patients with cognitive complaints are more likely to participate in a study like this as are patients who are concerned and educated about the possible cognitive effects of HIV. Patients with poor cognitive function may be less likely to participate as they may forget to attend their appointment or be less willing to participate due to poor decision making as a result of executive impairment. Despite attempts to reduce the likelihood of a patient not attending for neuropsychological testing (phone calls and/or text reminders of the appointment time, date and place) numerous patients did not attend at all or failed to attend serial appointments prior to finally successfully entering the study.

### 3.7 Conclusion

This study found that 59.6% of a clinical population with a positive screen for cognitive impairment met diagnostic criteria for HAND. The predominant neurocognitive profile was an amnesic, dysexecutive syndrome and patients exhibited rapid forgetting as well as apraxia. These findings support the candidate's hypothesis that there are degenerative and cortical features to HAND. Longitudinal follow up will be required to elucidate this further and to examine if any of the patients with non-diagnostic abnormalities on neuropsychological testing develop further abnormalities in the future and fulfil diagnostic criteria for HAND.



The findings of cortical deficits on both the neuropsychological batteries and the neurological examination support my hypothesis that the late effects of HIV on the brain are neurodegenerative. This hypothesis can only be reliably confirmed with longitudinal neuropsychological follow-up and extensive neuropathological analysis of this well characterised cohort of patients with HIV. If, however, there were other biomarkers of neurodegeneration such as cortical atrophy on MR imaging and/or protein fragments (amyloid or tau) in the CSF this would be a valuable insight and provide further support to the hypothesis that some of the late effects of HIV on the brain are due to neurodegeneration.

## 4 Magnetic Resonance Imaging of the Brain in HAND

### 4.1 Introduction

#### 4.1.1 Outline of the chapter

This chapter focuses on the subset of patients who underwent magnetic resonance imaging of their brain. The underlying hypothesis of the study is that HAND has both inflammatory and degenerative phases. Diffusion tensor imaging was used to examine for both inflammatory and degenerative changes whilst voxel based morphometry was utilised to assess for focal grey matter atrophy, a marker of neurodegeneration. The candidate expected to find that the HIV positive patients had evidence of focal grey matter atrophy and that there would be a correlation between the regions of atrophy and the neuropsychological tests that assess the function of that region. The candidate also expected to find that there would be evidence of disruption of the white matter microstructural integrity in the HIV positive patients.

#### 4.1.2 Voxel Based Morphometry (VBM)

Voxel based morphometry is an automated technique to assess cerebral structural changes. It is a voxel wise comparison of local tissue volumes. Statistical tests are performed across all voxels to identify volume differences between groups of patients (367). It is useful to detect focal atrophy or volume changes within specific regions. The technique was first published in 1995 by Wright et al who described a novel technique for characterising regional grey and white matter differences in structural MR images (368). They used rigid reorientation by eye, semi-automatic scalp editing and

segmentation. Ashburner et al later updated the methodology to include non-linear spatial normalisation and automatic segmentation (369). The technique was further updated with optimisation of grey matter normalisation and improved segmentation and deformation normalisation (370-372). VBM has been used in dementia (Alzheimer's disease, Frontotemporal dementia) (373-375), movement disorders (376, 377), amyotrophic lateral sclerosis (378, 379), epilepsy (380), multiple sclerosis (381-383) and psychiatric disorders (384-387). The benefits of VBM is that it is not biased to any one particular structure and it gives a comprehensive assessment of anatomical changes throughout the brain (369). It has also been used in HIV.

#### 4.1.3 Diffusion Tensor Imaging (DTI)

DTI measures the diffusion of water molecules in white matter. Movement of water can be anisotropic (directionally dependent) with diffusion greater along the length of the fibre (longitudinal direction) than perpendicular to it (radial or transverse direction), as myelin may restrict diffusion (388). With DTI, this degree of anisotropy and fibre direction can be mapped on a voxel by voxel basis, enabling in vivo assessment of white matter tract integrity (389). A diffusion tensor is a mathematical model which contains diffusion from a minimum of six directions. This allows diffusivity in any direction as well as the direction of maximum diffusivity to be calculated. The tensor matrix is ellipsoid and the principal axis is oriented in the direction of maximum diffusivity. Three eigenvalues are calculated via an algebraic procedure. These represent diffusivity in the three principal axes of the ellipsoid (major, medium and minor axes which are also known as eigenvectors) (389, 390). Diffusion anisotropy is the extent to which the shape of the tensor ellipsoid deviates

from that of a sphere; mathematically, this translates to the degree to which the three tensor eigenvalues differ from one another (390).

Diffusion along the major axis is assumed to reflect diffusivity parallel to the white matter tract and is termed axial or longitudinal diffusivity (AD). The two minor axes can be averaged to determine radial or transverse diffusivity (RD), which reflects diffusion perpendicular to the white matter (391). Mean diffusivity (MD) reflects the average diffusion from all three directions. Fractional anisotropy is derived from the standard deviation of the three eigenvalues and ranges in value from zero to one, with zero being isotropy and one being maximum anisotropy. It estimates how directionally constrained diffusion is along tracts hence it is used as an indicator of fibre coherence. It provides a measure of the general shape of the ellipsoid (389, 392). AD is a measure of axonal integrity and abnormalities in RD may indicate disruption of myelin integrity (393-396).



**Table 4-1 Interpretation of DTI Measures**

| DTI Measure     | Interpretation                                             |
|-----------------|------------------------------------------------------------|
| Decreases in FA | Indicates general loss of white matter integrity (397-399) |
| Increases in MD | Indicates general increases in diffusion of water (400)    |
| Changes in AD   | Indicates axonal degeneration (393, 394, 401-404)          |
| Increases in RD | Indicates demyelination (391, 393, 395, 396, 401)          |

#### 4.1.4 Volumetric Findings in HIV

##### **4.1.4.1 Early Findings on MRI in HIV/AIDS**

Cerebral atrophy is a feature of HIV encephalitis (HIVE) and was common in the pre-HAART era. Hyper-intensities may be found on T2-weighted and fluid attenuated inversion recovery (FLAIR) images in patients with HIVE. They may be diffuse and symmetrical within the white matter or may present with patchy scattered lesions within both the white and grey matter (100). Levy et al reviewed the computed topography (CT) brain imaging of 200 patients with AIDS and found diffuse cerebral atrophy in 75/200 (37.5%) (405). Grafe et al performed magnetic resonance imaging (MRI) of ten formalin fixed brains of patients who had died secondary to an AIDS related illness. Atrophy was demonstrated in eight of the ten brains examined. Foci of

increased signal intensity on MRI correlated with infarction and necrosis. MRI also detected severe ventriculitis and focal gliosis. This study highlighted the relevance of post-mortem findings to the interpretation of MRI brain findings in living patients (406). A subsequent study compared post-mortem brain MRI and neuropathological examination in seven asymptomatic HIV positive individuals, eight seronegative controls with a similar cause of death and six patients who died of AIDS in the absence of focal cerebral changes. Cerebral atrophy was well evaluated by MRI but they did not find any correlation between neuropathological myelin pallor and diffuse white matter signal abnormalities on MRI. They concluded that MRI was unlikely to have any diagnostic or prognostic value in the early stages of HIV (407). A further study correlating post-mortem MRI findings and neuropathology, carried out by Heindel et al, found a statistically significant relationship between the severity of CNS HIV infection and the MRI volume estimates of grey matter and abnormal white matter. They also demonstrated a significant relationship between cortical grey matter volumes and cortical synaptic density (408).

Flowers et al demonstrated that MRI was an invaluable tool for the assessment of encephalopathy in patients with AIDS (409). McArthur et al performed MRI brain scans on 50 HIV negative men, 85 HIV positive asymptomatic men and 14 HIV positive men with symptomatic HIV disease. They found white matter hyper-intensities but no correlation to HIV serostatus, neurological abnormalities, CD4 T cell count, alcohol or drug use, hypertension or smoking. They suggested that the white matter hyper-intensities were non-specific but given that only 14 of their patient group had symptomatic HIV disease it is possible that the study was not sufficiently powered to

detect the significance of these abnormalities in neurologically symptomatic HIV infection (410). Elovaara et al demonstrated a relationship between cerebral atrophy in HIV infection and duration of infection, neurological symptoms and cognitive abnormalities. They also found slight atrophy in cognitively intact patients along with increased intrathecal synthesis of immunoglobulin G and HIV antibodies and they suggested that subcortical white matter was an early target for HIV (411). All of these studies have demonstrated that cerebral atrophy is common in HIV dementia but also in HIV positive patients without overt cognitive disturbance.

Jarvik et al reviewed the MRI brain findings of 30 patients with AIDS and found four distinct patterns within their study group. Patients with toxoplasmosis and progressive multifocal leukoencephalopathy had multiple areas of high signal. Large confluent hyper-intensities within the white matter were seen in those with HIV. Cerebral atrophy and ventricular enlargement was seen in chronic HIV infection. The final pattern was that of solitary high signal intensity lesions in a non-viral opportunistic infection setting (412). Broderick et al demonstrated cerebral atrophy and abnormal signal intensity within the splenium in patients with AIDS dementia complex. They also demonstrated abnormal signal intensity in the deep white matter and although there were no statistically significant differences between those with and without dementia, those with AIDS dementia complex were more likely to have more severe grades of white matter abnormality (413).



#### **4.1.4.2 Quantitative MR Imaging in HIV**

Regional anatomical differences in cerebral atrophy have been demonstrated in HIV infection. Specific grey matter volume reduction in the basal ganglia and the posterior cortex was shown by Aylward et al who also found that basal ganglia volume differentiated HIV positive patients with and without dementia (414, 415). A longitudinal study by Hall et al demonstrated a correlation between worsening cognitive function and increase in atrophy as assessed by quantitative MRI measures of atrophy (bicaudate/brain ratio and bifrontal/brain ratio) (416). Stout et al also conducted a longitudinal study of MRI changes in HIV positive men. They found cerebral atrophy in both medically symptomatic and asymptomatic patients. Ventricular enlargement occurred only in those with advanced HIV infection (CDC stage C) and white matter atrophy was accelerated in CDC stage C. The caudate nucleus was the only grey matter region to sustain accelerated volume loss in stage C disease. This volume loss correlated with declining CD4<sup>+</sup> count (417). Archibald et al demonstrated decreases in the volumes of cerebral and cerebellar white matter, caudate nucleus, hippocampus and the cerebral cortex (418). Thompson et al found evidence of cortical thinning in the primary sensory, motor and premotor cortices in patients with AIDS. There was a correlation between prefrontal and parietal atrophy with lower CD4<sup>+</sup> T cell count. Cognitive or motor deficits correlated with prefrontal and parietal atrophy (419). Chiang et al found 10-15% atrophy bilaterally in the putamen, globus pallidus, thalamus, medial and basal frontal lobes, corpus callosum and the posterior limb of the internal capsule. Atrophy was most severe (15-20% loss) in the primary and association sensorimotor areas. There was relative sparing of the temporal lobes. No correlation was demonstrated between grey matter atrophy and



cognitive impairment but there was a correlation between volume loss and cognitive impairment on the white matter mask. There were correlations between CD4<sup>+</sup> T cell count and atrophy of the medial and basal frontal lobes, genu of the corpus callosum, middle part of the cingulate gyrus, primary and association sensorimotor areas, putamen and globus pallidus using the white matter mask (420).

Kuper et al demonstrated grey matter atrophy in the anterior cingulate and temporal cortices as well as white matter atrophy in the midbrain which increased with cognitive decline. They also found that prefrontal grey matter atrophy correlated with longer disease duration and that basal ganglia atrophy was correlated with motor dysfunction. Lower CD4<sup>+</sup> T cell count was associated with occipital grey matter atrophy (421). Cardenas et al examined the effect of HAART on atrophy and found that patients on HAART who were virally suppressed exhibited ongoing white matter volume loss (422). In a study by Ances et al they did not find any difference in measures of atrophy in the amygdala, caudate and corpus callosum between HIV positive patients on HAART and those naive to HAART (423). Becker et al also demonstrated persistence of subcortical atrophy in those on HAART (424). The CHARTER group demonstrated that a lower nadir CD4<sup>+</sup> T cell count was related to most measures of structural brain damage and that detectable CSF HIV RNA was related to less total white matter. Co-infection with hepatitis C was associated with more abnormal white matter while longer duration of HAART therapy was associated with loss of white matter and increased sulcal volumes (425). Heaps et al demonstrated atrophy in the white matter, thalamus and grey matter of South African patients infected with clade C HIV (426). Steinbrink et

al also demonstrated a significant correlation between the severity of global cerebral atrophy, basal ganglia signal changes and cognitive impairment (427).

Ances et al suggested that a gradual decline in caudate volume occurred after self-reported seroconversion (423). Ragin et al detected cortical grey matter atrophy in patients with HIV for less than one year (428). Total cerebral volume, including grey and white matter volume and volumes of the parietal, temporal and frontal lobes and the hippocampus, were most strongly associated with nadir CD4 and duration of infection while basal ganglia volumes were related most strongly to current HIV RNA (429). Kallianpur et al found a relationship between detectable HIV DNA in peripheral blood and enlargement of lateral ventricles and volumetric reductions of caudate, putamen, thalamus, hippocampus, nucleus accumbens, brainstem, total cortical grey matter and cerebral white matter (430).

Pfefferbaum examined the effects of alcohol and HIV infection on cerebral volume and found that HIV positive patients had reduced thalamic and frontal volumes which correlated with nadir CD4 count and a history of an AIDS defining illness. Patients who were HIV negative and had a history of alcoholism had volume deficits of the globus pallidus, hippocampus, amygdala and thalamus but those who were HIV positive, had a history of alcoholism and a history of an AIDS defining illness had smaller callosal volumes and larger Sylvian and third ventricular volumes than those who were HIV positive, had a history of alcoholism but without any AIDS defining illness. They also found that longer sobriety and less lifetime alcohol consumption were predictive of attenuated brain volume abnormalities in HIV positive and negative patients with

alcoholism (431). Given that alcohol dependence and excess are often a co-morbid issue for patients with HIV infection, it is important to be aware of the additive effect of alcohol on brain atrophy especially in the setting of advanced immunosuppression.

Thames et al examined the relationship between verbal fluency tasks and basal ganglia volumes and found that performance on phonemic word generation significantly predicted caudate volume, whereas performance on phonemic switching predicted putamen volume (432).

Towgood et al found that HIV positive subjects had atrophy of the medial and superior gyri compared to HIV negative subjects and found an independent effect of aging in fronto-temporal grey and white matter. They also found that the younger HIV positive subjects had a reduction in the volume of grey matter in the medial and superior frontal region and the older HIV positive subjects had a reduction in the volume of grey matter in the superior frontal gyrus compared to age matched HIV negative subjects despite similar cognitive performance (433). Becker et al demonstrated that HIV positive subjects had greater grey matter atrophy in the posterior and inferior temporal lobe, parietal cortex and cerebellum. They also demonstrated that aging had a significant effect on superior temporal and inferior frontal volumes. There was no significant interaction between HIV and age (434). Chang et al examined the impact of apolipoprotein E (APOE)  $\epsilon 4$  on cognition and atrophy and found that HIV positive subjects with APOE- $\epsilon 4$  genotype exhibited premature atrophy, most notable in the white matter and bilateral putamen (435).



Table 4-2 Summary of MRI Studies in HIV

| Author and Year            | Patients                                                                                                                     | Findings                                                                                                                                                                               |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Levy et al (1986) (405)    | 200 patients with AIDS                                                                                                       | Diffuse cerebral atrophy (75/200)                                                                                                                                                      |
| Grafe et al (1990) (406)   | 10 formalin fixed brains of patients who had died due to AIDS                                                                | Atrophy in 8/10<br>Foci of increased signal intensity on MRI correlated with infarction and necrosis<br>Severe ventriculitis (1/2 cases)<br>Focal gliosis (2/4 cases)                  |
| Hassine et al (1995) (407) | Formalin fixed brains from:<br>7 asymptomatic HIV positive<br>6 patients who had died due to AIDS<br>8 HIV negative controls | Asymptomatic HIV positive subjects were significantly more atrophic than the seronegative controls and significantly less atrophic than those with AIDS                                |
| Heindel et al (1994) (408) | Formalin fixed brains from 17 patients who had died due to AIDS                                                              | No association between MRI volumes and gliosis<br>Statistically significant association between severity of CNS HIV infection and MRI volumes.                                         |
| Jarvik et al (1988) (412)  | 30 patients with AIDS                                                                                                        | Large confluent white matter hyperintensities in HIV<br>Cerebral atrophy and ventricular enlargement in chronic HIV infection                                                          |
| Flowers et al (1990) (409) | 47 patients with AIDS                                                                                                        | High signal intensity on T2 in 29/47<br>Focal white matter lesions in 23/47<br>Diffuse white matter process in 6/47<br>Marked cerebral atrophy in 17/47<br>Cerebellar atrophy in 18/47 |



|                              |                                                                                            |  |                                                                                                                                                                                                                                                                                                                                                                                         |
|------------------------------|--------------------------------------------------------------------------------------------|--|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                              |                                                                                            |  | Brainstem atrophy in 7/47                                                                                                                                                                                                                                                                                                                                                               |
| McArthur et al (1990) (410)  | 50 HIV negative men<br>85 HIV positive asymptomatic men<br>14 HIV positive symptomatic men |  | White matter hyperintensities were found in 24% of the HIV negative men, 26% of the asymptomatic group and 17% of those with AIDS                                                                                                                                                                                                                                                       |
| Elovaara et al (1990) (411)  | 72 HIV positive subjects<br>34 HIV negative subjects                                       |  | Atrophy was more severe and more frequent in HIV positive subjects and correlated to duration of infection, neurological symptoms and cognitive abnormalities.                                                                                                                                                                                                                          |
| Broderick et al (1993) (413) | 45 patients with AIDS; 10 met criteria for AIDS dementia complex                           |  | Cerebral atrophy and high signal intensity in the splenium was associated with AIDS dementia complex                                                                                                                                                                                                                                                                                    |
| Aylward et al (1993) (414)   | 8 HIV positive men with HIV dementia<br>16 HIV positive men<br>10 HIV negative men         |  | Total brain volume was less in HIV positive subjects<br>Basal ganglia volume differentiated HIV positive patients with and without dementia<br>Concluded that HIV infection caused generalized brain atrophy, but that the clinical features of HIV dementia developed with selective basal ganglia atrophy, consistent with the characterization of HIV dementia as <b>subcortical</b> |
| Aylward et al (1995) (415)   | 8 HIV positive men with HIV dementia<br>16 HIV positive men<br>10 HIV negative men         |  | HIV dementia is associated with grey matter volume reduction in basal ganglia and posterior cortex as well as generalised volume reduction of white matter<br><b>Subcortical atrophy</b>                                                                                                                                                                                                |
| Hall et al (1996) (416)      | 47 HIV positive subjects<br>MRIs at 6 monthly intervals over 30 months                     |  | Increase in atrophy over time associated with worsening cognitive function<br><b>Subcortical atrophy</b>                                                                                                                                                                                                                                                                                |

|                              |                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                             |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Stout et al (1998) (417)     | 86 HIV positive subjects<br>23 HIV negative subjects<br>At least 2 MRIs 6 months apart                                           | Progressive atrophy in the grey and white matter<br><b>Subcortical atrophy</b>                                                                                                                                                                                                                                                                              |
| Archibald et al (2004) (418) | 21 HIV positive subjects<br>19 HIV negative subjects<br>Ante mortem MRI and post-mortem histopathology for HIV positive subjects | Significant decrease in the volumes of cerebral and cerebellar white matter, caudate nucleus, hippocampus and, to a lesser extent, cerebral cortex<br>Volume of cerebral white-matter tissue with elevated signal was also increased – this predicted the presence of HIV at autopsy<br><b>Cortical and subcortical atrophy (subcortical &gt; cortical)</b> |
| Thompson et al (2005) (419)  | 26 subjects with AIDS<br>14 HIV negative subjects                                                                                | In AIDS, primary sensory, motor and premotor cortices were 15% thinner.<br>Thinner frontopolar and language cortex correlated with lower CD4 counts.<br>Prefrontal and parietal tissue loss correlated with cognitive/motor deficits<br><b>Cortical atrophy</b>                                                                                             |
| Chiang et al (2007) (420)    | 26 subjects with AIDS<br>14 HIV negative subjects                                                                                | 15-20% atrophy in the primary and association sensorimotor areas<br>Atrophy of these regions, particularly in the white matter, correlated with cognitive impairment and lower CD4 counts<br><b>Cortical atrophy</b>                                                                                                                                        |
| Cardenas et al (2009) (422)  | 39 HIV positive subjects on HAART<br>30 HIV negative subjects<br>MRI at baseline and at 24 months                                | Greater loss of white matter from the entire brain as well as white matter of the frontal, temporal, and parietal lobes, compared to HIV negative<br>Greater rates of grey matter loss in HIV positive subjects (detected by deformation morphometry) – correlated with higher viral loads<br><b>Cortical and subcortical atrophy</b>                       |
| Cohen et al (2010) (429)     | 69 HIV positive asymptomatic subjects                                                                                            | Individuals with ADC exhibited decreased total grey matter and parietal cortex volumes                                                                                                                                                                                                                                                                      |

|                             |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-----------------------------|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                             | 13 HIV positive subjects with AIDS dementia complex                                              | Overall cerebral volume, including grey and white matter volume and volumes of the parietal, temporal, and frontal lobes and the hippocampus, were most strongly associated with nadir CD4 count and duration of infection whilst basal ganglia volumes were related to plasma HIV RNA<br><b>Cortical and subcortical atrophy</b>                                                                                                                                              |
| Kuper et al (2011) (421)    | 48 HIV positive subjects (28 with and 20 without cognitive deficits)<br>48 HIV negative subjects | VBM showed grey matter decrease in the anterior cingulate and temporal cortices along with white matter reduction in the midbrain region<br>Prefrontal grey matter atrophy in HIV was associated with longer disease duration, while motor dysfunction was associated with basal ganglia grey matter atrophy<br>Lower CD4 count correlated with decrease of occipital grey matter<br><b>Provide evidence for atrophy of nigro-striatal and fronto-striatal circuits in HIV</b> |
| Becker et al (2011) (424)   | 84 HIV positive subjects<br>71 HIV negative subjects                                             | Significant atrophy in both the caudate and putamen<br>The volume of the basal ganglia was inversely associated with the time since first seropositivity<br><b>Subcortical atrophy</b>                                                                                                                                                                                                                                                                                         |
| Jernigan et al (2011) (425) | 251 HIV positive subjects                                                                        | Lower CD4 nadir was associated with lower volumes of total white and subcortical grey matter and more abnormal white matter<br><b>Subcortical atrophy</b>                                                                                                                                                                                                                                                                                                                      |
| Ances et al (2012) (423)    | 26 HIV positive subjects on HAART<br>26 HIV positive subjects naïve to                           | HIV positive subjects had significant reductions within the amygdala, caudate and corpus callosum                                                                                                                                                                                                                                                                                                                                                                              |



|                               |                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                               | HAART                                                                          | No effect of HAART                                                                                                                                                                                                                                                                                                                                                                                            |
|                               | 26 HIV negative subjects                                                       | <b>Subcortical pattern of atrophy</b>                                                                                                                                                                                                                                                                                                                                                                         |
| Heaps et al (2012) (426)      | 28 HIV positive subjects<br>23 HIV negative subjects                           | Significantly lower volumes in the total white matter, thalamus and total grey matter<br><b>Cortical and subcortical atrophy</b>                                                                                                                                                                                                                                                                              |
| Ragin et al (2012) (428)      | 43 HIV positive subjects<br>21 HIV negative subjects                           | Significant reductions in total and cortical grey matter<br><b>Cortical and subcortical atrophy</b>                                                                                                                                                                                                                                                                                                           |
| Towgood et al (2012) (433)    | 20 HIV positive subjects aged 20 – 40<br>20 HIV positive subjects aged 50 – 75 | Reduced grey matter volume on MRI within the medial and superior frontal gyri<br>Significant ageing effects in fronto-temporal grey and white matter, independent of the effect of HIV<br><b>Cortical atrophy</b>                                                                                                                                                                                             |
| Becker et al (2012) (434)     | 84 HIV positive subjects<br>76 HIV negative subjects                           | HIV-related grey matter loss included the posterior and inferior temporal lobes, the parietal lobes and the cerebellum<br><b>Cortical atrophy</b>                                                                                                                                                                                                                                                             |
| Kallianpur et al (2013) (430) | 35 HIV positive subjects<br>12 HIV negative subjects                           | Subjects with detectable HIV DNA demonstrated decreased volumes of cerebellar and total subcortical grey matter<br>Only the detectable HIV DNA group showed significant enlargement of lateral ventricles and volumetric reductions of caudate, putamen, thalamus, hippocampus, nucleus accumbens, brainstem, total cortical grey matter and cerebral white matter<br><b>Cortical and subcortical atrophy</b> |



#### 4.1.5 DTI Findings in HIV

White matter tract integrity in HIV has been interrogated using diffusion tensor imaging (DTI). Pomara et al performed DTI in six HIV positive patients and nine healthy controls. Abnormal fractional anisotropy was found in the white matter of the frontal lobes and internal capsules of the HIV positive patients without any difference in mean diffusivity (436). They suggested that DTI may be more sensitive than conventional MRI methods for detecting subtle white matter disruptions in HIV disease. Filippi et al assessed ten HIV positive patients with mild atrophy on MR brain imaging and found decreased diffusion anisotropy in the splenium and genu of the corpus callosum (assessed by fractional anisotropy) and increased diffusion constant of the subcortical white matter in the frontal and parieto-occipital lobes (assessed by mean diffusivity) (437). Thurnher et al also demonstrated a statistically significant reduction in FA in the genu of the corpus callosum (438). Reductions in FA of the splenium of the corpus callosum have been demonstrated by Wu et al and correlated with dementia severity and deficits in motor speed. They also demonstrated increased MD values in the splenium which correlated with motor speed deficits (439). Wright et al found a reduction in MD in the corpus callosum and centrum semiovale (392). Ragin et al found significant relationships between increased MD measures in the putamen and verbal memory and overall cognitive impairment while reduced FA values were associated with visual memory and working memory. FA values within the caudate nucleus were associated with visual memory. There were relationships also between subcortical FA measures and CD4 count (440). Lower FA values in the regions of the superior corona radiata, the corpus callosum and the cingulum have been associated with poorer performance on prospective memory tasks in South African HIV positive

patients (441). Lower FA values have also been demonstrated in other studies (442, 443).

Gongvatana et al showed significantly higher MD and lower FA throughout the white matter of HIV positive subjects. Co-infection with hepatitis C was associated with decreased FA in the rostral middle frontal gyrus, medial orbitofrontal cortex, fusiform gyrus, inferior temporal gyrus and banks of the superior temporal sulcus and increased MD in the inferior parietal cortex, precuneus cortex, entorhinal cortex, parahippocampal gyrus, fusiform gyrus, inferior temporal gyrus, banks of the superior temporal sulcus and occipital lobes. Older age was associated with decreased FA in the fusiform gyrus, inferior temporal gyrus, superior temporal sulcus, insular cortex, all frontal white matter tracts, supramarginal gyrus, superior parietal cortex and inferior parietal cortex. Patients on HAART had increased FA values in the parahippocampal gyrus, fusiform gyrus and banks of the superior temporal sulcus thus suggesting better white matter integrity. Subjects with higher current CD4 count had higher FA measures in parietal lobe regions (444). High RD values have been demonstrated in the inferior cingulate, occipital forceps and superior longitudinal fasciculus of HIV positive patients naïve to HAART compared to those on HAART indicative of myelin disruption while HIV positive patients with AIDS had high AD values, indicative of axonal compromise, in posterior callosal regions, fornix and superior cingulate bundle (445). Chen et al did not demonstrate any effect of HAART on DTI measures. They also showed that RD was more affected than AD in HIV infection and suggested that demyelination may be the prominent disease progression in white matter (446). Nir et al also demonstrated that RD was more affected than any other DTI measure in a study of HIV positive patients

over the age of 60. They did not find an effect of age but found pervasive decreases in FA values and an increase in all diffusivity measures (447).

Zhu et al used a tract based spatial statistical approach to DTI analysis and demonstrated increased MD in the posterior aspects of both hemispheres in HIV positive neurologically asymptomatic subjects while those with mild cognitive impairment exhibited increased MD in prefrontal regions and decreased FA in the genu of the corpus callosum, the bilateral anterior corona radiata and cingulate gyrus. These results correlated with duration of infection. Speed of information processing and verbal fluency scores correlated with either reduced FA or increased MD in the body and splenium of the corpus callosum, the left anterior limb of the internal capsule, the left anterior, left superior and bilateral posterior corona radiata, the left posterior thalamic radiation, bilateral external capsule, the left superior longitudinal fasciculus and bilateral superior fronto-occipital fasciculus. The HIV positive subjects had nadir CD4<sup>+</sup> T cell counts less than 200 and had been on HAART for at least 12 consecutive weeks prior to MRI so this study highlighted that there is ongoing CNS injury in partial immune reconstitution, even in those who are neurologically asymptomatic (448).



#### 4.1.6 Summary

DTI has been shown to be a very sensitive method for the detection of white matter abnormalities. Overall the studies have shown increases in MD and decreases in FA in HIV infection. Early volumetric studies demonstrated global cerebral atrophy and prominent involvement of basal ganglia and white matter. In the post HAART era and with the evolution of imaging techniques more extensive atrophy has been demonstrated affecting both nigro-striatal and fronto-striatal circuits and frontal, parietal, temporal and occipital cortices. The pattern of HIV-associated brain loss may be changing from a subcortical to a cortical disease in the HAART era.

## 4.2 Study Aims

**Aim 1:** To examine patterns of focal grey matter changes in patients with cognitive impairment and HIV infection and their correlation to patient characteristics and neuropsychological test results.

**Aim 2:** To evaluate changes in white matter integrity in patients with cognitive impairment and HIV infection.

## 4.3 Methodology

### 4.3.1 Study Design and Recruitment

A cross-sectional observational study was carried out. Fifty patients with a positive screen for cognitive impairment who had undergone detailed neuropsychological assessment were recruited. Ideally, patients with HIV and normal cognition would also



have been recruited but ethical approval only allowed for recruitment and study of patients with positive screens for cognitive impairment and who had undergone detailed neuropsychological testing. Recruitment commenced on 18 January 2012 and was completed on 29 April 2013. Study participants were given a patient information leaflet (see Appendix 10) and patients provided written informed consent (see Appendix 11). Control MRI data from healthy volunteers who participated in two separate studies (a study on the imaging features of amyotrophic lateral sclerosis compared to healthy controls and a bank of healthy volunteer data from the Centre for Advanced Medical Imaging) was used for comparison. Control participants did not have a known history of HIV infection and had consented to having their anonymised imaging used for other studies at the time of initial participation. All imaging of patients and healthy volunteers was carried out at the Centre for Advanced Medical Imaging (CAMI) at St. James's Hospital under uniform scanning conditions, using identical pulse sequences and receiver coils.

#### 4.3.2 Inclusion and Exclusion Criteria

Patients were included in the study if they were HIV positive, over the age of 18, attending the outpatient clinic in St. James's Hospital, had a positive screen for cognitive impairment and had undergone detailed neuropsychological assessment, capable of giving informed consent, had sufficient use of the English language to be able to participate and were willing to voluntarily participate in the study.

Patients were excluded if they were HIV negative, under the age of 18, had insufficient English to partake or were unwilling to participate voluntarily in the study. If patients

had any of the following they were also excluded for standard MRI safety reasons: pregnancy, cerebral aneurysm clip, cochlear implant, the presence of metal in the eye or orbit, implanted spine stabilization rods, cardiac pacemaker, implanted insulin pump, implanted narcotics pump, implanted nerve stimulators, weight greater than 250 kg or severe claustrophobia.

#### 4.3.3 Ethical Approval and Data Protection

The study had full ethical approval from the combined ethics committee of the Adelaide and Meath Hospitals Incorporating the National Children's Hospital (AMNCH) and St. James's Hospital (SJH). All patients who participated in the study were given detailed patient information leaflets to read and were given the opportunity to ask any questions that they had prior to agreeing to participate in the study. The voluntary nature of participation was specifically emphasised and that refusal to participate would not affect their ongoing medical care in any way. Written informed consent was obtained from all participants. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

A password protected computerised database using Microsoft Access 2010 (R) was utilised. All identifying information was treated in a strictly confidential manner. Data was pseudo-anonymised. Each participant maintained the unique study identifying number assigned to them in the prevalence study within the database but for the MRIs each patient was assigned a new unique hospital medical record number by CAMI. This was separate to the patient's clinical hospital medical record number. The consent forms were stored in the neurology research office but did not contain any reference

to the unique study number. These documents were only accessed by the candidate. The MRI data was stored in CAMI and on an encrypted external hard drive which was only used by the candidate.

#### 4.3.4 Data Collection and Data Recorded

Demographic data collected in the prevalence study and the neuropsychological test results were included for the respective participants who agreed to take part in the MRI study and entered into the study database. CD4 T cell count, HIV viral load and antiretroviral therapy history were updated if any changes had occurred in the intervening period as well as any changes in clinical medical conditions.

#### 4.3.5 Missing Data

Nadir CD4<sup>+</sup> T cell count was not available for one patient who was diagnosed outside of Ireland. One patient was unable to tolerate the full imaging protocol and scanning was stopped due to claustrophobia.

#### 4.3.6 Acquisition of MRI Data

All patients had their MRI brain scans performed in CAMI on a 3 Tesla (T) Philips Achieva system with a gradient strength of 80 mT/m and a slew rate of 200 T/m/s. An eight channel receive only head coil was utilised. A 3D inversion recovery prepared spoiled gradient recalled echo sequence was used to obtain high resolution T1-weighted images of the brain in 160 slices, with a field of view (FOV) of 256 x 256 x 160 mm, spatial resolution of 1 x 1 x 1 mm, repetition time (TR) of 8.5ms, echo time (TE) of 3.9ms, flip angle = 8°, SENSE factor = 1.5 and acquisition time of 7 minutes 29 seconds.



T2-weighted turbo spin echo (TSE) imaging was performed in 30 slices with field of view of 230 x 184 x 149 mm, spatial resolution of 0.575 x 0.72 x 4 mm, TR of 3000 ms, TE of 80 ms, flip angle = 90°, TSE factor of 80 ms and acquisition time of 1 minute 48 seconds. Fluid attenuated inversion recovery (FLAIR) imaging was performed in 30 slices with FOV of 230 x 183 x 149 mm, spatial resolution of 0.65 x 0.87 x 4 mm, TR of 11000 ms, TE of 125 ms, TSE factor of 30 ms and acquisition time of 5 minutes and 8 seconds. Diffusion tensor imaging (DTI) was acquired using a spin echoplanar imaging (EPI) sequence with a 32-direction Stejskal-Tanner diffusion encoding scheme, FOV of 245 x 245 x 150 mm, spatial resolution of 2.5 x 2.5 x 2.5 mm, 60 slices with no interslice gap, TR of 8200 ms, TE of 59 ms, SENSE factor of 2, two b factors and a maximum b factor of 1100 s/mm<sup>2</sup>, spectral presaturation with inversion recovery (SPIR) fat suppression and dynamic stabilization in an acquisition time of 5 minutes 53 seconds. For full MRI protocols see appendices 12, 13, 14 and 15.

T1 weighted, T2 and FLAIR imaging data were individually, qualitatively reviewed by the candidate for the presence of unexpected intracranial pathology, such as space-occupying lesions, congenital malformations, and agenesis of corpus callosum, prior cerebrovascular events or findings indicative of hydrocephalus. A systematic image quality verification step was also undertaken to check for excessive motion abnormalities prior to feeding the raw data into the analysis pipelines.

#### 4.3.7 Processing of Data for Voxel Based Morphometry Analysis

The high resolution 3D T1-weighted datasets were converted from DICOM (Digital Imaging and Communications in Medicine) format to NIfTI (Neuroimaging Informatics



Technology Initiative) format using the dcm2nii program of the MRICron software package. Voxel based morphometry (VBM) analysis was conducted using the FMRIB Software Library (FSL) program (449). The FSL VBM methods have been described and the FSL-VBM platform is a well-recognised standardised tool for VBM analysis (369, 370, 450). It has been validated clinically (451).

All the images were visually inspected to ensure correct orientation (RPI orientation – right to left, posterior to anterior, inferior to superior). The images were deskulled and excess neck tissue removed. The brain extracted images were visually inspected to ensure accuracy. The brain extracted images were segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). A study specific grey matter template was created. The grey matter images were then affine registered to the GM ICBM (International Consortium for Brain Mapping) 152 template, concatenated and averaged. The averaged image was then flipped along the x-axis and the two mirror images were re-averaged to obtain a first-pass, study-specific "affine" GM template. The GM images were re-registered to this affine GM template using non-linear registration, concatenated into a 4D image called "template\_4D\_GM", averaged and flipped along the x-axis. Both mirror images were then averaged to create the final left-right, symmetric, study-specific non-linear GM template at  $2 \times 2 \times 2 \text{ mm}^3$  resolution in standard space. The 4D study template was visually inspected. The subjects' grey matter images were non-linearly registered to the study specific template and concatenated into a 4D image. The images were also modulated to compensate for any contraction or enlargement that may have occurred due to the non-linear component of the transformation. The modulated registered GM images

were concatenated into a 4D image and then smoothed with an isotropic Gaussian kernel with a sigma of 3mm. The modulated GM template was also visually inspected to ensure accuracy. Finally, a voxel-wise general linear model (GLM) was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space. The threshold-free cluster enhancement (TFCE) method was utilised to correct for multiple comparisons and statistical significance was set at  $p < 0.05$  (452). Corrections were made for mean centred age.

Figure 4-1 Structural Image Prior to VBM Pre-processing

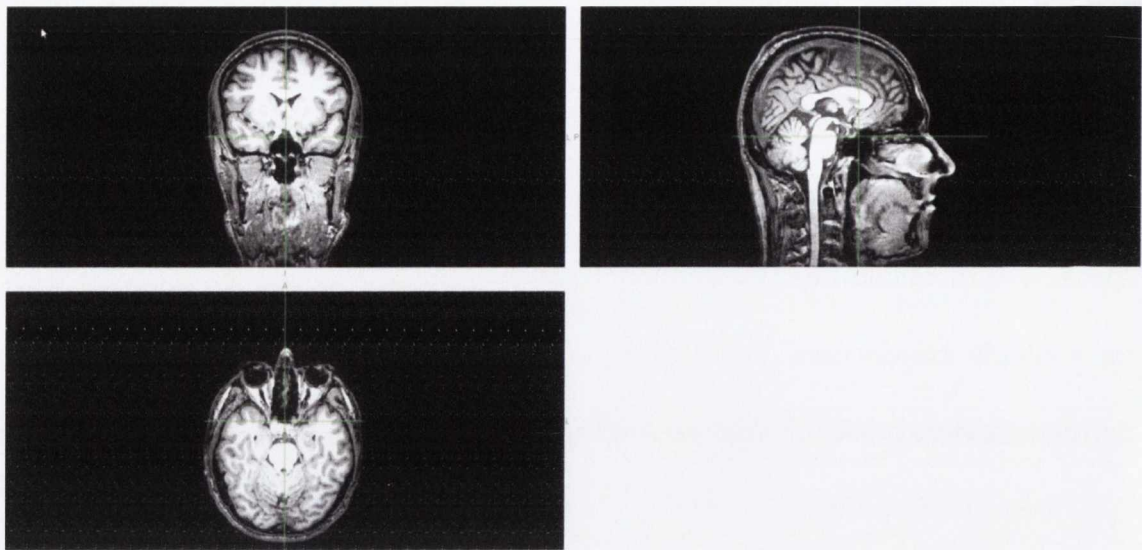


Figure 4-2 Process of brain extraction



Figure 4-3 Deskulled brain image from a study subject

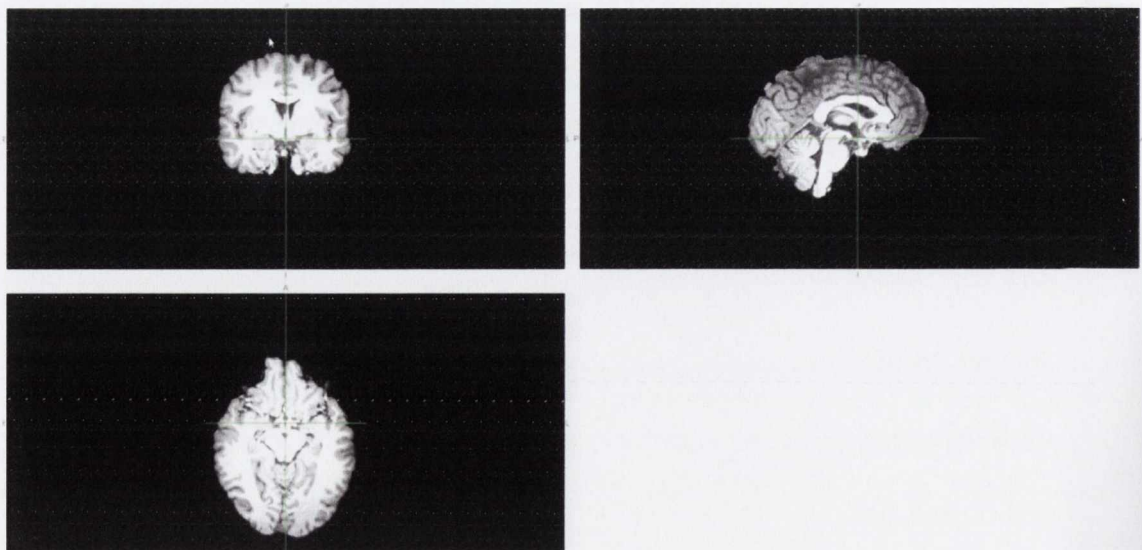




Figure 4-4 Segmentation of grey matter

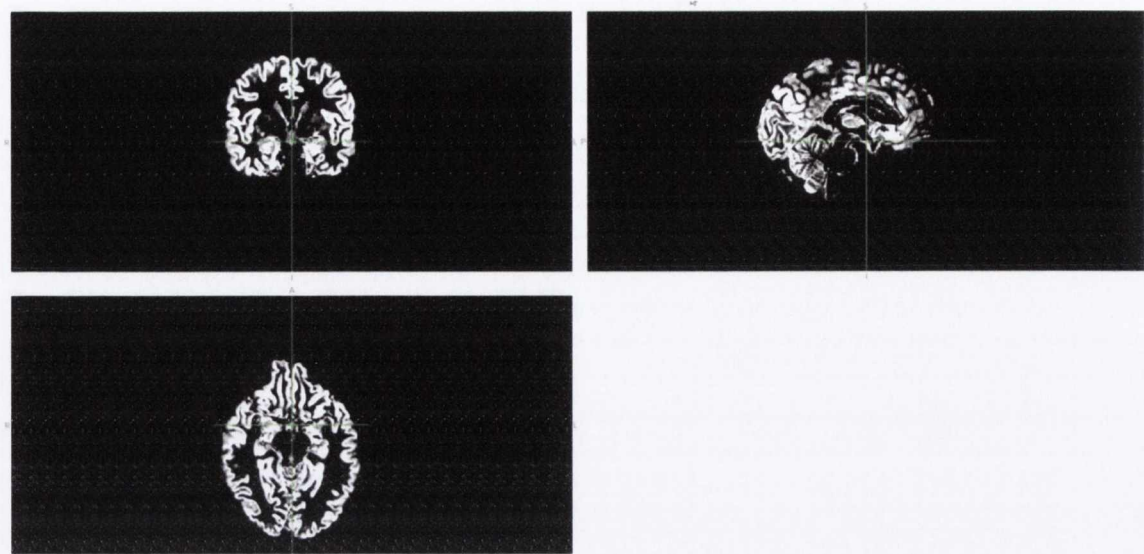


Figure 4-5 Grey matter study template

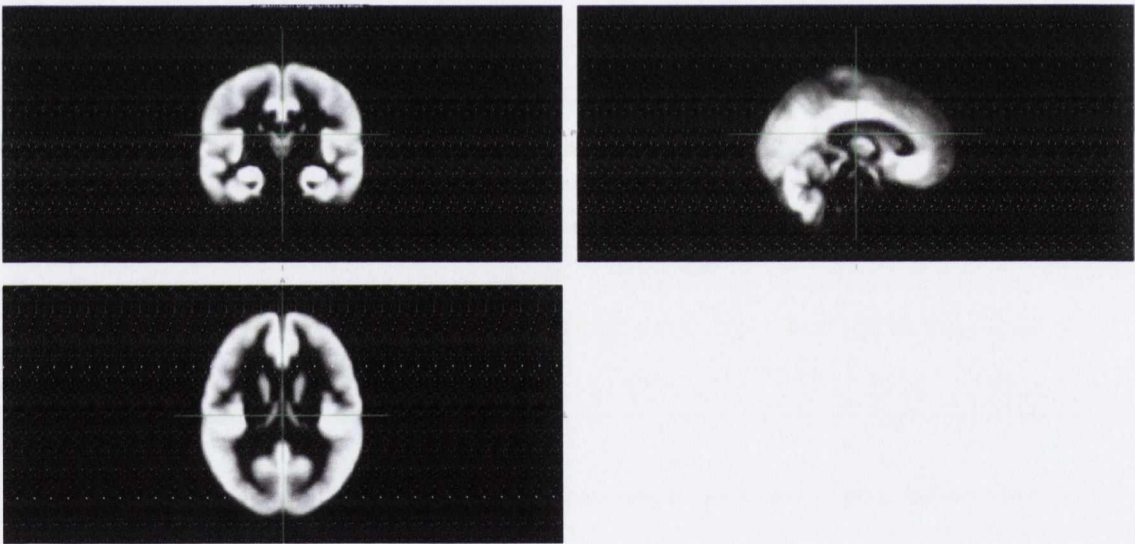




Figure 4-6 Grey matter of study subjects registered to template

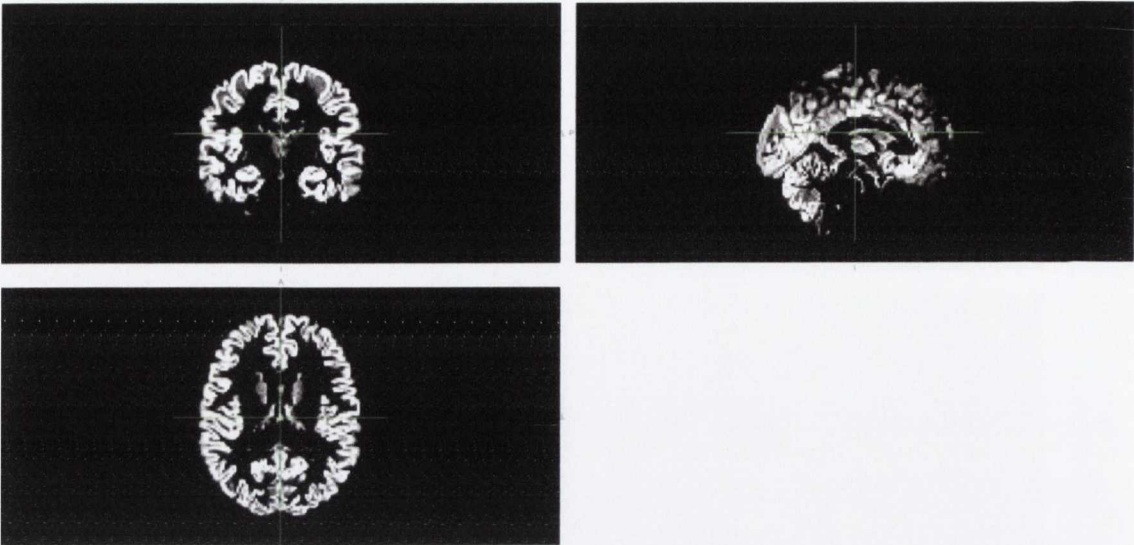
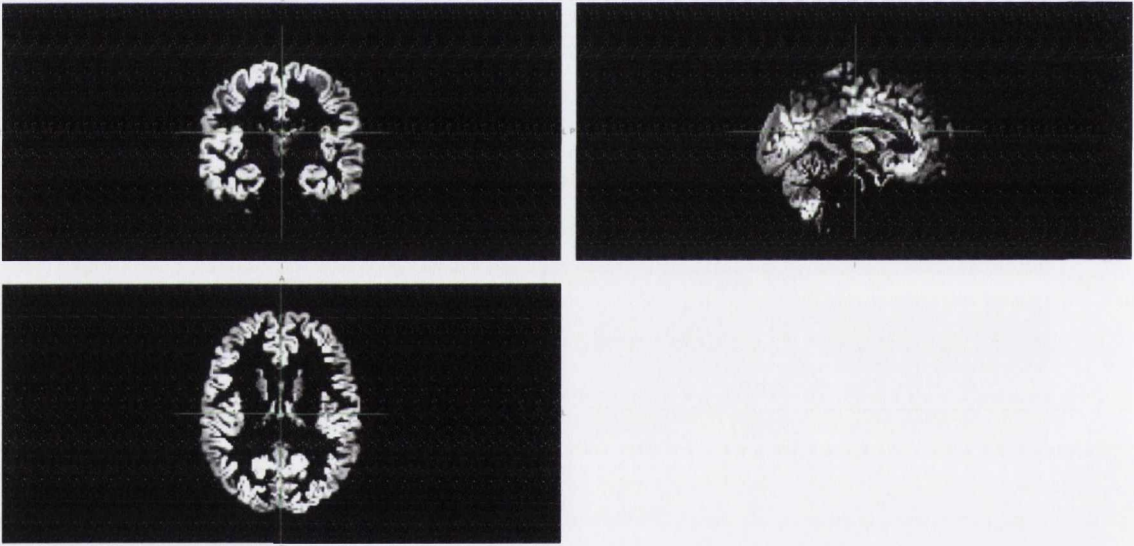


Figure 4-7 Modulation grey matter image



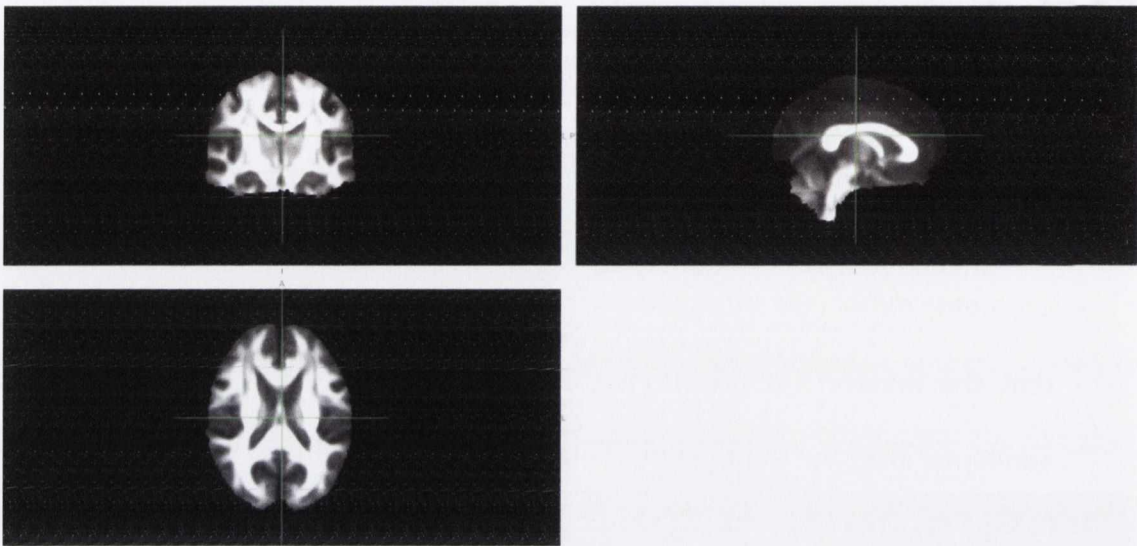
#### 4.3.8 Processing of Data for Diffusion Tensor Imaging (DTI) Analysis

ExploreDTI was used to perform the pre-processing steps for DTI analysis. ExploreDTI was released in 2009 (453). The T1-weighted high resonance structural images and the DTI images were converted from PAR REC format to NIfTI format and inspected for correct orientation. A MAT file was then generated. The images were corrected for EPI and susceptibility distortions by co-registering the DTI images to the T1-weighted structural images and subsequently for subject motion and eddy current induced geometric distortions. The data was re-inspected at the intermediate steps to ensure that the motion and distortion correction was performed correctly and that no additional artefacts were introduced into the data. The diffusion tensor and its three eigenvalues were calculated using log-linear regression in each voxel and using standard measures the three eigenvalues were used to calculate mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) data. Anisotropy was expressed as fractional anisotropy (FA) which was normalised to assume a range from 0 to 1. This data was then exported from ExploreDTI.

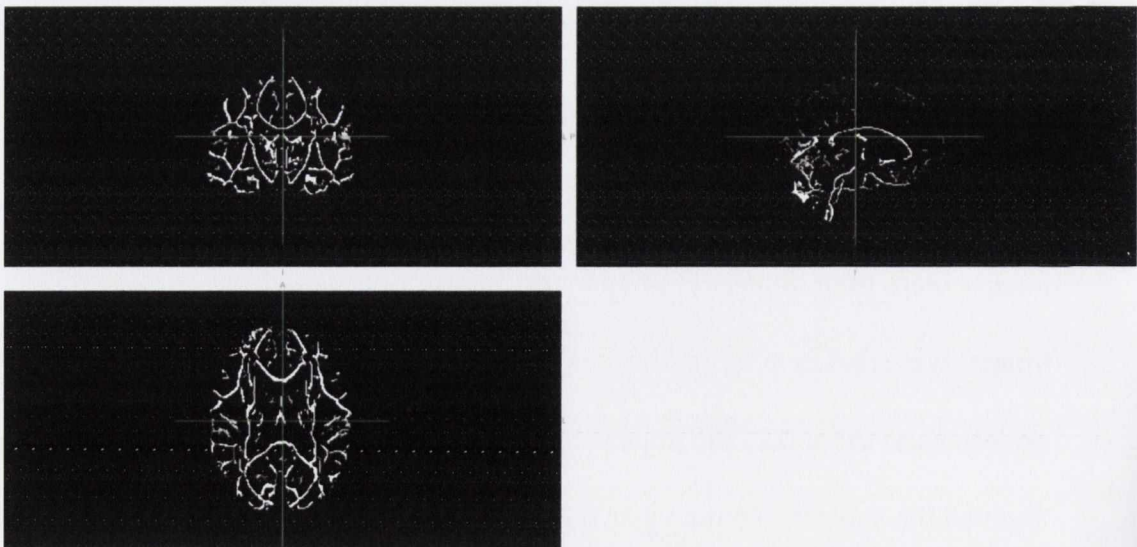
Tract based spatial statistics were then performed using the FSL platform (454). The images were non-linearly registered to a standard space FA template. The subjects FA images were then affine transformed to MNI152 space and then a mean of all the FA images was created and the images were skeletonised. The threshold for the mean FA skeleton was set at 0.2. The images were viewed to ensure accuracy of skeletonisation. Then all the subjects' FA data was projected onto the mean FA skeleton. Permutation-based nonparametric inference was used for comparison of diffusion parameters between the patient group and the healthy control group. The TFCE method was

applied for corrections of multiple comparisons and statistical significance was set at  $p < 0.05$ .

**Figure 4-8 Mean FA Image**



**Figure 4-9 Mean FA Skeleton**





#### 4.3.9 Role of the PhD Candidate

The candidate recruited and consented all of the study participants. The candidate was present for the MRI acquisition of all of the study participants and carried out all the post-processing and statistical analysis for VBM and DTI. The candidate created and maintained the study database.

### 4.4 Results

#### 4.4.1 Characteristics of the Study Group

Fifty patients who had a positive screen for cognitive impairment based on the BNCS and who had completed a detailed neuropsychological assessment underwent neuroimaging. Forty-one of the patients met criteria for HAND (27 ANI, 13 MND, 1 HAD). The remainder had abnormalities that did not fulfil diagnostic criteria for HAND on neuropsychological assessment. The majority were male (82%), Irish (70%), had a mean age of 45 years, had been diagnosed with HIV infection for a mean duration of 10.69 years, were on HAART (94%) and were virally suppressed (95.7%)(Table 4-3). The cognitive symptoms of the group are described in Table 4-4. The most common symptoms were forgetfulness, difficulties remembering names and word finding difficulties. The abnormal examination findings are described in Table 4-5. The most common examination abnormality in the study group was the presence of apraxia. The MRI data of fifty healthy volunteers was used for comparison purposes. The groups were matched exactly for gender and for age within plus/minus five years. There was no statistically significant difference on age between the patients and the healthy controls (see Table 4-6).

**Table 4-3 Characteristics of the Study Group**

| <b>Characteristics</b>               |               | <b>(n = 50)</b> |                   |
|--------------------------------------|---------------|-----------------|-------------------|
|                                      |               | <b><u>n</u></b> | <b><u>(%)</u></b> |
| <b>Handedness</b>                    | Right         | 47              | (94)              |
| <b>Gender</b>                        | Male          | 41              | (82)              |
| <b>Country of Birth</b>              | Ireland       | 35              | (70)              |
|                                      | Europe        | 4               | (8)               |
|                                      | Africa        | 10              | (10)              |
|                                      | South America | 1               | (2.0)             |
| <b>English as a first language</b>   | Yes           | 39              | (78)              |
| <b>Mode of Transmission</b>          | Heterosexual  | 19              | (38)              |
|                                      | MSM           | 22              | (44)              |
|                                      | IVDU          | 7               | (14)              |
|                                      | Other         | 2               | (4)               |
| <b>Virally Suppressed<br/>(n=47)</b> | Yes           | 45              | (95.7)            |
| <b>Hx of Hepatitis B</b>             | Yes           | 4               | (8)               |
| <b>Hx of Hepatitis C</b>             | Yes           | 9               | (18)              |
| <b>Hepatitis C PCR Positive</b>      | Yes           | 2               | (4)               |
| <b>Hypertension</b>                  | Yes           | 8               | (16)              |
| <b>Hypercholesterolaemia</b>         | Yes           | 5               | (10)              |
| <b>Diabetes</b>                      | Yes           | 3               | (6)               |
| <b>Hx of Syphilis</b>                | Yes           | 9               | (18)              |
| <b>Hx of Cryptococcal Meningitis</b> | Yes           | 1               | (2)               |
| <b>Hx of CNS TB</b>                  | Yes           | 2               | (4)               |
| <b>Hx of Toxoplasmosis</b>           | Yes           | 1               | (2)               |
| <b>Epilepsy</b>                      | Yes           | 3               | (6)               |
| <b>Neuropathy</b>                    | Yes           | 1               | (2)               |
| <b>Hx of Depression</b>              | Yes           | 14              | (28)              |
| <b>Methadone replacement therapy</b> | Yes           | 6               | (12)              |
| <b>Employment History</b>            | Employed      | 27              | (54)              |
|                                      | Student       | 4               | (8)               |
|                                      | Unemployed    | 16              | (32)              |
|                                      | Retired       | 3               | (6)               |
| <b>Smoking History</b>               | Smoker        | 21              | (42)              |
|                                      | Non smoker    | 17              | (34)              |
|                                      | Ex-smoker     | 12              | (24)              |
| <b>Alcohol History</b>               | None          | 8               | (16)              |

|                                     |                    |                    |      |
|-------------------------------------|--------------------|--------------------|------|
| <b>Units per week</b>               | 1 - 20 units       | 32                 | (64) |
|                                     | 21 - 40 units      | 5                  | (10) |
|                                     | > 40 units         | 2                  | (4)  |
|                                     | Hx of ADS          | 3                  | (6)  |
| <b>Illicit Substance Use</b>        | Never              | 38                 | (76) |
|                                     | Current Non IV Use | 6                  | (12) |
|                                     | Current IV Use     | 0                  | (0)  |
|                                     | Previous Hx        | 6                  | (12) |
| <b>Antiretroviral Therapy</b>       | Naïve              | 1                  | (2)  |
|                                     | On HAART           | 47                 | (94) |
|                                     | MTCT               | 2                  | (4)  |
| <b>Age (in years)</b>               | Mean (sd)          | 45.16 (10.6)       |      |
|                                     | Median (range)     | 44.25 (27-71)      |      |
| <b>Time since Diagnosis (years)</b> | Mean (sd)          | 10.69 (7.96)       |      |
|                                     | Median (range)     | 9.12 (0.95-28.6)   |      |
| <b>Nadir CD4 Count</b>              | Mean (sd)          | 220.5 (192.5)      |      |
|                                     | Median (range)     | 192.5 (9-848)      |      |
| <b>Current CD4 Count</b>            | Mean (sd)          | 610.8 (251.4)      |      |
|                                     | Median (range)     | 578.5 (147 - 1223) |      |
| <b>Years of Education</b>           | Mean (sd)          | 13.9 (3.9)         |      |
|                                     | Median (range)     | 14 (7-23)          |      |

MSM = Men who have sex with men, IVDU = Intravenous drug user, ADS = Alcohol dependence syndrome, HAART = Highly active anti-retroviral therapy, sd = standard deviation.

**Table 4-4 Cognitive Symptoms of the Study Group**

| <b>Symptoms</b>                 | <b>N</b> | <b>%</b> |
|---------------------------------|----------|----------|
| Word Finding difficulties       | 21       | 42       |
| Naming difficulties             | 23       | 46       |
| Facial recognition difficulties | 2        | 4        |
| Repeats                         | 17       | 34       |
| Forgetful                       | 33       | 66       |
| Misplaces objects               | 15       | 30       |
| Navigational issues             | 14       | 28       |
| Difficulties learning new info  | 9        | 18       |
| Errors at work                  | 14       | 28       |
| Safety concerns                 | 6        | 12       |
| Symptoms of dyspraxia           | 2        | 4        |
| Behavioural changes             | 1        | 2        |
| Personality changes             | 7        | 14       |
| Slower completing tasks         | 11       | 22       |
| Decreased concentration         | 10       | 20       |



**Table 4-5 Abnormal Examination Findings of the Study Group**

| Finding                                  | N  | %  |
|------------------------------------------|----|----|
| Cranial nerve abnormalities              | 2  | 4  |
| Hypertonia                               | 1  | 2  |
| Decreased power                          | 1  | 2  |
| Hyporeflexia                             | 8  | 16 |
| Hyper-reflexia                           | 2  | 4  |
| Co-ordination abnormalities              | 5  | 10 |
| Sensory abnormalities                    | 5  | 10 |
| Gait disturbance                         | 3  | 6  |
| Apraxia                                  | 10 | 20 |
| Reduction in rapid alternating movements | 4  | 8  |

**Table 4-6 Comparison of Age between Patients and Healthy Controls**

|     |                | Patients        | Healthy Controls | P value |
|-----|----------------|-----------------|------------------|---------|
| Age | Mean (sd)      | 45.16 (10.6)    | 41.48 (13.62)    | 0.135   |
|     | Median (range) | 44.25 (27 - 71) | 41 (22 - 66)     |         |

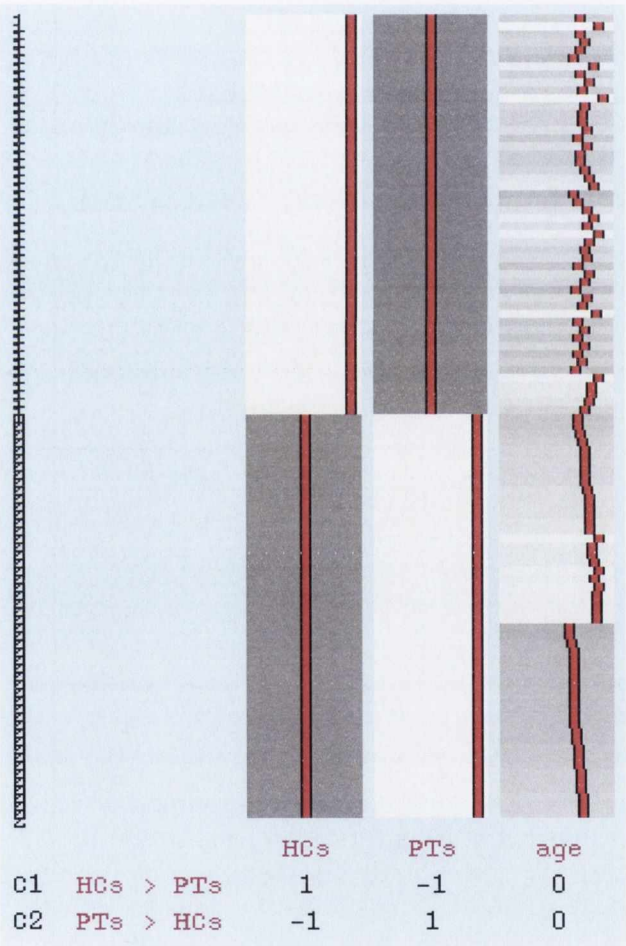
Sd = standard deviation

4.4.2 Results of VBM Analysis

In comparison to the HIV negative healthy controls, the HIV positive patients had atrophy in the anterior cingulate, left hippocampus, left temporal lobe, left central opercular cortex, left post-central gyrus, left supplementary motor area, right lateral occipital cortex, right precuneus, bilateral Heschl's gyrus (transverse temporal gyrus), bilateral insular cortex, bilateral parietal operculum, bilateral occipital poles and bilateral pre-central gyrus ( $p<0.05$ ). Significant results are displayed overlaid on a Montreal Neurological Institute (MNI) template with regions of significant difference illustrated on a spectrum of red to yellow with yellow being the most significant. The Harvard Oxford cortical structural atlas supplied with the FSL program was utilised to delineate cortical anatomy (455-458). MNI co-ordinates are displayed with each image. Anterior cingulate was the only region which remained

different between the groups at  $p < 0.01$ . The design matrix utilised is shown in Figure 4-10. Age was co-varied for in the matrix. As the groups were matched exactly for gender it was not co-varied for in the group analysis. There were no significant correlations to clinical or neuropsychological data (CD4 count, duration of infection, years of education, country of birth, illicit drug use, scores on the anxiety and depression subscales of the hospital anxiety and depression scale and scores on all the subsets of the RBANS test) co-varied for age.

Figure 4-10 Design matrix for VBM analysis



HCS=healthy controls, PTs=patients, c1=contrast 1 (to test if healthy controls had greater grey matter volumes than the HIV positive patients group), c2=contrast 2 (to test if the HIV positive group had greater grey matter volumes than the healthy controls).

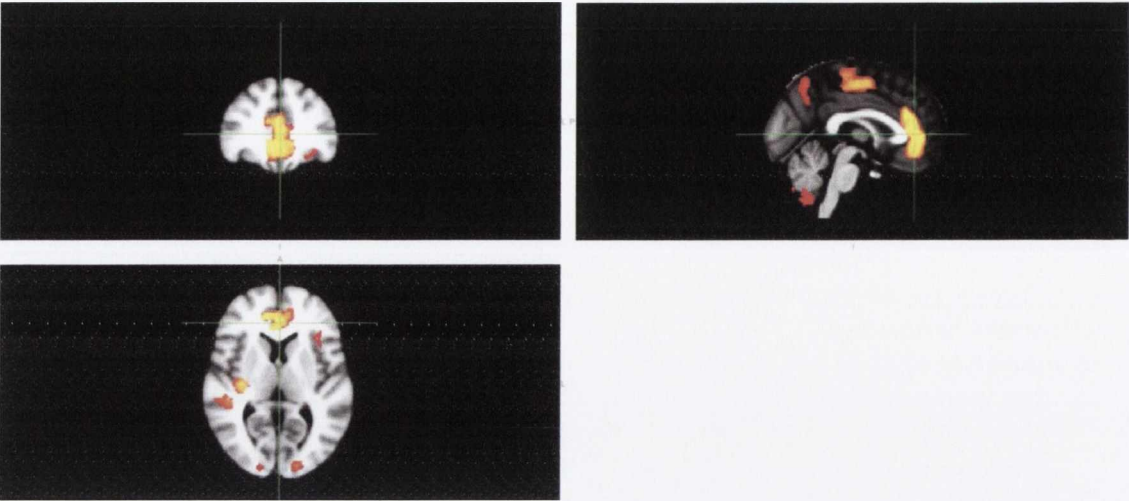
**Table 4-7 Normal function of grey matter regions that were abnormal in this study**

| Region                   | Location                                                                                                                                                                            | Function                                                                                                                                                                                     |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Anterior cingulate       | Lies between the cingulate sulcus and the corpus callosum; part of the limbic system (459).                                                                                         | It is part of a circuit involved in a form of attention that serves to regulate both cognitive and emotional processing; executive role (459).                                               |
| Hippocampus              | Medial temporal lobe                                                                                                                                                                | Consolidation of information from short-term memory to long-term memory and spatial navigation; acquisition, temporary storage, and retrieval of explicit memory for facts and events (460). |
| Temporal lobe            | Includes superior, middle and inferior temporal, lateral occipitotemporal, fusiform, lingual, parahippocampal and hippocampal convolutions and the transverse gyri of Heschl (461). | Auditory and visual processing, learning and memory, regulation of behaviour and emotion (461).                                                                                              |
| Heschl's gyrus           | Transverse temporal gyri (primary auditory cortex)                                                                                                                                  | Processes incoming auditory information (462).                                                                                                                                               |
| Pre-central gyrus        | Anterior to the central sulcus (463).                                                                                                                                               | Primary motor region (463).                                                                                                                                                                  |
| Post-central gyrus       | Posterior to the central sulcus and above the lateral fissure (463).                                                                                                                | Primary somatosensory cortex (463).                                                                                                                                                          |
| Supplementary motor area | Anterior to the primary motor cortex (463).                                                                                                                                         | Motor planning (464).                                                                                                                                                                        |
| Precuneus                | Posterior region of the medial parietal cortex, buried in the inter-hemispheric fissure and encased by the sagittal sinus and bridging veins (465).                                 | Episodic memory, visuo-spatial imagery and self-processing (465).                                                                                                                            |
| Insular cortex           | Lies beneath the temporal, frontal and parietal opercula (466)                                                                                                                      | Somatosensation, thermal and pain perception, viscerosensation, gustation, self-awareness, cognitive functioning and interpersonal experience (466).                                         |



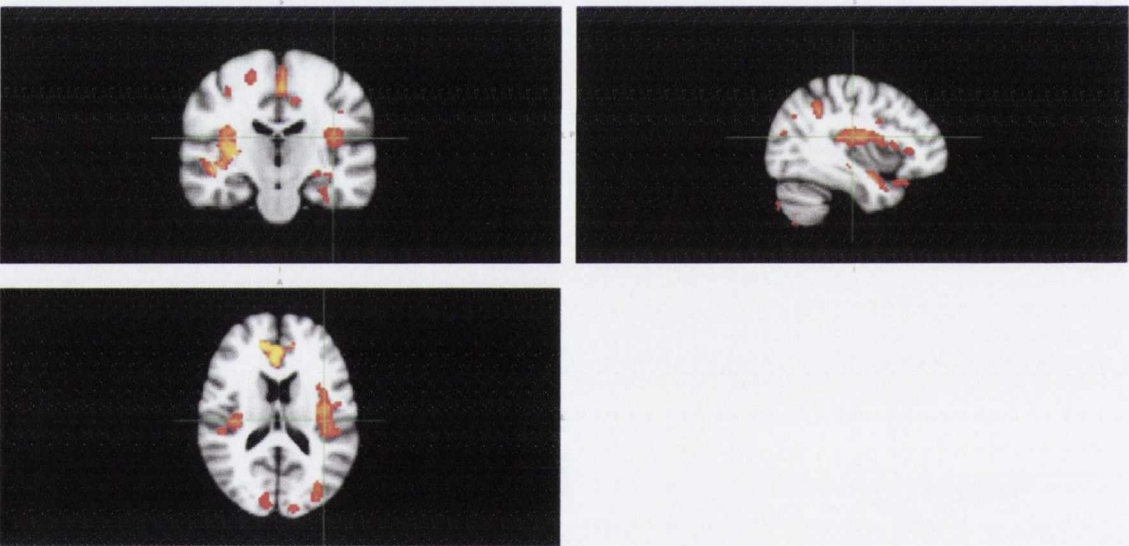
|                  |                                                                                                               |                                                                  |
|------------------|---------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| Operculum        | Cortex that borders the sylvian fissure and covers the insula; frontal, parietal and temporal opercula (461). | Perception of spoken language and motor aspects of speech (467). |
| Occipital cortex | Termini of the geniculocalcarine pathways (461).                                                              | Visual perception and recognition (461).                         |

Figure 4-11 Anterior Cingulate



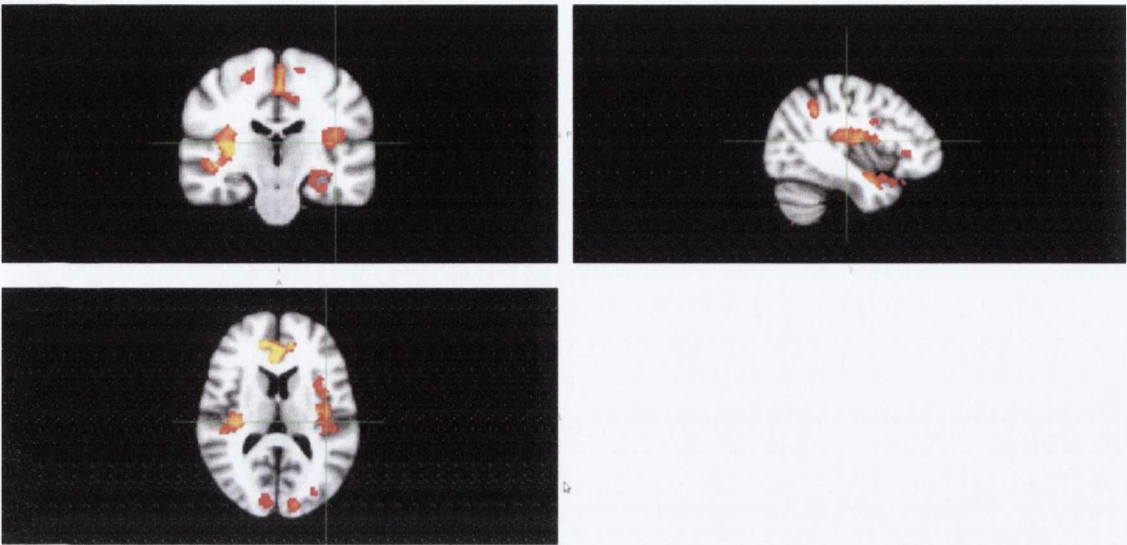
x=45, y=82, z=39 (p<0.05).

Figure 4-12 Left central opercular cortex



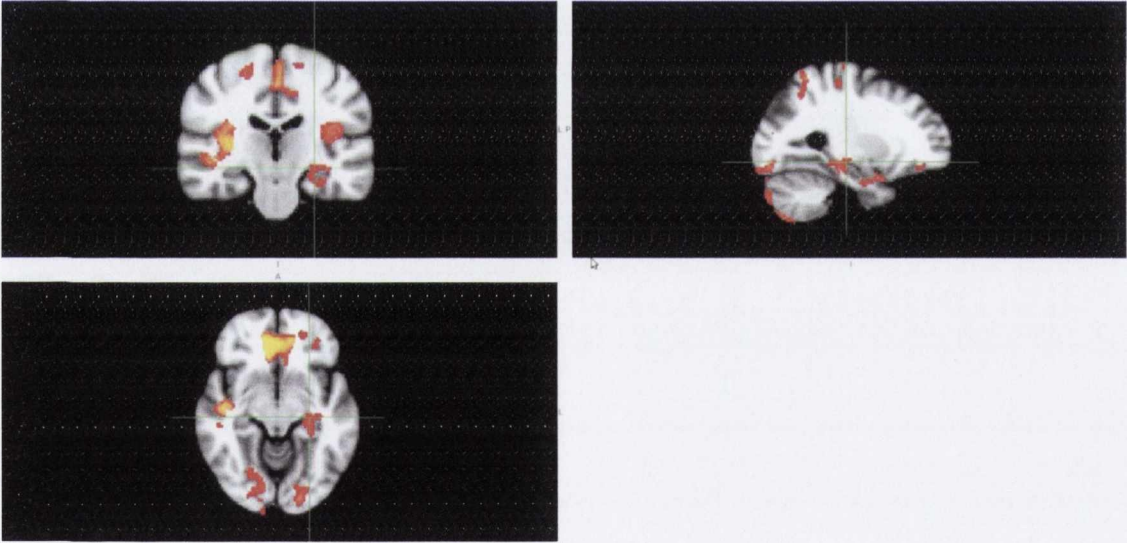
x=64, y=53, z=44 (p<0.05).

Figure 4-13 Left Heschl's gyrus and central opercular cortex



x=65, y=52, z=42 ( $p<0.05$ ).

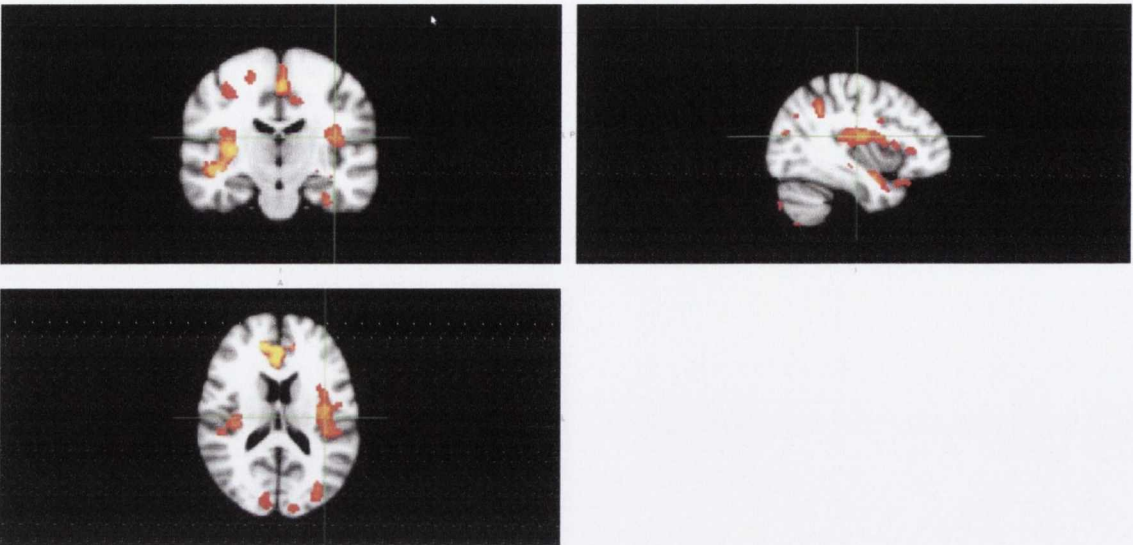
Figure 4-14 Left hippocampus



x=58, y=52, z=31 ( $p<0.05$ ).

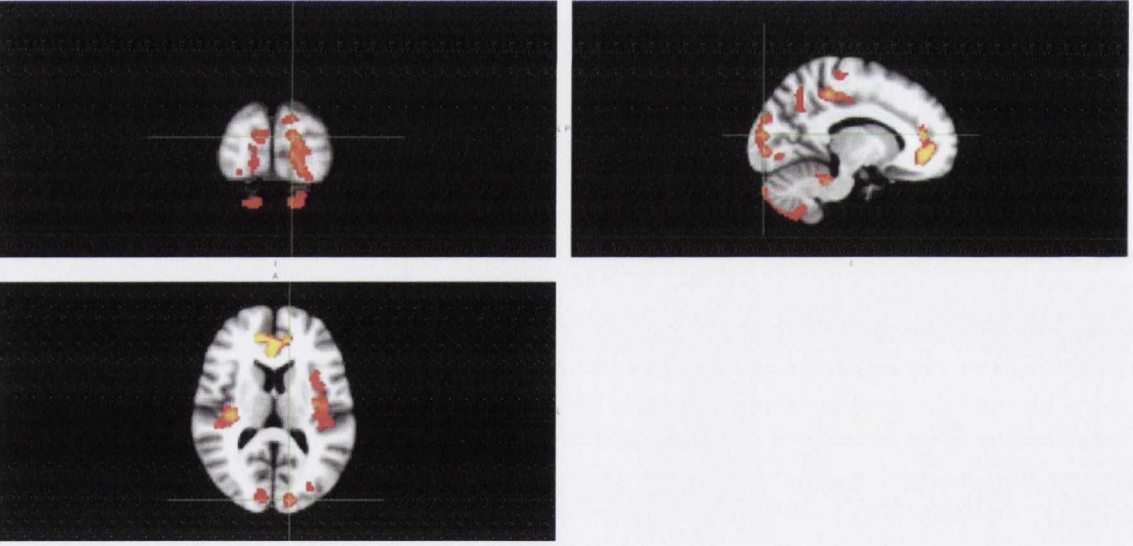


Figure 4-15 Left insular cortex and parietal operculum



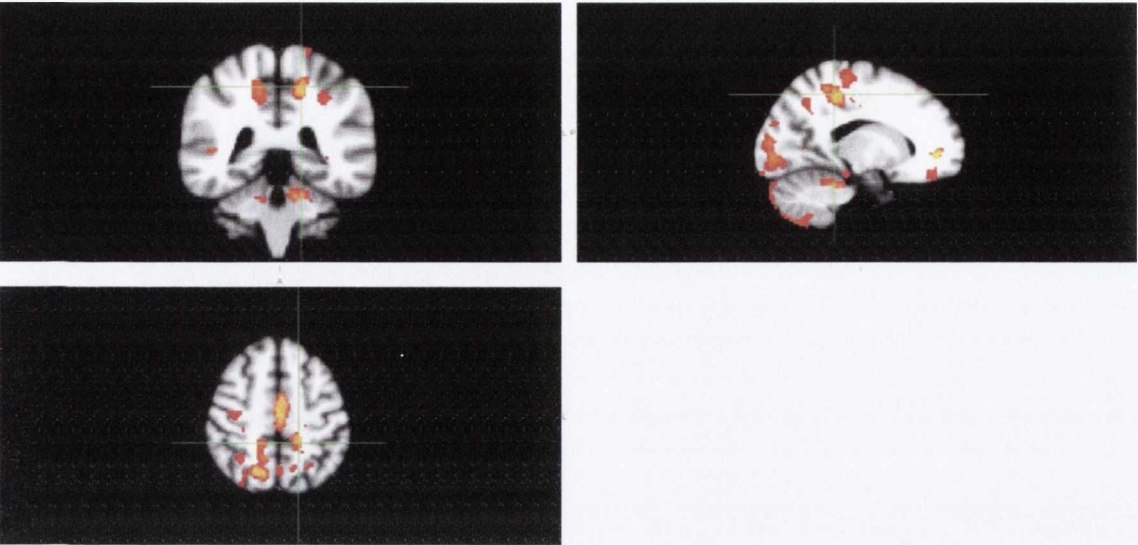
x=64, y=54, z=44 ( $p<0.05$ ).

Figure 4-16 Left occipital pole



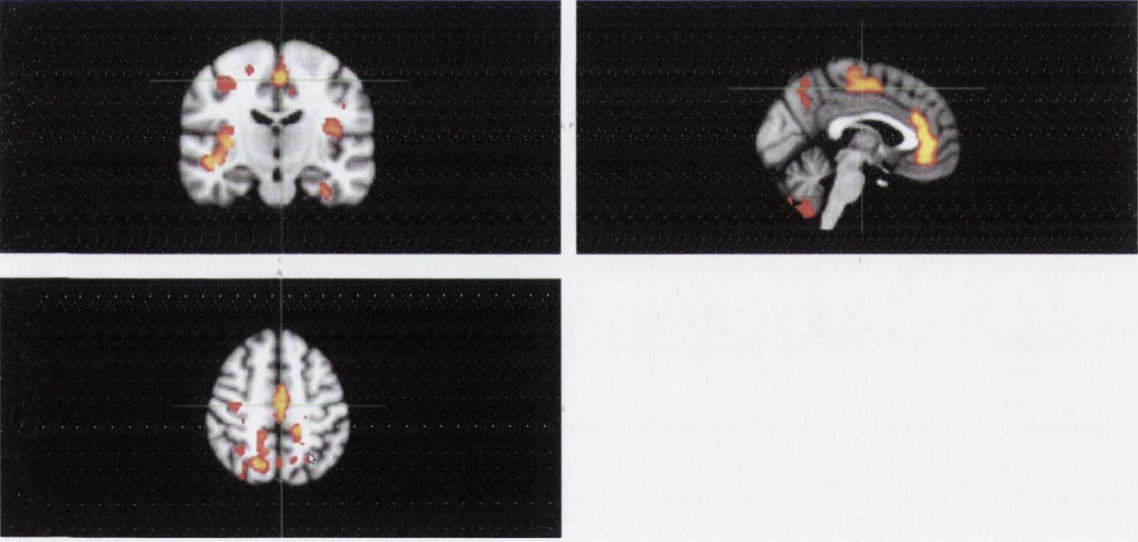
x=51, y=17, z=42 ( $p<0.05$ ).

Figure 4-17 Left post-central gyrus



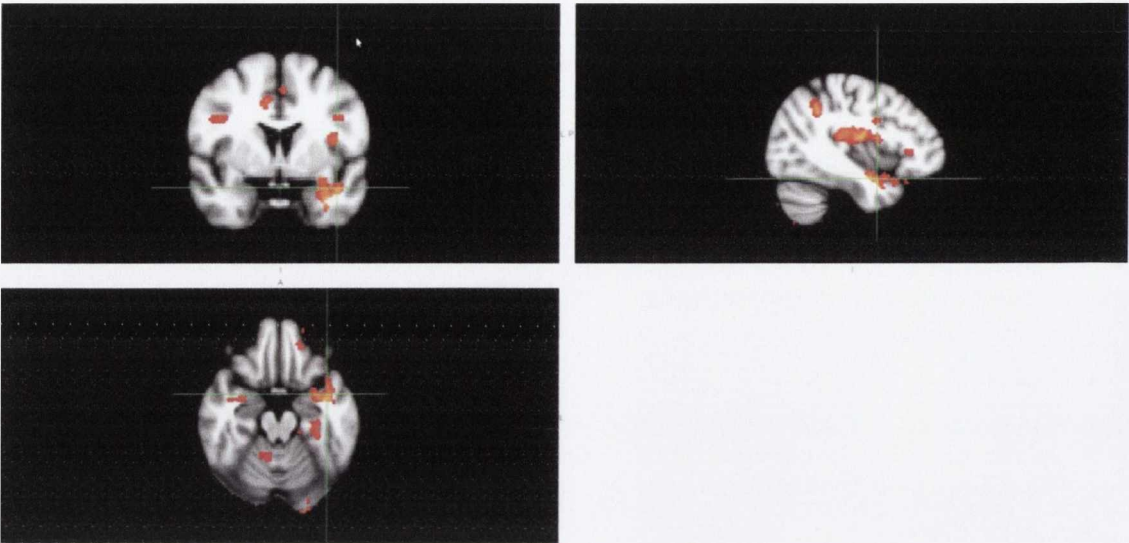
x=53, y=43, z=61 ( $p<0.05$ ).

Figure 4-18 Left pre-central gyrus and left supplementary area



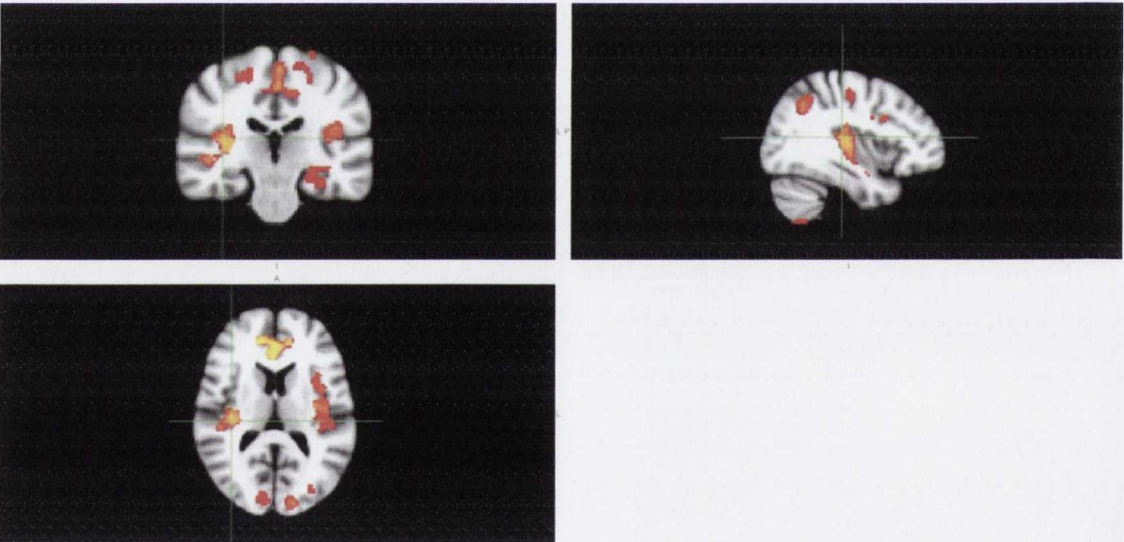
x=46, y=55, z=61 ( $p<0.05$ ).

Figure 4-19 Left temporal lobe



x=65, y=64, z=26 (p<0.05).

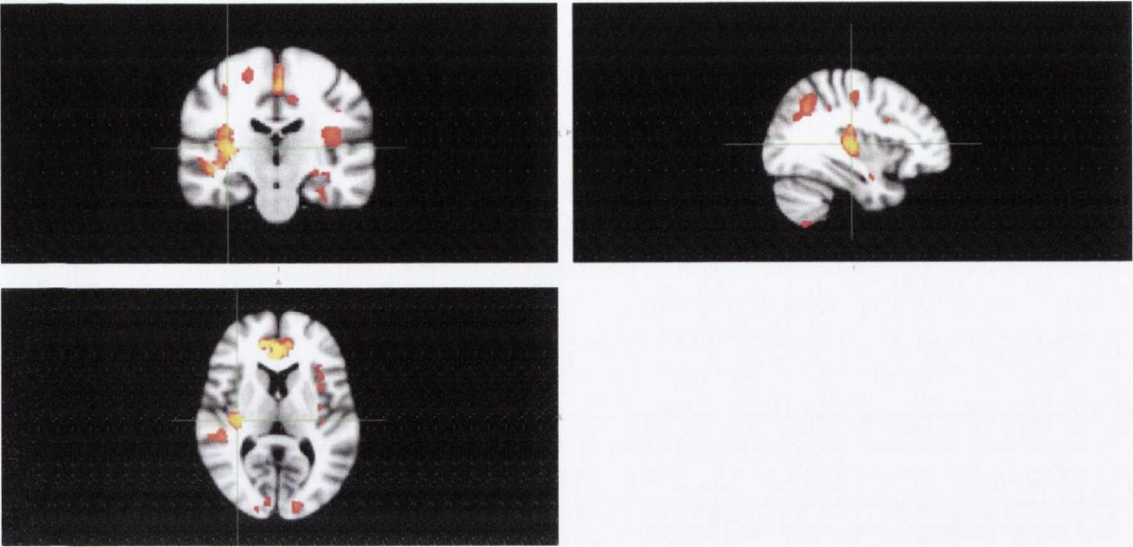
Figure 4-20 Right Heschl's gyrus and insular cortex



x=26, y=51, z=42 (p<0.05).

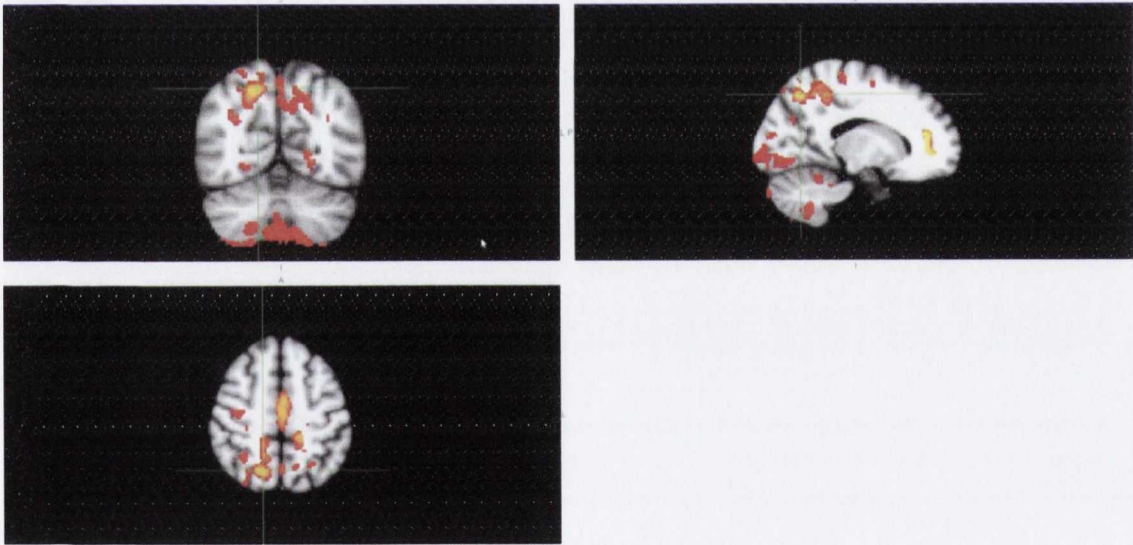


Figure 4-21 Right insular cortex



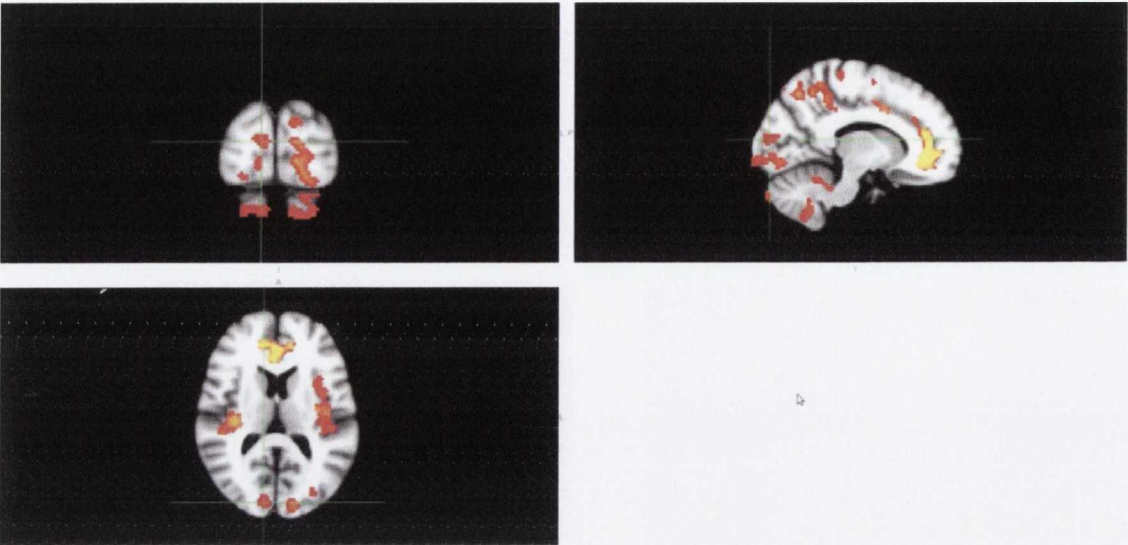
x=27, y=53, z=40 ( $p<0.05$ ).

Figure 4-22 Right lateral occipital cortex



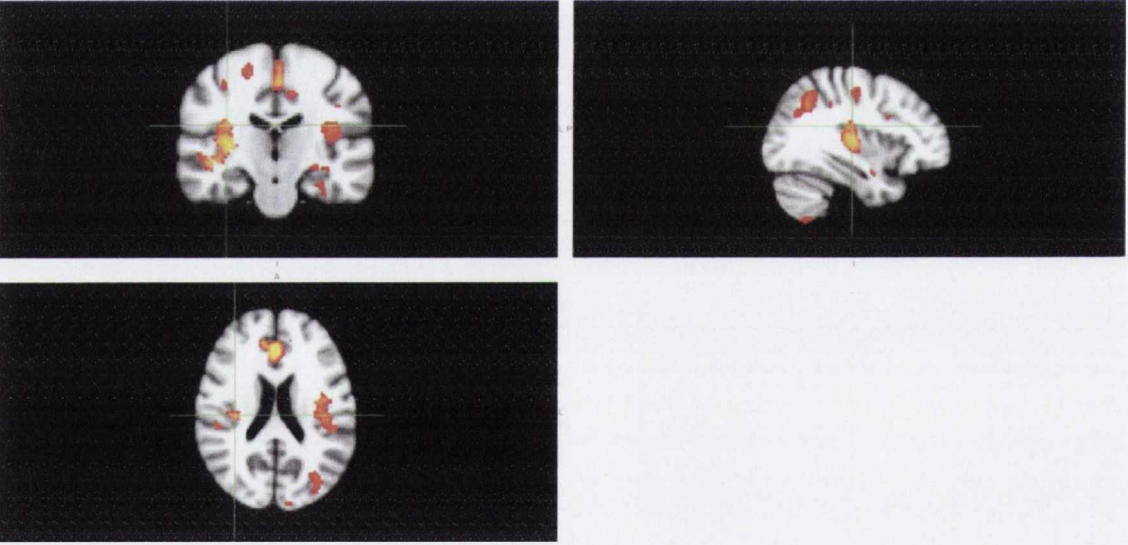
x=37, y=31, z=61 ( $p<0.05$ ).

Figure 4-23 Right occipital pole



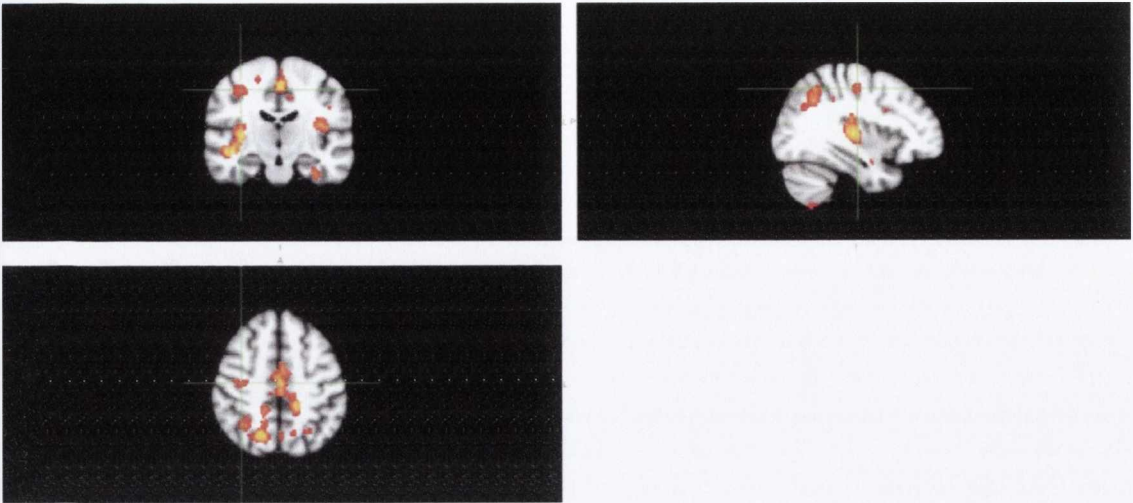
x=39, y=18, z=42 ( $p<0.05$ ).

Figure 4-24 Right parietal operculum



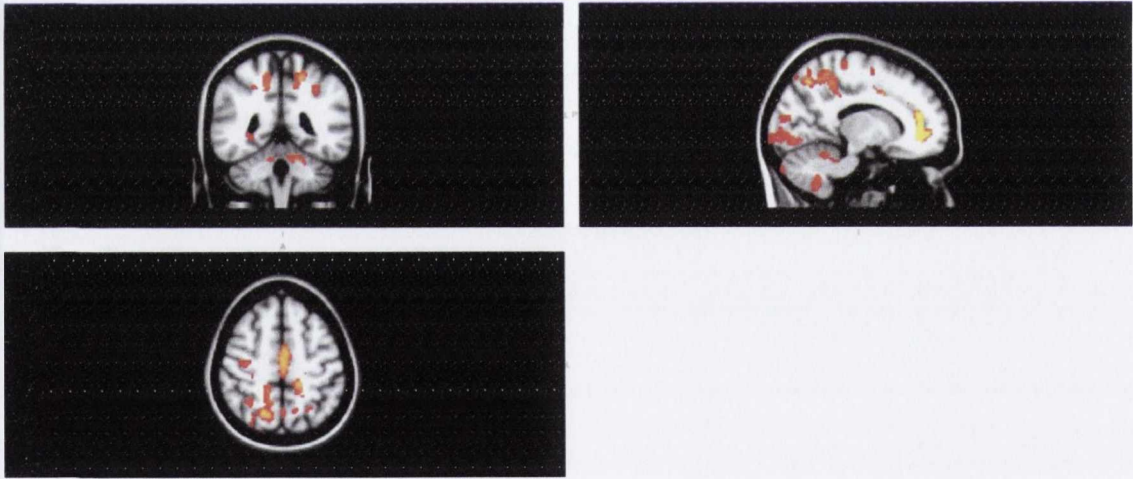
x=27, y=53, z=46 ( $p<0.05$ ).

Figure 4-25 Right pre-central gyrus



x=27, y=55, z=60 ( $p<0.05$ ).

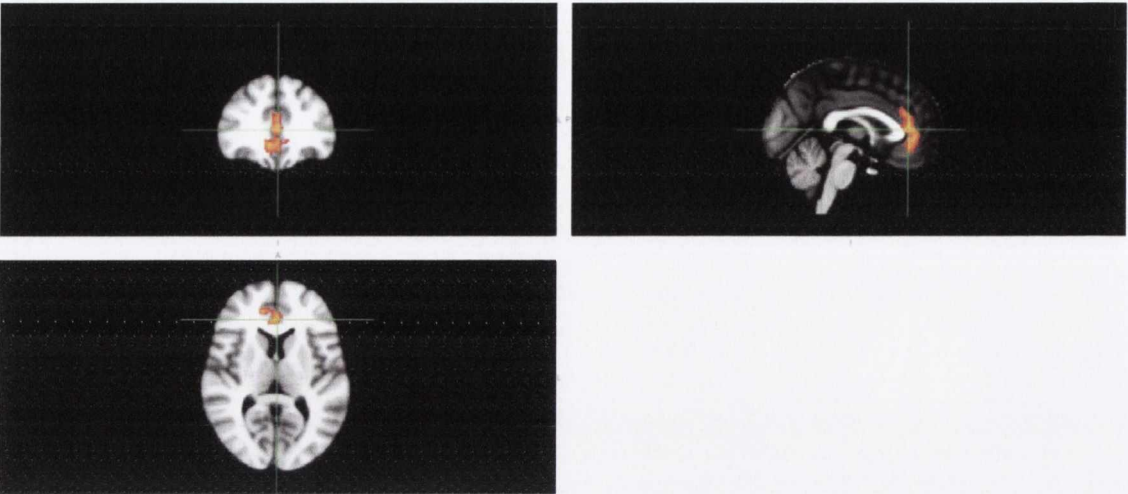
Figure 4-26 Right precuneus



x=38, y=41, z=61 ( $p<0.05$ ).



Figure 4-27 Anterior cingulate at  $p<0.01$



$x=45, y=81, z=40$  ( $p<0.01$ ).

#### 4.4.3 Results of DTI Analysis

Compared to HIV negative healthy controls, HIV positive patients had abnormalities in measures of FA, AD, MD and RD. There was a statistically significant reduction in FA (FA values less than 0.02) in the genu of the corpus callosum, bilateral anterior thalamic radiation, bilateral cingulum hippocampus, bilateral corticospinal tracts, bilateral inferior fronto-occipital fasciculi, bilateral superior longitudinal fasciculi, bilateral anterior corona radiata, bilateral forceps minor and forceps major ( $p < 0.01$ ). These results remained statistically significantly different at  $p < 0.0005$ . There was a statistically significant decrease in axial diffusivity in the left anterior thalamic radiation, left corticospinal tract and left forceps minor ( $p < 0.01$ ). There was a statistically significant increase in axial diffusivity in the left forceps major ( $p < 0.05$ ). The HIV positive group had a statistically significant increase in mean diffusivity in the genu of the corpus callosum, body of corpus callosum, bilateral anterior thalamic radiation, bilateral superior longitudinal fasciculi, right anterior corona radiata and right inferior fronto-occipital fasciculus ( $p < 0.01$ ). The increase in MD in the genu of the corpus callosum and bilateral inferior fronto-occipital fasciculi remained significant at  $p < 0.0005$ . The HIV positive group had increased radial diffusivity in the body of the corpus callosum, bilateral forceps minor, bilateral forceps major, left anterior thalamic radiation, left inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus and right superior longitudinal fasciculus ( $p < 0.01$ ) which remained statistically significant at  $p < 0.0005$ . The Johns Hopkins University (JHU) white matter tractography atlas and ICBM-DTI-81 white-matter labels atlas were utilised to identify tracts (468, 469). The corrected statistical maps were overlaid on an MNI brain template and the mean FA skeleton. The normal tracts are

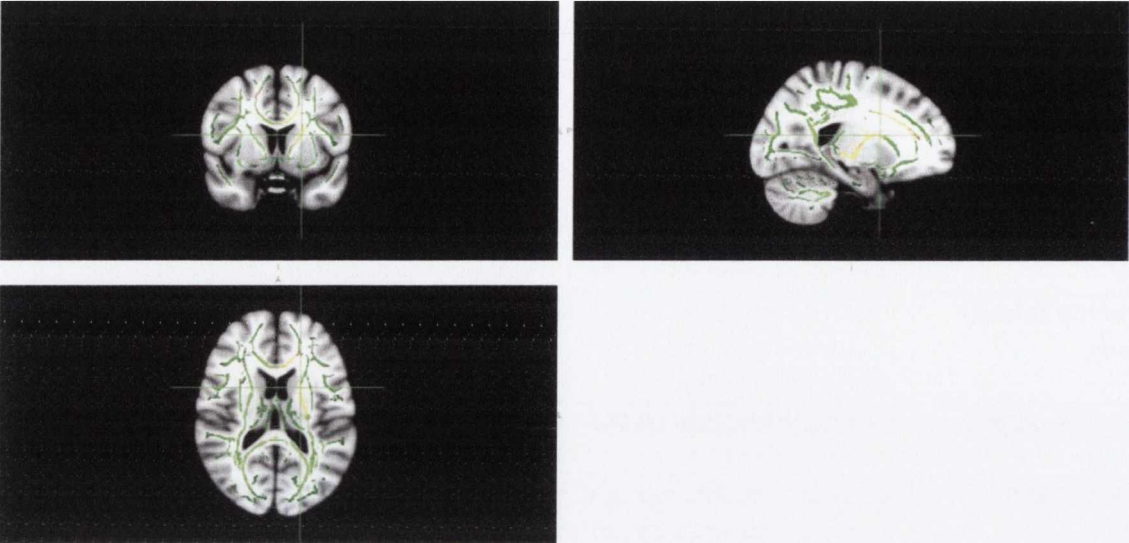
displayed in green with areas of abnormal diffusion displayed on a spectrum of red to yellow.



**Table 4-8 Normal connections and functions of white matter tracts that were abnormal**

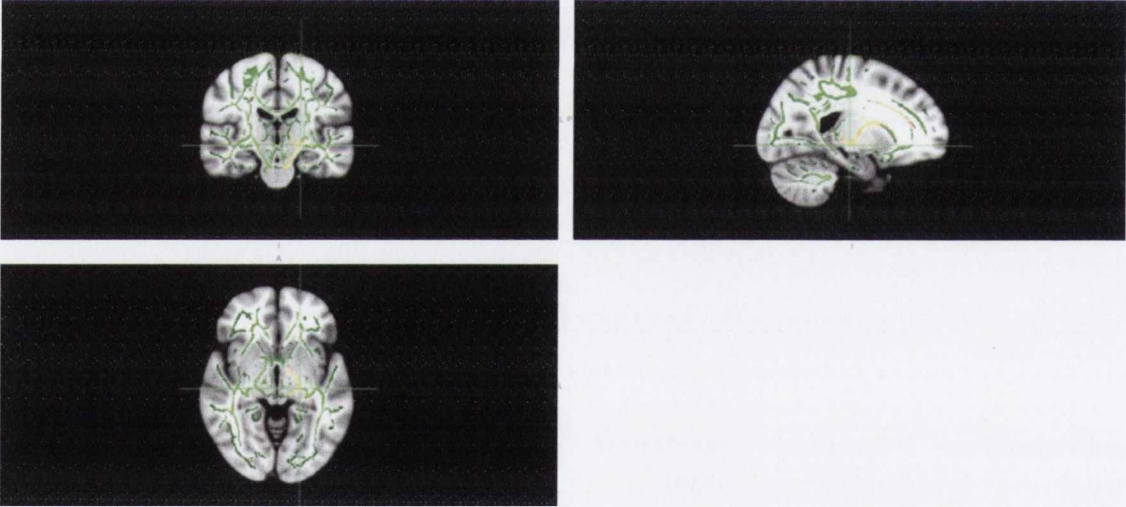
| Tract                                | Function                                                                                                                                        | Location                                                                                                                                                                                                                                                               |
|--------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Forceps Minor                        | Relays information intra-hemispherically and across corpus callosum                                                                             | Emerges from each side of the genu of the corpus callosum to reach the medial wall of the frontal lobe (464)                                                                                                                                                           |
| Forceps Major                        | Relays information intra-hemispherically and across corpus callosum                                                                             | Emerges from the splenium of the corpus callosum to reach the medial wall of the occipital lobe (464)                                                                                                                                                                  |
| Body of the corpus callosum          | Inter-hemispheric sensorimotor and auditory connectivity (389).                                                                                 | Connects neocortical areas between both cerebral hemispheres (464).                                                                                                                                                                                                    |
| Genu of the corpus callosum          | Relays information across hemispheres                                                                                                           | Fibres extend from the medial frontal lobe                                                                                                                                                                                                                             |
| Anterior thalamic radiation          | Relay sensory and motor data to precentral and postcentral cortex (389).                                                                        | Projection fibres - connects lateral thalamic nuclei to cerebral cortex through internal capsule (389).                                                                                                                                                                |
| Cingulum                             | Relays information from the cingulate gyrus to the entorhinal cortex.<br>Visceromotor control, visuospatial processing and memory access (389). | Association fibres - Runs from frontal lobe to temporal lobe (470).                                                                                                                                                                                                    |
| Corticospinal                        | Motor function to contralateral body (389).                                                                                                     | Brainstem tracts - Connects cerebral motor cortex to medulla then descends into contralateral spinal cord.                                                                                                                                                             |
| Superior longitudinal fasciculus     | Integration of auditory and speech nuclei (389).                                                                                                | Supero-lateral side of the putamen and forms a large arc (arcuate fasciculus). Association fibres - connections between the frontal, parietal, occipital, and temporal lobes including language-related areas (Broca's, Geschwind's and Wernicke's territories) (470). |
| Inferior longitudinal fasciculus     | Integration of auditory and speech nuclei (389).                                                                                                | Association fibres - Connects ipsilateral temporal and occipital lobes (389).                                                                                                                                                                                          |
| Inferior fronto-occipital fasciculus | Integration of auditory and visual association cortices with prefrontal cortex (389).                                                           | Association fibres – connects ipsilateral frontal and occipital lobes; ipsilateral frontal and posterior parietal and temporal lobes; intermingles with uncinate fasciculus (389).                                                                                     |
| Anterior corona radiata              | Motor function                                                                                                                                  | Projection fibres connect the internal capsule with areas of cerebral cortex. The anterior portion primarily contains fibres from prefrontal cortex (389, 470).                                                                                                        |

Figure 4-28 Left anterior thalamic radiation (decreased AD)



x=110, y=132, z=87 (p<0.01).

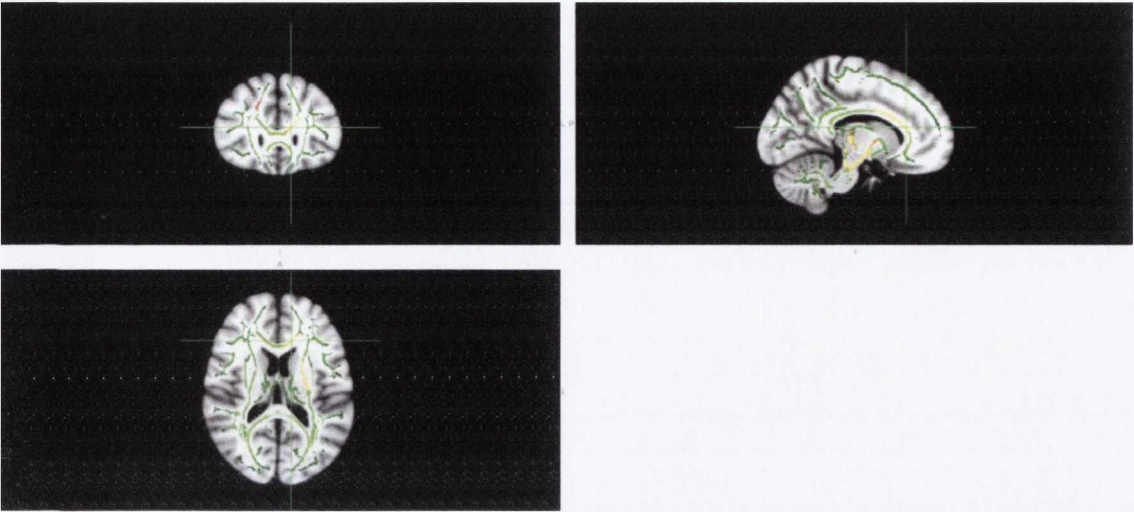
Figure 4-29 Left corticospinal tract (decreased AD)



x=110, y=106, z=67 (p<0.01).

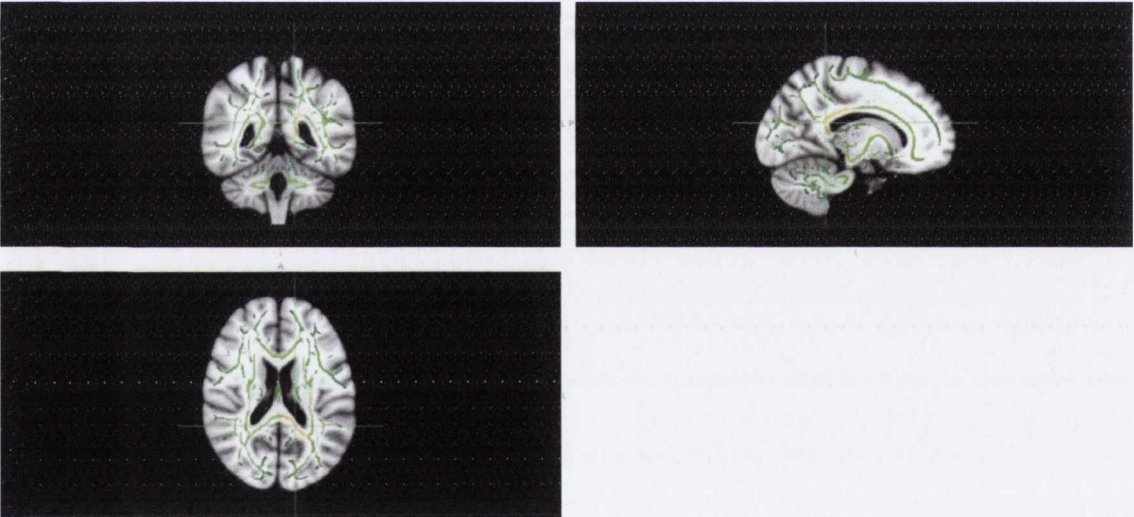


Figure 4-30 Left forceps minor (decreased AD)



x=101, y=154, z=87 ( $p<0.01$ ).

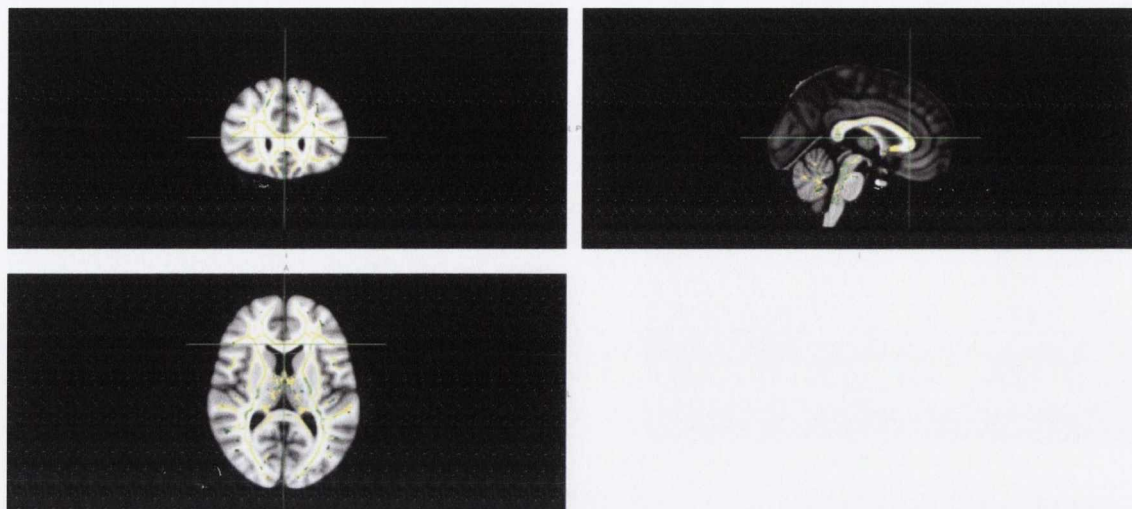
Figure 4-31 Left forceps major (increased AD)



x=104, y=82, z=91 ( $p<0.05$ ).

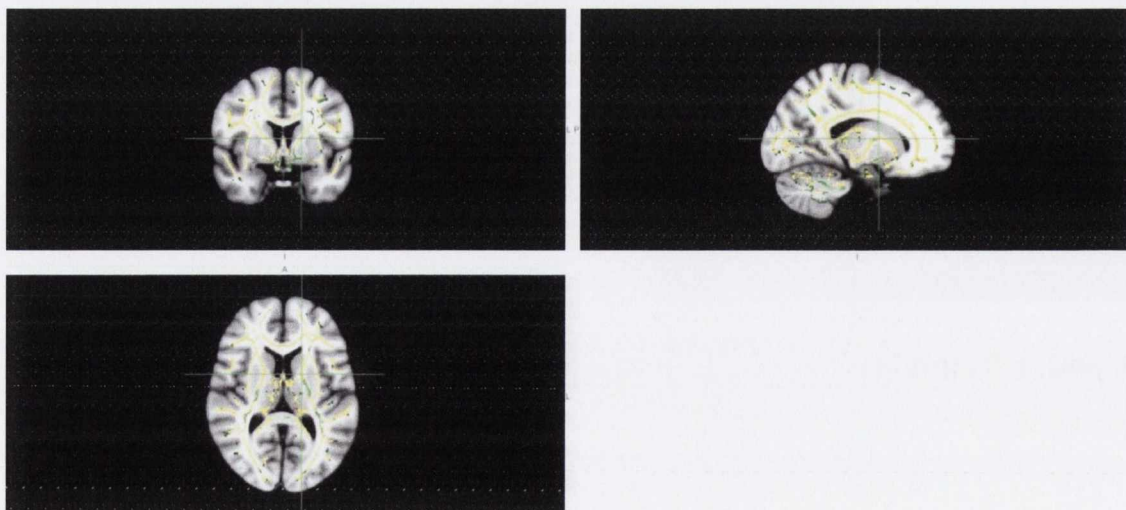


**Figure 4-32 Genu of corpus callosum (decreased FA)**



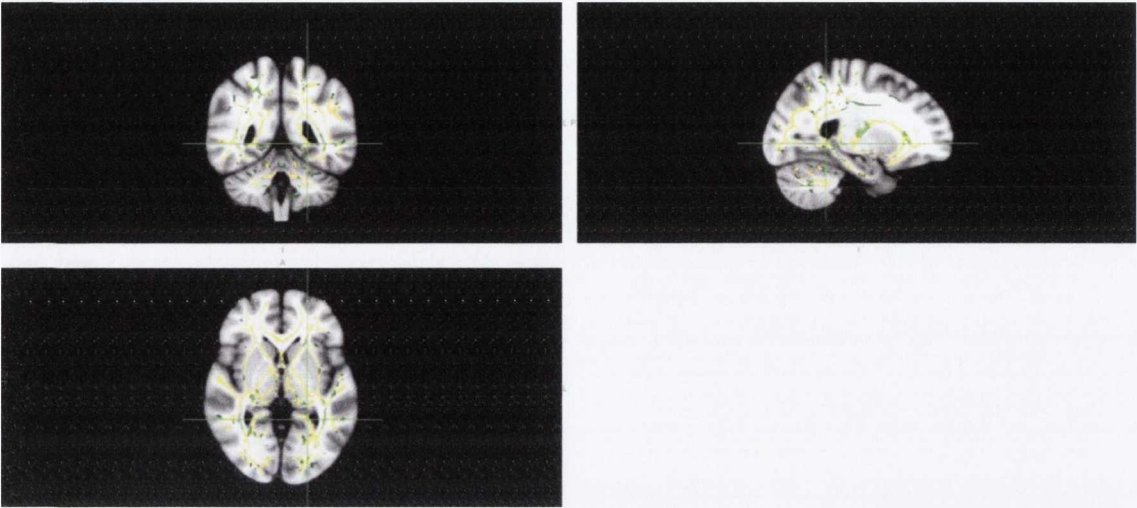
x=89, y=153, z=82 ( $p < 0.01$ ).

**Figure 4-33 Left anterior thalamic radiation (decreased FA)**



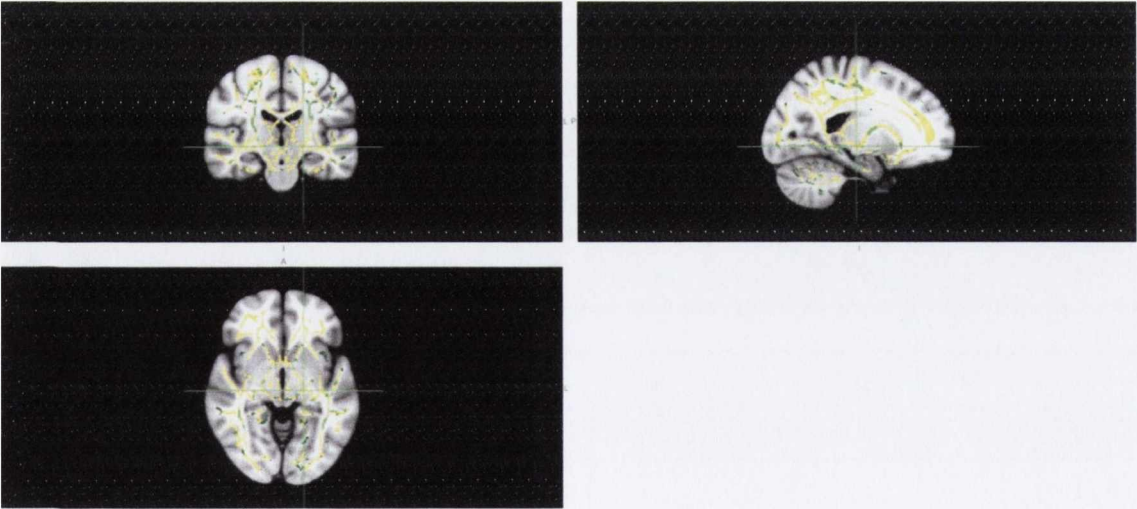
x=106, y=128, z=82 ( $p < 0.01$ ).

Figure 4-34 Left cingulum (hippocampus) (decreased FA)



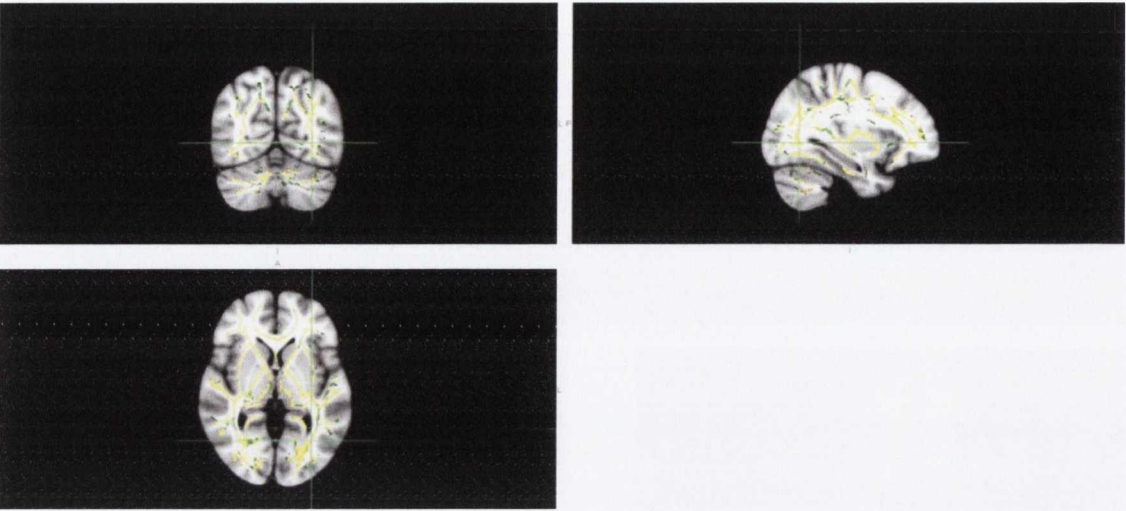
x=113, y=80, z=72 (p<0.01).

Figure 4-35 Left corticospinal tracts (decreased FA)



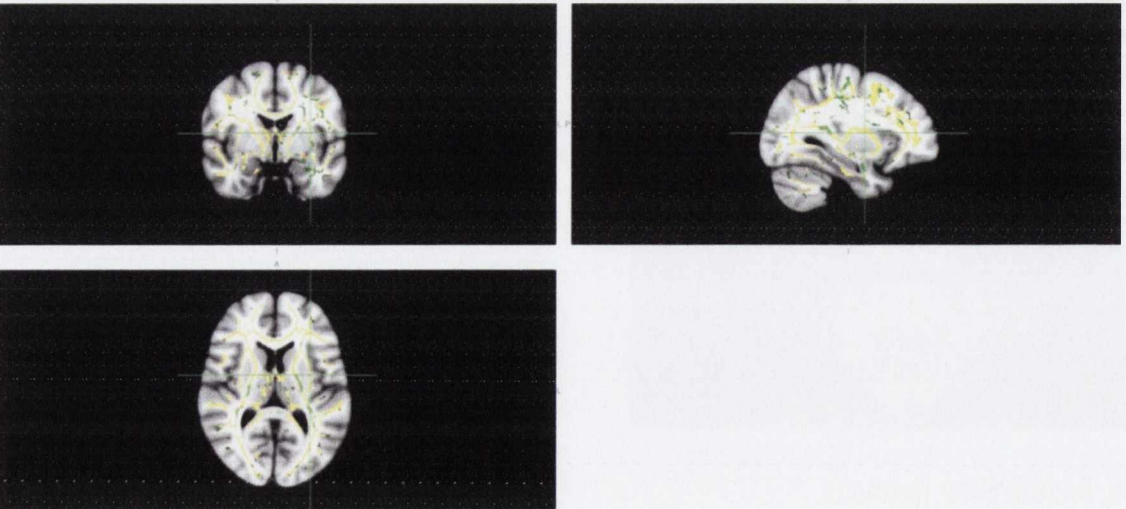
x=109, y=106, z=67 (p<0.01).

**Figure 4-36 Left inferior fronto-occipital fasciculus (decreased FA)**



$x=122, y=62, z=73$  ( $p<0.01$ ).

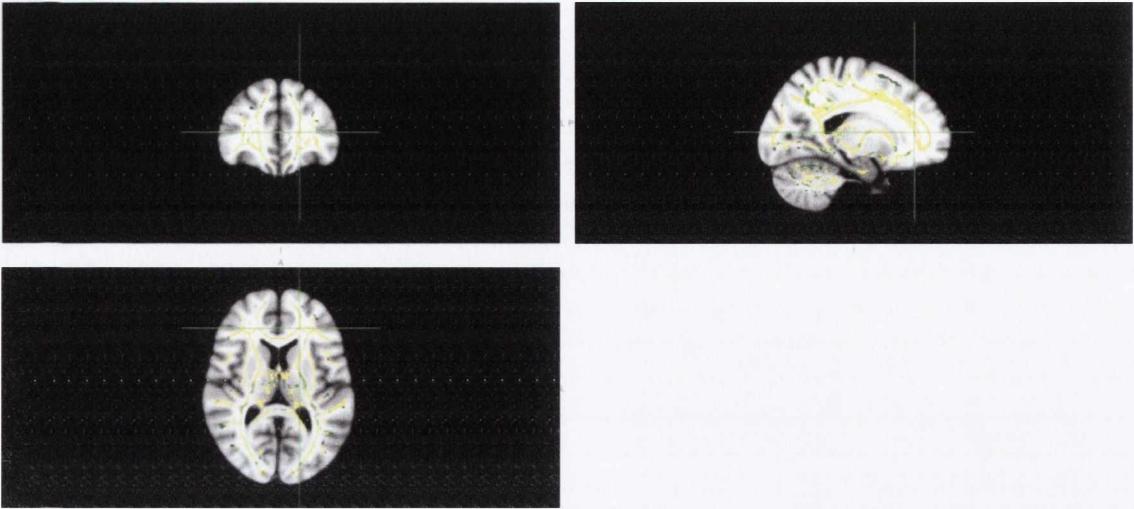
**Figure 4-37 Left superior longitudinal fasciculus (decreased FA)**



$x=121, y=123, z=82$  ( $p<0.01$ ).

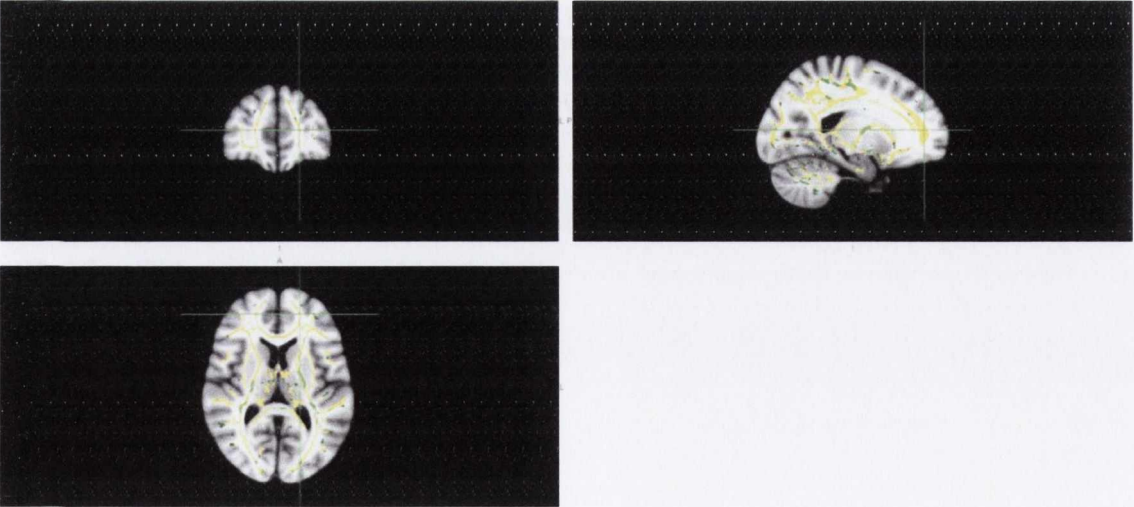


Figure 4-38 Left anterior corona radiata (decreased FA)



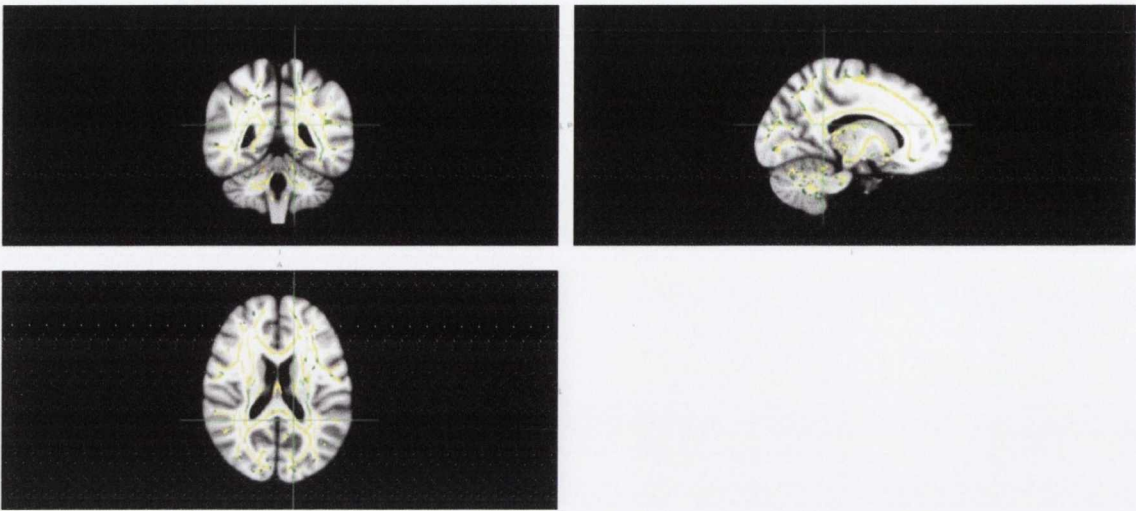
x=108 y=163, z=82 (p<0.01).

Figure 4-39 Left forceps minor (decreased FA)



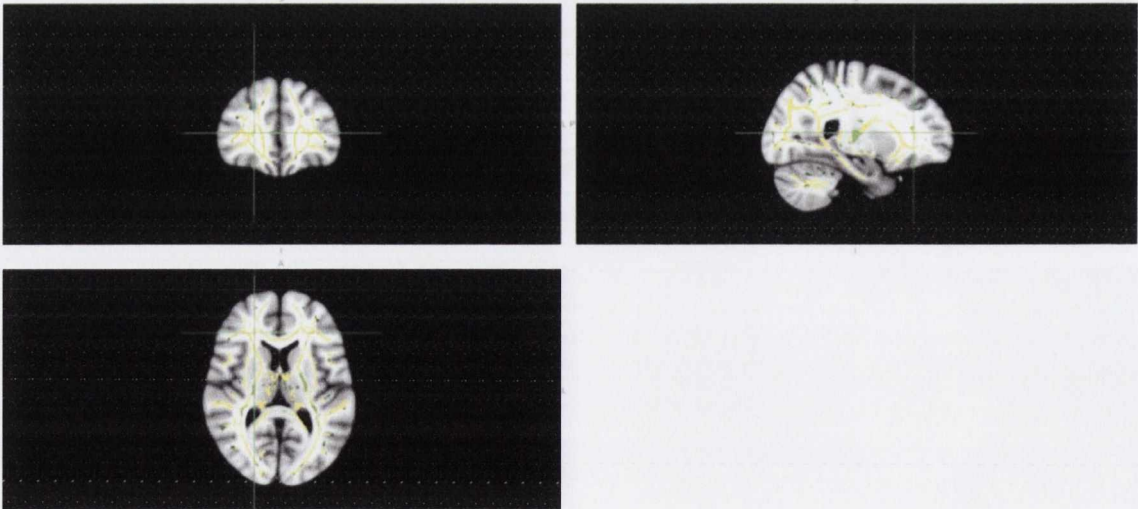
x=109, y=174, z=82 (p<0.01).

**Figure 4-40 Left forceps major (decreased FA)**



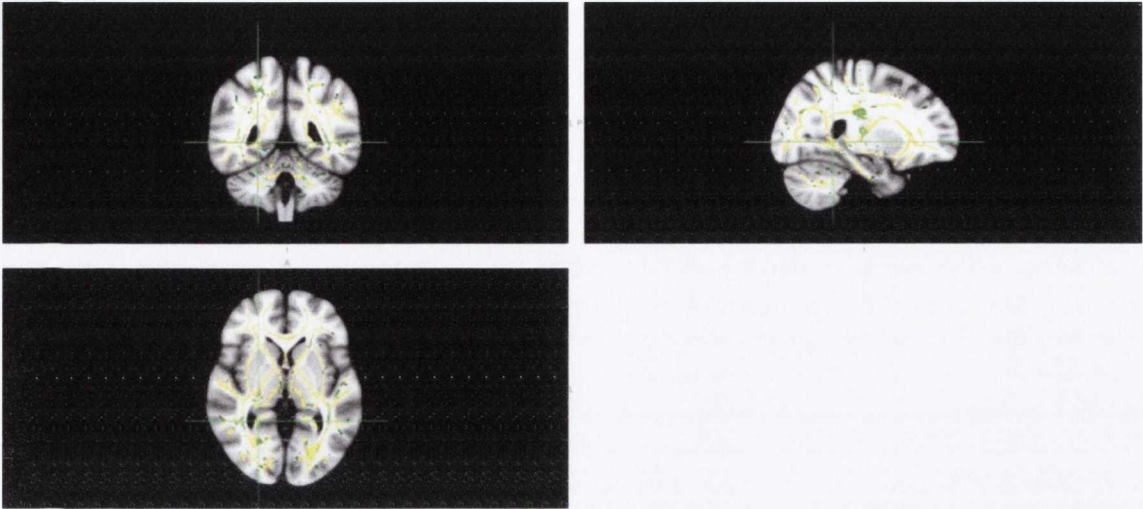
x=104, y=82, z=91 ( $p<0.01$ ).

**Figure 4-41 Right anterior thalamic radiata (decreased FA)**



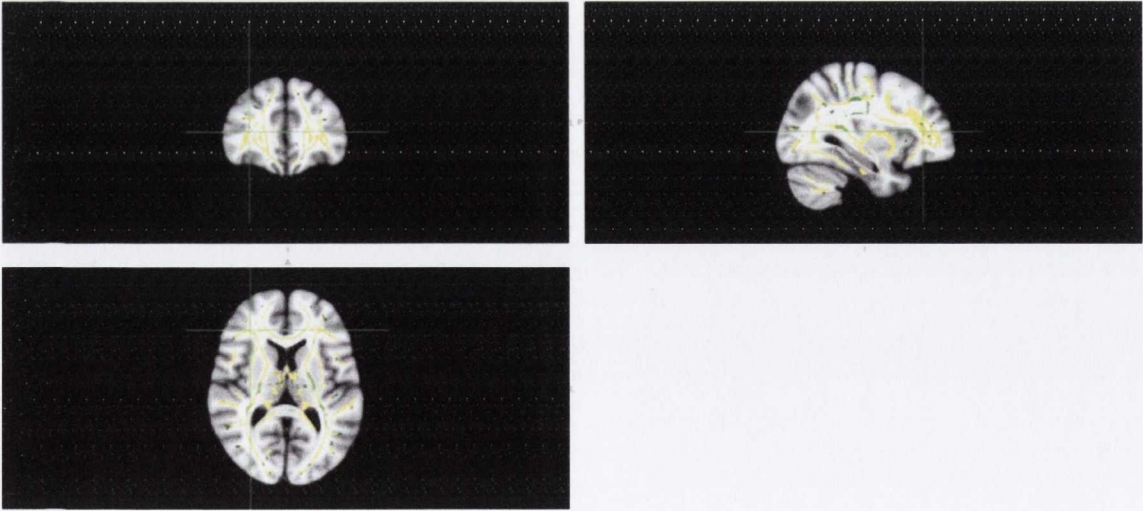
x=66, y=160, z=82 ( $p<0.01$ ).

**Figure 4-42 Right cingulum (hippocampus) (decreased FA)**



x=65, y=80, z=73 (p<0.01).

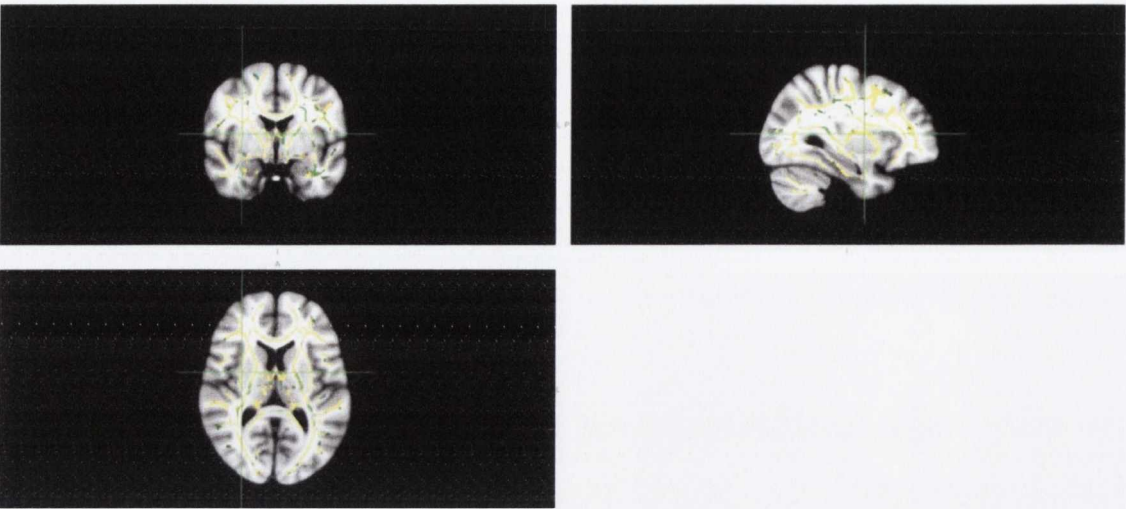
**Figure 4-43 Right inferior fronto-occipital fasciculus (decreased FA)**



x=57, y=162, z=82 (p<0.01).

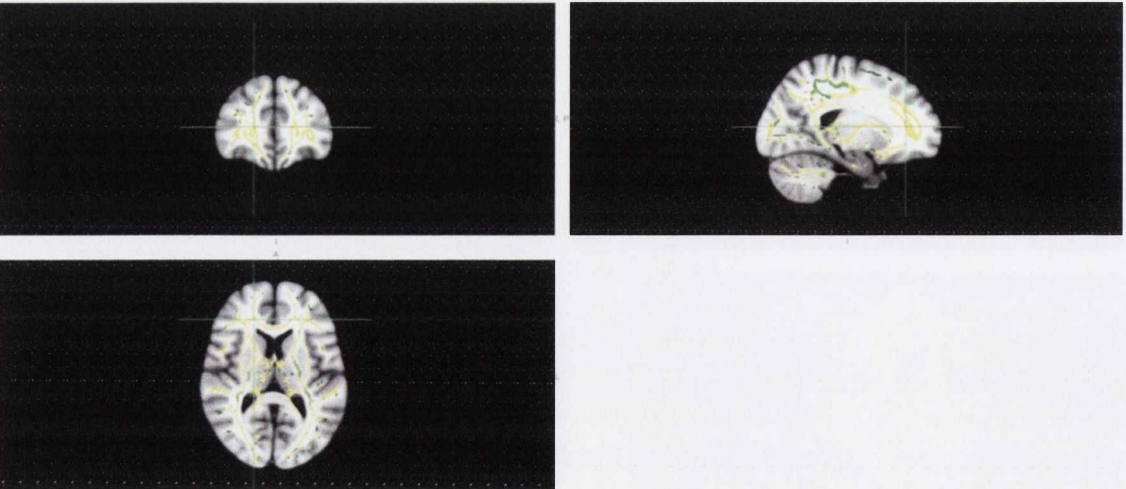


**Figure 4-44 Right superior longitudinal fasciculus (decreased FA)**



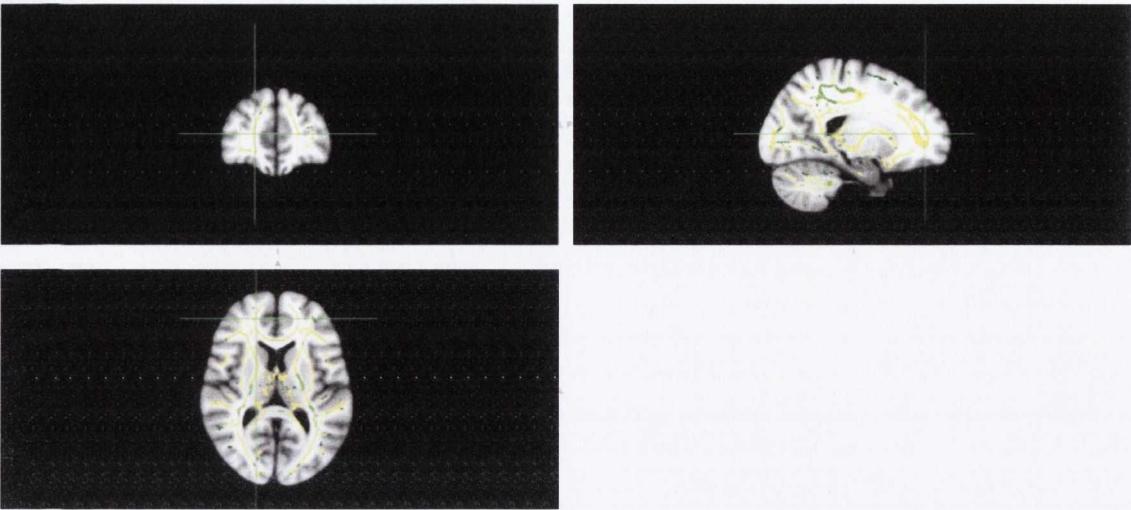
x=58, y=124, z=82 (p<0.01).

**Figure 4-45 Right anterior corona radiata (decreased FA)**



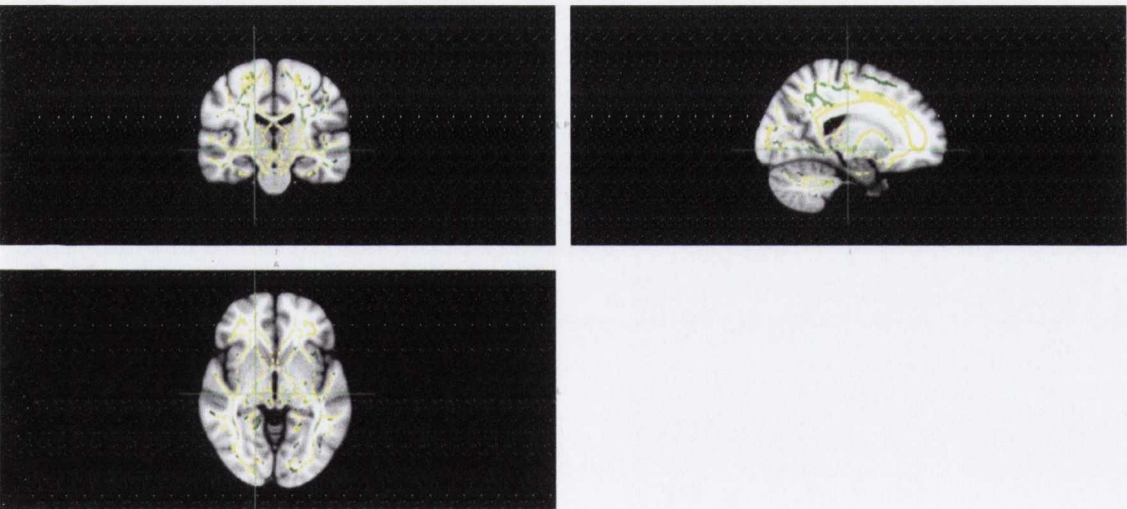
x=70, y=162, z=82 (p<0.01).

Figure 4-46 Right forceps minor (decreased FA)



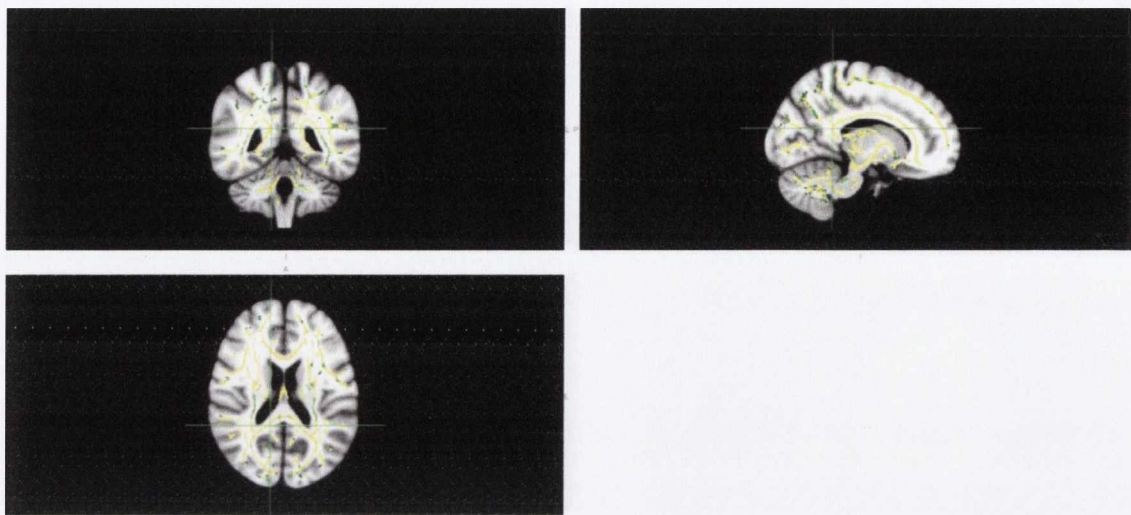
x=70, y=173, z=82 (p<0.01).

Figure 4-47 Right corticospinal tracts (decreased FA)



x=71, y=106, z=68 (p<0.01).

Figure 4-48 Right forceps major (decreased FA)



$x=77, y=82, z=91$  ( $p<0.01$ ).

Figure 4-49 Image of FA analysis at  $p<0.0005$

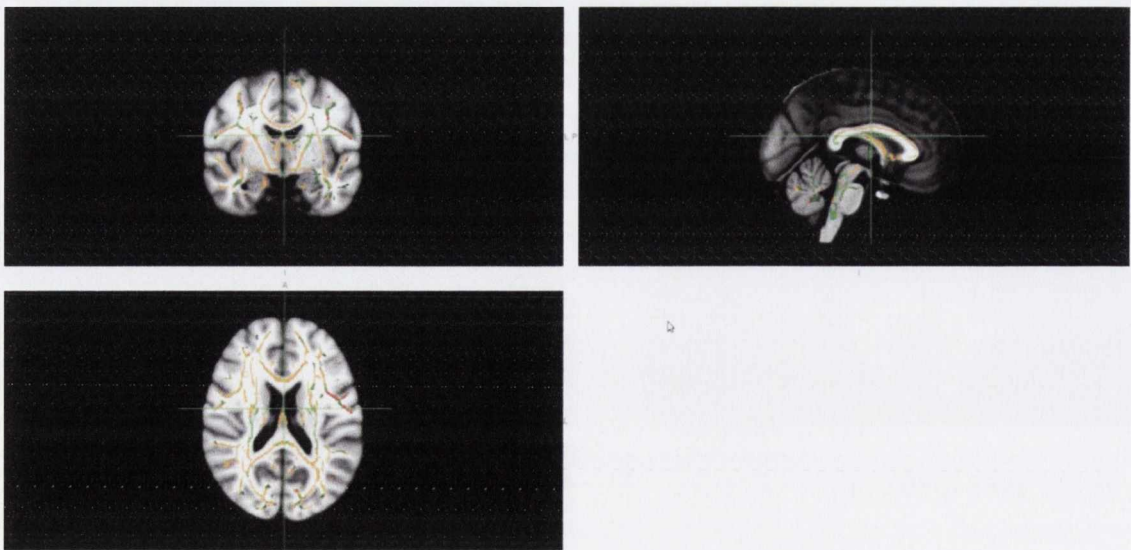
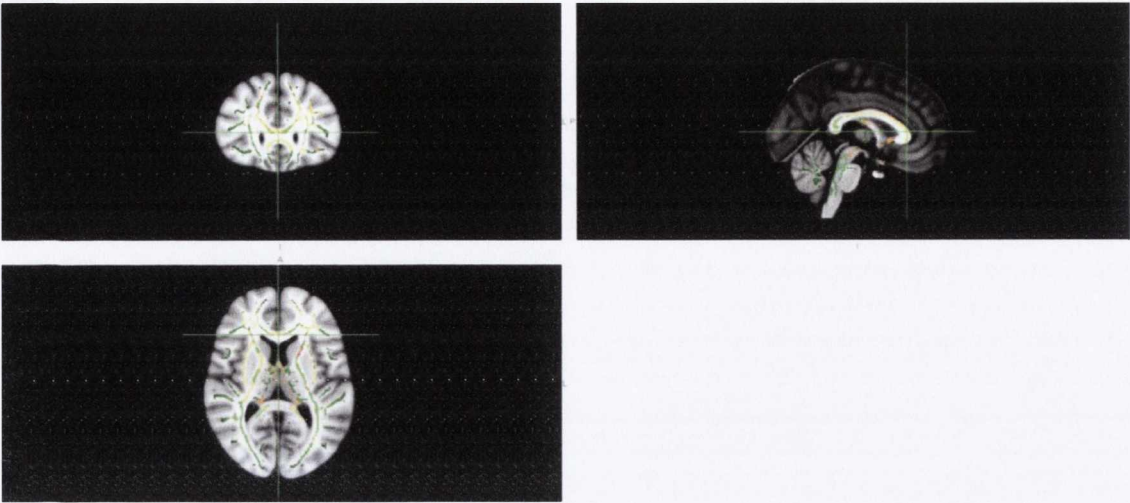


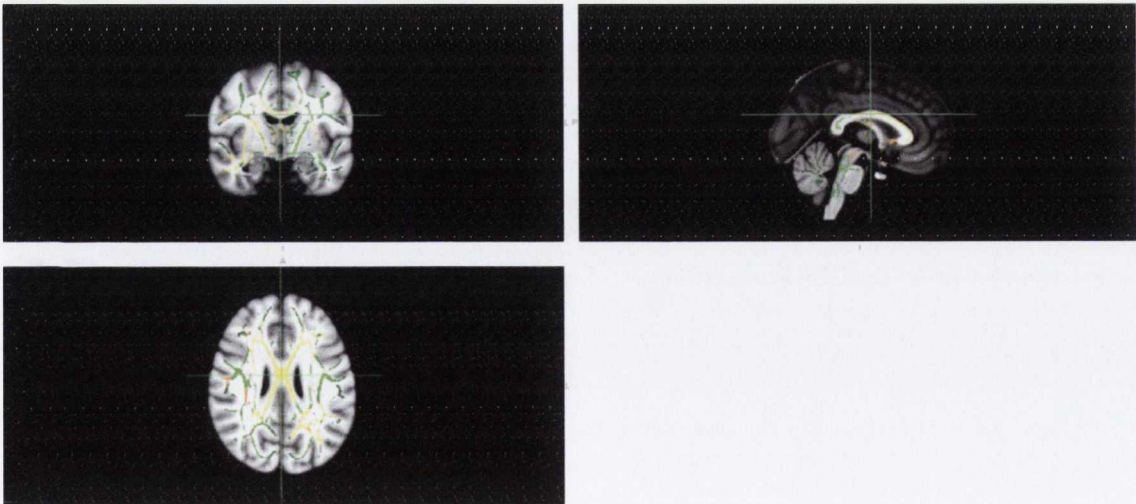


Figure 4-50 Genu of corpus callosum (increased MD)



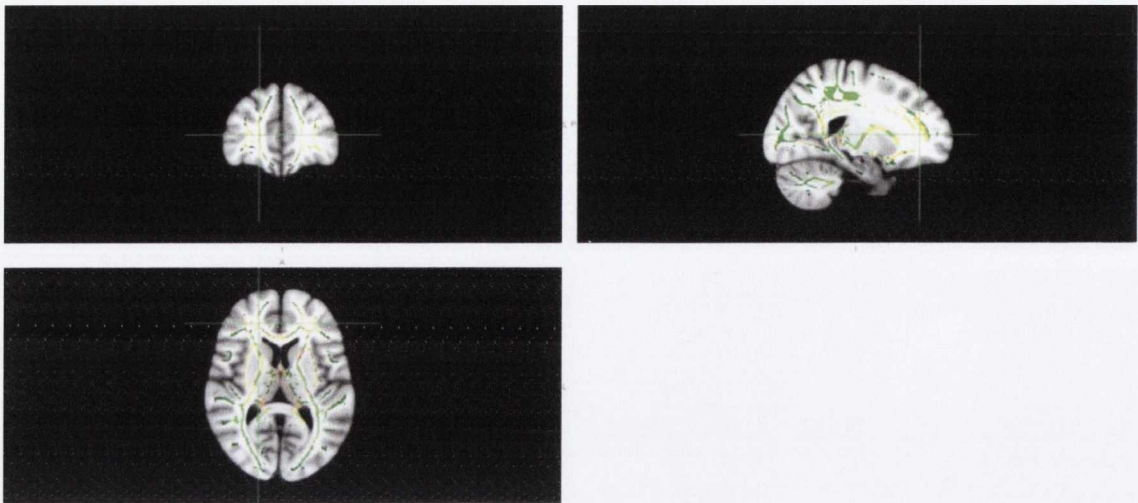
x=89, y=154, z=81 (p<0.01).

Figure 4-51 Body of corpus callosum (increased MD)



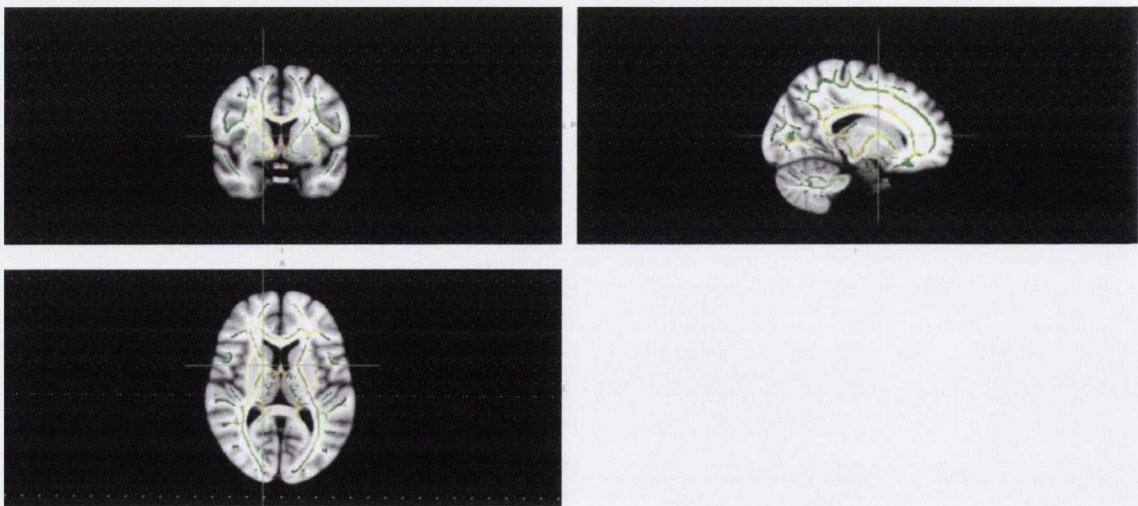
x=89, y=119, z=98 (p<0.01).

Figure 4-52 Right anterior corona radiata (increased MD)



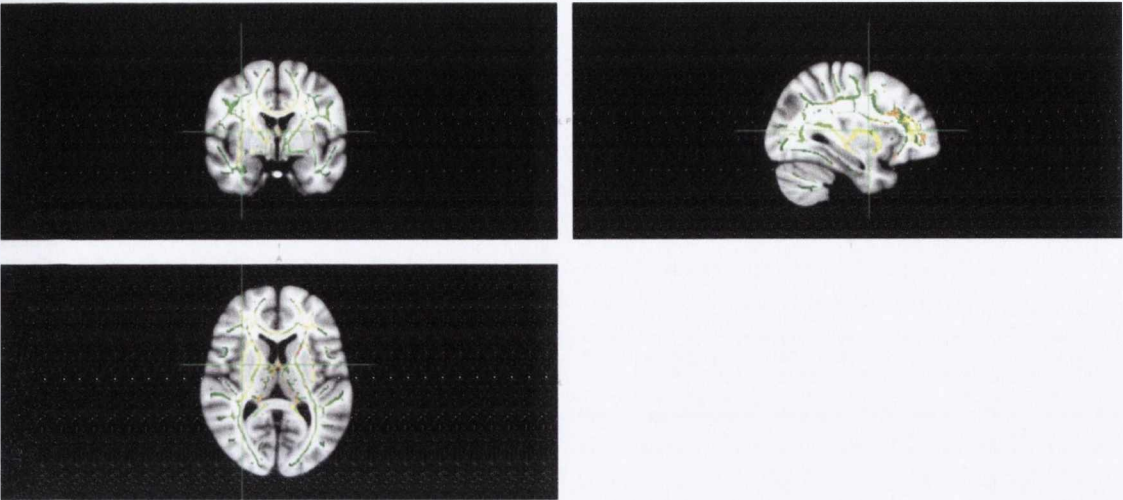
x=69, y=167, z=81 ( $p<0.01$ ).

Figure 4-53 Right anterior thalamic radiation (increased MD)



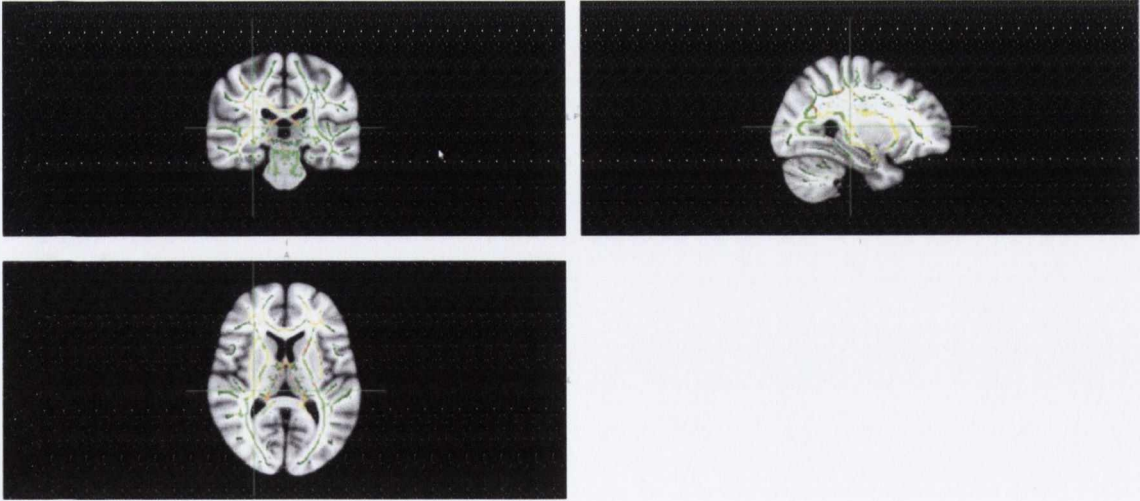
x=73, y=130, z=81 ( $p<0.01$ ).

Figure 4-54 Right superior longitudinal fasciculus (increased MD)



x=57, y=125, z=81 (p<0.01).

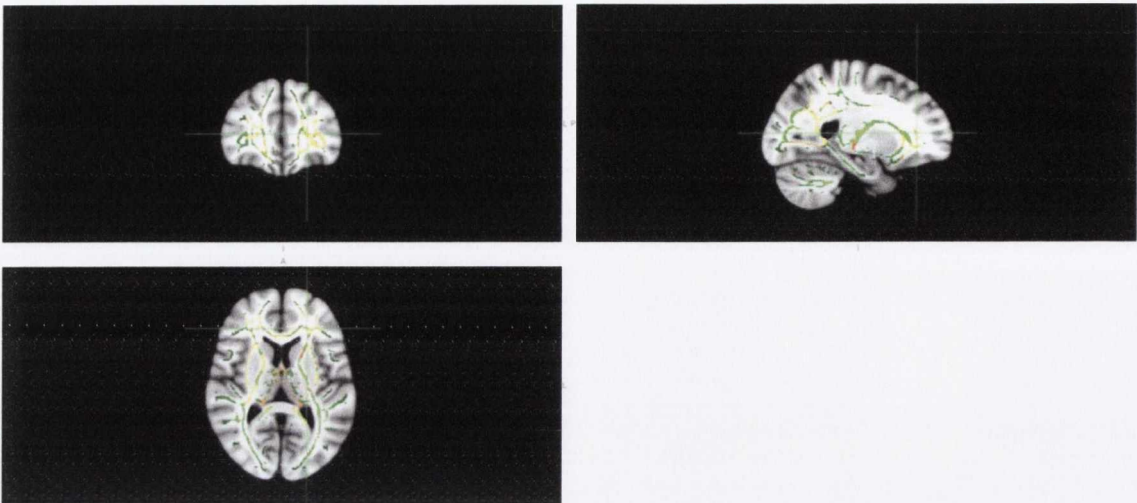
Figure 4-55 Right inferior fronto-occipital fasciculus (increased MD)



x=61, y=99, z=81 (p<0.01).

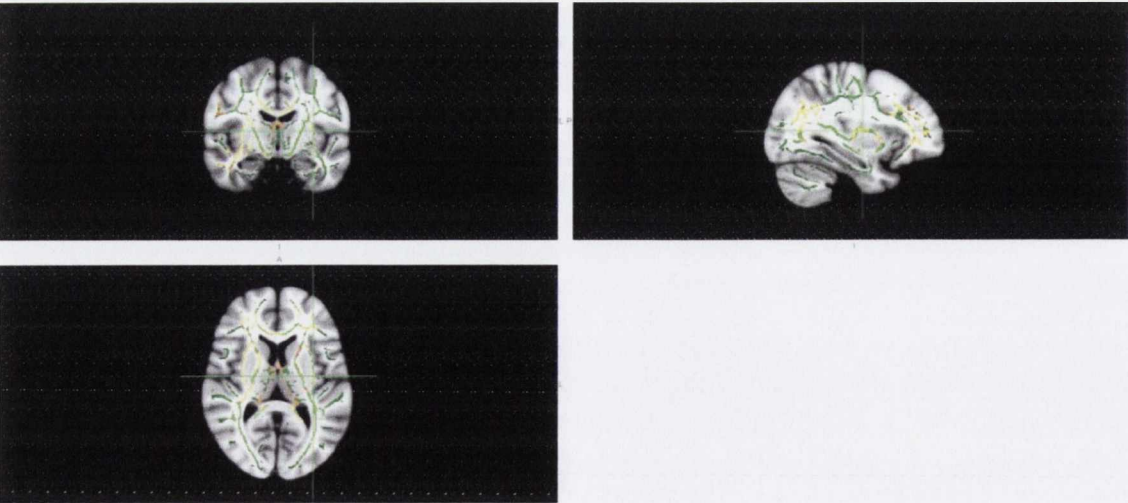


**Figure 4-56 Left anterior thalamic radiation (increased MD)**



$x=113, y=161, z=81$  ( $p<0.01$ ).

**Figure 4-57 Left superior longitudinal fasciculus (increased MD)**



$x=122, y=116, z=81$  ( $p<0.01$ ).

Figure 4-58 Image of MD analysis at  $p<0.0005$

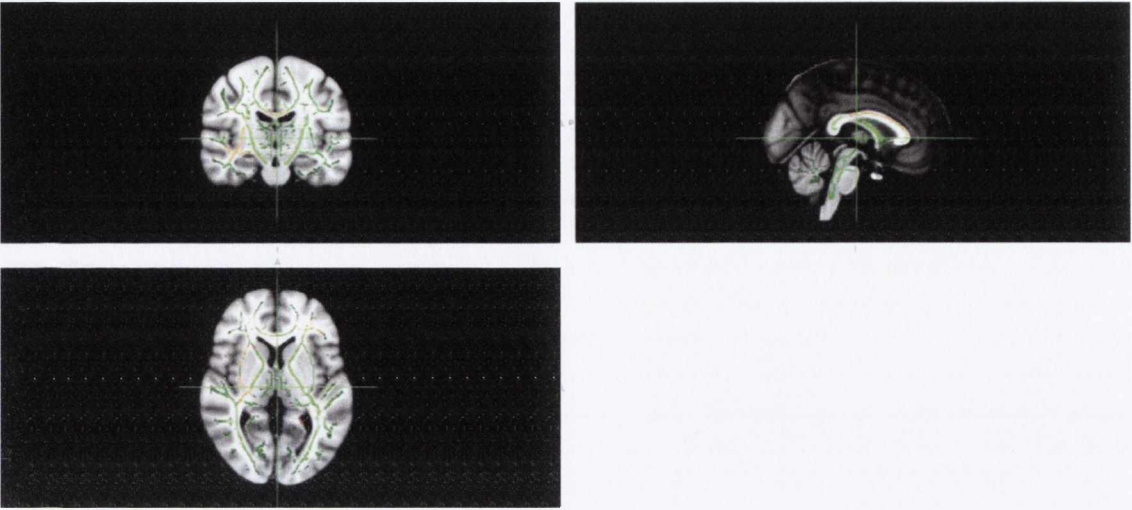
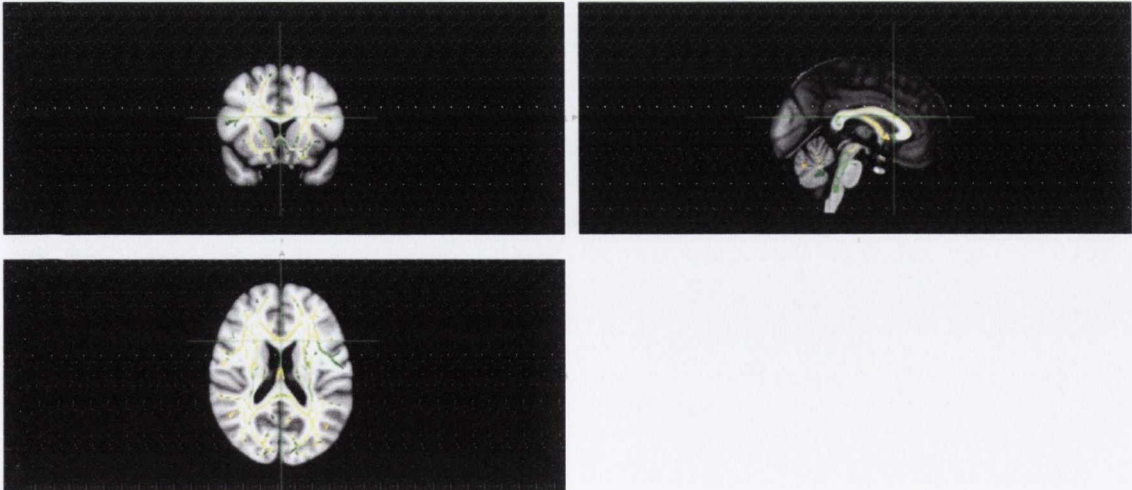
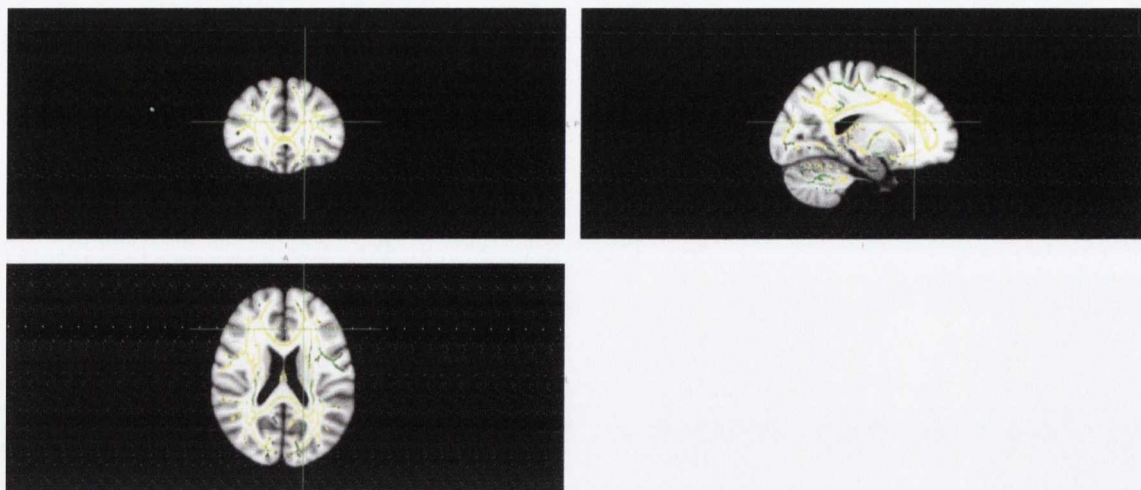


Figure 4-59 Body of corpus callosum (increased RD)



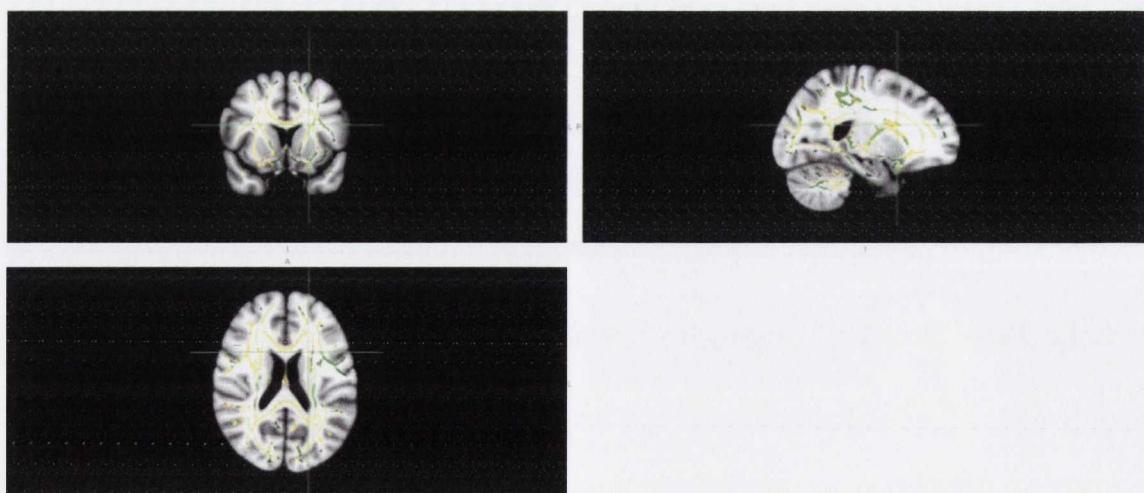
x=90, y=142, z=91 ( $p<0.01$ ).

**Figure 4-60 Left forceps minor (increased RD)**



x=108, y=156, z=92 (p<0.01).

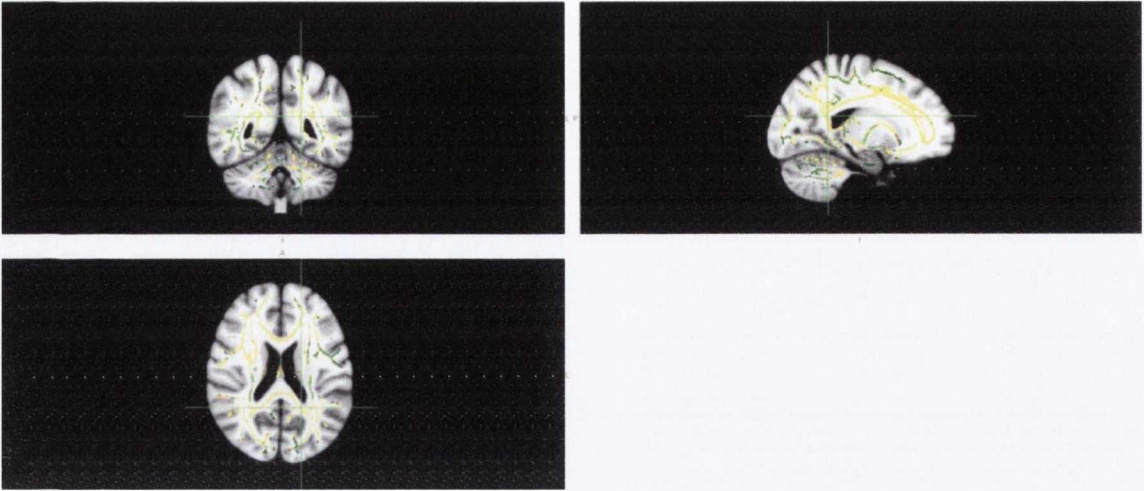
**Figure 4-61 Left anterior thalamic radiation (increased RD)**



x=111, y=138, z=92 (p<0.01).

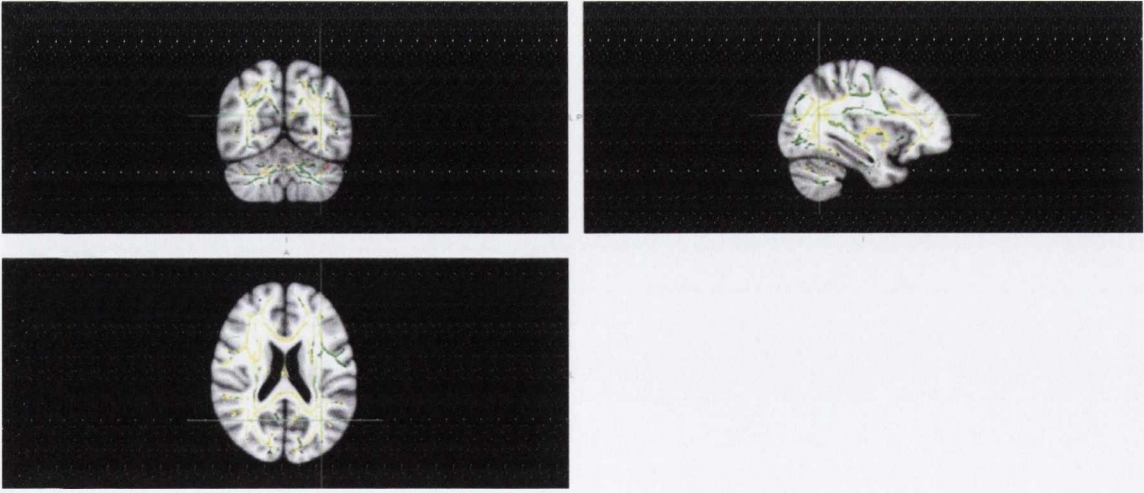


Figure 4-62 Left forceps major (increased RD)



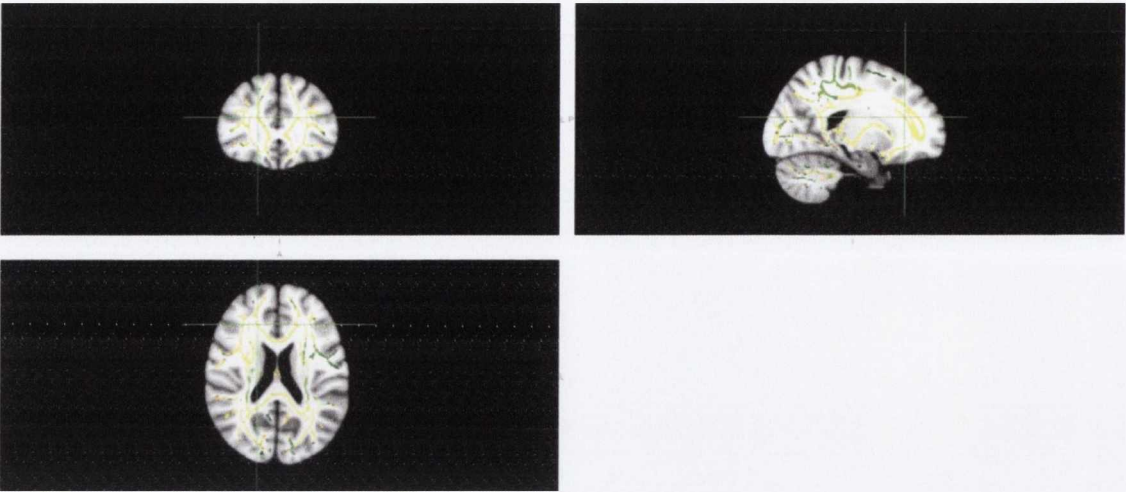
x=108, y=78, z=92 (p<0.01).

Figure 4-63 Left inferior longitudinal fasciculus (increased RD)



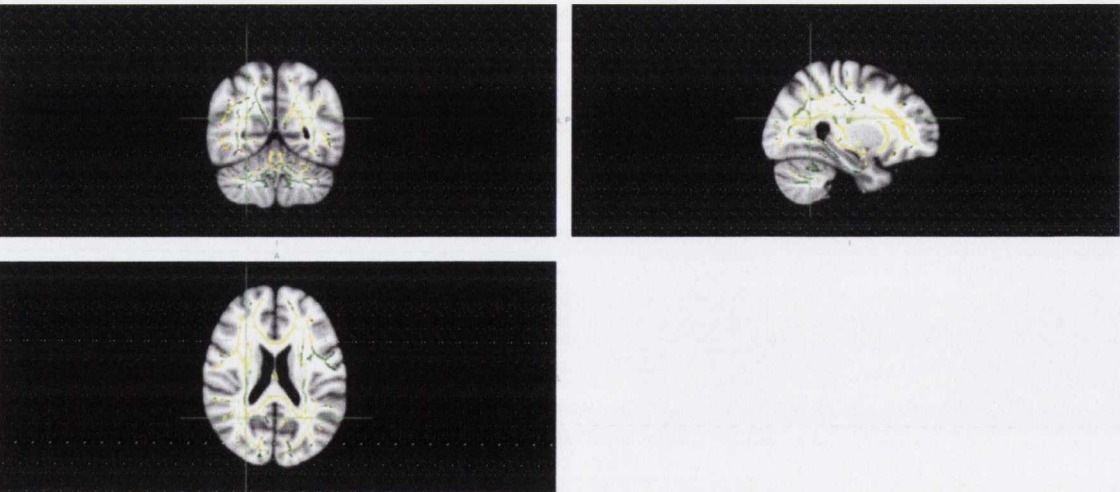
x=123, y=66, z=92 (p<0.01).

Figure 4-64 Right forceps minor (increased RD)



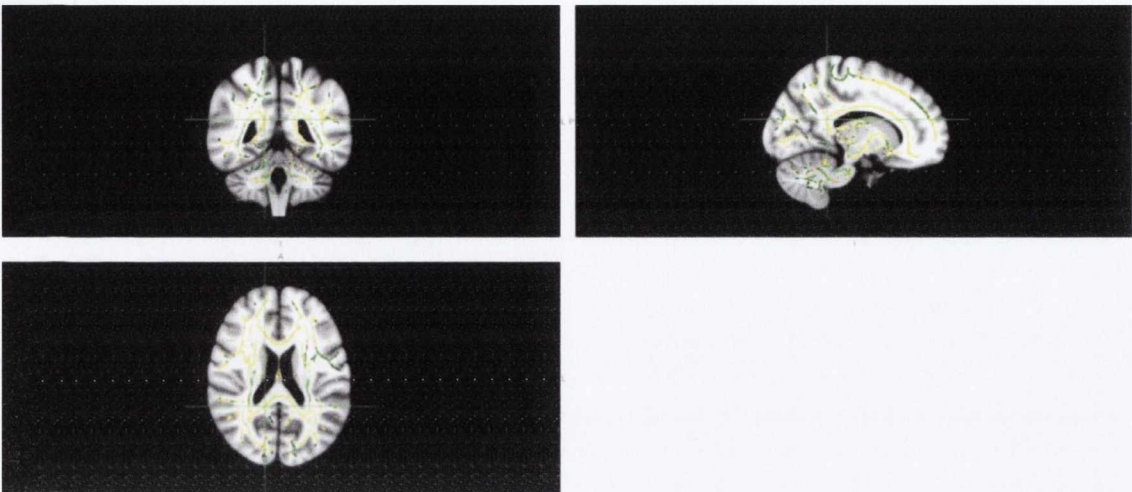
x=70, y=157, z=92 (p<0.01).

Figure 4-65 Right inferior fronto-occipital fasciculus (increased RD)



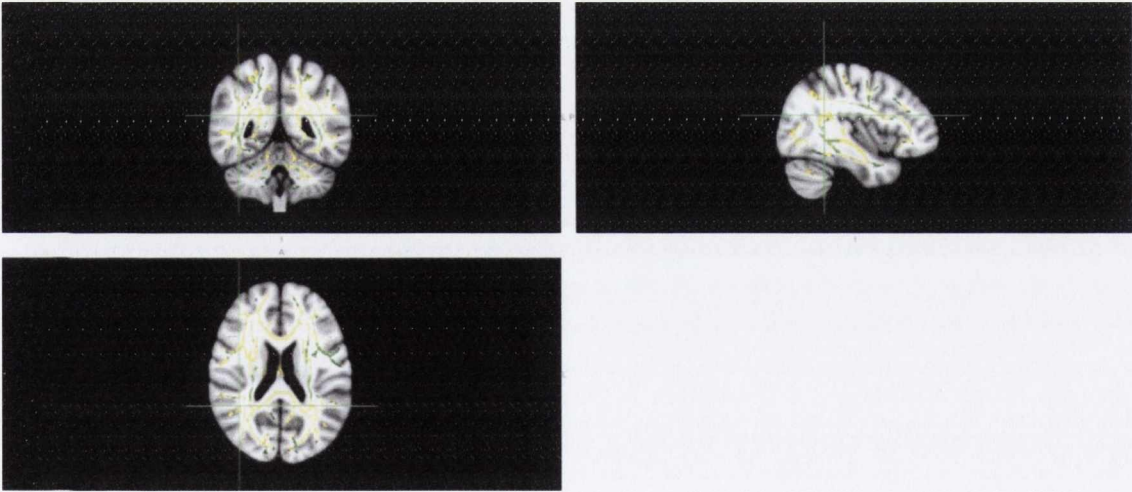
x=62, y=71, z=92 (p<0.01).

Figure 4-66 Right forceps major (increased RD)



x=76, y=82, z=92 (p<0.01).

Figure 4-67 Right superior longitudinal fasciculus (increased RD)



x=50, y=79, z=92 (p<0.01).



Figure 4-68 Image of RD analysis at  $p<0.0005$

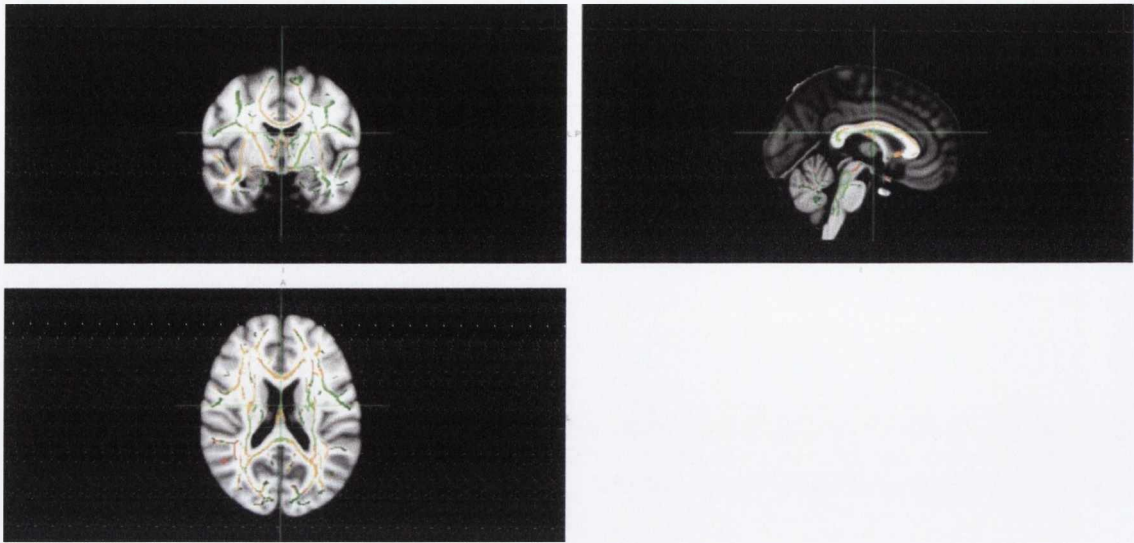


Table 4-9 Summary of DTI Findings

|                                      | FA (p<0.0005)         | AD (p<0.01)    | MD (p<0.01)                | RD (p<0.0005)         |
|--------------------------------------|-----------------------|----------------|----------------------------|-----------------------|
| Forceps minor                        | Decreased bilaterally | Decreased left |                            | Increased bilaterally |
| Forceps major                        | Decreased bilaterally | Increased Left |                            | Increased bilaterally |
| Genu corpus callosum                 | Decreased             |                | Increased (p<0.0005)       |                       |
| Body of corpus callosum              |                       |                | Increased                  | Increased             |
| Anterior thalamic radiation          | Decreased bilaterally | Decreased left | Increased bilaterally      | Increased Left        |
| Cingulum (hippocampus)               | Decreased Left        |                |                            |                       |
| Corticospinal tracts                 | Decreased bilaterally | Decreased left |                            |                       |
| Inferior fronto-occipital fasciculus | Decreased bilaterally |                | Increased Right (p<0.0005) | Increased Right       |
| Superior longitudinal fasciculus     | Decreased bilaterally |                | Increased bilaterally      | Increased Right       |
| Inferior longitudinal fasciculus     |                       |                |                            | Increased Left        |
| Anterior corona radiata              | Decreased bilaterally |                | Increased Right            |                       |

#### 4.4.4 Correlations of Neuropsychological Data to DTI Measures

Correlations were performed between all four DTI measures and clinical and neuropsychological measures co-varied for age. There were positive correlations between higher FA values and higher scores on the list learning score on the RBANS, figure copy score on the RBANS, figure recall score on the RBANS and retention (savings) of list memory. List learning score correlated with corpus callosum, bilateral forceps minor, bilateral forceps major, right inferior fronto-occipital fasciculus, left superior longitudinal fasciculus and right cingulum (hippocampus) ( $p < 0.05$ ). Figure copy score correlated with the body of the corpus callosum, bilateral forceps minor, bilateral forceps major, left longitudinal fasciculus, bilateral superior longitudinal fasciculus, right inferior fronto-occipital fasciculus and left inferior longitudinal fasciculus ( $p < 0.05$ ). Figure recall score correlated with left cingulate gyrus, bilateral forceps minor, bilateral superior longitudinal fasciculi, right anterior thalamic radiation, right inferior fronto-occipital fasciculus, right inferior longitudinal fasciculus and splenium of the corpus callosum ( $p < 0.01$ ). Retention of list items correlated with the body and splenium of the corpus callosum, bilateral forceps minor, bilateral forceps major, bilateral superior longitudinal fasciculi and bilateral anterior thalamic radiation ( $p < 0.05$ ). There were no correlations between measures of axial diffusivity and clinical or neuropsychological measures. There were negative correlations between figure copy score, figure recall score, list learning and list recall score on the RBANS and mean diffusivity (i.e. the higher the score the lower the MD). Higher scores on figure copy score correlated with lower MD in the body of the corpus callosum, left cingulum, right forceps minor, bilateral superior longitudinal fasciculus, splenium of corpus callosum and bilateral forceps major ( $p < 0.05$ ). Higher scores on list recall correlated with lower MD in the right anterior thalamic



radiation ( $p<0.05$ ). Higher scores on figure recall correlated with lower MD in bilateral forceps minor, bilateral superior longitudinal fasciculus and left forceps major ( $p<0.05$ ). Higher scores on list recall correlated with lower MD in the right anterior thalamic radiation ( $p<0.05$ ). There were negative correlations between figure copy score, figure recall score, line orientation score and list recall score and radial diffusivity (i.e. the higher the score the lower the RD). Higher scores on figure copy correlated with lower RD in bilateral forceps minor, right fronto-occipital fasciculus, bilateral anterior thalamic radiation, right inferior longitudinal fasciculus, left cingulum, left superior longitudinal fasciculus and left forceps major ( $p<0.05$ ). Higher scores on figure recall correlated with lower RD in bilateral forceps minor, bilateral superior longitudinal fasciculus and bilateral inferior longitudinal fasciculus ( $p<0.01$ ). Higher scores on line orientation correlated with lower RD in bilateral forceps minor, right inferior fronto-occipital fasciculus, bilateral anterior thalamic radiation and the body of the corpus callosum ( $p<0.05$ ). Higher scores on list recall correlated with lower RD in bilateral forceps minor, bilateral anterior thalamic radiation and right inferior fronto-occipital fasciculus ( $p<0.05$ ).

Table 4-10 Summary of Correlations to DTI measures for List Learning Sub-score on RBANS

|                                      | FA (p<0.05)          | MD (p<0.05)      | RD (p<0.05)            |
|--------------------------------------|----------------------|------------------|------------------------|
| Forceps minor                        |                      |                  |                        |
| Forceps major                        | Positive bilaterally |                  | Negative (bilaterally) |
| Body of corpus callosum              | Positive bilaterally |                  |                        |
| Anterior thalamic radiation          | Positive             |                  |                        |
| Cingulum (hippocampus)               |                      | Negative (right) | Negative (bilaterally) |
| Inferior fronto-occipital fasciculus | Positive (right)     |                  |                        |
| Superior longitudinal fasciculus     | Positive (left)      |                  | Negative (right)       |

Positive means that there was a positive correlation between the DTI value and the list learning score i.e. with higher list learning scores there were higher FA values, negative means that there was a negative correlation between the DTI value and the list learning score i.e. with higher list learning scores there were lower MD or RD values.

Table 4-11 Summary of Correlations to DTI Measures for List Recall Sub-score on RBANS

|                                      | MD (p<0.05)      | RD (p<0.05)            |
|--------------------------------------|------------------|------------------------|
| Forceps minor                        |                  |                        |
| Anterior thalamic radiation          |                  | Negative (bilaterally) |
| Inferior fronto-occipital fasciculus | Negative (right) | Negative (bilaterally) |
|                                      |                  | Negative (right)       |

Negative means that there was a negative correlation between the DTI value and the list recall score i.e. with higher list recall scores there were lower MD or RD values.

Table 4-12 Summary of correlations to DTI measures for Figure Copy Sub-score on RBANS

|                                      | FA (p<0.05)            | MD (p<0.05)            | RD (p<0.05)            |
|--------------------------------------|------------------------|------------------------|------------------------|
| Forceps minor                        | Positive (bilaterally) | Negative (right)       | Negative (bilaterally) |
| Forceps major                        | Positive (bilaterally) | Negative (bilaterally) | Negative (left)        |
| Splenium of corpus callosum          |                        | Positive               |                        |
| Body of corpus callosum              | Positive               |                        |                        |
| Anterior thalamic radiation          |                        |                        | Negative (bilaterally) |
| Cingulum (hippocampus)               |                        |                        | Negative (left)        |
| Corticospinal tracts                 |                        |                        |                        |
| Inferior fronto-occipital fasciculus | Positive (right)       |                        | Negative (right)       |
| Superior longitudinal fasciculus     | Positive (bilaterally) | Negative (bilaterally) | Negative (left)        |
| Inferior longitudinal fasciculus     | Positive (left)        |                        | Negative (right)       |
| Anterior corona radiata              |                        |                        |                        |

Positive means that there was a positive correlation between the DTI value and the figure copy score i.e. with higher figure copy scores there were higher FA values, negative means that there was a negative correlation between the DTI value and the figure copy score i.e. with higher figure copy scores there were lower MD or RD values.



Table 4-13 Summary of Correlations to DTI Measures for Figure Recall Sub-score on RBANS

|                                      | FA (p<0.01)            | MD (p<0.05)            | RD (p<0.01)            |
|--------------------------------------|------------------------|------------------------|------------------------|
| Forceps minor                        | Positive (bilaterally) | Negative (bilaterally) | Negative (bilaterally) |
| Forceps major                        |                        | Negative (left)        |                        |
| Splenium of corpus callosum          | Positive               |                        |                        |
| Anterior thalamic radiation          | Positive (right)       |                        |                        |
| Inferior fronto-occipital fasciculus | Positive (right)       |                        |                        |
| Superior longitudinal fasciculus     | Positive (bilaterally) | Negative (bilaterally) | Negative (bilaterally) |
| Inferior longitudinal fasciculus     | Positive (left)        |                        | Negative (bilaterally) |
| Anterior corona radiata              | Decreased bilaterally  |                        |                        |

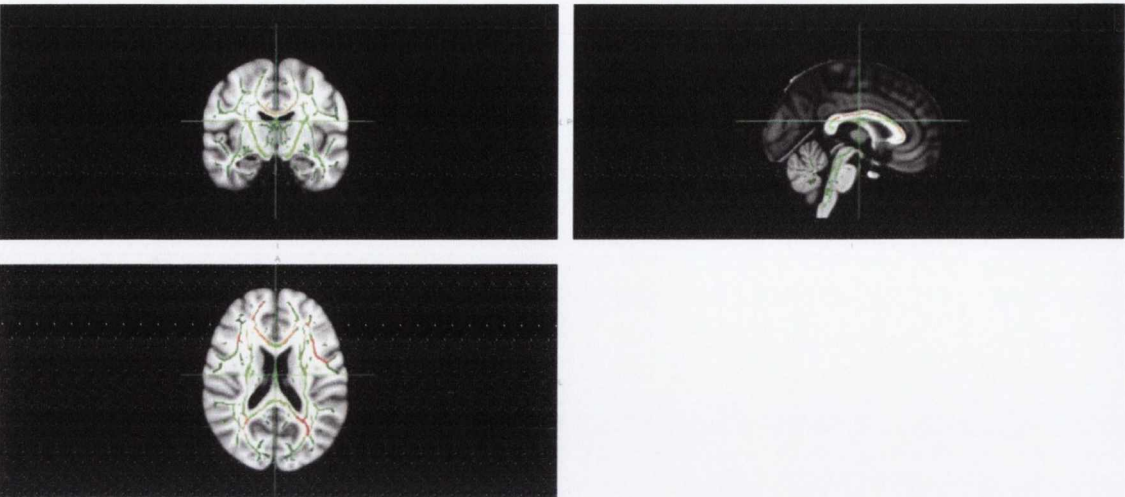
Positive means that there was a positive correlation between the DTI value and the figure recall score i.e. with higher figure recall scores there were higher FA values, negative means that there was a negative correlation between the DTI value and the figure recall score i.e. with higher figure recall scores there were lower MD or RD values.

Table 4-14 Summary of Correlations to DTI Measures for List Retention

| FA (p<0.05)                      |                        |
|----------------------------------|------------------------|
| Forceps minor                    | Positive (bilaterally) |
| Forceps major                    | Positive (bilaterally) |
| Splenium of corpus callosum      | Positive               |
| Body of corpus callosum          | Positive               |
| Anterior thalamic radiation      | Positive (bilaterally) |
| Superior longitudinal fasciculus | Positive (bilaterally) |

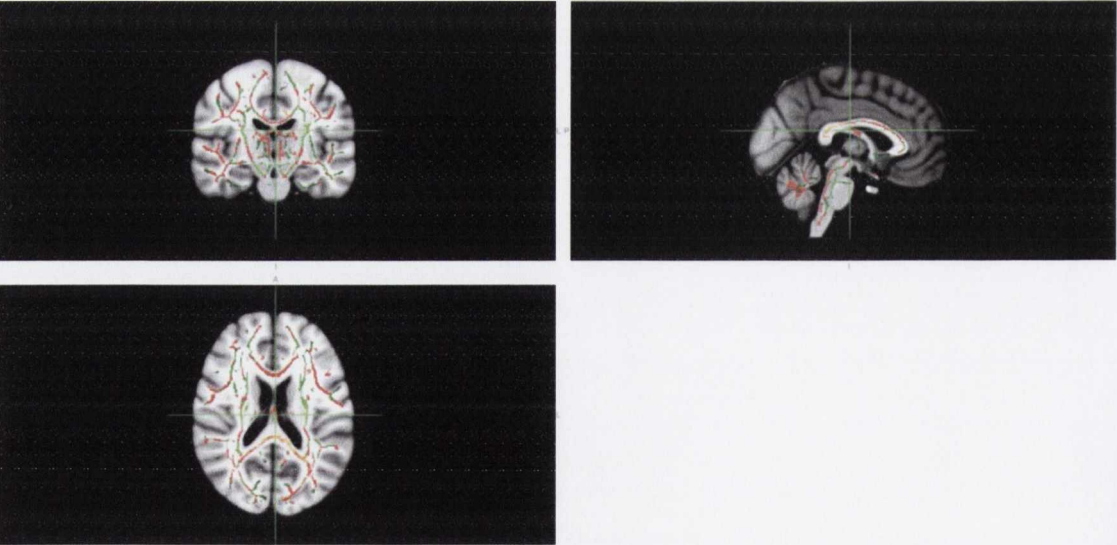
Positive means that there was a positive correlation between the FA value and the list retention score i.e. with higher list retention scores there were higher FA values.

Figure 4-69 Correlation between FA values and List Learning Sub-score on the RBANS



$p < 0.05$

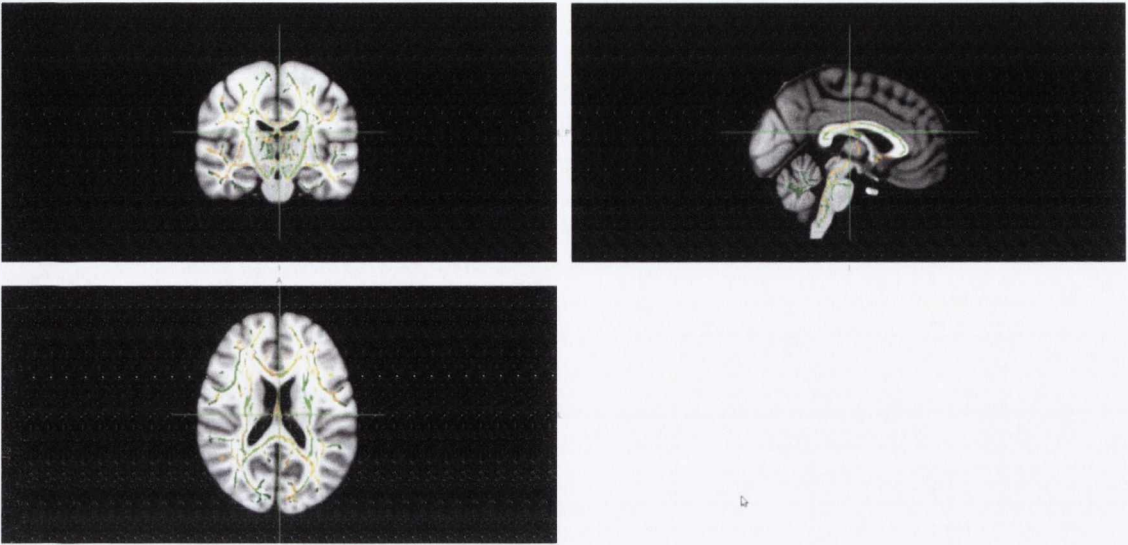
Figure 4-70 Correlation between FA values and Figure Copy Sub-score on the RBANS



$p < 0.05$

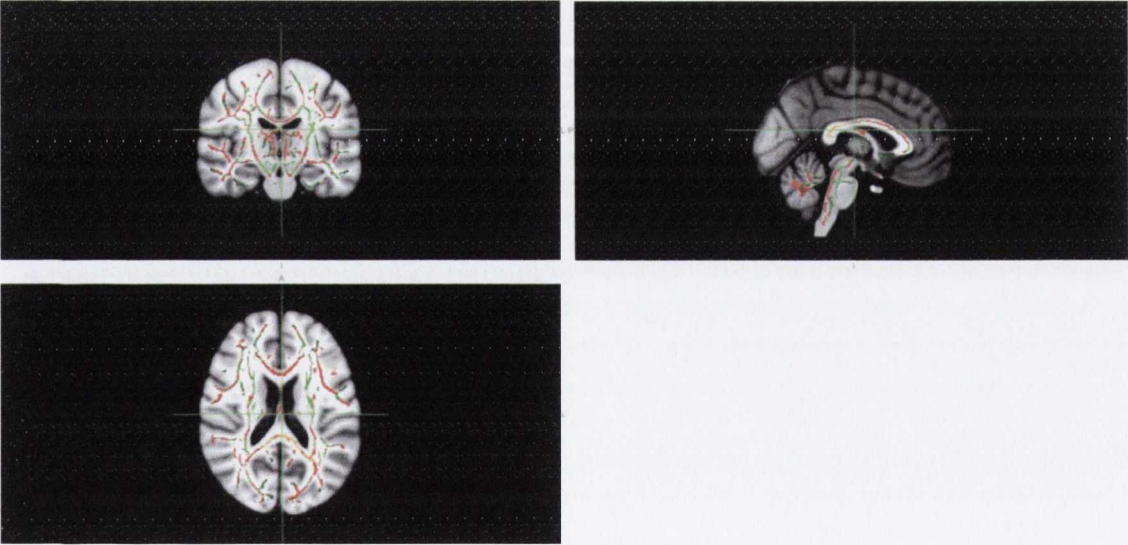


**Figure 4-71 Correlation between FA values and Figure Recall Sub-score on the RBANS**



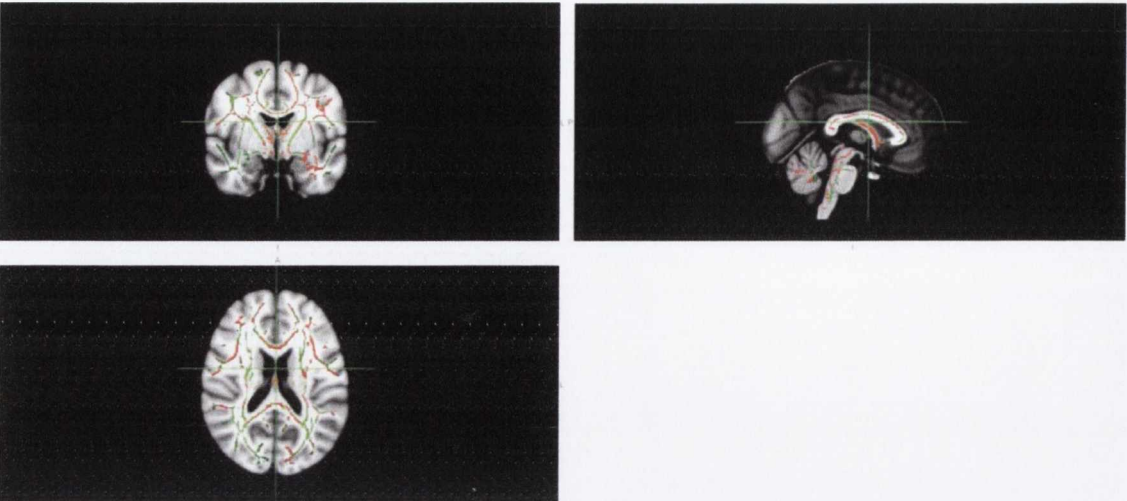
$p < 0.01$

**Figure 4-72 Correlation between FA values and Retention of List Memory Items**



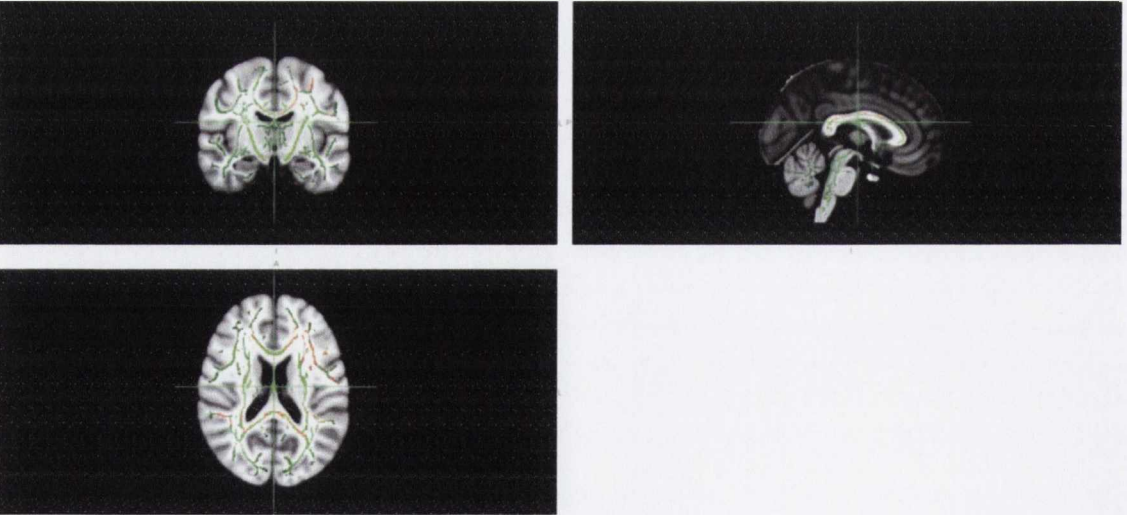
$p < 0.05$

Figure 4-73 Correlation between MD values and Figure Copy Sub-score on RBANS



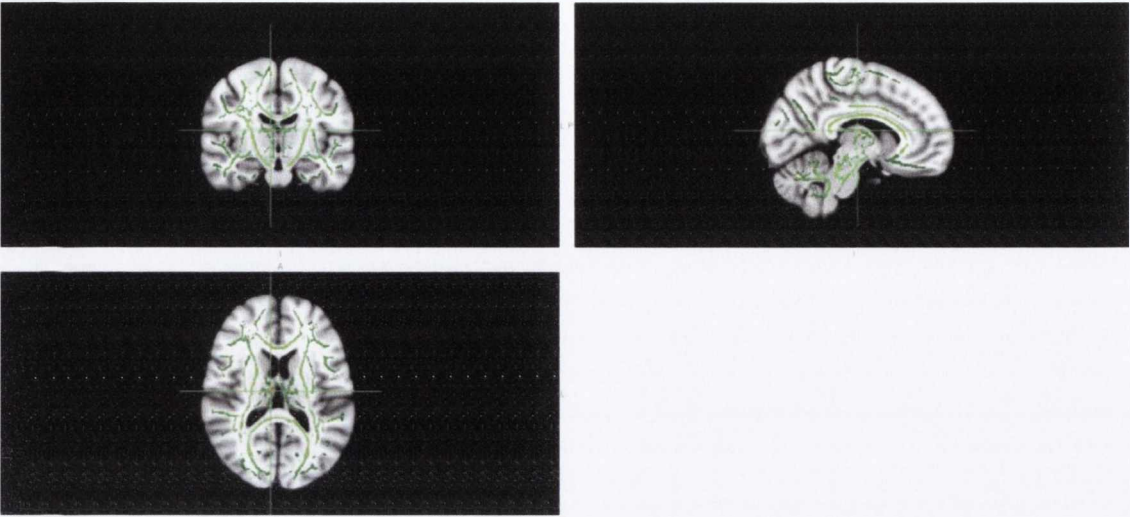
$p < 0.05$

Figure 4-74 Correlation between MD values and Figure Recall Sub-score on RBANS



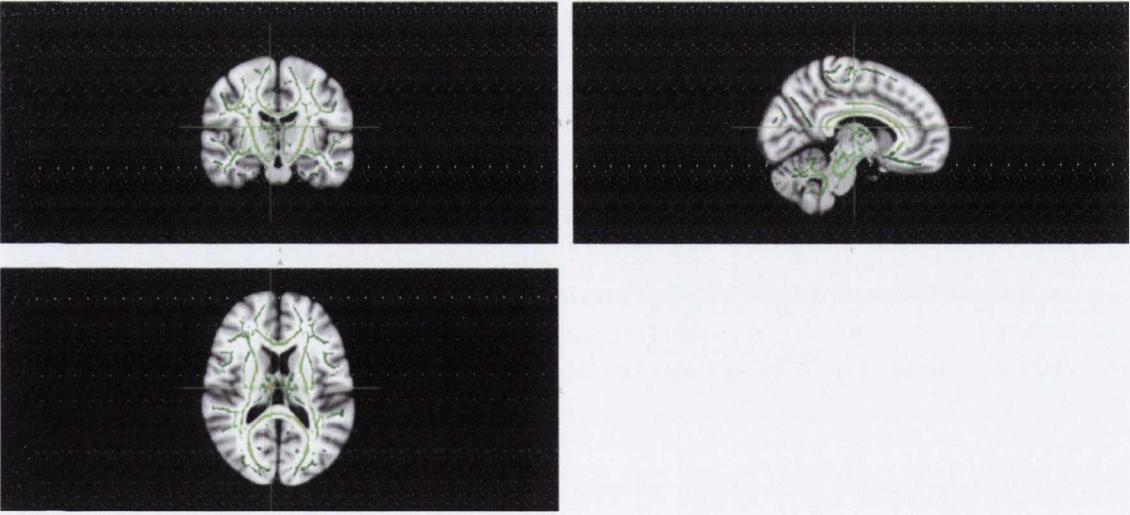
$p < 0.05$

Figure 4-75 Correlation between MD values and List Learning Sub-score on RBANS



$p < 0.05$

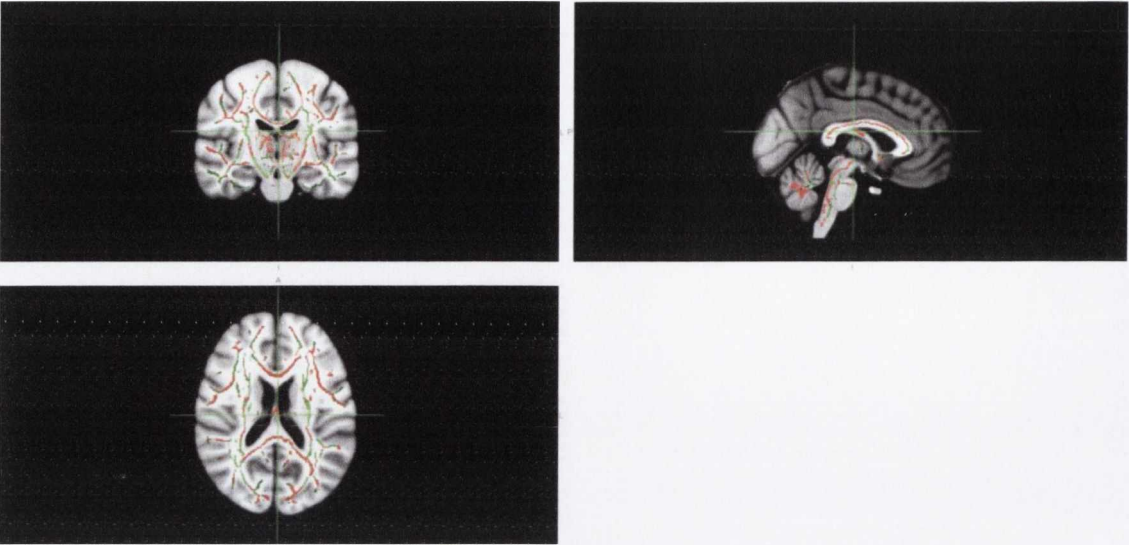
Figure 4-76 Correlation between MD values and List Recall Sub-score on RBANS



$p < 0.05$

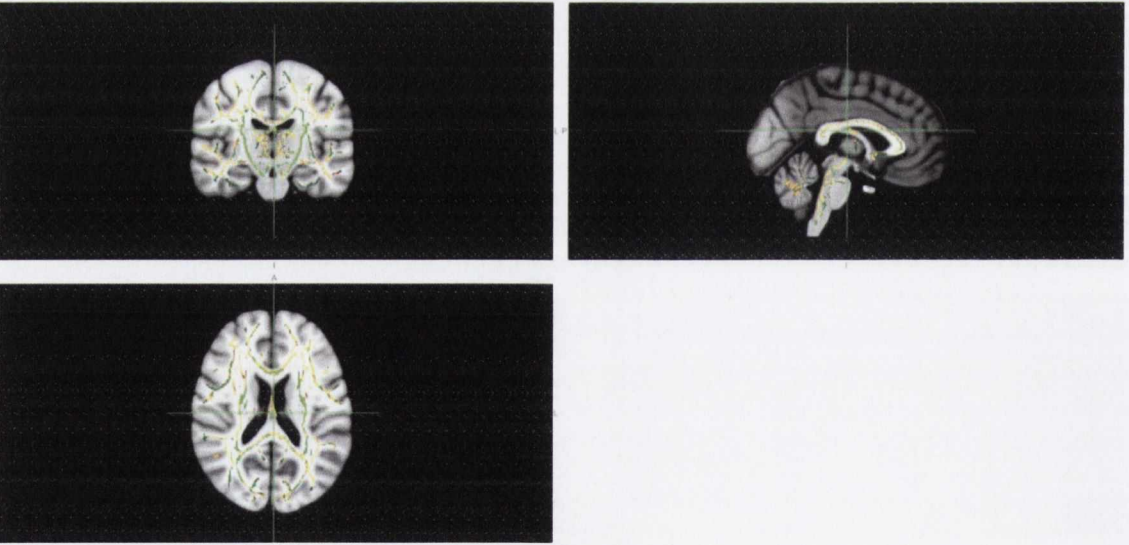


Figure 4-77 Correlation between RD values and Figure Copy Sub-score on RBANS



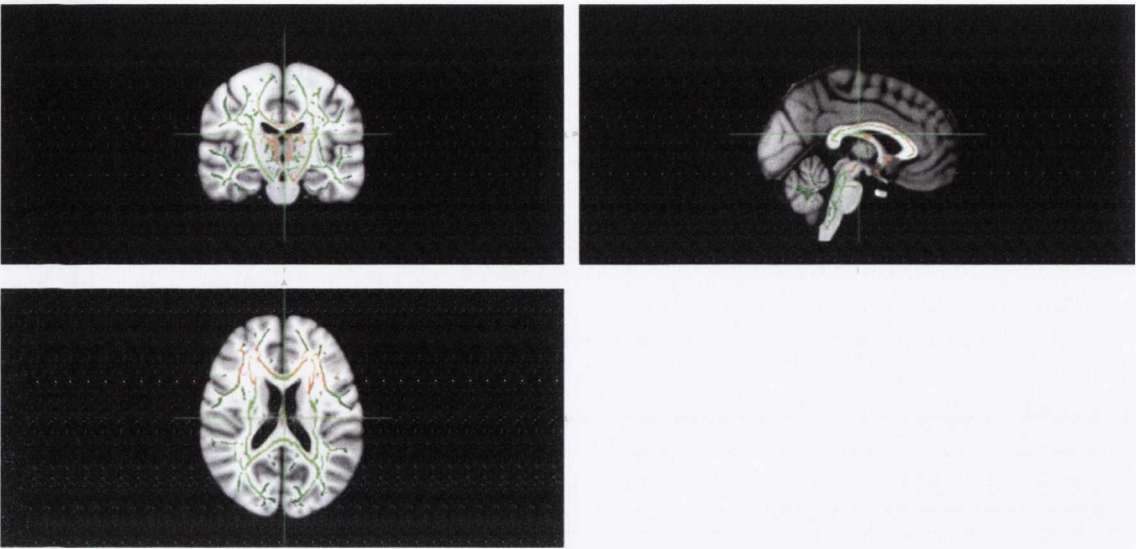
$p < 0.05$

Figure 4-78 Correlation between RD values and Figure Recall Sub-score on RBANS



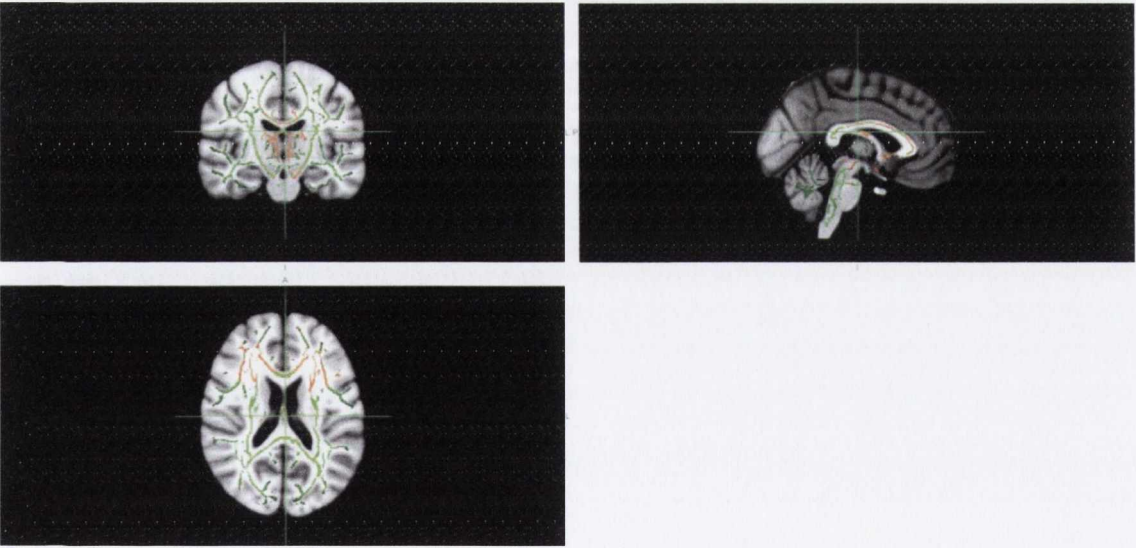
$p < 0.01$

Figure 4-79 Correlation between RD values and List Learning Sub-score on RBANS



$p < 0.05$

Figure 4-80 Correlation between RD values and List Recall Sub-score on RBANS



$p < 0.05$

## 4.5 Discussion

### 4.5.1 Discussion of VBM Results

Grey matter atrophy was demonstrated in this cohort of patients in the anterior cingulate, left hippocampus, left temporal lobe, left central opercular cortex, left post-central gyrus, left supplementary motor area, right lateral occipital cortex, right precuneus, bilateral Heschl's gyrus, bilateral insular cortex, bilateral parietal operculum, bilateral occipital poles and bilateral pre-central gyrus. The majority of the patients in this cohort were on HAART (94%) and were virally suppressed (95.7%) yet still demonstrated focal grey matter atrophy similarly to other studies in the post-HAART era (419-422, 433, 434). Grey matter atrophy has also been demonstrated within the first year of HIV infection (428). There were ten patients within the group who did not meet diagnostic criteria for HAND but nevertheless had abnormalities on neuropsychological assessment. This study highlights that at a group level there were focal areas of grey matter atrophy not only in patients who met diagnostic criteria for HAND but also in those patients who did not meet criteria suggesting that atrophy of grey matter regions occurs in HIV even prior to fulfilling diagnostic criteria for HAND.

There were no significant correlations to clinical or neuropsychological data. This may be due to the fact that the HIV negative control group did not undergo neuropsychological assessment and hence correlations could only be performed within the patient group. It may be due to a lack of power to detect differences in cortical atrophy patterns between patients with ANI, MND and HAD as most of the patients with HAND were in the ANI and MND groups or a lack of power to detect differences in



cortical atrophy patterns associated with specific neuropsychological sub-scores or domains. It may also be a reflection of the fact that there were multiple areas of focal grey matter volume loss found and these may not necessarily relate to one specific neuropsychological sub-score and may actually relate to overall cognitive function in the setting of HIV. While specific cognitive domains can be regionally localised, they are also dependent on complex networks involving multiple lobes and deficits can be far more complex to determine and therefore sub-scores on neuropsychological testing may not correlate to one specific cortical region particularly in a study of this size with only fifty participants. It may also be due to the fact that we have demonstrated widespread atrophy in this cohort and therefore it is not possible to detect correlations to specific neuropsychological sub-scores due to the widespread nature of the atrophy. Previous studies have demonstrated relationships to CD4 count (bilateral lateral temporal, prefrontal and parietal cortical thinning (419), occipital atrophy (421) and frontal atrophy (429)), to disease duration (parietal, temporal and frontal lobes (429), posterior temporal (422) and bilateral inferior frontal (421)), to cognitive and motor deficits (prefrontal and parietal atrophy (419)) and detectable HIV in peripheral blood (caudate, putamen, thalamus, hippocampus, nucleus accumbens, brainstem, total cortical grey matter (430)). It is likely that the underlying cognitive deficits evident in this cohort of patients are secondary to both grey and white matter involvement.

Despite the lack of correlation to specific neuropsychological scores there was atrophy in regions consistent with the neuropsychological deficits found in the patient cohort. Within the subset of the cohort who had MR imaging, 82% (n=41) had abnormalities in

delayed memory, 78% (n=39) had abnormalities in immediate memory, 54% had abnormalities in attention (n=27), 42% had abnormalities in language (n=21) and 20% had abnormalities in visuospatial skills (n=13). There was evidence of atrophy in the left hippocampus and left temporal lobe, both of which are involved in learning and memory (460, 461). The hippocampus in particular is involved in the consolidation of information from short-term memory to long-term memory (460). The anterior cingulate is involved in executive control of attention and was atrophied in this cohort of patients (459). There was also atrophy of the precuneus which is involved in episodic memory (465). There was significant atrophy of the pre and post central gyri despite the majority of patients having normal motor function but they had evidence of apraxia which could be secondary to this cortical loss. There was atrophy of the left supplementary motor area (SMA). This is involved in motor planning (464) and there have been case reports of ideomotor apraxia without bucco-facial apraxia associated with left mesial hemisphere infarction involving the SMA (471). Recent structural and functional imaging studies demonstrated a relationship between apraxia and the pre-supplementary area (472). Ideomotor apraxia has also been associated with lesions of the premotor cortex (473). Apraxia was the most common examination abnormality in this group of patients. The occipital lobes are involved in visual perception (461) and 20% of the patients demonstrated difficulties with visuospatial skills. Despite the lack of correlation to specific neuropsychological scores, the pattern of atrophy is consistent with the cognitive deficits found in this group of patients suggesting that there is an anatomical basis and correlate for the cognitive profile seen in this cohort.

#### 4.5.2 Discussion of DTI Results

This study demonstrated diffuse white matter integrity disruption in this cohort of HIV positive patients across four different DTI measures. Abnormalities in FA and MD are not related to specific white matter pathology but are sensitive markers of white matter integrity. Decreases in FA measures showed the most abnormalities in this study and increases in MD were also demonstrated (Table 4-9) in this study. Previous studies have also demonstrated reductions in FA and increases in MD measures in HIV infection (436, 438-441, 443-445, 448). The findings of this study suggest that there is widespread white matter integrity loss in HIV positive patients with cognitive impairment and contribute further to the studies already conducted. Reductions in AD were restricted to the left forceps minor, left anterior thalamic radiation and left corticospinal tracts. Reductions in AD have been shown to correlate to axonal degeneration (402, 403). Increases in RD measures are associated with myelin pathology (391, 404) and were shown in forceps minor and major bilaterally, body of the corpus callosum, left anterior thalamic radiation, right superior longitudinal fasciculus, right inferior fronto-occipital fasciculus and left inferior longitudinal fasciculus.

Pfefferbaum et al demonstrated increased axial and radial diffusivity. The abnormalities in RD were seen in patients who were naïve to HAART while patients on HAART showed normal RD measures at those sites leading the authors to speculate that the increased radial diffusivity may be due to inflammation in the naïve group which normalises or disappears when treated with HAART. High axial or longitudinal diffusivity was seen in patients with AIDS suggestive of axonal compromise in more



advanced immune suppression (445). The majority of patients in the candidate's study were on HAART and were virally suppressed hence it was not possible to assess for differences between those naïve to or on HAART.

The findings of this study suggest that in HAND there is evidence of both axonal and demyelination abnormalities on DTI and lends further support to previous studies (445, 446). The neuropathological findings of HAND support this as it has been demonstrated that HIV has a predilection for white matter (100) and both axonal loss and myelin pallor have been found at autopsy (99). The abnormalities in RD were more widespread than the decreases in AD measures similarly to a study by Chen et al (446) who postulated therefore that the underlying pathology may be driven by demyelination. Similarly Nir et al also found that HIV related deficits were more strongly related to RD than to any other DTI measure (447). Demyelination is usually driven by inflammation and these findings of abnormal radial diffusivity support the candidate's hypothesis that there are inflammatory changes in HAND in addition to degenerative changes which would be supported by the changes in axial diffusivity measures in HIV positive patients in this cohort. In the anterior thalamic radiation and the left forceps minor there was both decreased axial diffusivity and increased radial diffusivity suggesting that abnormalities in myelination and axonal disruption were occurring at the same site spontaneously.

With regard to correlation to clinical and neuropsychological data there were correlations between some neuropsychological scores and increased FA, decreased MD and decreased RD suggesting that higher scores correlated with better white

matter integrity. These were particularly evident for list learning, figure copy and recall sub-scores on the RBANS. Interestingly there were no correlations to length of diagnosis, nadir CD4 or current CD4 T cell count. List learning showed positive correlations to FA and negative correlations to RD in forceps minor bilaterally and right inferior fronto-occipital fasciculus. Forceps minor is involved in the relay of information intra-hemispherically (464) and the inferior fronto-occipital fasciculus integrates auditory and visual association cortices with the pre-frontal cortex (389). As these areas were correlated with higher scores on these tests it suggests that both of these tracts are important for list learning. Higher figure copy scores correlated positively with FA and negatively with RD bilaterally in the forceps minor, forceps major (left only for RD), right inferior fronto-occipital fasciculus and superior longitudinal fasciculus (bilaterally for FA and left only for RD). Higher figure recall scores correlated positively with FA and negatively with RD in bilateral forceps minor, bilateral superior longitudinal fasciculus and bilateral inferior longitudinal fasciculus (left only for FA). Forceps major and minor relay information intra-hemispherically and are correlated in this study with higher scores on these two tests (visuospatial and visual memory) (464). The longitudinal fasciculi are involved in the integration of auditory and speech nuclei but are correlated with higher scores suggesting that they play a role in this visuospatial task and visual memory (389). The inferior fronto-occipital fasciculus plays an important role in visuospatial tasks as it integrates the visual association cortex with the pre-frontal cortex (389).

#### 4.5.3 Strengths of the study

The patients were well characterised from a neurological and neuropsychological perspective. Excellent MR imaging quality was achieved. Validated and widely accepted techniques were used for MRI analysis.

#### 4.5.4 Limitations of the study

There were no HIV positive patients who had entirely normal cognitive testing within the study cohort to examine the effect of HIV alone, in the setting of normal cognition, on grey matter volume or DTI measures. Also the controls used were drawn from two separate studies but were age and gender matched to the study cohort. DTI has some limitations assessing fibre integrity in regions with extensive fibre crossing as FA values may be naturally low in these regions (474). In order to overcome this issue we used multiple measures of DTI. The study was cross-sectional in nature and whilst it gives an excellent snapshot of the neuroimaging features of this cohort at this point in time, a longitudinal study is required to assess the rate of atrophy over time and to further assess correlation to neuropsychological scores over time.

### 4.6 Conclusion

Focal grey matter atrophy and diffuse white matter integrity loss were demonstrated in a cohort of HIV positive patients with cognitive impairment, the majority of whom met criteria for HAND. There was evidence of inflammatory changes on DTI in the form of increased radial diffusivity and degenerative changes on DTI and VBM as represented by decreased axial diffusivity and focal grey matter atrophy respectively.



There were significant correlations to clinical data only for DTI measures and not for VBM results. The correlations to neuropsychological data were associated with increased FA and decreased MD and RD suggesting increased white matter integrity with higher scores. VBM and DTI are sensitive biomarkers of neurodegeneration and white matter tract integrity respectively. The findings of this study all support the candidate's hypothesis of an inflammatory degenerative continuum underlying the neuropathogenesis of HAND however longitudinal follow-up is required to further evaluate the role of neurodegeneration and to assess the nature of the disruption of the white matter microstructural integrity over time.

## **5 Cerebrospinal Fluid Findings in an Irish Cohort of HIV Positive Patients with Cognitive Impairment**

### **5.1 Outline of the chapter**

This chapter focuses on the subset of patients who underwent cerebrospinal fluid examination. The underlying hypothesis of the study is that HAND has both inflammatory and degenerative phases. Flow cytometry was used to examine the immune status of these patients. Cytokine analysis was used to examine for biomarkers of neuroinflammation and analysis of tau and beta amyloid was used as biomarkers of neurodegeneration. The candidate expected to find that the HIV positive patients had evidence of immune activation in addition to biomarkers of neuroinflammation and neurodegeneration.

### **5.2 Role of Immunity**

#### **5.2.1 Role of T and B Lymphocytes**

Progression of HIV infection to AIDS is associated with a gradual decline in CD4 T cell numbers that results in immunodeficiency, increased susceptibility to opportunistic infections and may eventually lead to death if untreated (475). CD4 T cells are the primary cell type affected in HIV infection.

The adaptive immune response consists of T and B lymphocytes. T cells can be divided into naïve, central and effector memory and terminally differentiated subsets on the basis of expression of cell surface of receptors CD27 and CD45RA. Naïve T lymphocytes

give rise to a heterogeneous population of antigen-specific T cells. Effector T lymphocytes are found in settings of active antigenic stimulation (e.g., during primary viral infection) and are capable of eliminating viruses or tumours whereas memory T lymphocytes remain present in the absence of antigenic stimulation and have the capacity to expand rapidly upon secondary challenge (476). Memory T cells facilitate immunosurveillance. Memory T cell trafficking in peripheral tissues enables rapid protection from re-infection while memory cells present in the secondary lymphoid organs are mobilised (477). Central memory T cells can produce interleukin (IL)-2 and proliferate extensively, whereas effector memory T cells are less proliferative and produce effector cytokines such as interferon (IFN)- $\gamma$ . Both CD4<sup>+</sup> and CD8<sup>+</sup> central memory cells are the predominant cell type in secondary lymphoid organs, whereas effector memory T cells predominate in peripheral compartments (477). T cells are activated by dendritic cells. This involves the integration of multiple signals including antigen, co-stimulatory molecules, cytokines and chemokines. Higher levels of inflammation and antigen favour the generation effector memory cells rather than central memory cells (477-480).

Memory CD4<sup>+</sup> activated T lymphocytes are the primary targets of HIV. Up to 80% of the gut-associated lymphoid tissue CD4<sup>+</sup> T cells can be depleted in the first 3 weeks of primary HIV infection. Follicular helper cells, a subset of CD4<sup>+</sup> T cells, interact with antigen specific B cells to promote the production of memory B cells and plasma cells. CD4<sup>+</sup> T cells produce a number of effector cytokines including IFN- $\gamma$  and TNF- $\alpha$  and chemokines such as Regulated And Normal T cell Expressed and Secreted (RANTES)



and Macrophage Inflammatory Protein (MIP)- 1 $\alpha$  and  $\beta$  (481). CD8<sup>+</sup> T cells are cytotoxic and release antiviral cytokines including IFN- $\gamma$ , TNF- $\alpha$ , RANTES, MIP-1 $\alpha$  and  $\beta$ , perforin and granzymes (481). With HIV disease progression, the CD4<sup>+</sup> and CD8<sup>+</sup> T-cell compartments present an accumulation of highly differentiated memory T cells and a profound loss of naive T cells (481-483).

B cells produce antigen specific antibodies and give rise to plasma cells. As HIV progresses there is an excess of activated and terminally differentiated cells and a reduction in the number of naïve and resting memory B cells (484-486).

#### 5.2.2 Lymphocyte subtyping in CSF in HIV

Marcondes et al examined the T cell infiltrates in the brains of rhesus monkeys infected with simian immunodeficiency virus (SIV). These monkeys showed evidence of CNS disease with abnormal brainstem auditory and auditory evoked potentials. At autopsy 11 weeks post infection with SIV there were over five-fold more activated CD8<sup>+</sup> T cells in the brains of the infected monkeys and those cells expressed mRNA for cytolytic and proinflammatory molecules including granzymes A and B, perforin and IFN- $\gamma$  (487).

Shacklett et al performed flow cytometry on paired blood and CSF samples from 19 HIV positive and 8 HIV negative subjects. They hypothesised that lymphocyte extravasation was driven by lymphocyte activation, expression of adhesion molecules

and increased vascular permeability and was coupled with chemokine-mediated trafficking to inflammatory sites within the CNS (488). Neuenburg et al characterised T cell phenotypes in 74 paired blood and CSF samples of HIV positive and negative subjects and concluded that the proportion of CD4<sup>+</sup> T cells and memory T cells among T cells in CSF was higher compared to blood regardless of HIV infection. In HIV infection, activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells in CSF were more abundant than in HIV negative controls. They also found that combination antiretroviral therapy reduced T cell activation in CSF and blood (489).

Von Geldern et al found a shift from naïve to effector memory CD8<sup>+</sup> T cells in the CSF of treatment naïve HIV positive subjects. The HIV load correlated with CD8<sup>+</sup> T cells in the CSF and effector memory CD8<sup>+</sup> T cells correlated positively with viral replication in the CSF (490). Sinclair et al evaluated the effect of HAART on activation of T cells in CSF and blood, and interactions of this activation with CSF HIV-1 RNA concentrations by examining three groups: those off HAART, those on HAART with > 500 copies/ml of HIV-1 RNA and those on HAART with < 500 copies/ml of HIV-1 RNA. CD8 T-cell activation in CSF and blood was highly correlated across all subjects and was highest in those who were off HAART and lowest in those with less than 500 copies/ml of HIV-1 RNA. The similarity of CD8 T-cell activation in blood and CSF led the authors to suggest that these cells move from blood to CSF (491).

Sadagopal et al characterised HIV-specific CD8<sup>+</sup> T cells in CSF and blood from seven HAART naïve subjects with normal neuropsychological testing and found that HIV

specific CD8<sup>+</sup> T cells were 2.4 fold higher in CSF than in blood but HIV RNA concentrations were lower. HIV specific CD8<sup>+</sup> T cells were at comparable maturation states as assessed by CD45RO and CCR7 staining in CSF and blood. They postulated that the higher CD8<sup>+</sup> T cell levels and lower HIV RNA in the CSF could be secondary to increased migration to and/or preferential expansion of HIV-specific T cells within the CNS (114). During chronic viral infections, T cells may become exhausted due to constant antigen exposure and can be associated with enhanced programmed death 1 (PD-1) expression. PD-1 expression in CSF and blood of 15 HIV positive subjects was examined by Sadagopal et al. PD-1 expression was higher on HIV-specific CD8<sup>+</sup> T cells than on total CD8<sup>+</sup> T cells in both CSF and blood. PD-1 expression on CSF T cells correlated positively with CSF HIV-1 RNA and inversely with blood CD4<sup>+</sup> T-cell counts. This led the authors to suggest that HIV-1 infection results in higher PD-1 expression on CSF T cells. PD-1 expression was higher on T cells in CSF than on those in blood in both HIV positive and negative subjects. The authors postulated that the higher PD-1 expression level on CSF T cells does not reflect cellular exhaustion but may actually be a mechanism to down-regulate immune-mediated tissue damage within the CNS (492).

CD4<sup>+</sup> T cells are the main cell constituent of CSF in HIV negative individuals whereas in patients with HIV, CD8<sup>+</sup> T cells were the major cellular component of CSF (115). Rawson et al examined the role of peripheral CD8<sup>+</sup> T cells in cognitive impairment in 144 HIV positive subjects, 44 of whom had cognitive impairment based on the Brief NeuroCognitive Screen. The subjects with neurocognitive impairment were 8% more likely to have inversion of CD4:CD8 ratio and higher median peak CD8 cell counts



reported compared to non-impaired subjects (493). Kowarik et al examined the immune cell subtyping in the CSF of patients with various neurological diseases including HIV, multiple sclerosis, bacterial and viral meningitis, Lyme disease, stroke, vasculitis Guillain-Barre syndrome and sarcoidosis. T cells were the predominant population in the CSF with CD4<sup>+</sup> T cells being more prevalent than CD8<sup>+</sup> T cells. In HIV patients, CD4<sup>+</sup> and CD8<sup>+</sup> T cells were significantly altered and the CD4/CD8 ratio was reduced. B cells and plasmablasts were consistently elevated in inflammatory diseases. Natural Killer cells were slightly elevated in neuroinflammation (494). Cepok et al found that B cell and plasmablast levels were elevated in the CSF of HIV positive subjects and that plasmablasts were more prevalent early in HIV infection (495)

### 5.2.3 Role of Natural Killer Cells

Natural killer (NK) cells are part of the innate immune response along with mast cells, phagocytes, macrophages, neutrophils, dendritic cells, basophils, eosinophils and gamma-delta cells. NK cells have both cytotoxic and cytokine-producing functions and are widespread throughout both lymphoid and non-lymphoid tissues. CD4<sup>+</sup> T cells and NK cells interact in the lymph nodes and T cell derived IL-2 promotes NK cell proliferation, cytotoxicity and cytokine production. NK cells promote the maturation of dendritic cells via IFN- $\gamma$  and TNF which in turn activate NK cells via IL-12. NK cells can also promote the priming of CD4<sup>+</sup> T helper type 1 cells by secreting IFN-  $\gamma$  (496). HIV infection is associated with a functional impairment of NK cells. There is an inverse correlation between NK cell-mediated suppression of HIV replication and viraemia. NK cells in HIV have reduced stores of perforin and granzyme A thus rendering them less

cytotoxic. Persistent HIV viraemia may result in aberrant expression of several inhibitory and activating NK cell surface receptors. There are changes in the expression of some activated markers on NK cell surfaces which may suggest that there is NK cell exhaustion and anergy secondary to chronic stimulation. There may be expansion of NK cell populations expressing the activating NKG2C receptor and decreased expression of the inhibitory NKG2A receptor in the setting of HIV infection and this may alter the balance of NK cells responses towards activation (497).

#### 5.2.4 Role of Natural Killer like T Cells (NK like cells)

These cells share properties of both T cells and NK cells and are capable of potent perforin/granzyme-mediated killing of a variety of tumour target cells. Their cytotoxicity is unrestricted and does not require prior antigenic exposure. They express IFN- $\gamma$ , IL-4 and IL-13 (498).

#### 5.2.5 Role of Gamma/Delta T Cells

HIV can also infect gamma/delta ( $\gamma\delta$ ) T cells (499), populations of innate lymphocytes that recognise non-peptide antigens and play central roles both in the initiation and regulation of adaptive immune responses (500). Human  $\gamma\delta$  T cells can be classified into three major subsets according to differential usage of T cell receptor for antigen (TCR)  $\gamma$  and  $\delta$ -chains. V $\gamma$ 9V $\delta$ 2 cells (hereafter referred to as V $\delta$ 2 T cells) are the predominant subset in blood, accounting for 1-5% of peripheral T cells (501). T cells expressing V $\delta$ 1 TCR chains predominate in mucosal tissues. V $\delta$ 3 T cells make up the majority of V $\delta$ 1- and V $\delta$ 2-negative  $\gamma\delta$  T cells in both tissues. V $\delta$ 1 T cells are expanded and V $\delta$ 2 T cells

are numerically and functionally impaired in blood and mucosal tissues from HIV-infected individuals (502-505).

Activated V $\delta$ 2 T cells produce proinflammatory cytokines and chemokines, kill infected cells, secrete growth factors for epithelial cells, and present antigens to alpha-beta ( $\alpha\beta$ ) T cells (501). V $\delta$ 2 T cells proliferate in response to HIV-infected cells, kill HIV-infected cells, suppress HIV replication and produce HIV-inhibiting chemokines *in vitro* (506, 507). V $\delta$ 2 effector-memory cell numbers are reduced and their proliferative, cytotoxic and cytokine responses are impaired in HIV-infected individuals compared to controls (502-504). In one clinical trial, treatment of HIV-infected patients naïve to HAART with the V $\delta$ 2 T cell-inducing drug zoledronate and the T cell growth factor interleukin-2 (IL-2) resulted in V $\delta$ 2 cell expansion and improved HIV-specific CD8 T cell responses (508). These studies provide evidence that V $\delta$ 2 T cells mediate direct immunity against HIV. They also have indirect anti-viral activities, being able to activate monocytes, dendritic cells, B cells, neutrophils and  $\alpha\beta$  T cells (501, 509-513) and may combat opportunistic infections and neoplasms that arise in HIV infection (505).

V $\delta$ 1 T cells appear to play a role in the progression of HIV infection. V $\delta$ 1 T cells expressing the activating natural killer (NK) G2C receptor are expanded in HIV infection (514, 515) and these cells respond to human leukocyte antigen (HLA) E, which is induced on HIV-infected CD4 T cells, leading to enhanced V $\delta$ 1 cytotoxicity (516). V $\delta$ 1 cells can also lyse uninfected CD4 T cells (517) and therefore contribute to the decline in CD4 T cells that occurs in HIV infection.



The above studies indicate that  $\gamma\delta$  T cells play roles both in immunity against HIV and in HIV-associated immunodeficiency and are dysregulated in HIV-infected patients.  $\gamma\delta$  T cells are among the first cells to sense pathogens and they respond rapidly by direct cytotoxicity and selective activation of adaptive immune responses. Perella et al examined levels of  $\gamma\delta$  cells in CSF of patients with AIDS related dementia and patients with other non-inflammatory neurological diseases and did not demonstrate any significant difference between the two groups (518).

### **5.3 Role of Amyloid and Tau**

Tau protein is a microtubule associated phosphoprotein which promotes microtubule assembly and stabilisation. It is found in high concentrations within axons of the central nervous system. In humans, tau consists of at least six isoforms of related amino acid sequences that are produced from a single gene by alternative mRNA splicing and that are expressed in a stage- and cell type-specific manner. Tau is also a component of the paired helical filaments associated with Alzheimer's disease and other disorders of the CNS (519). Elevated concentrations of tau may be detected in cerebrospinal fluid of patients with neuronal damage due to acute ischaemic stroke (520), Creutzfeldt-Jakob disease (521), Alzheimer's disease (522, 523) and fronto-temporal dementia (524, 525). Elevated tau is thought to be related to neuronal loss, which occurs in these conditions.

Beta-amyloid<sub>(1-42)</sub> is created by the cleavage of the amyloid precursor protein (APP). APP can be cleaved by alpha, beta or gamma secretases into monomers of between 38 to 43 amino acids. Beta-amyloid<sub>(1-42)</sub> contains 42 amino acids and is created via cleavage by  $\beta$  or  $\gamma$  secretases. Beta-amyloid<sub>(1-42)</sub> monomers associate to form neurotoxic oligomers resulting in insoluble fibrillar amyloid plaques which are one of the pathological hallmarks of Alzheimer's disease (526). Clearance of beta-amyloid<sub>(1-42)</sub> is regulated by insulin degrading enzyme, neprilysin, matrix metalloproteinase 9 and plasminogen (527-529). Beta-amyloid can activate microglia to produce cytokines and neurotoxins, hence promoting neurodegeneration. Microglia also express receptors that promote the clearance and phagocytosis of beta-amyloid, such as class A scavenger receptor, CD36 and receptor for advanced-glycosylation end products (RAGE) (529). In Alzheimer's disease the clearance of neurotoxic beta-amyloid is disrupted and plaques form. Hickmann et al have shown that age and progression of Alzheimer's disease affect the ability of microglia to clear beta-amyloid (529).

HIV also disrupts the clearance of beta-amyloid. Gp120 induces release of TNF- $\alpha$  and IL-1 $\beta$  from microglia and these cytokines stimulate increased cleavage of APP by  $\beta$  and  $\gamma$  secretases. Tat inhibits neprilysin and increases the aggregation of beta-amyloid<sub>(1-42)</sub> into neuronal endolysosomes through endocytosis (530).

The results of analysis of tau and beta-amyloid<sub>(1-42)</sub> levels in CSF in HAND have been conflicting as was discussed in chapter 1 and the findings of studies to date are summarised in Table 5-1.

**Table 5-1 Summary of Studies Assessing CSF Amyloid and Tau**

| Reference                    | Subjects                                                                                                                              | Major Findings                                                                                                                                                                                                                                                          |
|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Peluso et al 2013 (531)      | 92 Primary HIV infection<br>25 HIV negative controls                                                                                  | P-tau was increased in 66/92 with primary HIV<br>No difference in total tau<br>Beta-amyloid was increased in 33/92                                                                                                                                                      |
| Steinbrink et al 2013 (427)  | 94 HIV positive                                                                                                                       | Correlation between increase in CSF tau and severity of periventricular white matter abnormalities and basal ganglia abnormalities.<br>Total tau correlated with neuropsychological results.                                                                            |
| Krut et al 2013 (532)        | 21 HAD<br>12 Bacterial meningitis<br>13 Lyme neuroborreliosis<br>10 HSV encephalitis<br>21 Alzheimer's disease<br>42 Healthy controls | Decreased beta-amyloid in HIV.<br>Lower concentration of soluble APP compared to Alzheimer's disease.                                                                                                                                                                   |
| Du Pasquier et al 2013 (533) | 49 HIV positive<br>29 Alzheimer's disease                                                                                             | HIV – increase in total tau.<br>No difference in p-tau.                                                                                                                                                                                                                 |
| Gisslen et al 2009 (534)     | 86 HIV positive<br>- 21 ADC<br>- 25 CNS OI<br>- 40 asymptomatic<br>64 HIV negative including 21 with Alzheimer's disease              | Soluble APP levels were highly correlated and decreased in patients with ADC and CNS OI.<br>CNS OI – decreased beta-amyloid.<br>Total tau was increased in some patients with ADC but did not differ significantly from asymptomatic patients.<br>No increase in p-tau. |
| Clifford et al 2009 (163)    | 49 with HAND (11 with HAD)<br>21 HIV positive asymptomatic<br>50 Healthy controls<br>68 Alzheimer's disease                           | Decreased beta-amyloid in HAND compared to controls and HIV asymptomatic similarly to those with Alzheimer's disease.<br>Total tau and p-tau slightly lower in HIV than controls.                                                                                       |



|                            |                                                                                                                                            |                                                                                                           |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| Brew et al 2005 (162)      | 121 HIV positive<br>20 Alzheimer's disease<br>20 Healthy controls                                                                          | Decreased beta-amyloid in ADC.<br>Increased total tau in ADC stage 2.<br>Increased p-tau in ADC.          |
| Green et al 2000 (535)     | 24 ADC<br>10 HIV + lymphoma<br>20 CNS OI<br>22 HIV + miscellaneous neurological symptoms                                                   | 14 (18%) had increased total tau.<br>No correlation between degree of dementia or atrophy and tau levels. |
| Andersson et al 1999 (536) | 52 HIV positive <ul style="list-style-type: none"> <li>- 37 asymptomatic</li> <li>- 8 ADC</li> <li>- 7 with AIDS</li> </ul>                | Increased tau in ADC.                                                                                     |
| Ellis et al 1998 (537)     | 32 HIV positive <ul style="list-style-type: none"> <li>- 9 with HAND</li> <li>- 9 with peripheral neuropathy</li> </ul> 9 Healthy controls | No difference in tau levels.                                                                              |

P-tau= phosphorylated tau, HAD= HIV associated dementia, HSV = herpes simplex virus, ADC = AIDS Dementia Complex, CNS OI = central nervous system opportunistic infection, HAND = HIV associated neurocognitive disorder.

### 5.4 Study Aims

**Aim 1:** To investigate the role of inflammatory and immune mediated changes in HIV related cognitive impairment by enumerating and determining the activation and differentiation status of lymphocyte subpopulations and measuring levels of pro-inflammatory cytokines.

**Aim 1a:** To determine the frequency of T cells and B cells at different stages of differentiation in CSF and peripheral blood.

**Aim 1b:** To determine the frequency of innate cells (natural killer (NK) cells and gamma delta T cells) in CSF and peripheral blood.

**Aim 1c:** To determine the levels of pro-inflammatory cytokines in CSF.

**Aim 2:** To investigate the role of neurodegeneration in HAND by measuring the levels of total and phosphorylated tau and beta-amyloid<sub>(1-42)</sub> in CSF.

## **5.5 Methodology**

### **5.5.1 Study Design and Recruitment**

This was a cross-sectional observational study. Patients who had a positive screen for cognitive impairment based on the BNCS and had completed a detailed neuropsychological assessment were recruited. Recruitment commenced on 10 January 2012 and finished on 21 November 2013. Study participants were given a patient information leaflet (see Appendix 10) and patients provided written informed consent (see Appendix 16).

### **5.5.2 Inclusion and Exclusion Criteria**

Patients were included in the study if they were HIV positive, over the age of 18, attending the outpatient clinic in St. James's Hospital, had a positive screen for cognitive impairment and had undergone detailed neuropsychological assessment, capable of giving informed consent, had sufficient use of the English language to be able to participate and were willing to voluntarily participate in the study.

Patients were excluded if they were HIV negative, under the age of 18, had insufficient English to partake or were unwilling to participate voluntarily in the study. If patients had any of the following they were also excluded for standard safety reasons: an

international normalised ratio (INR) > 1.3 or other coagulopathy, platelet count < 50, infection at desired puncture site, obstructive / non-communicating hydrocephalus, an intracranial mass, elevated intracranial pressure (ICP) / papilloedema, decreased level of consciousness, partial / complete spinal block or acute spinal trauma.

### 5.5.3 Ethical Approval and Data Protection

The study had full ethical approval from the combined ethics committee of the Adelaide and Meath Hospitals Incorporating the National Children's Hospital (AMNCH) and St. James's Hospital (SJH). All patients who participated in the study were given patient information leaflets to read and were afforded the opportunity to ask any questions that they had prior to agreeing to participate in the study. The voluntary nature of participation was emphasised and that refusal to participate would not affect their ongoing medical care in any way. Written informed consent was obtained from all participants. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

### 5.5.4 Lumbar punctures

Lumbar punctures were performed in the GUIDE day ward under aseptic technique in the left lateral position after checking a full blood count and coagulation screen. Three bottles were collected for standard WCC, RCC, protein, glucose and HIV viral load (these were processed by the microbiology laboratory in St. James's Hospital and the viral load was assessed using Abbott Real Time<sup>TM</sup> HIV-1 assay in the Virus Reference Laboratory). One bottle was collected with 5mls for flow cytometry and 5 – 10mls was



collected in a polypropylene tube for analysis of amyloid and tau. Serum glucose was collected at the same time as the lumbar puncture.

#### 5.5.5 Preparation of Peripheral Blood and Cerebrospinal Fluid Mononuclear Cells

Preparation of blood and CSF samples was performed immediately after lumbar puncture. Whole blood was collected in standard heparinised tubes and then diluted in a 1:1 ratio with phosphate buffered saline (PBS). Lymphoprep<sup>TM</sup> was added to a universal container. The ratio of peripheral blood mononuclear cells (PBMC) to Lymphoprep<sup>TM</sup> was 1.25:1. The mix of blood and PBS was gently added to the universal container with Lymphoprep<sup>TM</sup> using a pipette in order to create an even interface between the Lymphoprep<sup>TM</sup> on the bottom and the diluted blood on top. This was then centrifuged at 2000 revolutions per minute (rpm) for 20 minutes without any brake (see Figure 5-1). The PBMCs were then removed and placed into a new universal container and PBS added. A sample was taken for counting prior to addition of PBS. This was centrifuged at 1200 rpm for ten minutes without any brake. The supernatant was discarded and the PBMC pellet re-suspended in a sufficient volume of PBS to yield a count of one million cells per millilitre.

CSF was centrifuged at 1800rpm for five minutes. Supernatant was then frozen at -80 degrees Celsius for subsequent analysis of cytokines. The CSF pellet was then re-suspended in PBS. Counting of both the PBMCs and CSF mononuclear cells was performed using disposable counting chambers. Twenty microliters of PBMCs and twenty microliters of ethidium bromide/acrydine orange (EBAO) were added to the

chamber. Live cells fluoresce green and dead or dying cells fluoresce orange when viewed under an ultraviolet microscope.

Mononuclear cells from CSF and blood were labelled with two panels of fluorochrome-labelled antibodies. The first panel was used to determine the phenotype of T cells using antibodies to CD3, CD4, CD8, C45RA and CD27. The second panel examined innate cell phenotypes and consisted of antibodies to CD56, CD3, CD19 and gamma delta ( $\gamma\delta$ ). Tubes were labelled for two unstained/negative controls, nine single stained compensation tubes (one tube was labelled for each fluorochrome that was used in the experiment), eighteen tubes were labelled for fluorescence minus one (FMO) and four tubes were labelled for PBMC and CSF from the study subjects. Table 5-2 shows the amount of each individual antibody that was required to be added to the appropriate tubes.

A negative/unstained control was used for both blood and CSF which just contained PBMCs and CSF respectively and no antibodies. Negative controls account for autofluorescence and are used to define negativity versus positivity for antibody staining. Each compensation tube contained the appropriate volume of one fluorochrome-labelled antibody in addition to one drop of OneComp eBeads<sup>TM</sup>. Compensation beads check that the fluorochrome-labelled antibodies are working and compensate the flow cytometer for spectral overlap. Each FMO tube contained all the fluorochrome-labelled antibodies in that panel except one. FMOs assist in determining where to set the gates. The tubes containing PBMCs and CSF mononuclear cells had all the fluorochrome-labelled antibodies added to them. Table 5-3 demonstrates the labels

given to each tube and what was added to each tube in the process of preparation for flow cytometry. The samples were all mixed using a pulse vortex. The samples were then incubated at room temperature in the dark for twenty minutes. The samples were then washed in PBS and centrifuged at 1800rpm for five minutes. The supernatants were decanted and the cell pellets re-suspended in 100µL of 4% paraformaldehyde. The samples were then incubated in the fridge for twenty minutes and subsequently centrifuged at 1800rpm for five minutes. Flow cytometry was performed on a CyAn™ ADP Analyzer (Beckman Coulter) and was either performed later that same day or the next day.

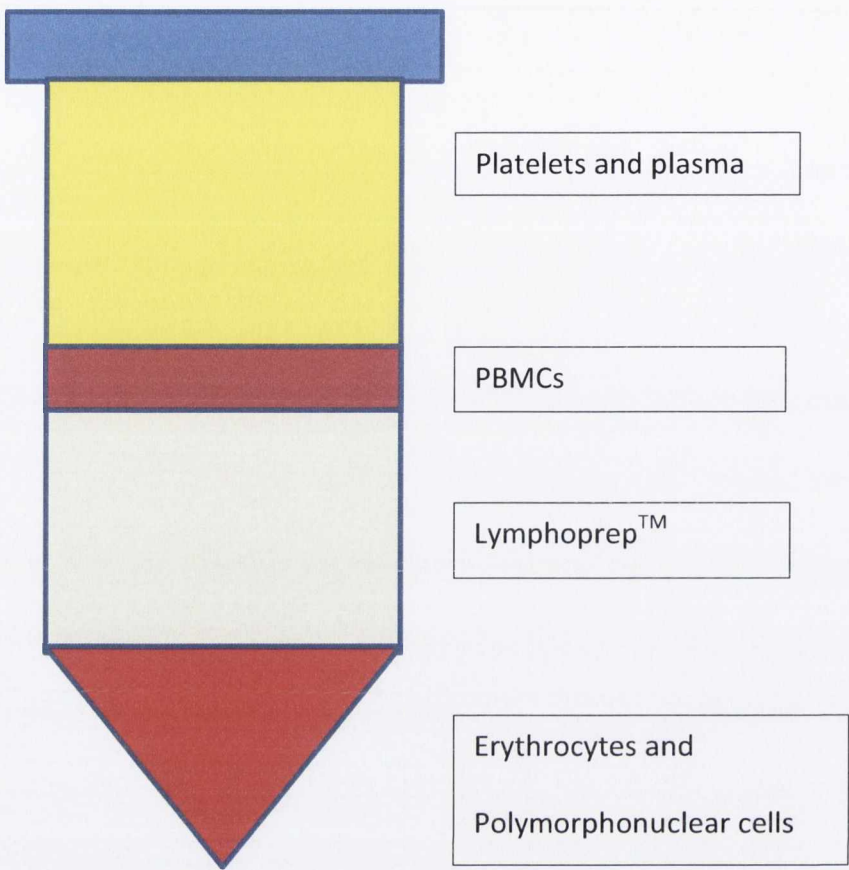
T lymphocytes were CD3<sup>+</sup>; CD4<sup>+</sup> T cells were CD3<sup>+</sup>CD4<sup>+</sup> and CD8<sup>+</sup> T cells were CD3<sup>+</sup>CD8<sup>+</sup>. Differentiation status of T lymphocytes was determined using CD45RA and CD27: CD45RA<sup>+</sup>CD27<sup>-</sup> (terminally differentiated), CD45RA<sup>-</sup>CD27<sup>+</sup> (central memory), CD45RA<sup>+</sup>CD27<sup>+</sup> (naïve) and CD45RA<sup>-</sup>CD27<sup>-</sup> (effector memory). NK cells were CD56<sup>+</sup>CD3<sup>-</sup> and NK like T cells were CD56<sup>+</sup>CD3<sup>+</sup>. Gamma delta cells were γδ<sup>+</sup>CD3<sup>+</sup>. B cells were CD19<sup>+</sup>.

Figure 5-2 demonstrates the gating strategy utilised in the experiment. The lymphocyte gate was centred on the lymphocytes and was used in subsequent analysis for compensation using FMOs and then the PBMC and CSF samples for analysis and determination of T cell and innate cell phenotypes. Figure 5-3 demonstrates examples of compensation using beads for panel 1. Figure 5-4 demonstrates examples of FMOs for panel 1. The gating strategy for differentiation of T lymphocytes is demonstrated in



Figure 5-5. Figure 5-6 demonstrates examples of compensation using beads for panel 2. Figure 5-7 demonstrates examples of FMOs for panel 2.

Figure 5-1 Density separation of PBMCs by centrifugal forces.



This figure demonstrates the principle of density separation after centrifugation of peripheral blood.

Table 5-2 Amount of fluorochrome-labelled antibody added

| Panel 1           | Amount of fluorochrome-labelled antibody added (µL) |
|-------------------|-----------------------------------------------------|
| CD3 Pacific Blue  | 1                                                   |
| CD45RA FITC       | 1                                                   |
| CD4 PE            | 1                                                   |
| CD27 APC Cy7      | 1                                                   |
| CD8 PeCy5         | 1                                                   |
| Panel 2           |                                                     |
| CD56 FITC         | 20                                                  |
| γδ PE             | 1                                                   |
| CD19 Pacific Blue | 5                                                   |
| CD3 PerCPCy 5     | 5                                                   |



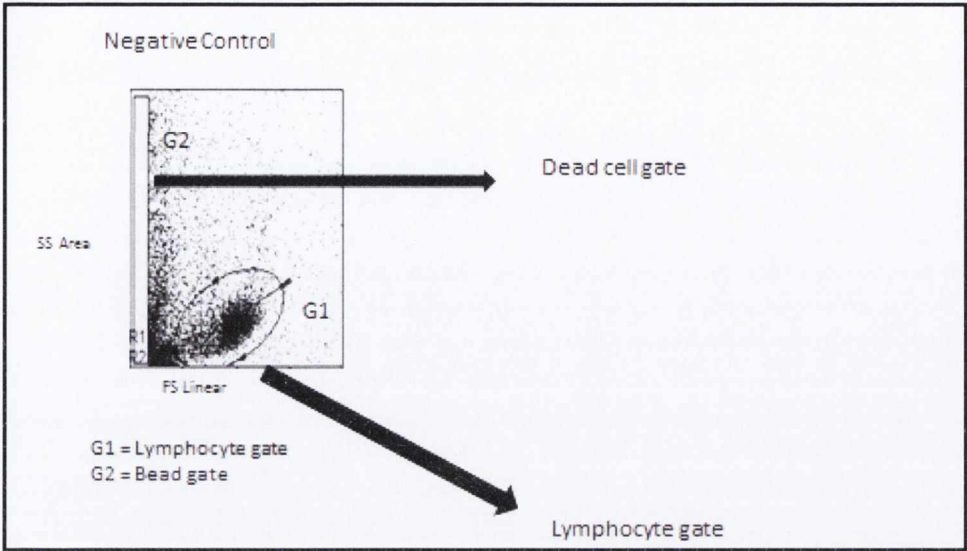
**Table 5-3 Protocol for Flow Cytometry Preparation**

|                   |                                                               |
|-------------------|---------------------------------------------------------------|
| <b>Panel 1</b>    |                                                               |
|                   | <b>PBMCs</b>                                                  |
| Negative Control  | PBMC Cells only                                               |
| Compensation 1 P1 | Beads + CD3PB                                                 |
| Compensation 2 P1 | Beads + CD45RA                                                |
| Compensation 3 P1 | Beads + CD4PE                                                 |
| Compensation 4 P1 | Beads + CD27APC Cy7                                           |
| Compensation 5 P1 | Beads + CD8 PeCy                                              |
| FMO 1 P1 PBMC     | PBMC Cells + CD45RA, CD4PE, CD27APC Cy7, CD8 PeCy5            |
| FMO 2 P1 PBMC     | PBMC Cells + CD3PB, CD4PE, CD27APC Cy7, CD8PeCy5              |
| FMO 3 P1 PBMC     | PBMC Cells + CD3PB, CD45RA, CD27APC Cy7, CD8PeCy5             |
| FMO 4 P1 PBMC     | PBMC Cells + CD3PB, CD45RA, CD4PE, CD8PeCy5                   |
| FMO 5 P1 PBMC     | PBMC Cells + CD3PB, CD4PE, CD45RA, CD27APC Cy7                |
| PBMC P1           | PBMC Cells + CD3PB, CD4PE, CD45RA, CD27APC Cy7, CD8PeCy5      |
|                   | <b>CSF</b>                                                    |
| Negative control  | CSF cells only                                                |
| FMO 1 P1 CSF      | CSF Cells + CD45RA, CD4PE, CD27APC Cy7, CD8 PeCy5             |
| FMO 2 P1 CSF      | CSF Cells + CD3PB, CD4PE, CD27APC Cy7, CD8PeCy5               |
| FMO 3 P1 CSF      | CSF Cells + CD3PB, CD45RA, CD27APC Cy7, CD8PeCy5              |
| FMO 4 P1 CSF      | CSF Cells + CD3PB, CD45RA, CD4PE, CD8PeCy5                    |
| FMO 5 P1 CSF      | CSF Cells + CD3PB, CD4PE, CD45RA, CD27APC Cy7                 |
| CSF P1            | CSF Cells + CD3PB, CD4PE, CD45RA, CD27APC Cy7, CD8PeCy5       |
| <b>Panel 2</b>    |                                                               |
|                   | <b>PBMCs</b>                                                  |
| Compensation 1 P2 | Beads + CD56FITC                                              |
| Compensation 2 P2 | Beads + $\gamma\delta$ PE                                     |
| Compensation 3 P2 | Beads + CD19PB                                                |
| Compensation 4 P2 | Beads + CD3PerCPCy5                                           |
| FMO 1 P2 PBMC     | PBMC Cells + $\gamma\delta$ PE, CD19PB, CD3PerCPCy5           |
| FMO 2 P2 PBMC     | PBMC Cells + CD56FITC, CD19PB, CD3PerCPCy5                    |
| FMO 3 P2 PBMC     | PBMC Cells + CD56FITC, $\gamma\delta$ PE, CD3PerCPCy5         |
| FMO 4 P2 PBMC     | PBMC Cells + CD56FITC, $\gamma\delta$ PE, CD19PB              |
| PBMC P2           | PBMC Cells + CD56FITC, $\gamma\delta$ PE, CD19PB, CD3PerCPCy5 |
|                   | <b>CSF</b>                                                    |
| FMO 1 P2 CSF      | CSF Cells + $\gamma\delta$ PE, CD19PB, CD3PerCPCy5            |
| FMO 2 P2 CSF      | CSF Cells + CD56FITC, CD19PB, CD3PerCPCy5                     |
| FMO 3 P2 CSF      | CSF Cells + CD56FITC, $\gamma\delta$ PE, CD3PerCPCy5          |
| FMO 4 P2 CSF      | CSF Cells + CD56FITC, $\gamma\delta$ PE, CD19PB               |
| CSF P2            | CSF Cells + CD56FITC, $\gamma\delta$ PE, CD19PB, CD3PerCPCy5  |

PBMCs=peripheral blood mononuclear cells, P1=panel 1, P2= panel 2, FMO=fluorescence minus one.

This table shows the labels of each tube on the left hand side and what was added to each tube in the process of preparation for flow cytometry on the right hand side.

Figure 5-2 Gating Strategy for Flow Cytometry



SS = side scatter, FS = forward scatter. This figure shows the gating strategy used in this experiment. The lymphocyte gate contained the lymphocytes and was used to compensate the boundaries for positive and negative cells with the subsequent FMOs (fluorescence minus one) and the phenotype of the lymphocytes with panels 1 and 2.

Figure 5-3 Compensation using beads for panel 1

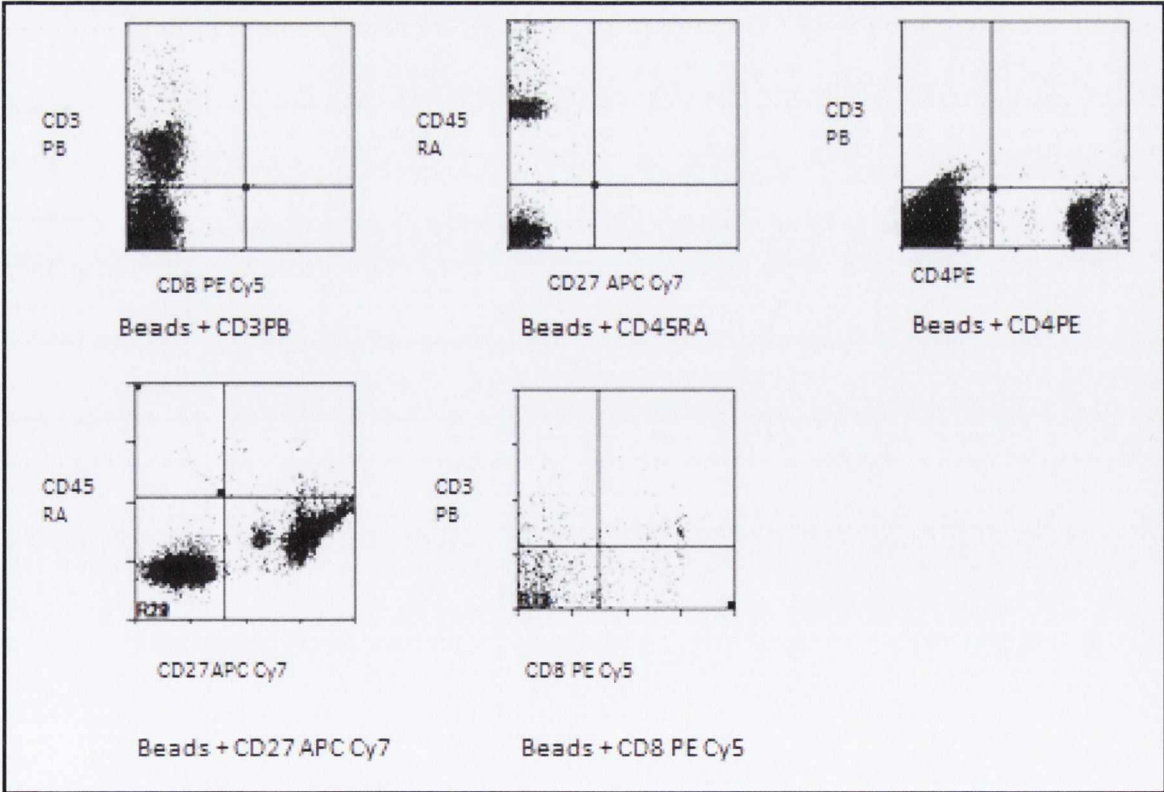


Figure 5-3 gives examples of compensation with each antibody in Panel 1.

Figure 5-4 FMOs used in Panel 1

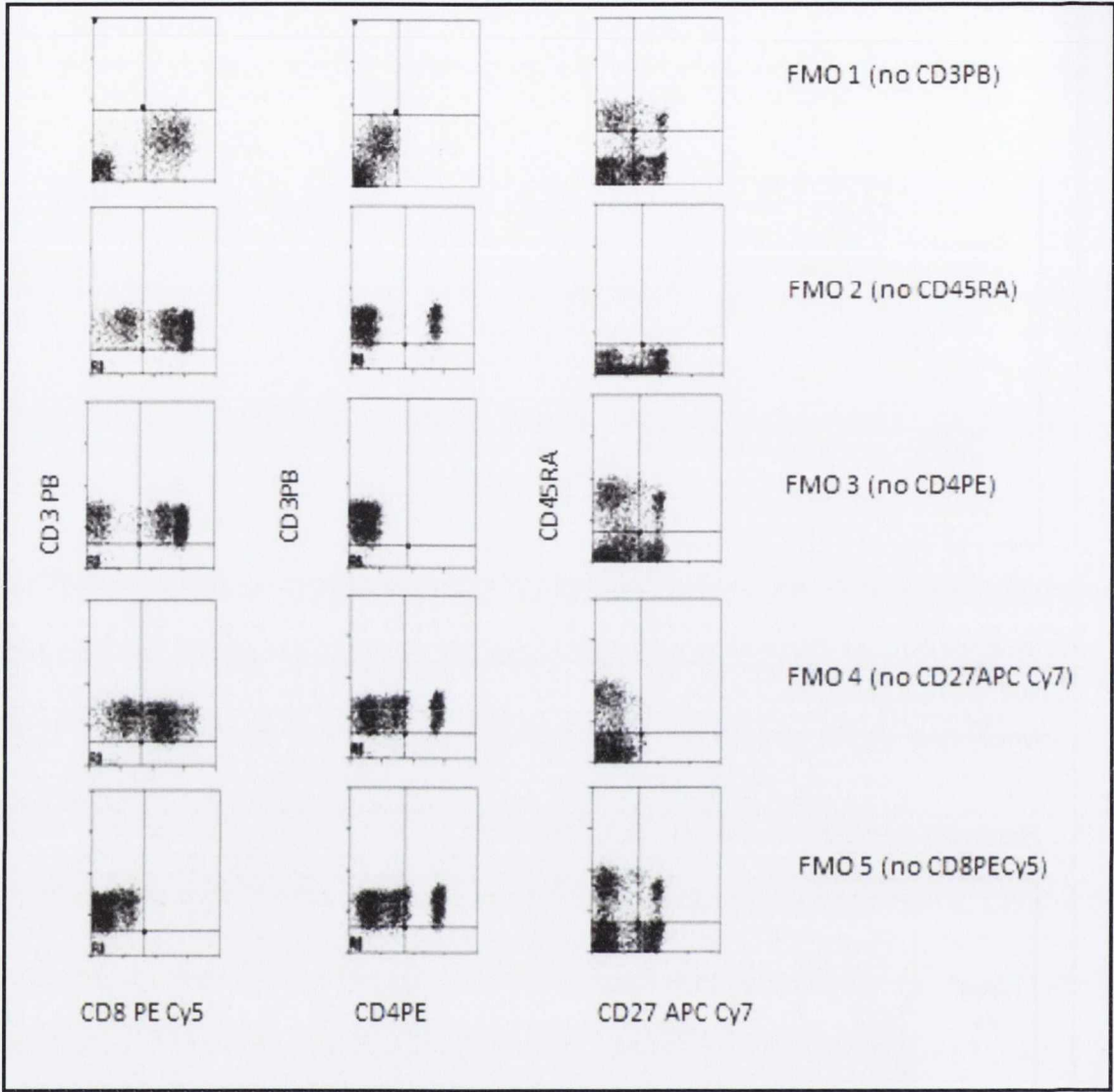


Figure 5-4 demonstrates examples of compensation using FMOs (fluorescence minus one) for panel 1. Each FMO contains all of the antibodies in the panel except one. The antibody which has been excluded from each FMO is denoted in brackets.



Figure 5-5 Differentiation of T lymphocytes gating strategy



Figure 5-5 demonstrates the differentiation of T lymphocytes gating strategy. CD3<sup>+</sup> T cells were gated onto a histogram with CD4 and CD8 to give CD3<sup>+</sup>CD4<sup>+</sup> and CD3<sup>+</sup>CD8<sup>+</sup> cells (an example is shown with percentages demonstrated). The CD3<sup>+</sup>CD4<sup>+</sup> and CD3<sup>+</sup>CD8<sup>+</sup> cells were then gated onto a histogram containing CD45RA and CD27 APC Cy7 to determine their respective differentiation status (examples shown with percentages).

Figure 5-6 Compensation using beads for panel 2

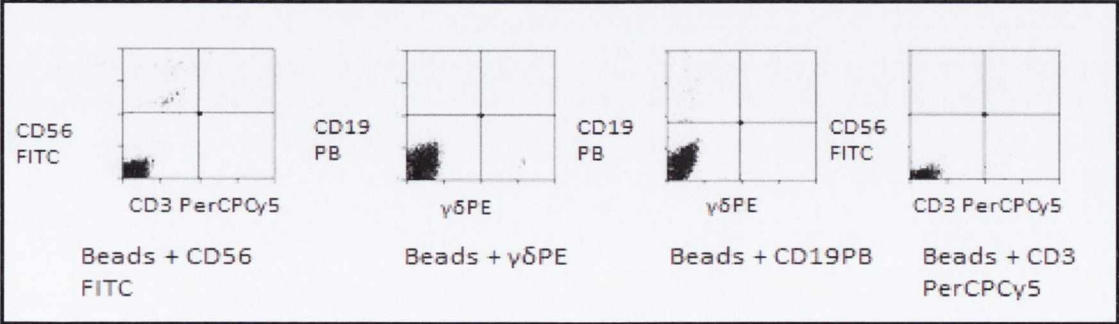


Figure 5-6 gives examples of compensation with each antibody in Panel 2.

Figure 5-7 FMOs used in Panel 2

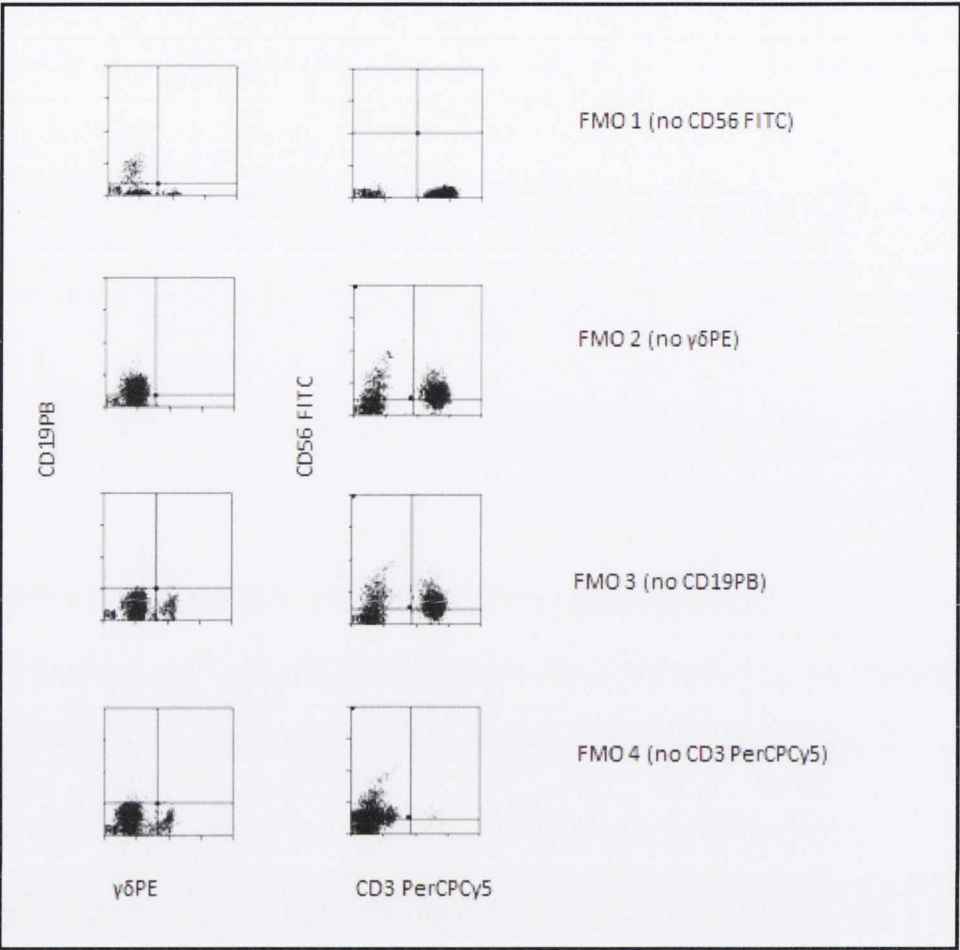


Figure 5-7 demonstrates examples of compensation using FMOs (fluorescence minus one) for panel 2.

#### 5.5.6 Data Collection and Data Recorded

Demographic data collected in the prevalence study and the neuropsychological test results were included for the respective participants who agreed to take part in the MRI study and entered into the study database. CD4<sup>+</sup> T cell count, HIV viral load and antiretroviral therapy history were updated if any changes had occurred in the intervening period as well as any changes in clinical medical conditions. CSF white cell count (WCC), red cell count (RCC), glucose, protein and CSF HIV viral load were recorded as were results of India ink staining and cryptococcal antigen testing where performed. The results of flow cytometry of PBMCs and CSF were expressed as the absolute number and percentage of CD3<sup>+</sup> T lymphocytes that express CD4<sup>+</sup>, CD8<sup>+</sup>, the absolute number and percentage of CD3<sup>+</sup>CD4<sup>+</sup> and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes that express CD45RA<sup>+</sup>, CD27<sup>+</sup>, CD45RA<sup>+</sup>CD27<sup>+</sup> and CD45RA<sup>+</sup>CD27<sup>-</sup>, the absolute number and percentage of lymphocytes that express CD56<sup>+</sup>CD3<sup>+</sup> and CD56<sup>+</sup>CD3<sup>-</sup>, the absolute number and percentage of lymphocytes that express gamma delta ( $\gamma\delta$ ) and CD3 ( $\gamma\delta$ <sup>+</sup>CD3<sup>+</sup>) and the absolute number and percentage of lymphocytes that express CD19<sup>+</sup>. Beta-amyloid<sub>(1-42)</sub>, total tau and phosphorylated tau levels in CSF were recorded. Cytokine levels of TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-10 and IL-17A in CSF were also recorded.

#### 5.5.7 Missing Data

Technical difficulties were experienced during flow cytometry analysis of two patients and unfortunately the data could not be used for analysis as the machine failed to compensate accurately hence most histograms showed up double positive cells due to



lack of adequate compensation. Data is missing for one patient's CSF on panel one as the computer crashed immediately after acquisition of CSF sample as the file was being saved.

#### 5.5.8 Analysis of $\beta$ -amyloid<sub>(1-42)</sub>, total tau and phosphorylated tau

Beta-amyloid<sub>(1-42)</sub> was measured using INNOTEST (R)  $\beta$ -AMYLOID<sub>(1-42)</sub> assay. It is a solid-phase enzyme immunoassay in which the amyloid peptide is captured by a first monoclonal antibody, 21F12. CSF samples were added and incubated with a biotinylated antibody, 3D6bio. This antigen-antibody complex was then detected by a peroxidase-labelled streptavidin. After addition of substrate working solution, samples will develop a colour. The colour intensity is a measure for the amount of human  $\beta$ -amyloid<sub>(1-42)</sub> protein in the sample.

Total tau was measured using the INNOTEST (R) hTAU Ag assay. It is a solid-phase enzyme immunoassay in which the human tau protein or fragments are captured by a first monoclonal antibody, AT120. CSF samples were added and incubated with two biotinylated antibodies, HT7bio and BT2bio. This antigen-antibody complex was then detected by a peroxidase-labelled streptavidin. After addition of substrate working solution, samples will develop a colour. The colour intensity is a measure for the amount of human total tau protein in the sample.

Phosphorylated tau was measured using the INNOTEST (R) PHOSPHO-TAU<sub>(181P)</sub> assay. It is a solid-phase enzyme immunoassay in which the phosphorylated tau protein or fragments are captured by a first monoclonal antibody, HT7. CSF samples are added

and incubated with a biotinylated antibody, AT270bio. This antigen-antibody complex is then detected by a peroxidase-labelled streptavidin. After addition of substrate working solution, positive samples will develop a colour. The colour intensity is a measure for the amount of human phosphorylated tau protein in the sample.

These assays were performed by the Department of Immunology in St. James's Hospital and they followed the manufacturer's instructions for the respective kits. The kits were designed for use on human CSF. The normal concentration of total tau cut off was <320pg/ml. The normal concentration for amyloid beta was >220pg/ml and <61 pg/mL was considered in the normal range for phosphorylated tau.

#### 5.5.9 Analysis of cytokines

CSF was analysed for the presence of TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-10 and IL-17A using two arrays from Meso Scale Discovery (MSD): Proinflammatory Panel 1 (human) Kit V-PLEX<sup>TM</sup> and V-PLEX Plus and Human IL-17A Kit V-PLEX<sup>TM</sup> and V-PLEX Plus. The assays are sandwich immunoassays. The plates are pre-coated with capture antibodies on independent and well defined spots. The plate was washed with 150 $\mu$ L/well of wash buffer. CSF samples were thawed on ice prior to analysis. Samples were run at three dilutions (1:2, 1:4 and 1:8). Dilutions were prepared in eppendorf tubes in the appropriate diluents and samples were then transferred directly to the MSD plate. All samples were measured in duplicate. To each well of the pre-coated MSD plate 50 $\mu$ l of diluted sample or calibrator was added. The plate was sealed and incubated at room temperature with shaking for two hours. The plates were again washed with 200 $\mu$ L/well of wash buffer. To each well 25 $\mu$ L of a solution containing detection

antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) was added. The plates were then incubated at room temperature for two hours whilst shaking. Analytes in the sample bind to capture antibodies immobilised on the working electrode surface: recruitment of the detection antibodies by the bound analytes completes the sandwich. The plates were again washed with 150µL/well of wash buffer. An MSD buffer was added (150µL) to each well which created the appropriate chemical environment for electrochemiluminescence. The plates were read on the MESO™ QuickPlex™ SQ120 Imager. A voltage was applied to the plates causing the captured labels to emit light. The instrument measures the intensity of the emitted light in order to provide a quantitative measure of analytes in the sample. V-PLEX assay kits have been validated (538). All data was analysed using MSD Discovery Workbench 4.0 data analysis software. The standard curves were fitted using a 4 parameter logistic fit with  $1/y^2$  weighting.

Figure 5-8 Layout of the Samples on the MSD Plate

|   | 1                 | 2                 | 3                 | 4                 | 5                 | 6                 | 7                 | 8                 | 9                  | 10                 | 11                 | 12                 |
|---|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|--------------------|
| A | S001<br>Standards | S001<br>Standards | U001<br>Patient 1 | U001<br>Patient 1 | U009<br>Patient 3 | U009<br>Patient 3 | U017<br>Patient 6 | U017<br>Patient 6 | U025<br>Patient 9  | U025<br>Patient 9  | U033<br>Patient 11 | U033<br>Patient 11 |
| B | S002<br>Standards | S002<br>Standards | U002<br>Patient 1 | U002<br>Patient 1 | U010<br>Patient 4 | U010<br>Patient 4 | U018<br>Patient 6 | U018<br>Patient 6 | U026<br>Patient 9  | U026<br>Patient 9  | U034<br>Patient 12 | U034<br>Patient 12 |
| C | S003<br>Standards | S003<br>Standards | U003<br>Patient 1 | U003<br>Patient 1 | U011<br>Patient 4 | U011<br>Patient 4 | U019<br>Patient 7 | U019<br>Patient 7 | U027<br>Patient 9  | U027<br>Patient 9  | U035<br>Patient 12 | U035<br>Patient 12 |
| D | S004<br>Standards | S004<br>Standards | U004<br>Patient 2 | U004<br>Patient 2 | U012<br>Patient 4 | U012<br>Patient 4 | U020<br>Patient 7 | U020<br>Patient 7 | U028<br>Patient 10 | U028<br>Patient 10 | U036<br>Patient 12 | U036<br>Patient 12 |
| E | S005<br>Standards | S005<br>Standards | U005<br>Patient 2 | U005<br>Patient 2 | U013<br>Patient 5 | U013<br>Patient 5 | U021<br>Patient 7 | U021<br>Patient 7 | U029<br>Patient 10 | U029<br>Patient 10 | U037<br>Patient 13 | U037<br>Patient 13 |
| F | S006<br>Standards | S006<br>Standards | U006<br>Patient 2 | U006<br>Patient 2 | U014<br>Patient 5 | U014<br>Patient 5 | U022<br>Patient 8 | U022<br>Patient 8 | U030<br>Patient 10 | U030<br>Patient 10 | U038<br>Patient 13 | U038<br>Patient 13 |
| G | S007<br>Standards | S007<br>Standards | U007<br>Patient 3 | U007<br>Patient 3 | U015<br>Patient 5 | U015<br>Patient 5 | U023<br>Patient 8 | U023<br>Patient 8 | U031<br>Patient 11 | U031<br>Patient 11 | U039<br>Patient 13 | U039<br>Patient 13 |
| H | S008<br>Standards | S008<br>Standards | U008<br>Patient 3 | U008<br>Patient 3 | U016<br>Patient 6 | U016<br>Patient 6 | U024<br>Patient 8 | U024<br>Patient 8 | U032<br>Patient 11 | U032<br>Patient 11 |                    |                    |

Figure 5-8 demonstrates the layout of the individual patients’ samples on the MSD plate utilised in the experiment.



Figure 5-9 Standard curves for all analytes

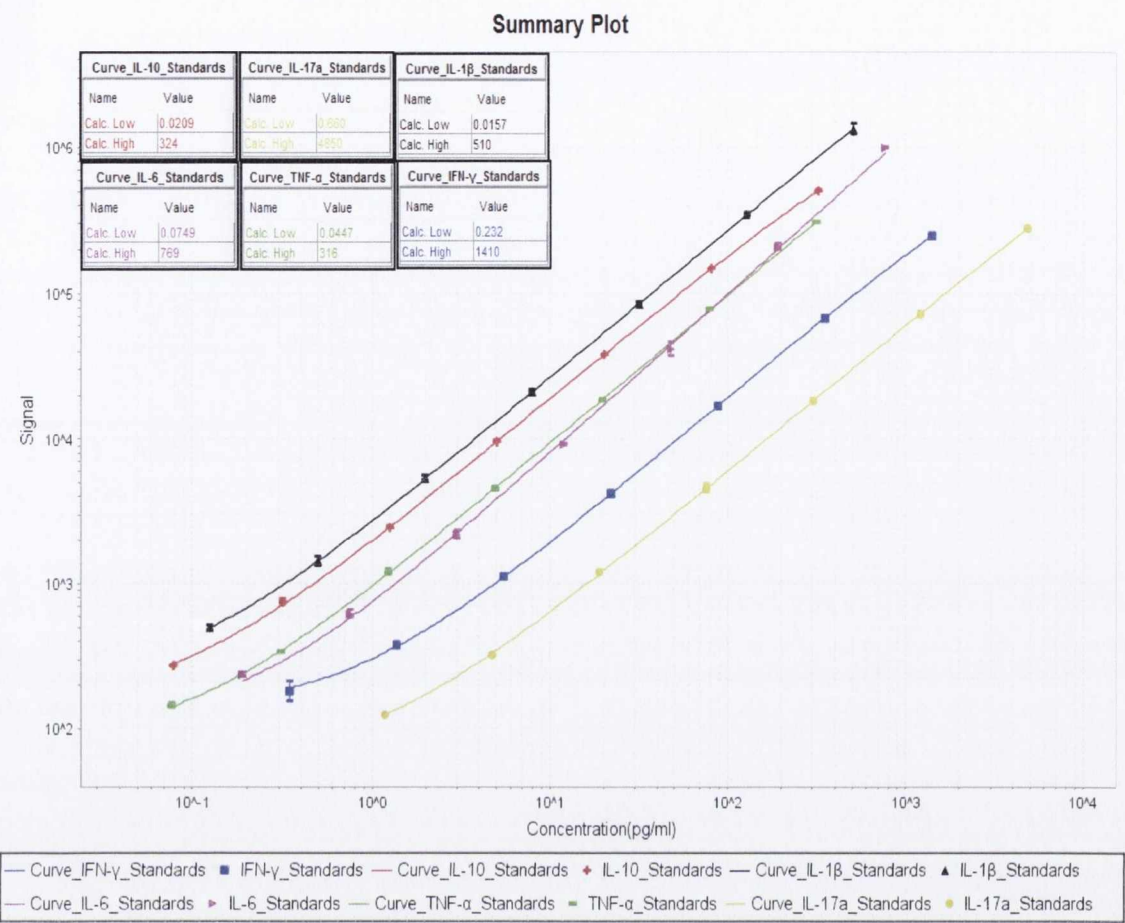


Figure 5-9 demonstrates the standard curves for all the analytes utilised in the experiment. IFN-γ = interferon gamma, IL-10 = interleukin 10, IL-1β=interleukin 1 beta, IL-6=interleukin 6, TNF-α=tumour necrosis factor alpha, IL-17a=interleukin 17a.

**Table 5-4 Limits of Detection (quoted by MSD and those achieved in the study)**

|               | MSD Quoted |                | Plate 1        |
|---------------|------------|----------------|----------------|
|               | Median     | Range<br>pg/ml | LLOD<br>Median |
| IFN- $\gamma$ | 0.2        | 0.05 - 0.62    | 0.32           |
| IL-1 $\beta$  | 0.04       | 0.02 - 0.27    | 0.02           |
| IL-6          | 0.06       | 0.01 - 0.11    | 0.08           |
| IL-10         | 0.03       | 0.01 - 0.15    | 0.02           |
| TNF- $\alpha$ | 0.04       | 0.01 - 0.13    | 0.04           |
| IL-17a        | 0.74       | 0.45 - 2.84    | 0.25           |

LLOD = Limits of detection

5.5.10 Role of the PhD Candidate

The candidate recruited all participants to the study and obtained informed consent from all study participants. The candidate performed all of the lumbar punctures. Blood and CSF preparation, flow cytometry acquisition and analysis and cytokine analysis were all performed by the candidate. Amyloid and tau analysis was performed by the Immunology Department at St James’s Hospital.

5.6 Results

5.6.1 Patient Characteristics

Thirteen patients were recruited and underwent lumbar puncture; eleven men and two women; nine of whom met criteria for ANI and three of whom met criteria for MND. One patient had neuropsychological abnormalities that did not meet diagnostic criteria for HAND. The majority were Irish (9/13, 69.2%). They were all on HAART and were all virally suppressed. They had a mean age of 46.9 years and were diagnosed with HIV for a mean length of 11.3 years.

All of the patients had less than 200 copies/ml of HIV RNA in their CSF. Cryptococcal antigen testing was negative in all of the patients as was TB staining and culture. CSF white cell count varied between less than one and three. CSF protein varied between 21 and 70 (mean 38.7 and median 38).



Table 5-5 Characteristics of the Study Group

| <u>Characteristics</u>            |               | <u>(n = 13)</u> |            |
|-----------------------------------|---------------|-----------------|------------|
|                                   |               | <u>n</u>        | <u>(%)</u> |
| Gender                            | Male          | 11              | 84.6       |
|                                   | Female        | 2               | 15.4       |
| Country of Birth                  | Ireland       | 9               | 69.2       |
|                                   | Europe        | 1               | 7.7        |
|                                   | Africa        | 2               | 15.4       |
|                                   | South America | 1               | 7.7        |
| English as a first Language       | Yes           | 10              | 76.9       |
| Mode of Transmission              | Heterosexual  | 6               | 46.1       |
|                                   | MSM           | 5               | 38.5       |
|                                   | IVDU          | 2               | 15.4       |
| Virally Suppressed (n=13)         | Yes           | 13              | 100        |
| Hx of Hepatitis B                 | Yes           | 1               | 7.7        |
| Hx of Hepatitis C                 | Yes           | 3               | 23.1       |
| Hx of Hypertension                | Yes           | 3               | 23.1       |
| Hx of Hypercholesterolaemia       | Yes           | 1               | 7.7        |
| Hx of Syphilis                    | Yes           | 1               | 7.7        |
| Hx of Toxoplasmosis               | Yes           | 1               | 7.7        |
| Hx of Epilepsy                    | Yes           | 2               | 15.4       |
| Hx of Neuropathy                  | Yes           | 1               | 7.7        |
| Hx of Depression                  | Yes           | 8               | 61.5       |
| Methadone replacement therapy     | Yes           | 1               | 7.7        |
| Use of Benzodiazepines            | Yes           | 3               | 23.1       |
| Employment History                | Employed      | 6               | 46.2       |
|                                   | Unemployed    | 7               | 53.8       |
| Smoking History                   | Smoker        | 4               | 30.8       |
|                                   | Non smoker    | 4               | 30.8       |
|                                   | Ex-smoker     | 5               | 38.4       |
| Alcohol History<br>Units per week | None          | 3               | 23         |
|                                   | 1-10 units    | 6               | 46.2       |

|                                       |                    |                    |      |
|---------------------------------------|--------------------|--------------------|------|
|                                       | 11-20 units        | 2                  | 15.4 |
|                                       | 21-30 units        | 1                  | 7.7  |
|                                       | Hx of ADS          | 1                  | 7.7  |
| <b>Illicit Substance Use</b>          | Never              | 9                  | 69.2 |
|                                       | Current Non IV Use | 1                  | 7.7  |
|                                       | Previous Hx        | 3                  | 23.1 |
| <b>Antiretroviral Therapy</b>         | On HAART           | 13                 | 100  |
| <b>Positive Screen for Anxiety</b>    | Yes                | 5                  | 38.5 |
| <b>Positive Screen for Depression</b> | Yes                | 1                  | 7.7  |
| <b>HAND</b>                           | Non diagnostic     | 1                  | 7.7  |
|                                       | ANI                | 9                  | 69.2 |
|                                       | MND                | 3                  | 23.1 |
|                                       | HAD                | 0                  | 0    |
| <b>Age (in years)</b>                 | Mean (sd)          | 46.9 (10.74)       |      |
|                                       | Median (range)     | 45 (30 - 67)       |      |
| <b>Time since Diagnosis (years)</b>   | Mean (sd)          | 11.3 (7.5)         |      |
|                                       | Median (range)     | 11.3 (1.33 - 26.5) |      |
| <b>Nadir CD4 Count</b>                | Mean (sd)          | 173.5 (108.6)      |      |
|                                       | Median (range)     | 138 (46 - 388)     |      |
| <b>Current CD4 Count</b>              | Mean (sd)          | 523.4 (212.5)      |      |
|                                       | Median (range)     | 434 (189 - 1026)   |      |
| <b>Years of Education</b>             | Mean (sd)          | 13.54 (3.7)        |      |
|                                       | Median (range)     | 14 (8 - 19)        |      |

Table 5-6 Description of Each Patient

| Patient Number | Age | Gender | Length of Dx | Nadir CD4 Count | Current CD4 Count | Viral Load | Classification |
|----------------|-----|--------|--------------|-----------------|-------------------|------------|----------------|
| 1              | 67  | M      | 5.3 years    | 90              | 643               | ND         | ANI            |
| 2              | 45  | M      | 10.5 years   | 106             | 386               | ND         | MND            |
| 3              | 45  | M      | 12.2 years   | 194             | 423               | ND         | Non diagnostic |
| 4              | 35  | M      | 2.6 years    | 388             | 541               | ND         | MND            |
| 5              | 48  | M      | 17.5 years   | Not known       | 632               | ND         | ANI            |
| 6              | 39  | M      | 11.7 years   | 138             | 189               | ND         | MND            |
| 7              | 30  | M      | 5 years      | 340             | 364               | ND         | ANI            |
| 8              | 43  | M      | 22.1 years   | Not known       | 1026              | < 50       | ANI            |
| 9              | 49  | F      | 26.4 years   | 240             | 611               | ND         | ANI            |
| 10             | 64  | M      | 11.7 years   | 108             | 434               | < 40       | ANI            |
| 11             | 38  | M      | 7 years      | 97              | 750               | ND         | ANI            |
| 12             | 55  | F      | 15.2 years   | 162             | 386               | ND         | ANI            |
| 13             | 52  | M      | 3.5 years    | 46              | 419               | ND         | ANI            |

M=male, F=female, Dx=diagnosis, ND=not detectable (Viral load is in copies per ml where an amount is given), ANI=asymptomatic neurocognitive impairment, MND=mild neurocognitive disorder, non-diagnostic=neuropsychological abnormalities that did not meet criteria for HAND.



### 5.6.2 Results of Flow Cytometry

Thirteen patients had flow cytometry performed on peripheral blood mononuclear cells (PBMCs) and cerebrospinal fluid (CSF) but data from two patients had to be excluded due to technical difficulties resulting in difficulty adequately compensating the data. Data from the remaining eleven patients is presented. Examples of results from flow cytometry of PBMCs and CSF are shown in Figure 5-10, Figure 5-11, Figure 5-12 and Figure 5-13. The breakdown of T lymphocytes in PBMCs in four patients showed a greater percentage of CD4<sup>+</sup> T cells than CD8<sup>+</sup> T cells, in six patients there was a greater percentage of CD8<sup>+</sup> T cells and in one patient there was an equal distribution of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. The breakdown of T lymphocytes in CSF showed a greater proportion of CD8<sup>+</sup> T cells in eight patients. In PBMCs the differentiation status of CD3/CD4 T cells showed that the greatest proportion was central memory cells followed by naïve, effector memory and terminally differentiated. The differentiation status of CD3/CD8 T cells in PBMCs showed that the largest group was naïve cells, followed by effector memory, central memory and terminally differentiated cells. The differentiation status of CD3/CD4 and CD3/CD8 T cells in CSF showed that the greatest proportion of cells was central memory, then effector memory, terminally differentiated and naïve cells.

Figure 5-10 Example of T cell differentiation status (PBMC)

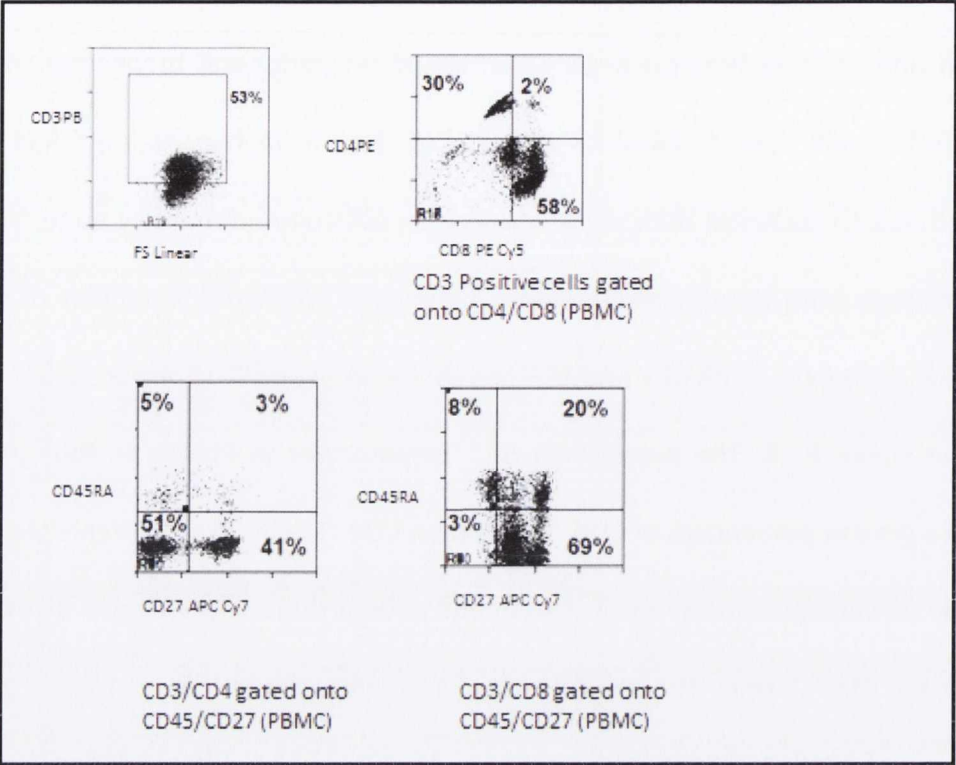


Figure 5-11 Example of T cell differentiation status (CSF)

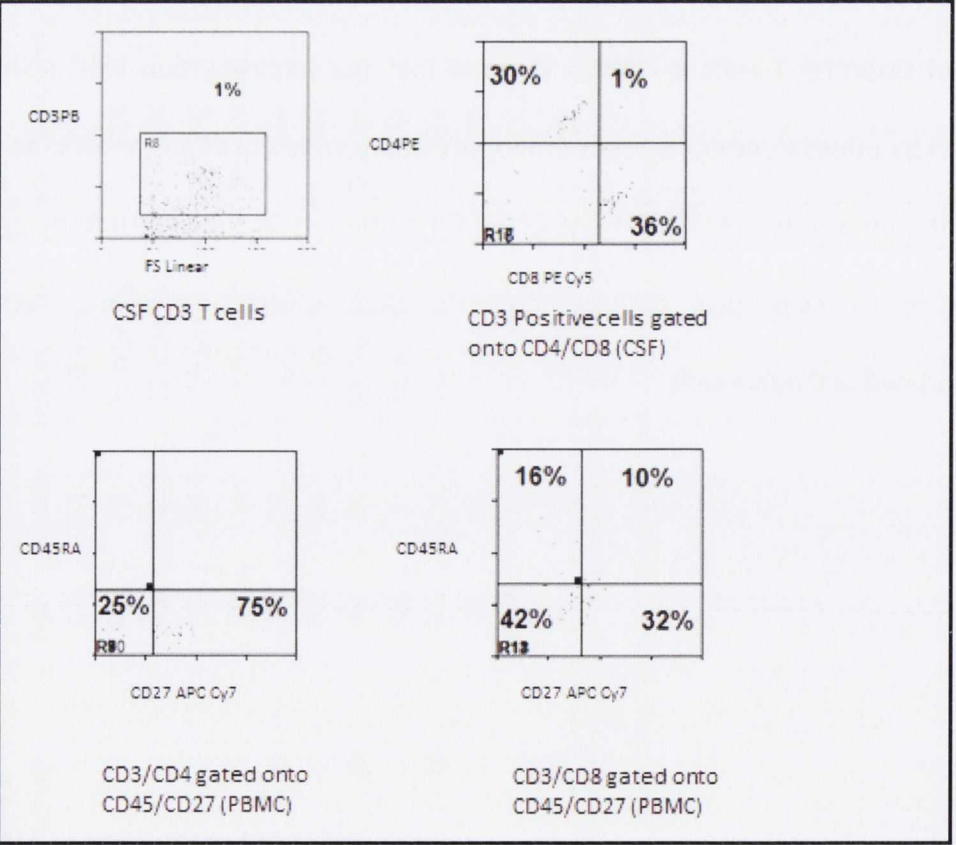


Figure 5-12 Example of results of PBMCs stained with antibodies from panel 2

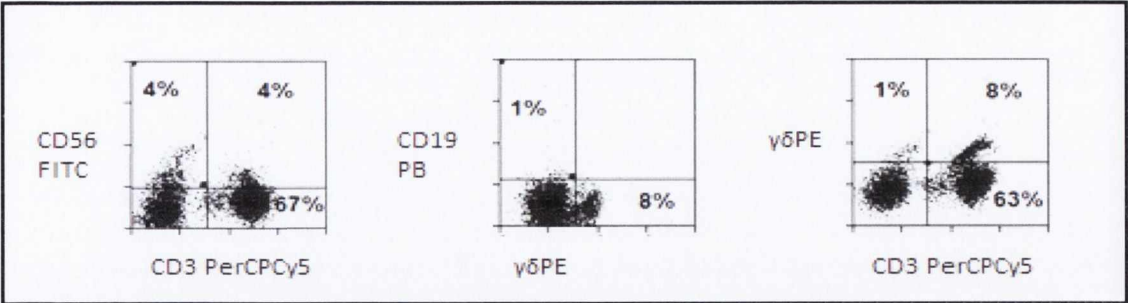


Figure 5-13 Example of results of CSF stained with antibodies from panel 2

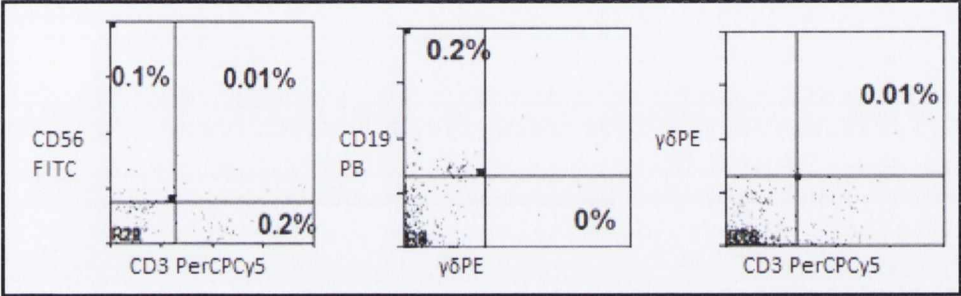




Figure 5-14 Percentage of T Lymphocytes in Peripheral Blood Mononuclear Cells

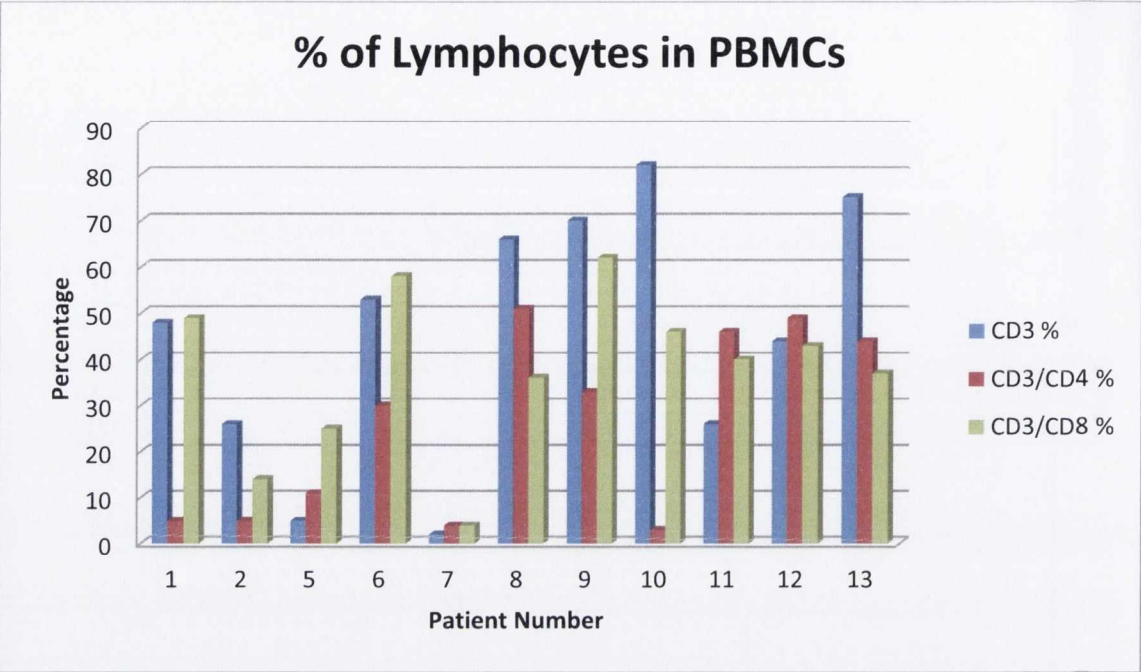


Figure 5-15 Number of T Lymphocytes per millilitre in Peripheral Blood Mononuclear Cells

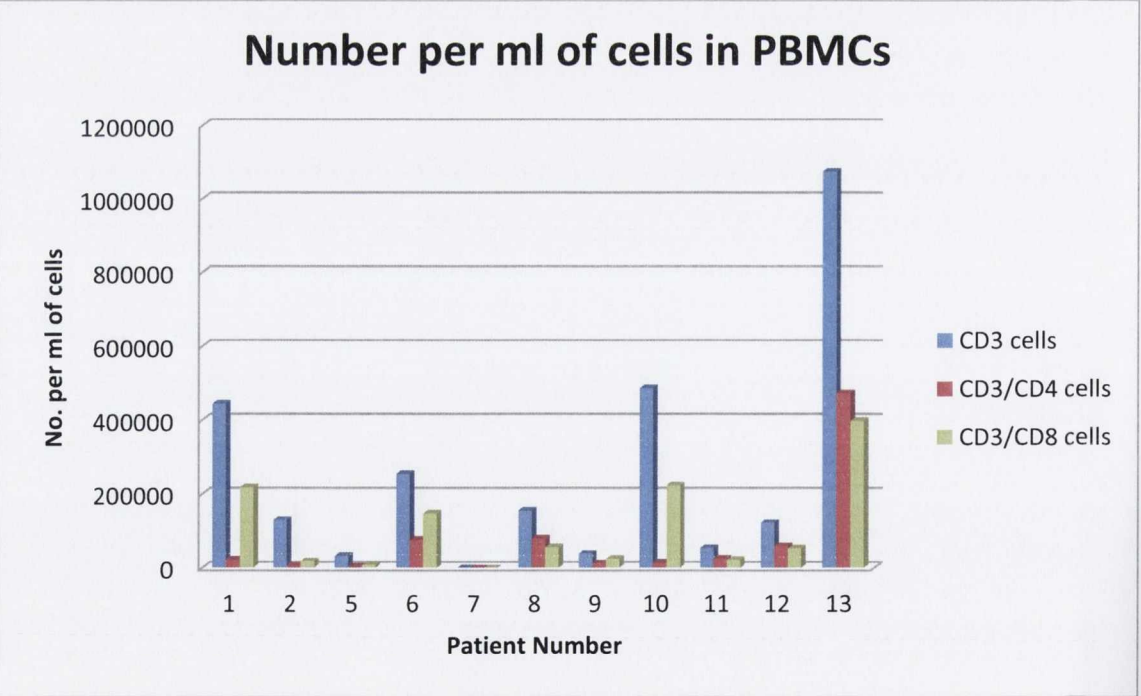


Figure 5-16 Percentage of T Lymphocytes in Cerebrospinal Fluid

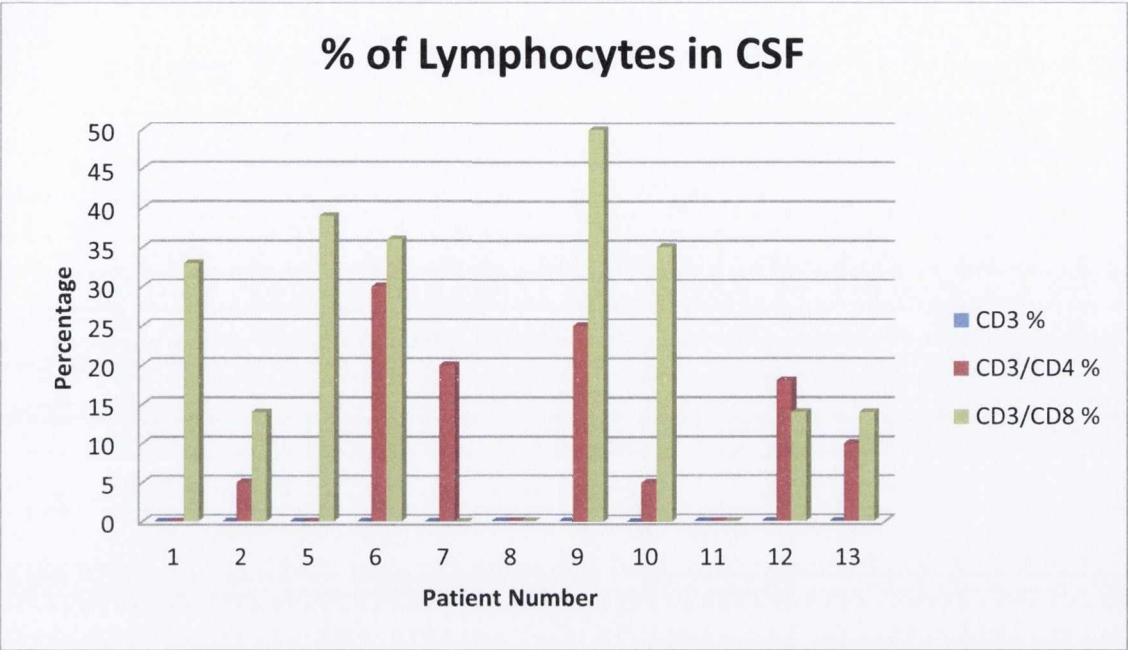


Figure 5-17 Number of T Lymphocytes per millilitre in CSF

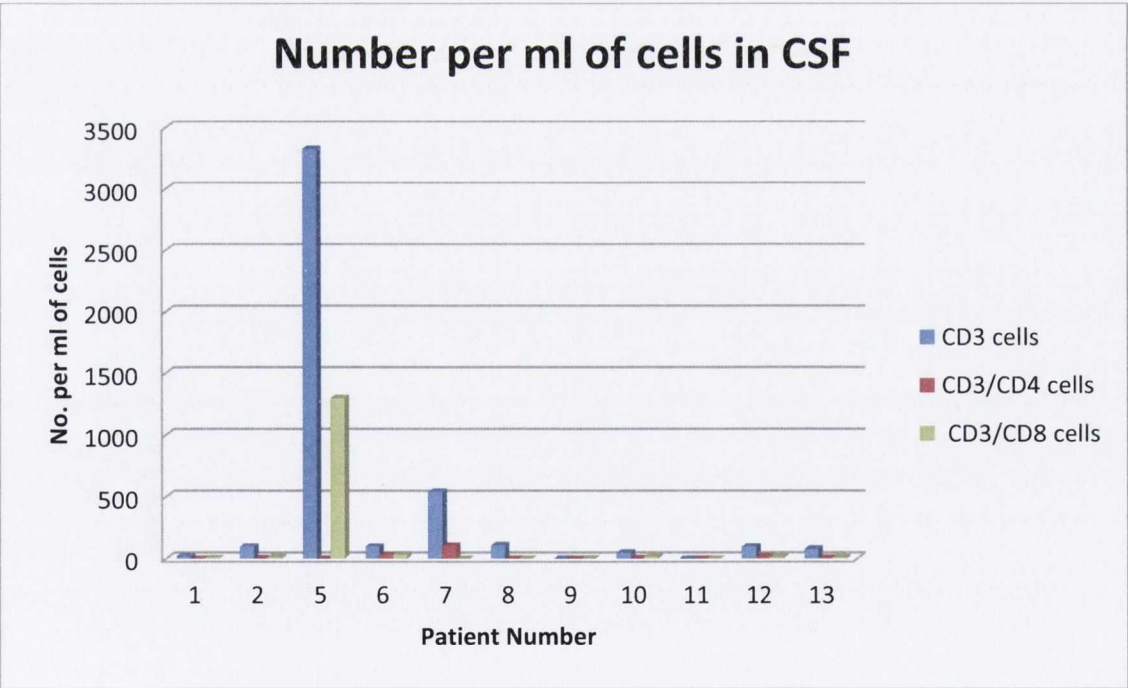


Figure 5-18 Number of T Lymphocytes per millilitre in CSF excluding Patient 5

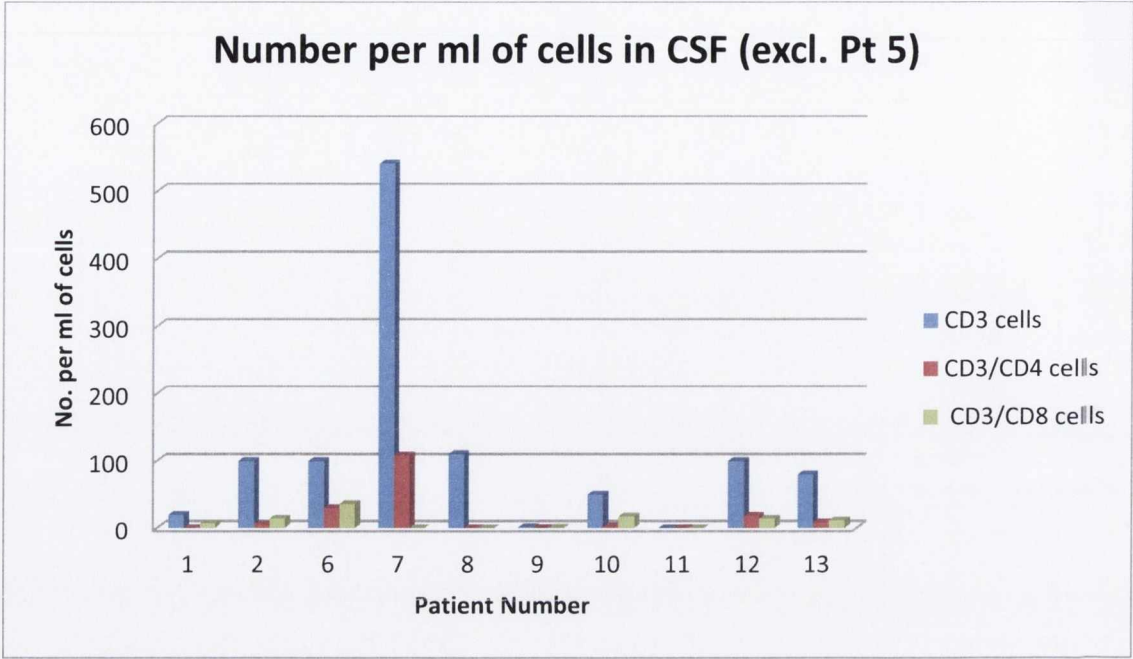


Figure 5-19 Differentiation of CD3/CD4 positive T cells in Peripheral Blood Mononuclear Cells (percentages)

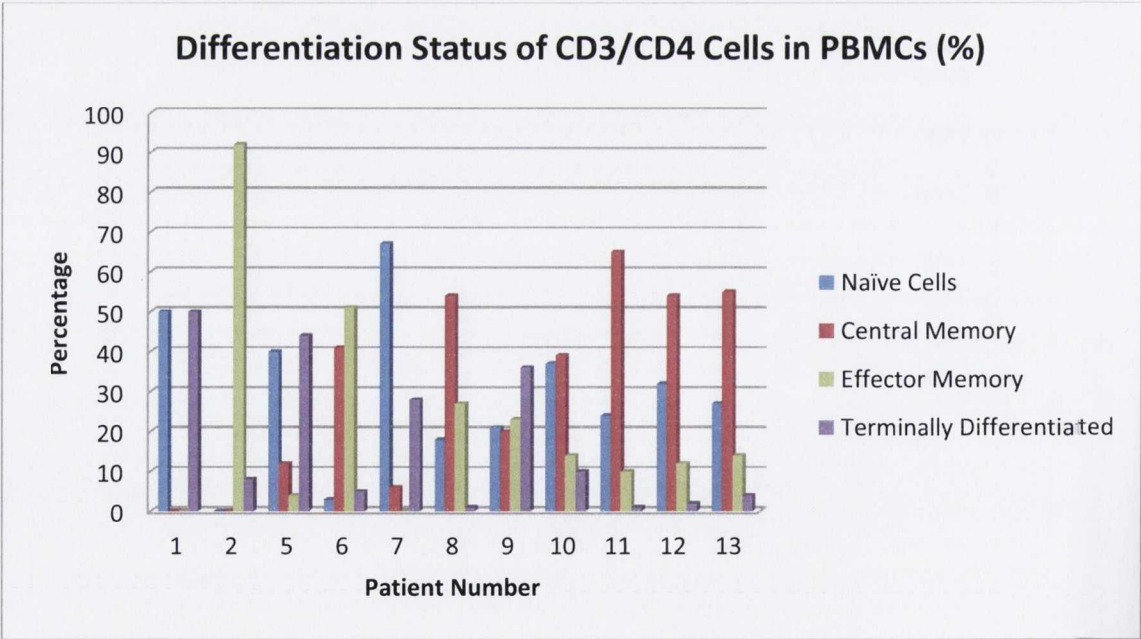




Figure 5-20 Differentiation of CD3/CD4 positive T cells in Peripheral Blood Mononuclear Cells (cells per millilitre)

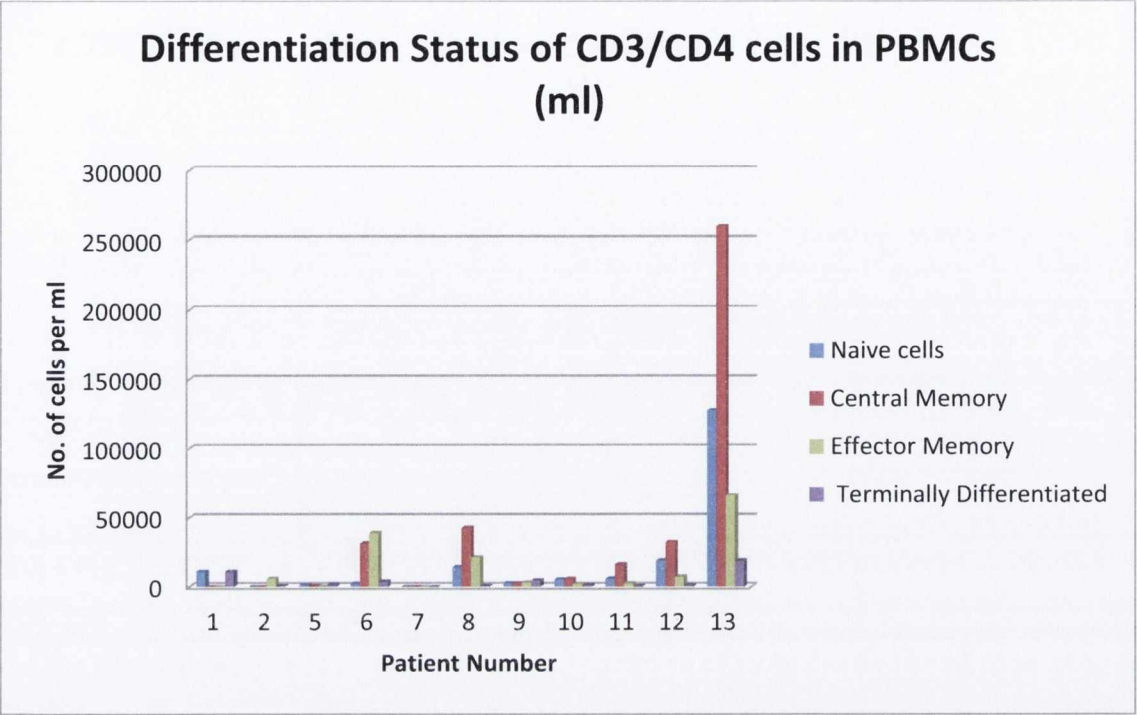


Figure 5-21 Differentiation of CD3/CD8 positive T cells in Peripheral Blood Mononuclear Cells (percentages)

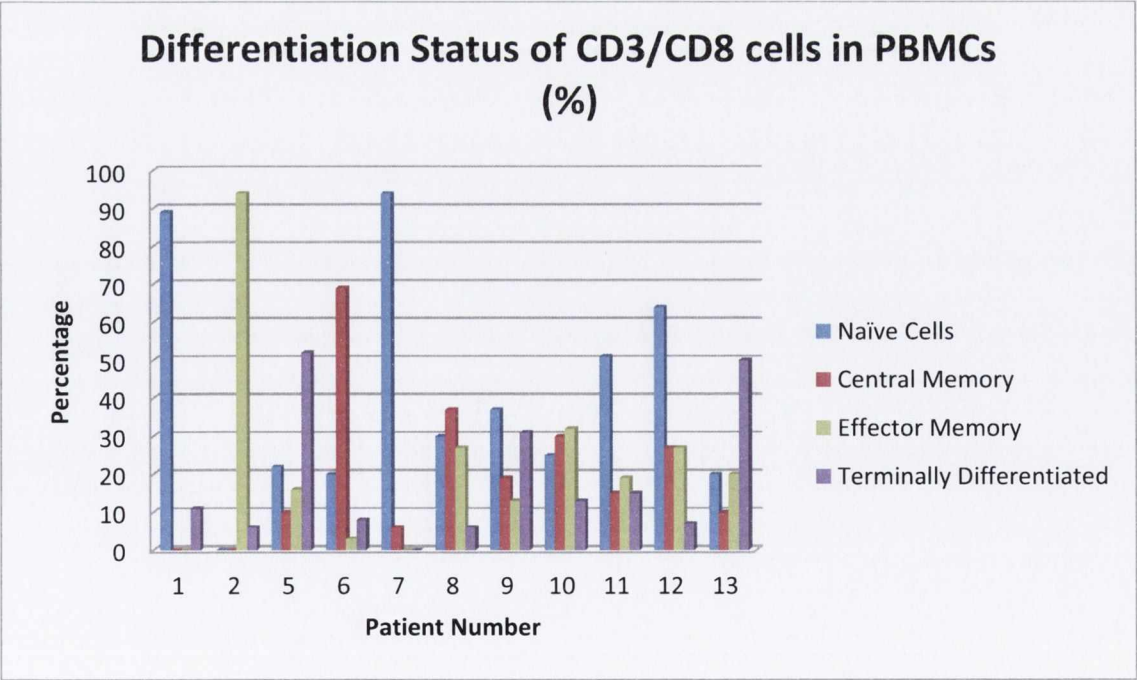


Figure 5-22 Differentiation of CD3/CD8 positive T cells in Peripheral Blood Mononuclear Cells (cells per millilitre)

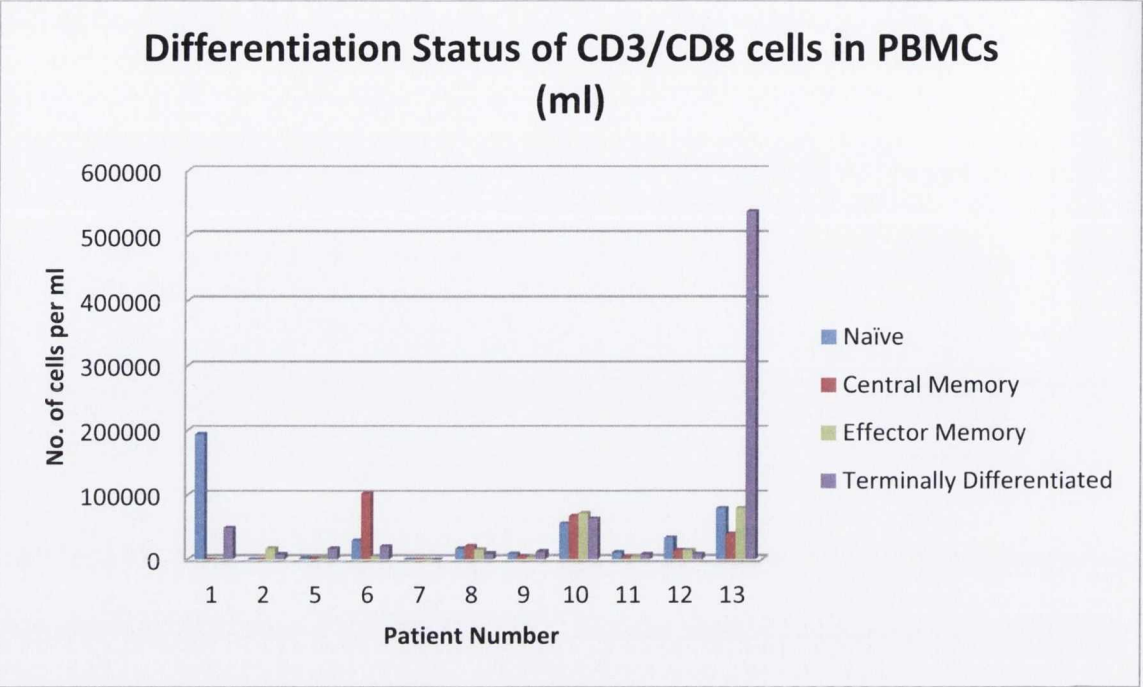


Figure 5-23 Differentiation of CD3/CD4 positive T cells in Cerebrospinal Fluid (percentages)

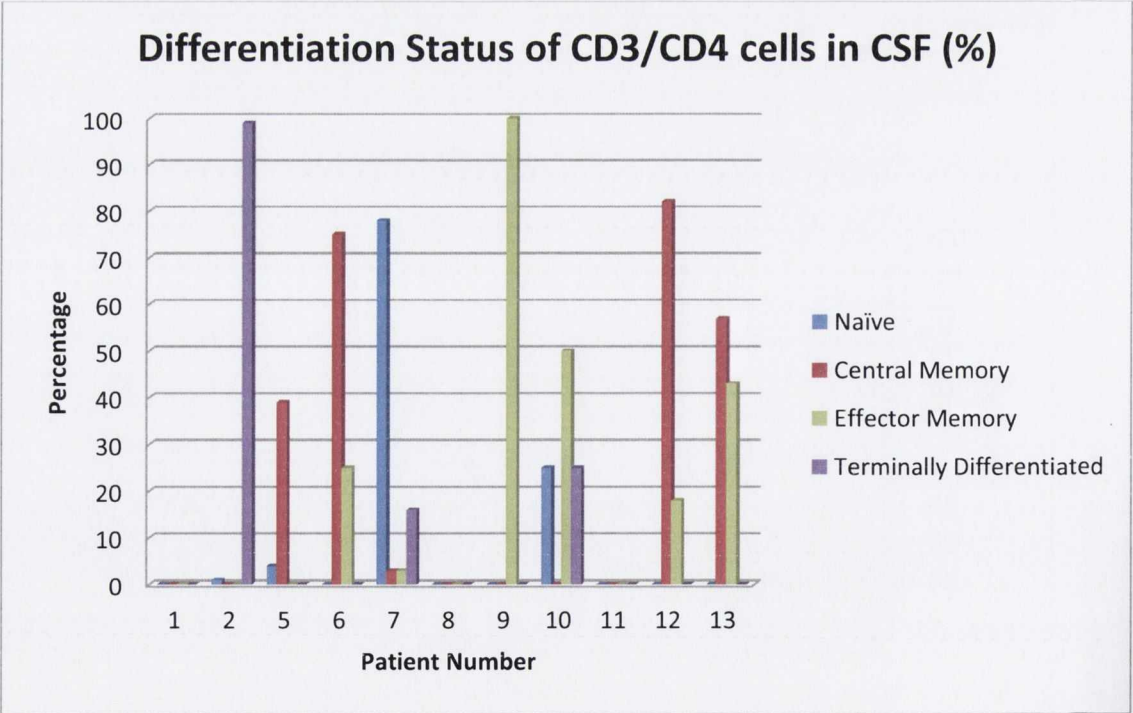


Figure 5-24 Differentiation of CD3/CD4 positive T cells in Cerebrospinal Fluid (cells per millilitre)

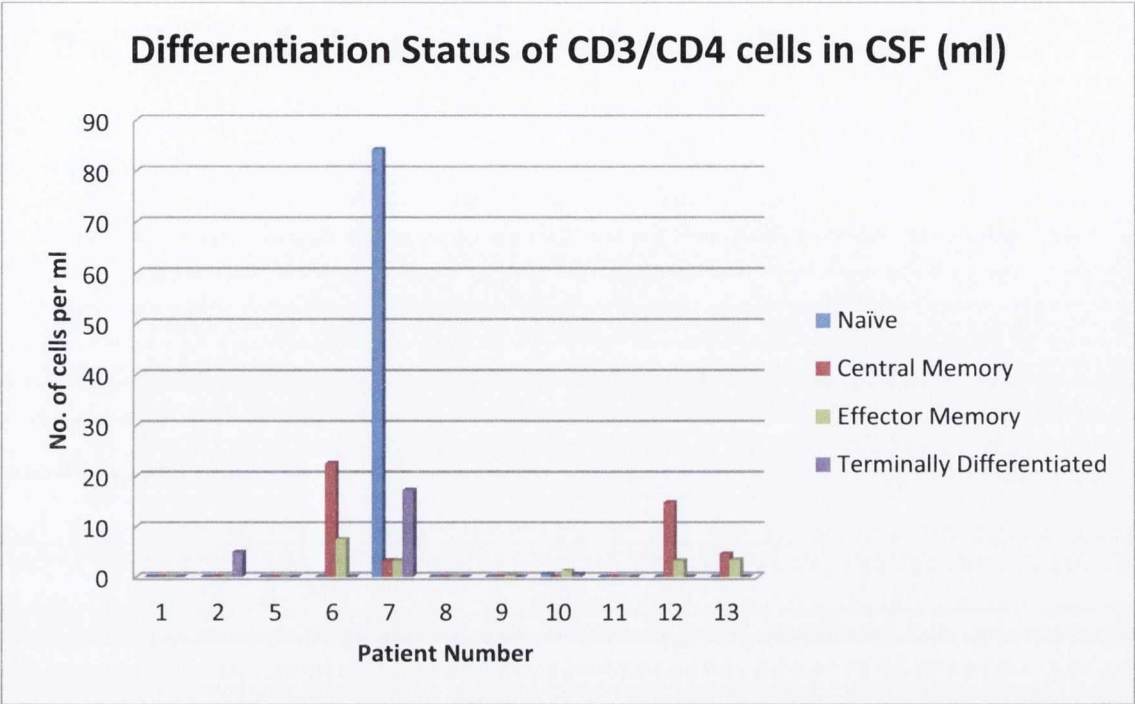


Figure 5-25 Differentiation of CD3/CD8 positive T cells in Cerebrospinal Fluid (percentages)

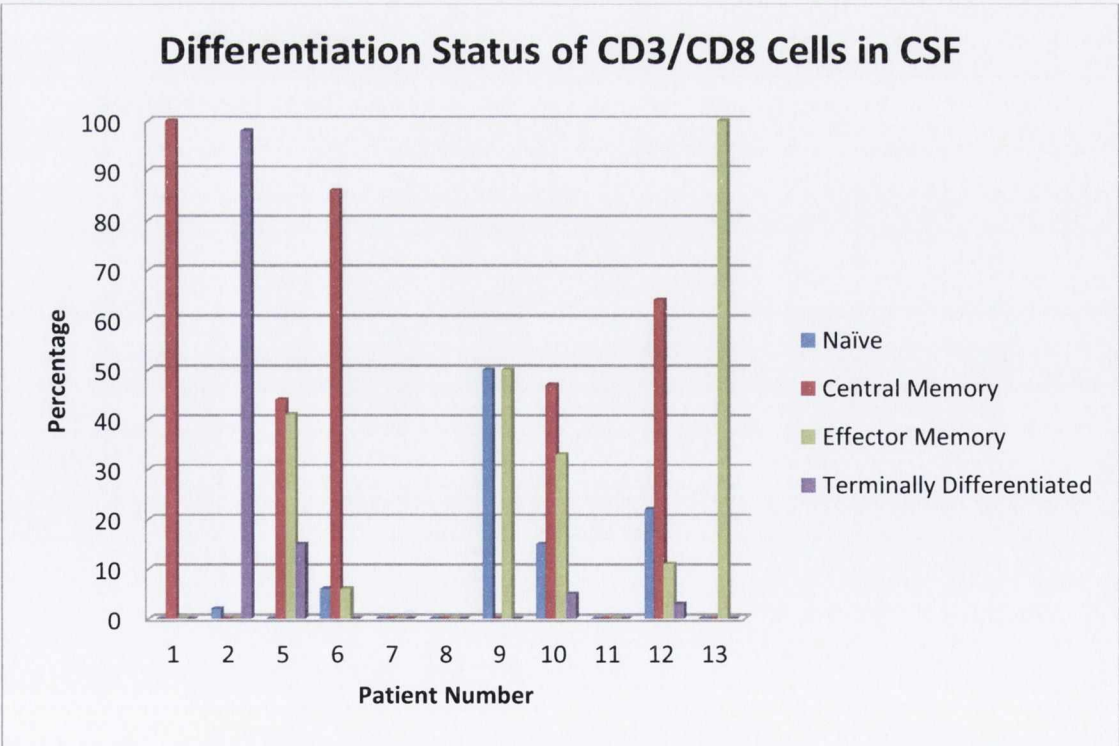




Figure 5-26 Differentiation of CD3/CD8 positive T cells in Cerebrospinal Fluid (cells per millilitre)

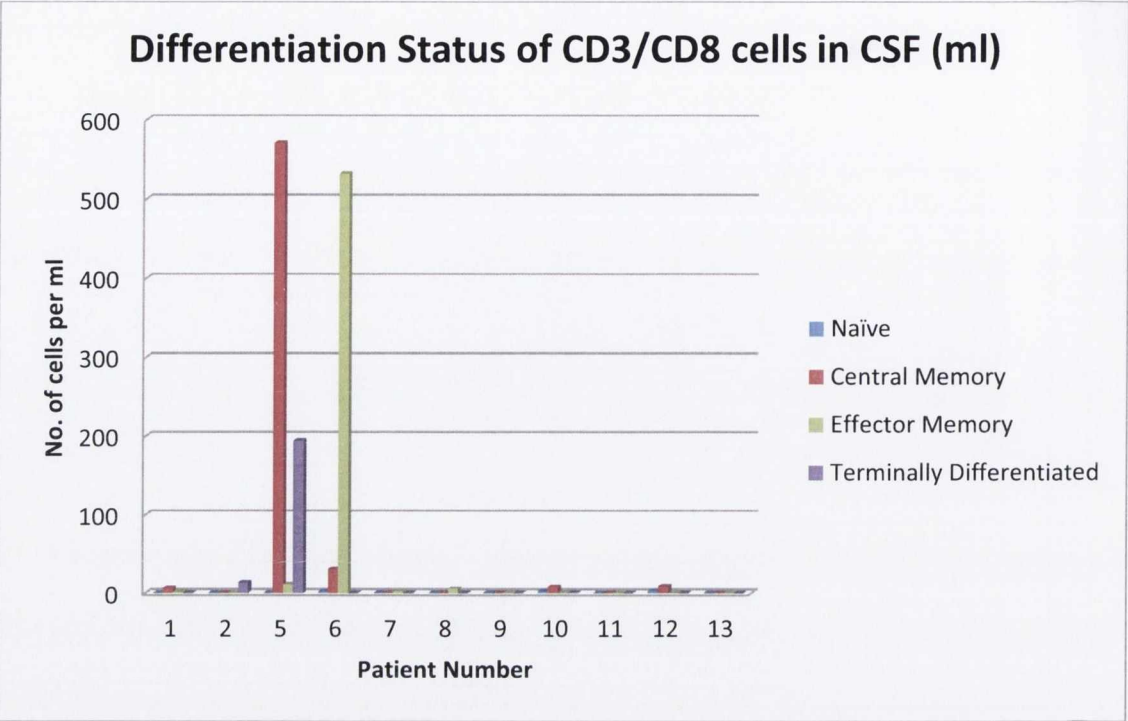


Figure 5-27 Percentage of NK (CD56<sup>+</sup>CD3<sup>-</sup>) cells and NK Like (CD56<sup>+</sup>CD3<sup>+</sup>) cells in Peripheral Blood Mononuclear Cells

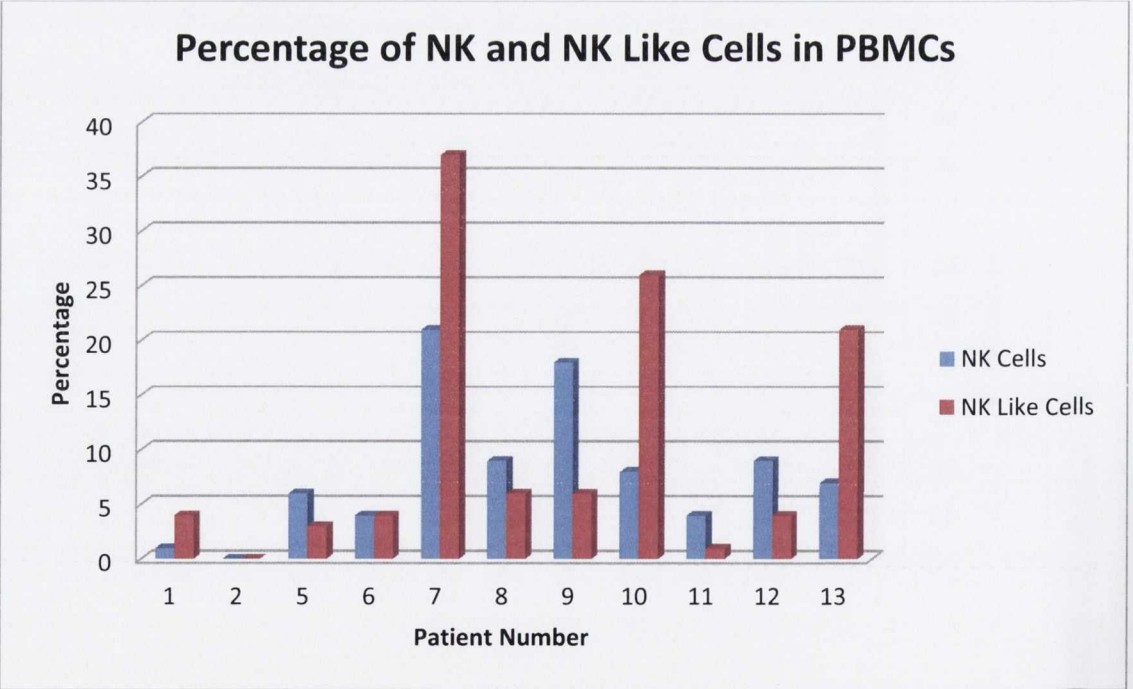


Figure 5-28 Percentage of NK (CD56<sup>+</sup>CD3<sup>-</sup>) cells and NK Like (CD56<sup>+</sup>CD3<sup>+</sup>) cells in Cerebrospinal Fluid

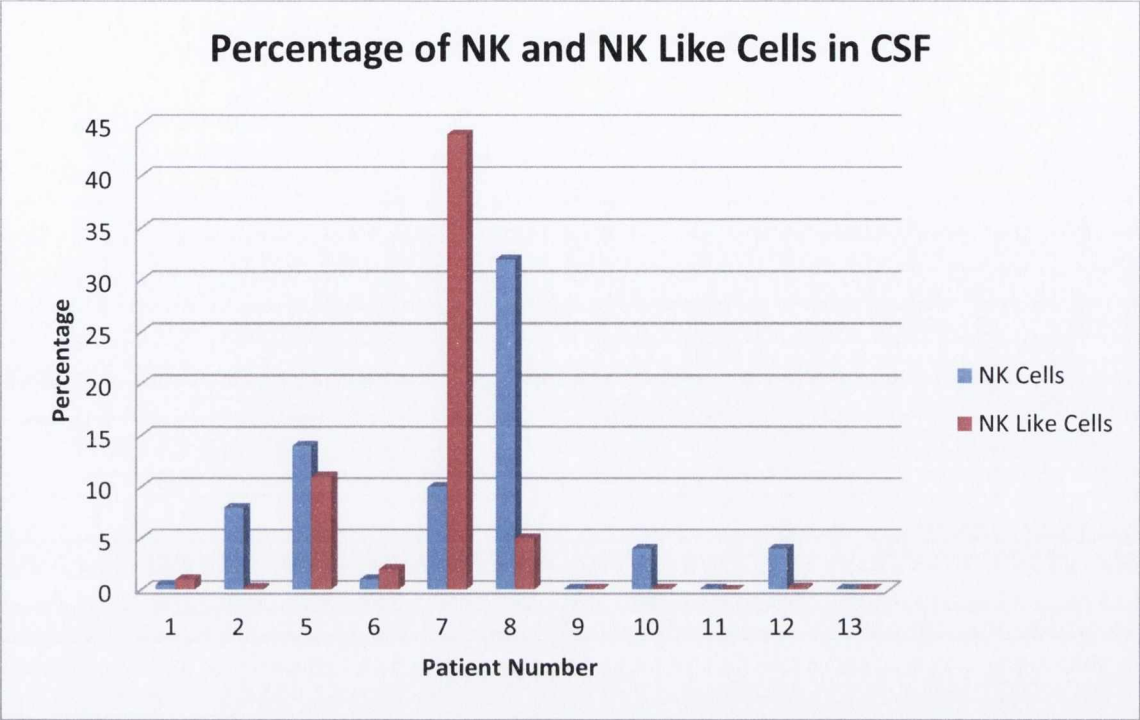


Figure 5-29 Number of NK (CD56<sup>+</sup>CD3<sup>-</sup>) cells and NK Like (CD56<sup>+</sup>CD3<sup>+</sup>) cells in Peripheral Blood Mononuclear Cells per millilitre

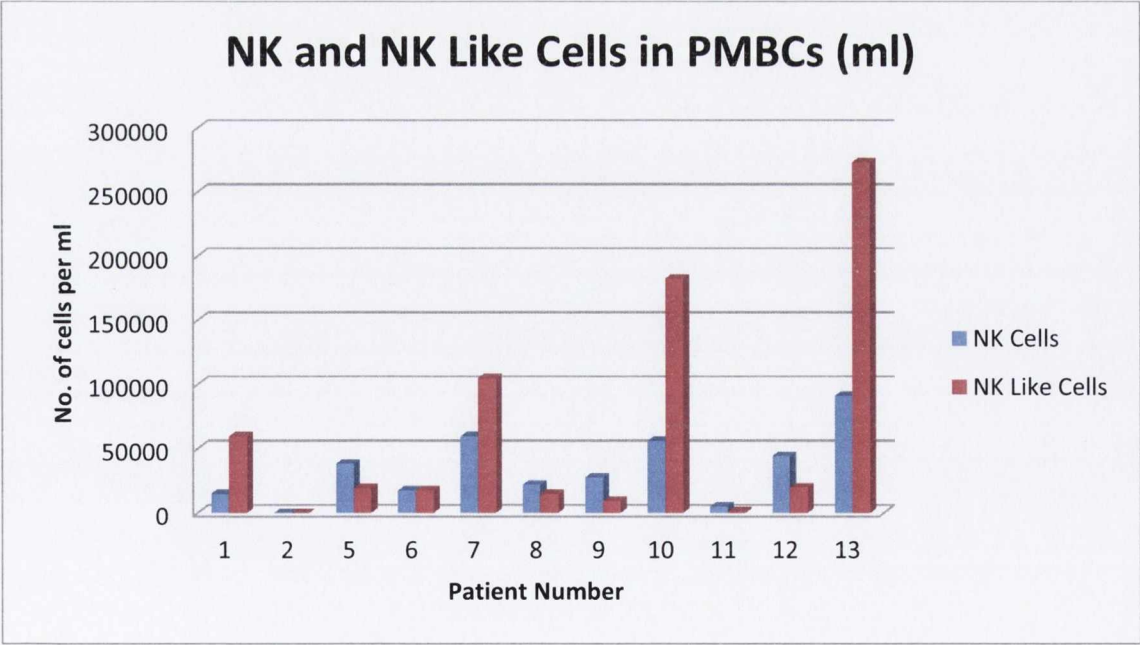


Figure 5-30 Number of NK (CD56<sup>+</sup>CD3<sup>-</sup>) cells and NK Like (CD56<sup>+</sup>CD3<sup>+</sup>) cells in Cerebrospinal Fluid per millilitre

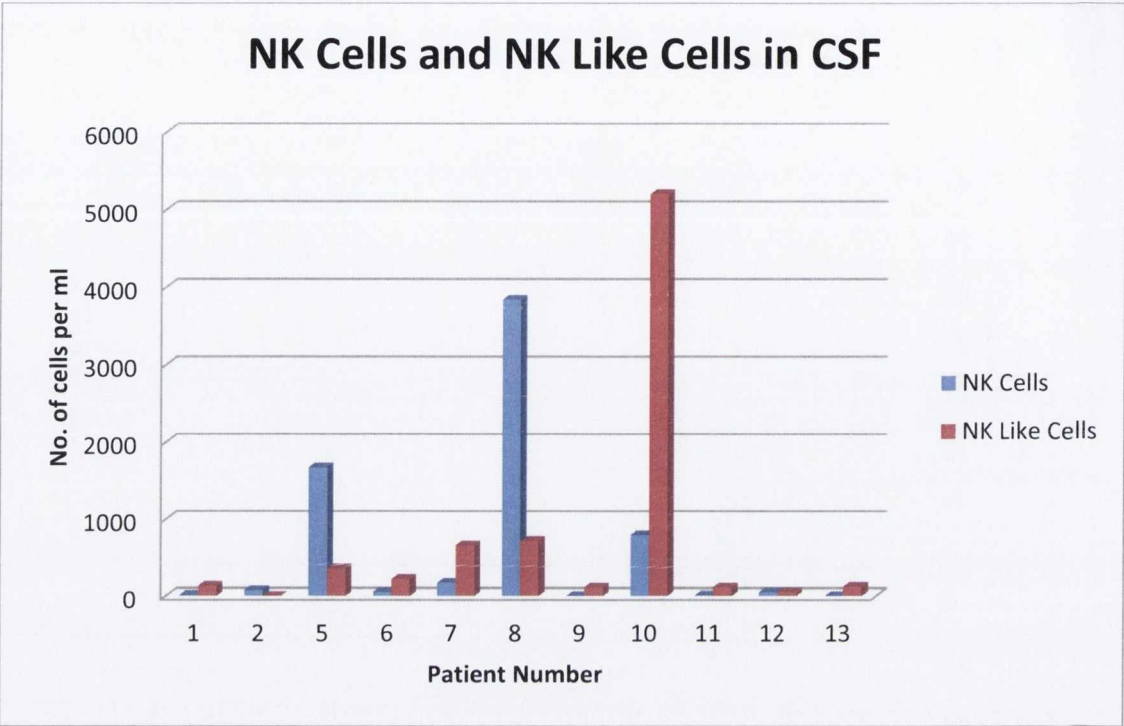


Figure 5-31 Percentage of  $\gamma\delta$  Cells in Peripheral Blood Mononuclear Cells

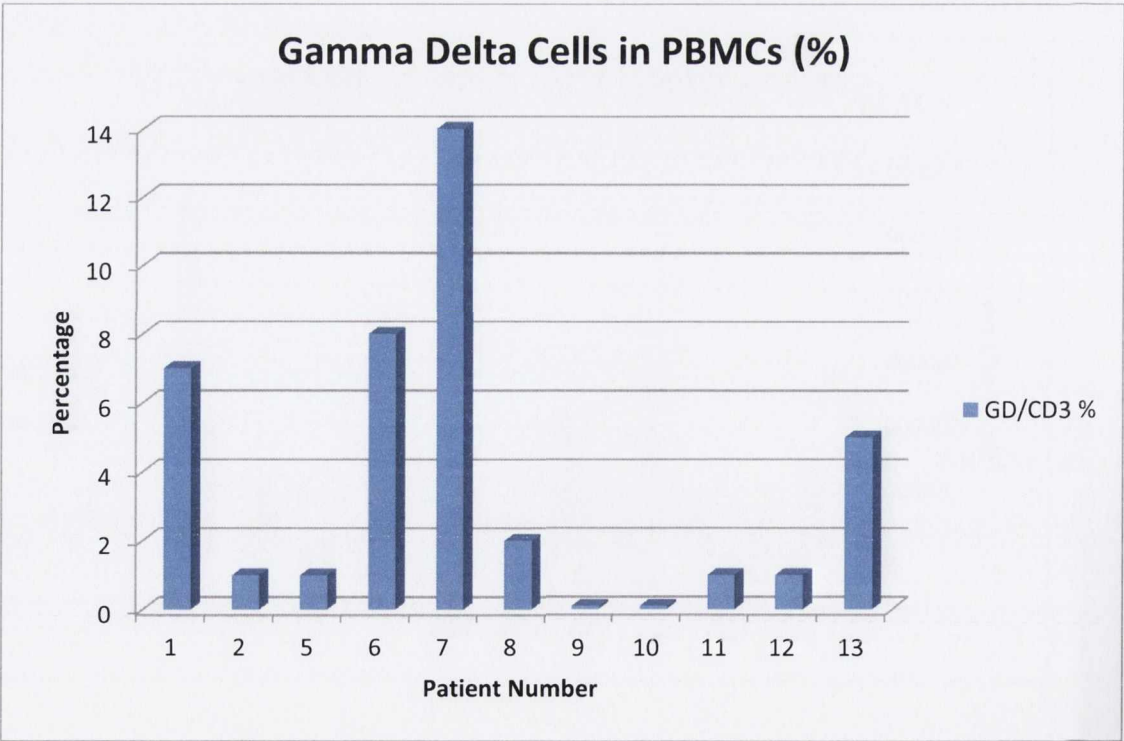




Figure 5-32 Percentage of  $\gamma\delta$  Cells in Cerebrospinal Fluid

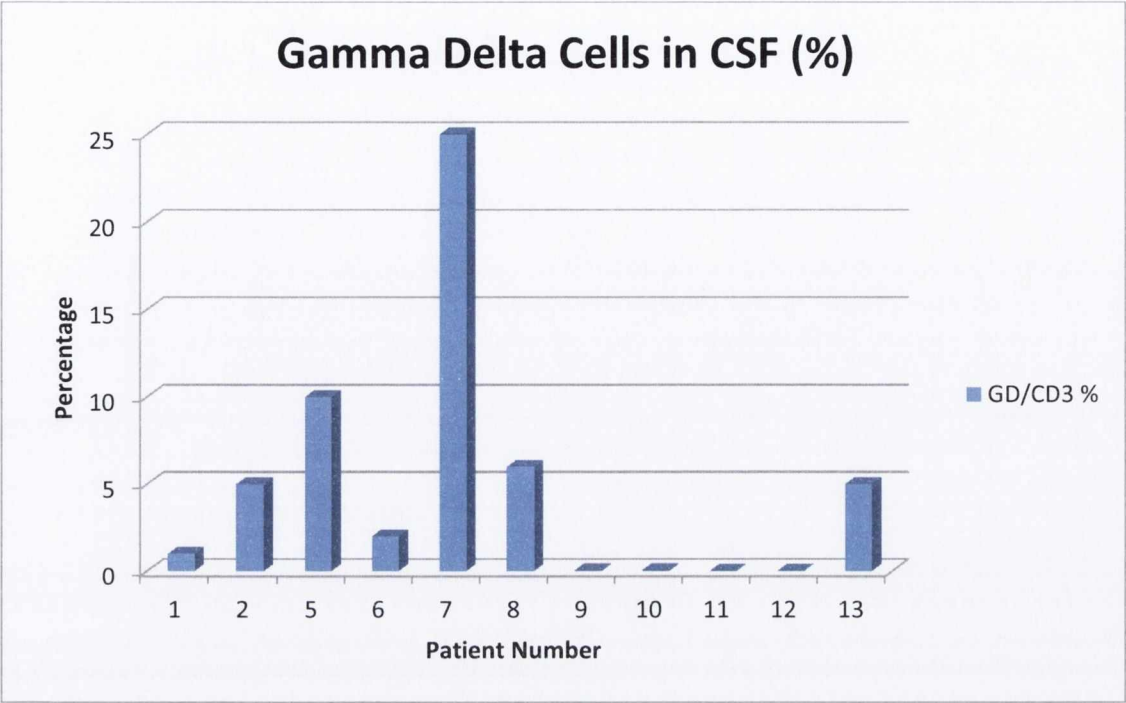


Figure 5-33 Number of  $\gamma\delta$  Cells in Peripheral Blood Mononuclear Cells (ml)

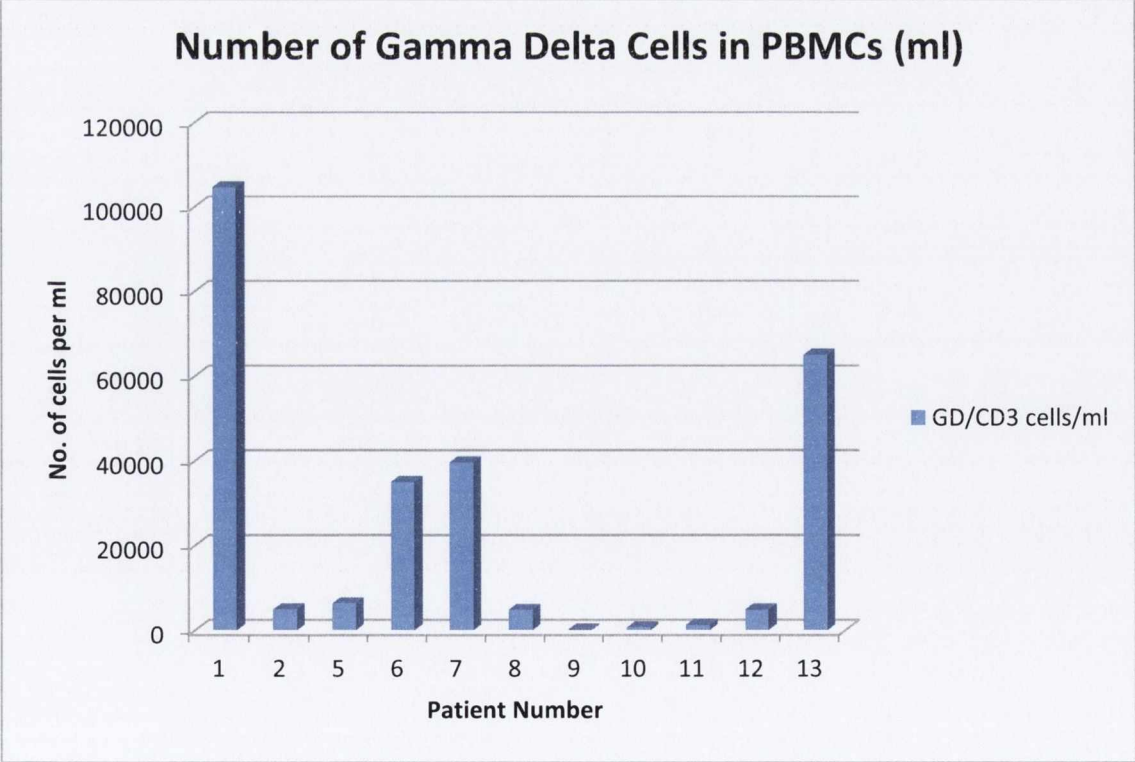


Figure 5-34 Number of  $\gamma\delta$  Cells in Cerebrospinal Fluid (ml)

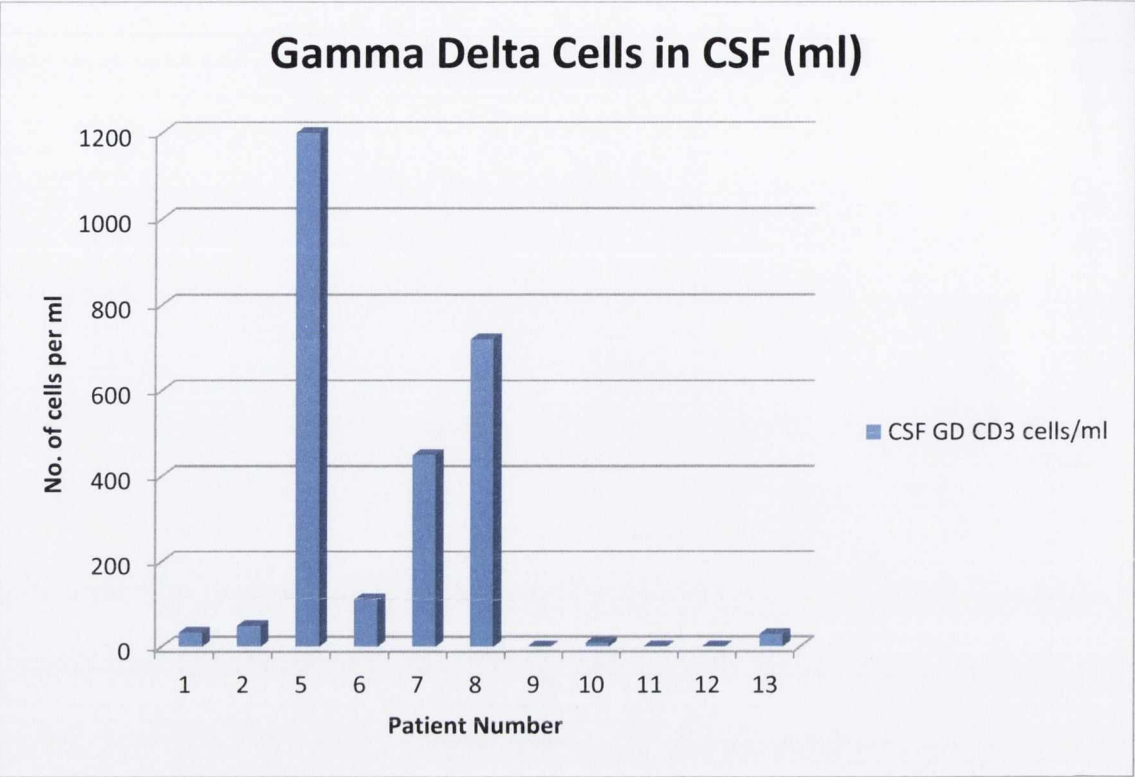


Figure 5-35 Percentage of B Cells in Peripheral Blood Mononuclear Cells

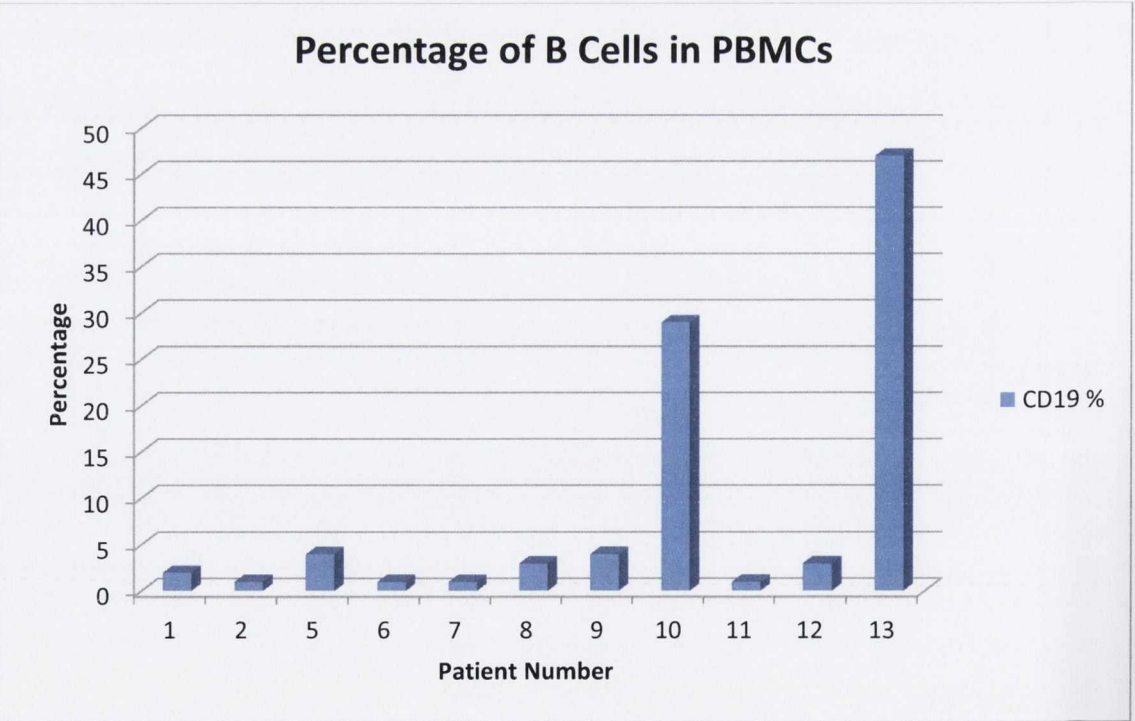


Figure 5-36 Percentage of B Cells in Cerebrospinal Fluid

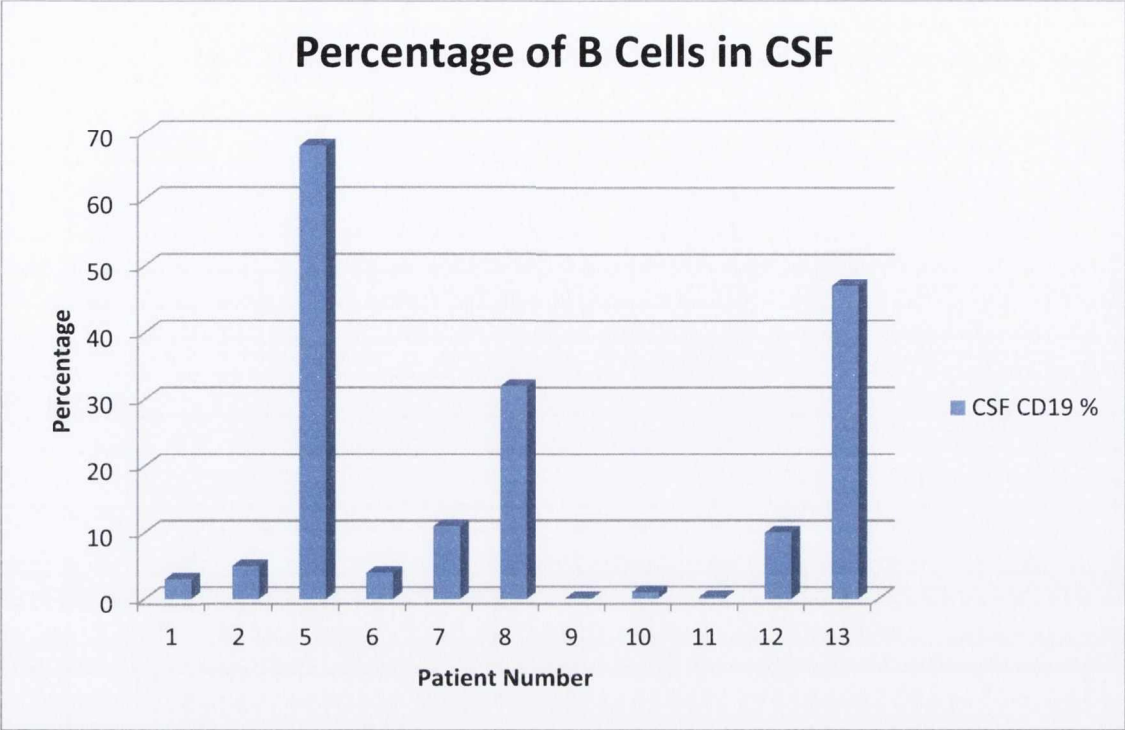


Figure 5-37 Number of B Cells in Peripheral Blood Mononuclear Cells per millilitre

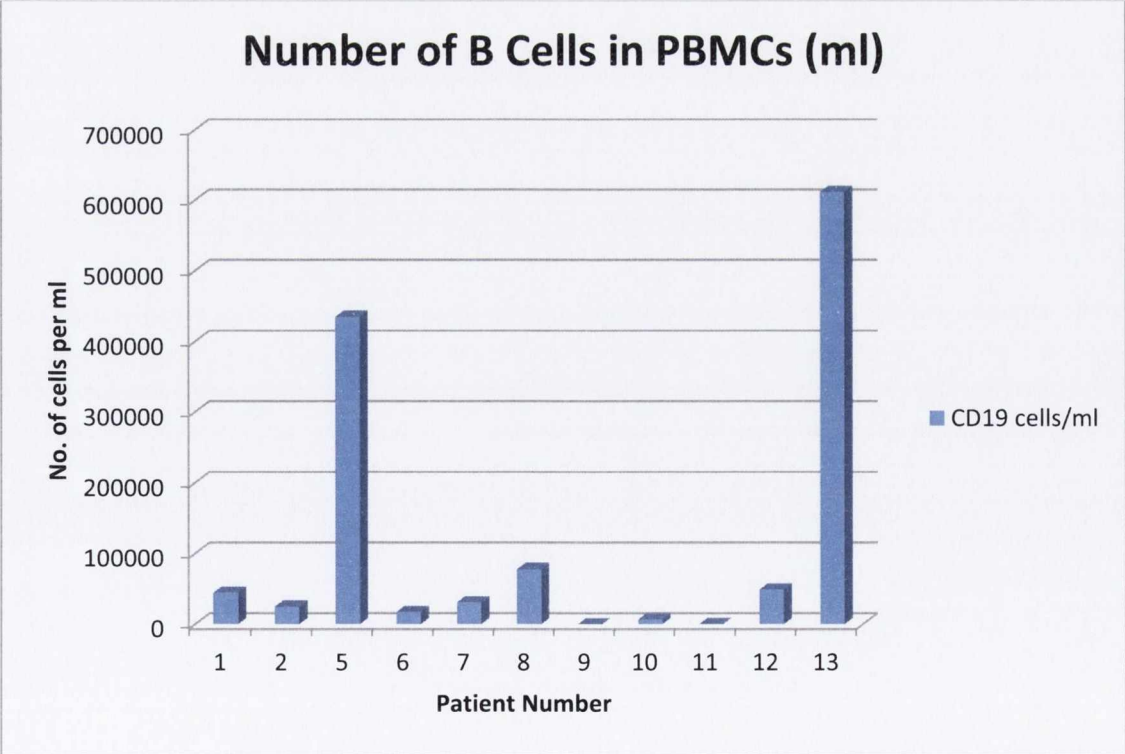




Figure 5-38 Number of B Cells in Cerebrospinal Fluid per millilitre

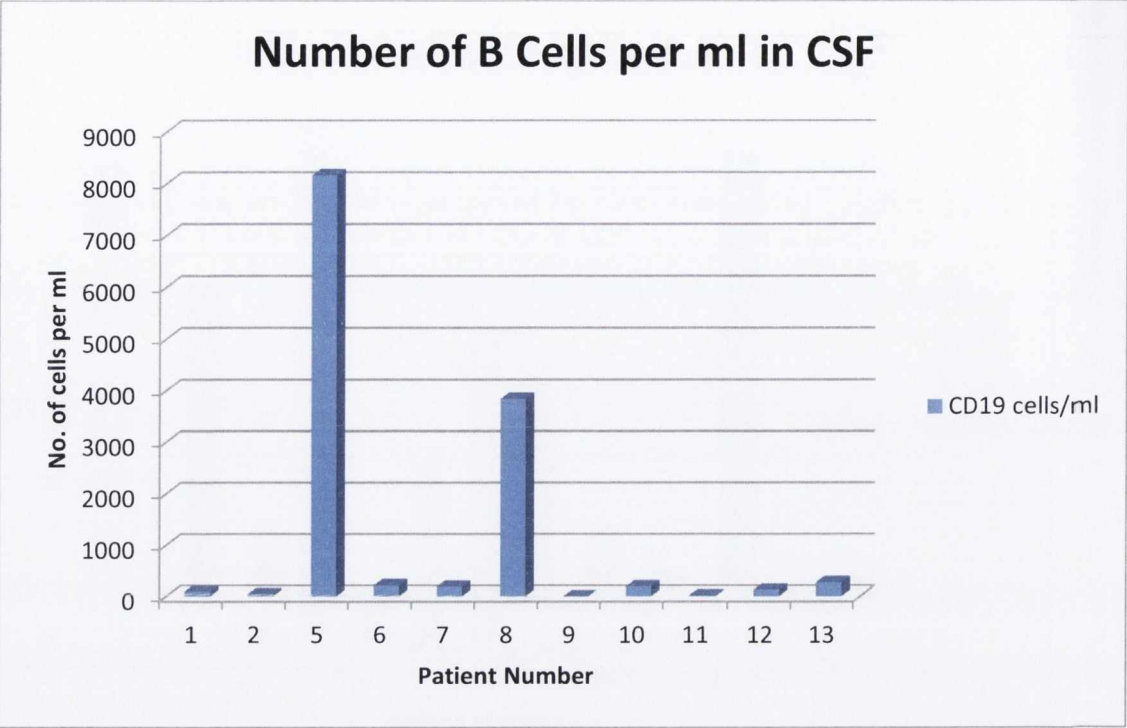


Table 5-7 Summary of Results of Flow Cytometry Data

|                                     | PBMCs          |                |                          | CSF            |                   |
|-------------------------------------|----------------|----------------|--------------------------|----------------|-------------------|
|                                     |                | %              | Cells / ml               | %              | Cells / ml        |
| CD3 T Cells                         | Mean (Std Dev) | 45.18 (27.5)   | 254048.18 (317007.6)     | 9.42 (9.568)   | 402.6 (981.6)     |
|                                     | Median (range) | 48 (2 - 82)    | 130000 (400 - 1074150)   | 10 (0 - 27)    | 100 (0 - 3328)    |
| CD3/CD4 T Cells<br>(% of CD3 cells) | Mean (Std Dev) | 25.55 (20.151) | 70293 (136498.9)         | 10.273 (11.13) | 15.609 (32.1)     |
|                                     | Median (range) | 30 (3 - 51)    | 22214.4 (16 - 472626)    | 5 (0 - 30)     | 2.5 (0 - 108)     |
| CD3/CD8 T Cells<br>(% of CD3 cells) | Mean (Std Dev) | 37.64 (17.6)   | 106167.7 (126774.2)      | 21.364 (17.88) | 127.05 (388.5)    |
|                                     | Median (range) | 40 (4 - 62)    | 52748.96 (16 - 397435.5) | 14 (0 - 50)    | 11.2 (0 - 1297.9) |
| CD3/CD4 T Cells                     |                |                |                          |                |                   |
| Naïve cells                         | Mean (Std Dev) | 29 (19.5)      | 17279.4 (37117.4)        | 9.818 (23.8)   | 7.72 (25.4)       |
|                                     | Median (range) | 27 (0 - 67)    | 5406.6 (0 - 127609)      | 0 (0 - 78)     | 0 (0 - 84.2)      |
| Central Memory                      | Mean (Std Dev) | 31.45 (24.45)  | 35588.8 (5698.8)         | 23.27 (33.4)   | 4.09 (7.55)       |
|                                     | Median (range) | 39 (0 - 65)    | 76034.5 (0 - 259944.3)   | 0 (0 - 82)     | 0 (0 - 22.5)      |
| Effector Memory                     | Mean (Std Dev) | 22.455 (27.26) | 13398.53 (21179)         | 21.7 (31.8)    | 1.72 (2.41)       |
|                                     | Median (range) | 14 (0 - 92)    | 2975.3 (0 - 66167.6)     | 3 (0 - 100)    | 0.2 (0 - 7.5)     |
| Terminally Differentiated           | Mean (Std Dev) | 17.18 (18.65)  | 4026.3 (5879.8))         | 12.72 (29.8)   | 11 (29.2)         |
|                                     | Median (range) | 8 (1 - 50)     | 1461.24 (4.5 - 18905)    | 0 (0 - 99)     | 0 (0 - 98)        |
| CD3/CD8 T Cells (PBMC)              |                |                |                          |                |                   |
| Naïve cells                         | Mean (Std Dev) | 41.1 (30.1)    | 39197.7 (57091.7)        | 8.63 (15.58)   | 0.76 (1.22)       |

|                                    |                |               |                       |                |                  |
|------------------------------------|----------------|---------------|-----------------------|----------------|------------------|
| Central Memory                     | Median (range) | 30 (0 - 94)   | 16822 (0 - 193753.99) | 0 (0 - 50)     | 0 (0 - 3.08)     |
|                                    | Mean (Std Dev) | 20.27 (20.15) | 22948.8 (33668.7)     | 31 (38.8)      | 56.9 (170.8)     |
|                                    | Median (range) | 15 (0 - 69)   | 4617.8 (0 - 101810.9) | 0 (0 - 100)    | 0 (0 - 571.08)   |
| Effector Memory                    | Mean (Std Dev) | 22.8 (26)     | 19151.9 (28630.3)     | 21.9 (31.8)    | 50.27 (159.85)   |
|                                    | Median (range) | 19 (0 - 94)   | 4426.56 (0 - 79487.1) | 6 (0 - 100)    | 0.2 (0 - 532.15) |
| Terminally Differentiated          | Mean (Std Dev) | 18.09 (18.07) | 66592.3 (157226.8)    | 11 (29.2)      | 19.06 (58.4)     |
|                                    | Median (range) | 11 (0 - 52)   | 12152 (0 - 537075)    | 0 (0 - 98)     | 0 (0 - 194.68)   |
| NK Cells                           |                |               |                       |                |                  |
| CD56 <sup>+</sup> CD3 <sup>-</sup> | Mean (Std Dev) | 7.9 (6.48)    | 34270.2 (27096)       | 6.7 (9.6)      | 611.236 (1189)   |
|                                    | Median (range) | 7 (0 - 21)    | 27720 (0 - 91140)     | 4 (0.1 - 32)   | 56 (0.6 - 3840)  |
| NK Like Cells                      |                |               |                       |                |                  |
| CD56 <sup>+</sup> CD3 <sup>+</sup> | Mean (Std Dev) | 10.2 (12.1)   | 63859.8 (88992.3)     | 5.8 (13.1)     | 702.5 (1510.6)   |
|                                    | Median (range) | 4 (0.1 - 37)  | 19200 (50 - 273420)   | 0.2 (0.1 - 44) | 136 (0.1 - 5200) |
| γδ Cells                           |                |               |                       |                |                  |
| γδ <sup>+</sup> CD3 <sup>+</sup>   | Mean (Std Dev) | 3.655 (4.4)   | 24356.4 (34046.8)     | 4.92 (7.4)     | 237.12 (394.9)   |
|                                    | Median (range) | 1 (0.1 - 14)  | 5000 (154 - 104664)   | 2 (0.01 - 25)  | 34 (0.14 - 1200) |
| B cells                            |                |               |                       |                |                  |
| CD19 <sup>+</sup>                  | Mean (Std Dev) | 8.727 (15.07) | 118224.5 (205574.46)  | 16.48 (22.7)   | 1202 (2562.8)    |
|                                    | Median (range) | 3 (1 - 47)    | 31240 (154 - 611940)  | 5 (0.1 - 68)   | 198 (2 - 8160)   |

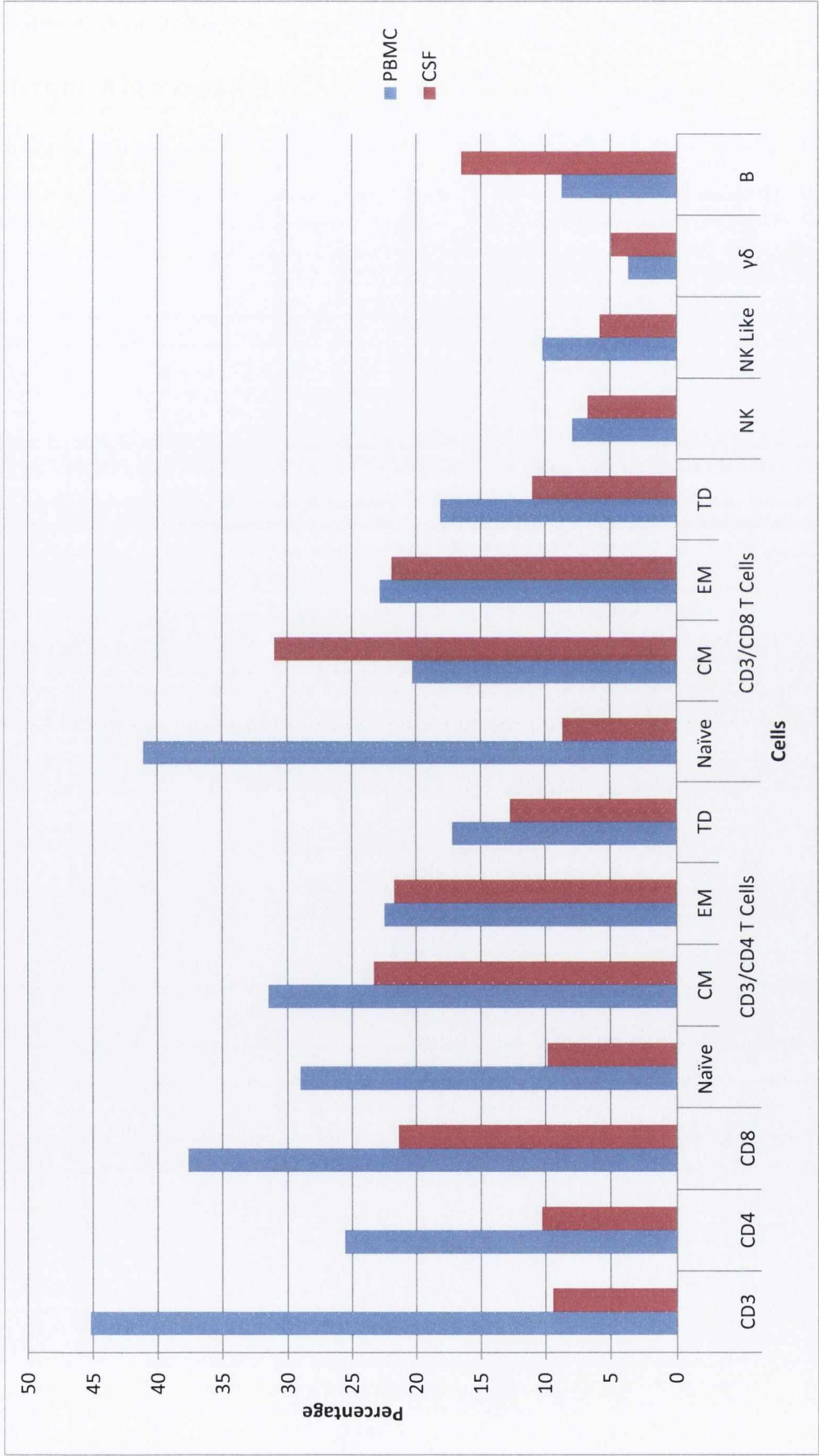


Table 5-8 Statistical Comparison of Percentages of Cells in Blood and CSF

|                                     |                | PBMCs          | CSF            | P Value |
|-------------------------------------|----------------|----------------|----------------|---------|
|                                     |                | %              | %              |         |
| CD3 T Cells                         | Mean (Std Dev) | 45.18 (27.5)   | 9.42 (9.568)   | 0.013   |
|                                     | Median (range) | 48 (2 - 82)    | 10 (0 - 27)    |         |
| CD3/CD4 T Cells<br>(% of CD3 cells) | Mean (Std Dev) | 25.55 (20.151) | 10.273 (11.13) | 0.051   |
|                                     | Median (range) | 30 (3 - 51)    | 5 (0 - 30)     |         |
| CD3/CD8 T Cells<br>(% of CD3 cells) | Mean (Std Dev) | 37.64 (17.6)   | 21.364 (17.88) | 0.017   |
|                                     | Median (range) | 40 (4 - 62)    | 14 (0 - 50)    |         |
| CD3/CD4 T Cells                     |                |                |                |         |
| Naïve cells                         | Mean (Std Dev) | 29 (19.5)      | 9.818 (23.8)   | 0.01    |
|                                     | Median (range) | 27 (0 - 67)    | 0 (0 - 78)     |         |
| Central Memory                      | Mean (Std Dev) | 31.45 (24.45)  | 23.27 (33.4)   | 0.008   |
|                                     | Median (range) | 39 (0 - 65)    | 0 (0 - 82)     |         |
| Effector Memory                     | Mean (Std Dev) | 22.455 (27.26) | 21.7 (31.8)    | 0.959   |
|                                     | Median (range) | 14 (0 - 92)    | 3 (0 - 100)    |         |
| Terminally Differentiated           | Mean (Std Dev) | 17.18 (18.65)  | 12.72 (29.8)   | 0.182   |
|                                     | Median (range) | 8 (1 - 50)     | 0 (0 - 99)     |         |
| CD3/CD8 T Cells (PBMC)              |                |                |                |         |
| Naïve cells                         | Mean (Std Dev) | 41.1 (30.1)    | 8.63 (15.58)   | 0.01    |
|                                     | Median (range) | 30 (0 - 94)    | 0 (0 - 50)     |         |
| Central Memory                      | Mean (Std Dev) | 20.27 (20.15)  | 31 (38.8)      | 0.475   |
|                                     | Median (range) | 15 (0 - 69)    | 0 (0 - 100)    |         |
| Effector Memory                     | Mean (Std Dev) | 22.8 (26)      | 21.9 (31.8)    | 0.953   |
|                                     | Median (range) | 19 (0 - 94)    | 6 (0 - 100)    |         |
| Terminally Differentiated           | Mean (Std Dev) | 18.09 (18.07)  | 11 (29.2)      | 0.074   |
|                                     | Median (range) | 11 (0 - 52)    | 0 (0 - 98)     |         |
| NK Cells                            |                |                |                |         |
| CD56 <sup>+</sup> CD3 <sup>-</sup>  | Mean (Std Dev) | 7.9 (6.48)     | 6.7 (9.6)      | 0.553   |
|                                     | Median (range) | 7 (0 - 21)     | 4 (0.1 - 32)   |         |
| NK Like Cells                       |                |                |                |         |
| CD56 <sup>+</sup> CD3 <sup>+</sup>  | Mean (Std Dev) | 10.2 (12.1)    | 5.8 (13.1)     | 0.182   |
|                                     | Median (range) | 4 (.01 - 37)   | 0.2 (.01 - 44) |         |

|                                      |                |               |               |       |
|--------------------------------------|----------------|---------------|---------------|-------|
| <b>γδ Cells</b>                      |                |               |               |       |
| <b>γδ<sup>+</sup>CD3<sup>+</sup></b> | Mean (Std Dev) | 3.655 (4.4)   | 4.92 (7.4)    | 0.798 |
|                                      | Median (range) | 1 (0.1 - 14)  | 2 (0.01 - 25) |       |
| <b>B cells</b>                       |                |               |               |       |
| <b>CD19<sup>+</sup></b>              | Mean (Std Dev) | 8.727 (15.07) | 16.48 (22.7)  | 0.139 |
|                                      | Median (range) | 3 (1 - 47)    | 5 (0.1 - 68)  |       |

Figure 5-39 Percentage of Cells in Blood and CSF





### 5.6.3 Results of Cytokine Analysis in CSF

Analysis of all thirteen patients is presented. There were low levels of IL-6 and TNF- $\alpha$  detected in all of the patients CSF. There were extremely low levels of IL-1 $\beta$  and IL-10 detected in eleven of the patients. The levels of IL-17 and IFN- $\gamma$  were below the limits of detection of the test.

Figure 5-40 Results of Interleukin-6 Analysis (IL-6)

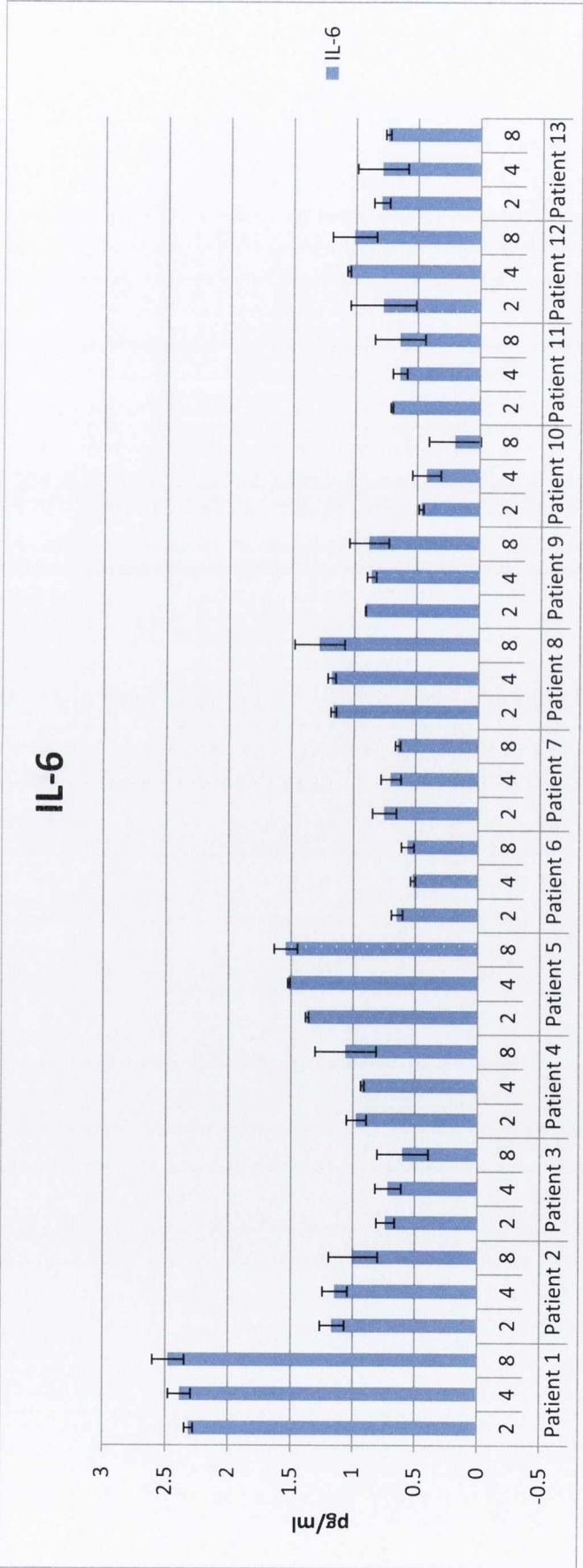


Figure 5-41 Results of Tumour Necrosis Factor Alpha (TNF-α) Analysis

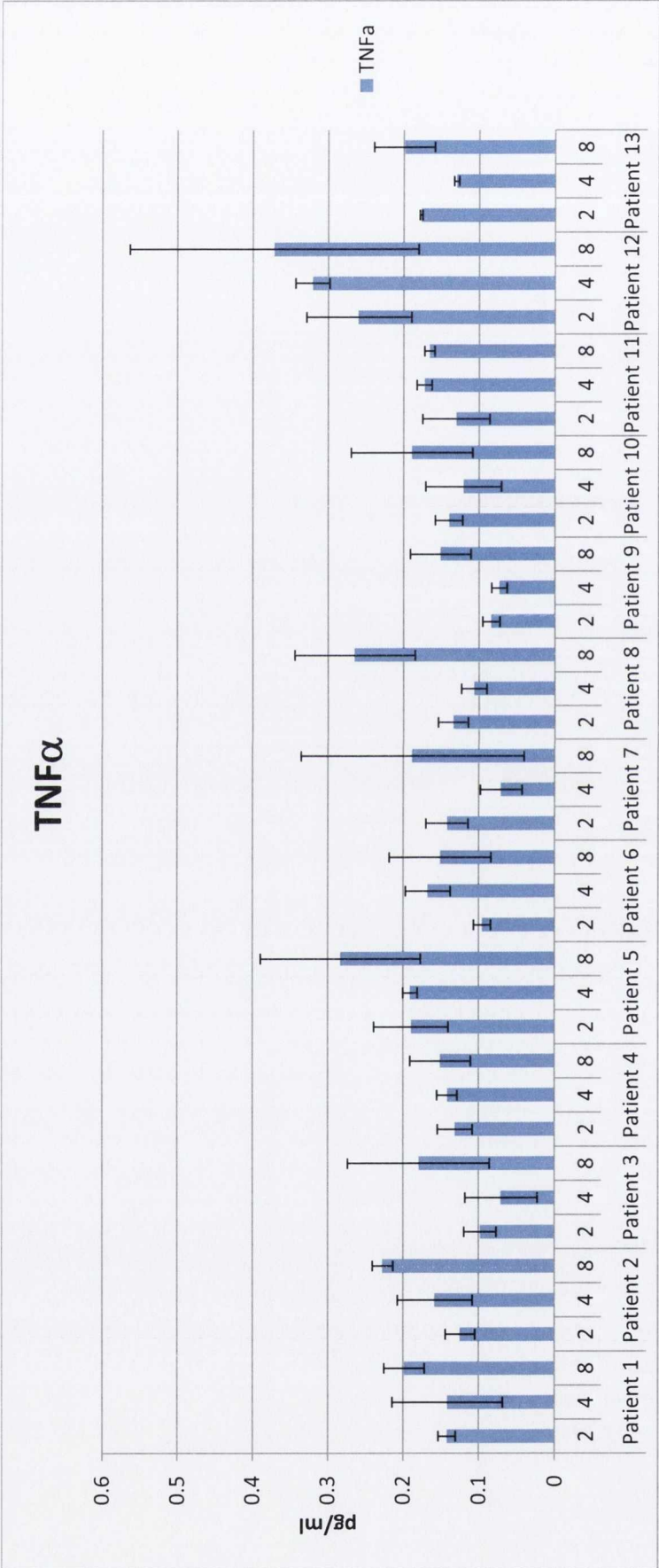




Figure 5-42 Results of Interleukin 1-beta (IL-1 $\beta$ ) Analysis

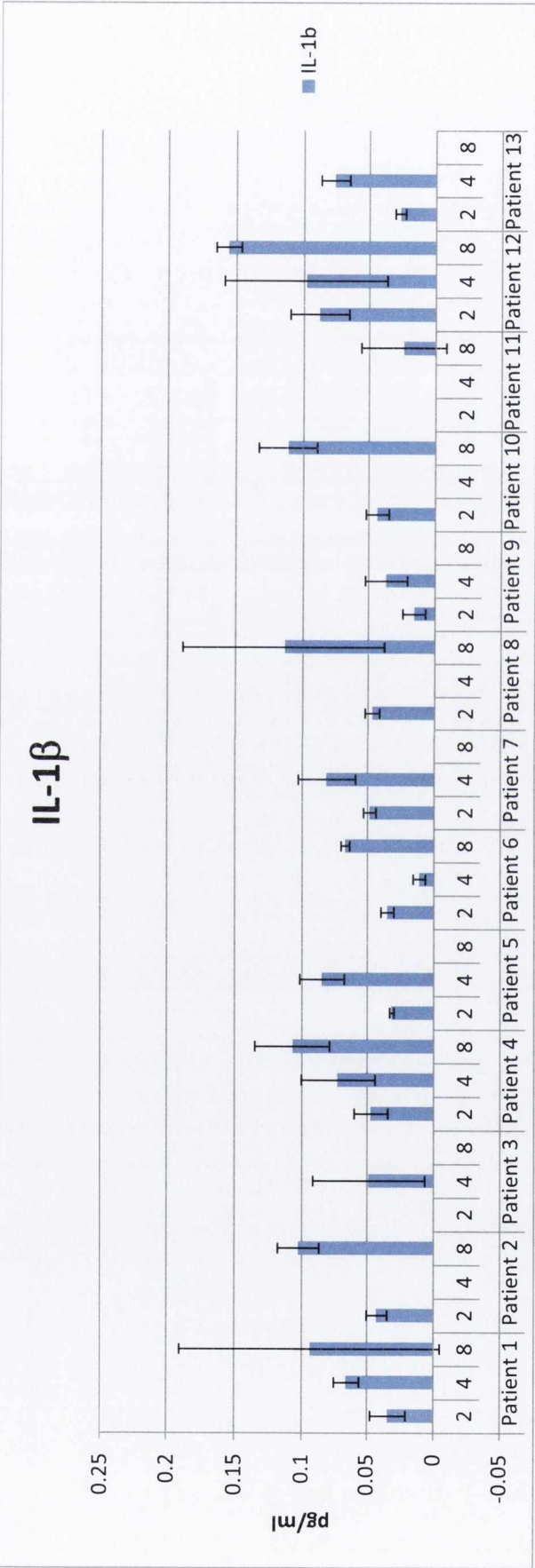


Figure 5-43 Results of Interleukin 17a (IL-17a) Analysis

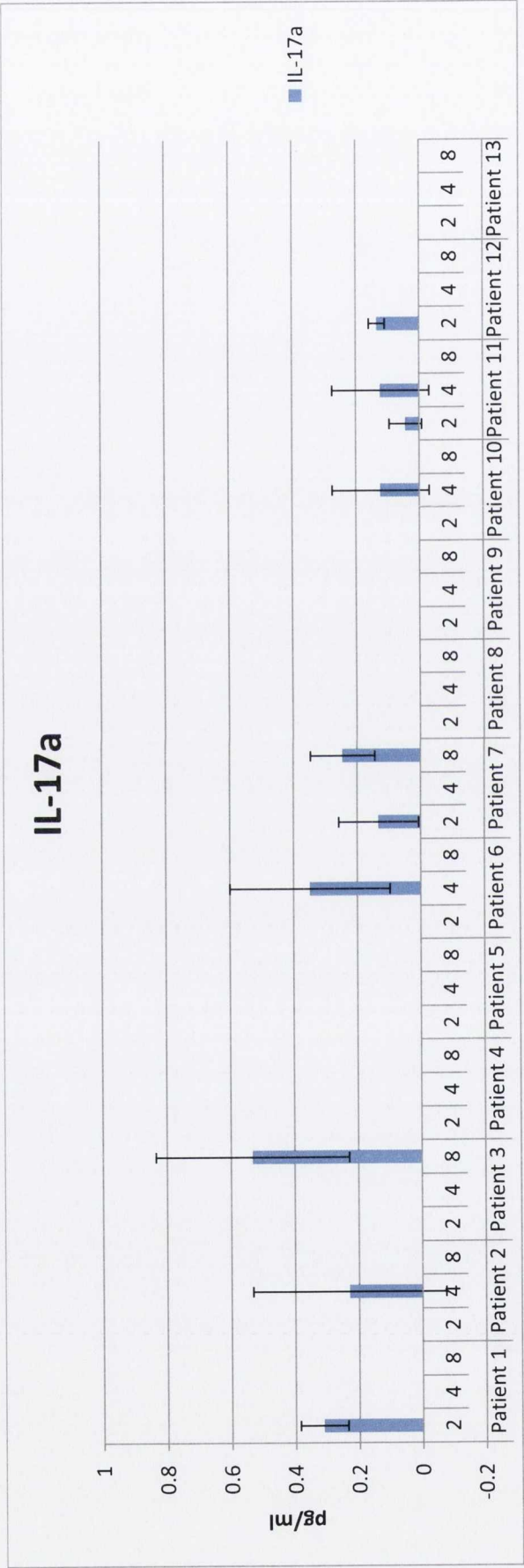


Figure 5-44 Results of Interleukin 10 (IL-10) Analysis

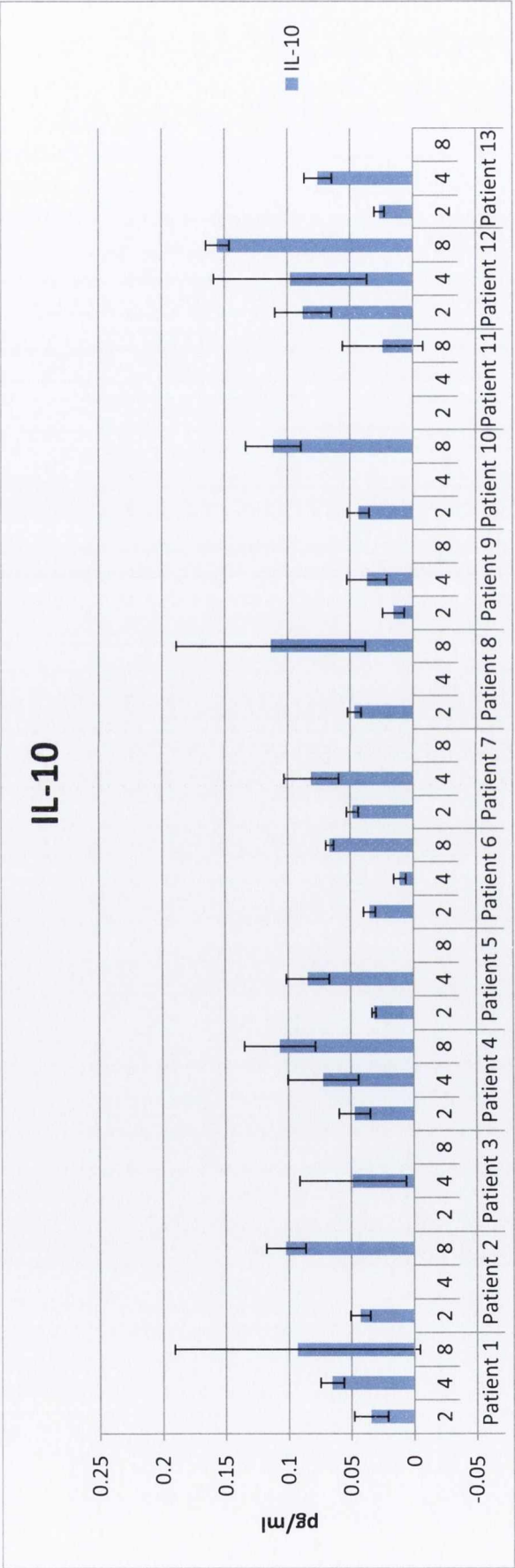




Figure 5-45 Results of Interferon gamma (IFN- $\gamma$ ) Analysis

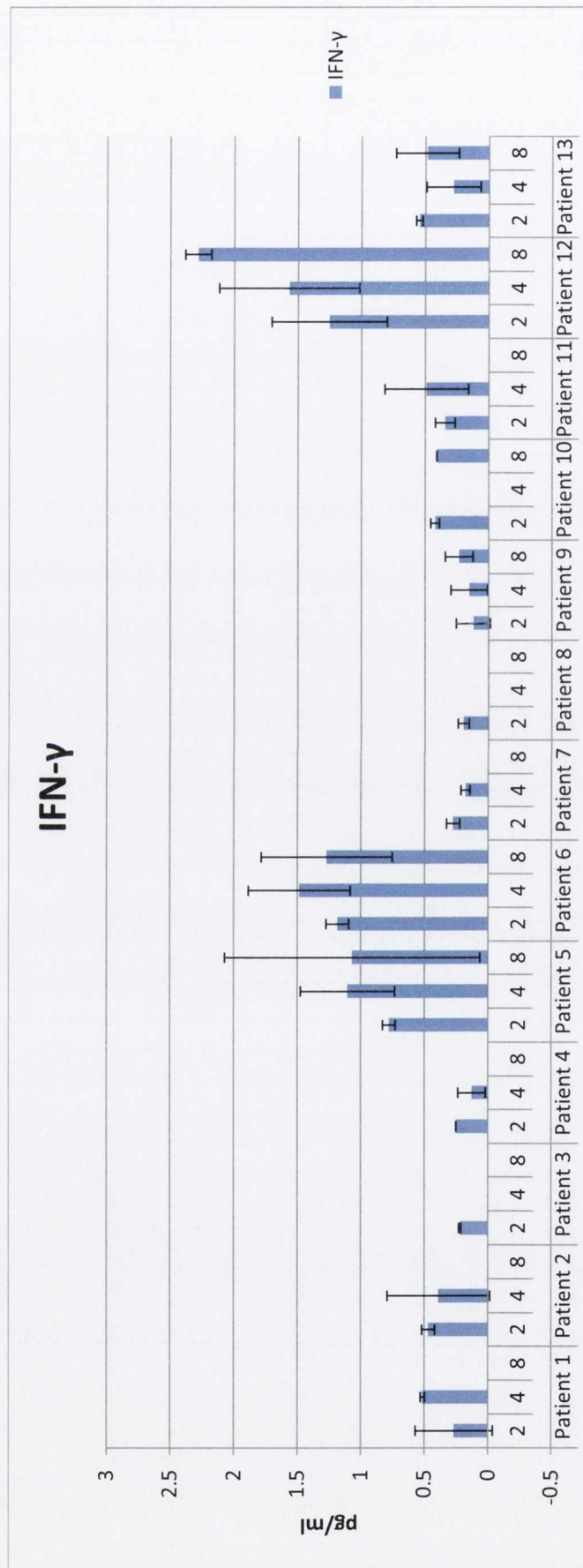
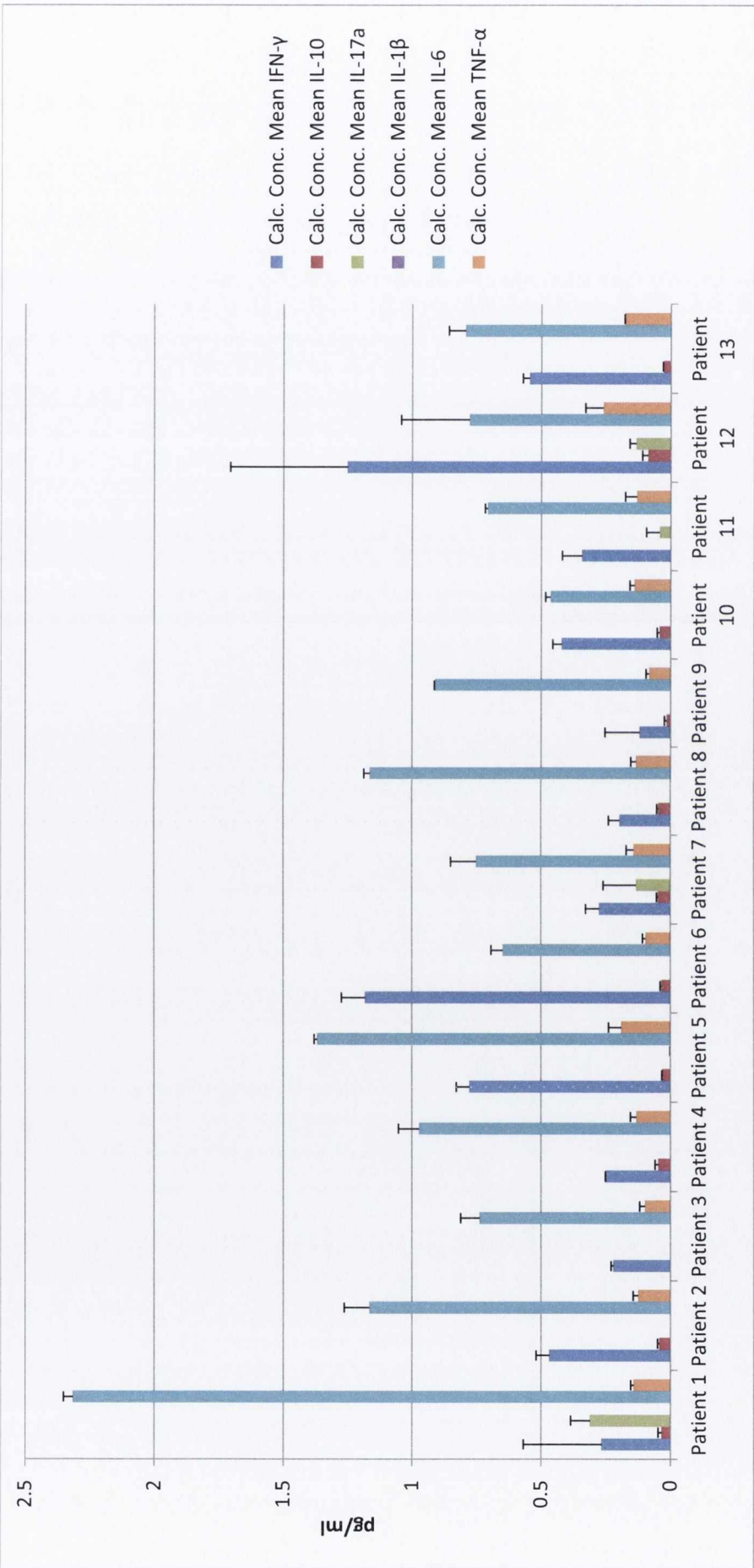


Figure 5-46 Summary of All Cytokine Analysis for All Patients at a Dilution of 1:2



Calc. Conc = calculated concentration

5.6.4 Results of Analysis of Amyloid and Tau Levels

Analysis of all thirteen patients is presented in Table 5-9. Levels of beta-amyloid<sub>(1-42)</sub>, total tau and phosphorylated tau were all within normal limits for the study group.

Table 5-9 Summary of Results of Beta-amyloid and Tau Analysis

|             | all results in pg/ml |           |                    |
|-------------|----------------------|-----------|--------------------|
|             | Beta-amyloid         | Total Tau | Phosphorylated Tau |
| Patient 1   | 978.812              | 204.01    | 54.938             |
| Patient 2   | 811.984              | 146.322   | 41.755             |
| Patient 3   | 889.945              | 180.059   | 44.831             |
| Patient 4   | 831.287              | 199.203   | 45.555             |
| Patient 5   | 919.939              | 217.113   | 52.406             |
| Patient 6   | 941.99               | 240.222   | 57.343             |
| Patient 7   | 777.546              | 164.126   | 36.239             |
| Patient 8   | 1010.077             | 294.248   | 57.109             |
| Patient 9   | 880.374              | 153.4     | 41.213             |
| Patient 10  | 601.237              | 200.71    | 48.63              |
| Patient 11  | 863.821              | 195.707   | 45.766             |
| Patient 12  | 1178.49              | 376.937   | 92.277             |
| Patient 13  | 1120.291             | 252.986   | 51.778             |
| POS control | 677.034              | 984.193   | 116.287            |
| NEG control | 259.06               | 180.028   | 36.441             |

5.7 Discussion

5.7.1 Discussion of Results of Flow Cytometry

HIV results in depletion of CD4<sup>+</sup> T cells and the usual ratio of CD4/CD8 cells is reversed in HIV infection. This can be corrected with HAART but often may not correct despite normalisation of CD4<sup>+</sup> T cell count (539). Half of the patients in the study group showed a greater proportion of CD8<sup>+</sup> T cells in PBMCs and more than half in CSF despite all of the patients being virally suppressed. The majority of CD4<sup>+</sup> and CD8<sup>+</sup> T



cells bore an effector memory phenotype in the CSF (CD45<sup>+</sup>CD27<sup>+</sup>) suggesting activated T cells migrating from secondary lymphoid tissue to target organ (brain). Although the main cell constituents within the CSF of HIV positive patients are CD8<sup>+</sup> cells, the activation status of these cells in this patient cohort suggests that despite viral suppression peripherally and centrally there are still memory cells present in the CSF attempting to control viral replication and possibly also suggesting that there is inflammation ongoing centrally to which CD8<sup>+</sup> T cells are responding or contributing as activated memory cells may release IFN- $\gamma$  and IL-2. CD8<sup>+</sup> T cells are cytotoxic and although they are essential to the control of viral replication it is possible that they may also have a detrimental effect as is in the case in the setting of CD8<sup>+</sup> encephalitis and immune reconstitution (116-118).

There were low frequencies found of NK cells (7.9%, 6.7%), NK like cells (10.2%, 5.8%) and gamma delta cells (3.65%, 4.1%) both in PBMCs and CSF. This suggests that there is activation of the innate immune response within the CNS in these HIV positive patients with cognitive impairment. There were higher levels of B cells in the CSF compared to PBMCs (16.5% vs 8.7%). Cepok et al found higher levels of B cells in the CSF of HIV positive patients (495). B cells play an essential role in the humoral immune response in neuroinflammation and serve as antigen presenting cells for T cells and the chemokine CXCL13 is the major determinant of B cell recruitment during neuroinflammation (540). B cells have been shown to play a role in acute CNS inflammation in relapsing remitting multiple sclerosis (RRMS) and clinically isolated syndrome (CIS) and have been found in the CSF of these patient groups in comparison to those with progressive MS (541). In this study group there were higher levels of B

cells in the CSF in comparison to the blood compartment suggesting that the elevation of B cells within the CSF was not just due to trafficking of B cells from the blood into the CSF.

#### 5.7.2 Discussion of Results of Cytokine Analysis

There were low levels detected in the CSF of all the patients of IL-6 and tumour necrosis factor alpha (TNF- $\alpha$ ). TNF- $\alpha$  is pro-inflammatory and is primarily released by activated macrophages, CD4<sup>+</sup> T cells and NK cells. TNF- $\alpha$  is involved in synaptic plasticity and scaling (542). IL-6 is proinflammatory. It can antagonise TNF- $\alpha$  and IL-1 and it can result in astrogliosis (543). Eleven of the patients also had extremely low levels of IL-1 $\beta$  and IL-10. IL-1 $\beta$  is a pro-inflammatory cytokine that is released primarily by microglia within the CNS but can also be released by astrocytes. IL-10 is an anti-inflammatory cytokine and supports B cell survival, proliferation and antibody production (543).

These results suggest that there is a very low level of neuroinflammation within this study group. This may be due to the fact that all the patients were virally suppressed and had been diagnosed for a mean length of eleven years. Inflammation may occur earlier in the disease process or may occur at levels below the limits of detection of available kits.

### 5.7.3 Discussion of Results of Tau and Amyloid

Tau and amyloid measurements were normal in all thirteen patients. This may be due to a sampling bias or may reflect that amyloid and tau levels are normal in HAND.

### 5.7.4 Limitations

The number of patients recruited was quite low. There were no HIV positive subjects with normal neuropsychological testing or HIV negative healthy volunteers recruited for comparison which makes interpretation of the results rather difficult. It was quite difficult to recruit patients for this study and many patients did not wish to participate despite the fact that a lumbar puncture is clinically indicated for all HIV positive patients. There were also patients who had already had clinical lumbar punctures performed for their cognitive impairment and therefore it was not ethical to repeat the lumbar puncture. There were a number of study participants who agreed to have a lumbar puncture but unfortunately repeatedly failed to attend their appointments despite phone and text reminders.

## 5.8 Conclusion

The main finding of this study was the presence of central and effector memory cells in the CSF of patients with HAND. This suggested that there was trafficking of activated T cells into the CSF of these patients with HAND despite viral suppression. This suggests that despite viral suppression and the lack of detectable levels of most cytokines, there is an active inflammatory process ongoing in the CSF of these patients. We did not detect any evidence of biomarkers of neurodegeneration. This study should be



continued with further patients recruited cross-sectionally and a longitudinal follow-up also to determine more precisely the aetiological role of intrathecal CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and B cells in the setting of HAND.

## **6 Discussion, Future Directions, Recommendations and Conclusion**

### **6.1 Summary of findings**

Chapter 2 discussed the findings of a prevalence study of 600 patients with HIV which showed that 51.5% of the study group had a positive screen for cognitive impairment based on the Brief NeuroCognitive Screen. A positive screen was associated with female gender, endorsement of cognitive symptoms, an African country of birth, English as a second language, heterosexual acquisition of HIV, lack of virological suppression, co-infection with hepatitis C, use of methadone replacement therapy, use of benzodiazepines, being on anti-depressants or anti-psychotic medications, unemployment, alcohol consumption of between 1 to 20 units of alcohol per week, a positive screen for anxiety on the HAD scale, a positive screen for depression on the HAD scale, being younger, a lower nadir CD4<sup>+</sup> T cell count ( $p=0.023$ ), a lower current CD4<sup>+</sup> T cell count and fewer years of education. On multivariate analysis a positive screen was associated with endorsement of cognitive symptoms, an African country of birth, use of benzodiazepines and alcohol consumption greater than 40 units per week.

The Hospital Anxiety and Depression Scale was used to screen for depression and anxiety. It showed that 9.1% of the study group had a positive screen for depression with a previous history of depression and the use of anti-psychotics being independent predictors of a positive screen for depression on multivariate analysis. It also showed that 24.5% of the study group had a positive screen for anxiety with female gender, a

previous history of depression, a previous history of anxiety and neuropathy being independent predictors of a positive screen for anxiety on multivariate analysis.

This study demonstrated that over half of the study group had a positive screen for cognitive impairment. This study group had not been previously assessed for cognitive impairment and this study highlighted the need for a structured cognitive screening program at our institution.

Chapter 3 discussed the neuropsychological profile of 104 patients with a positive screen for cognitive impairment who underwent a detailed neurological assessment. Of 104 patients with a positive screen for cognitive impairment, 62 patients (59.6%) met diagnostic criteria for HIV Associated Neurocognitive Disorders (HAND). The breakdown of HAND within the study group was as follows: asymptomatic neurocognitive impairment (ANI) 40/104 (38.5%), mild neurocognitive disorder 20/104 (19.2%) and HIV associated dementia 2/104 (1.9%). There were 32 patients (30.8%) who had abnormalities in one domain who did not meet diagnostic criteria for HAND.

The predominant neuropsychological profile was an amnestic, dysexecutive syndrome. Immediate memory and delayed memory were the most affected domains on the RBANS within patients who met criteria for HAND. On the ACE-R and MoCA memory, verbal fluency/language and executive domains were the most affected. The FAB showed executive impairment in 45% of patients with HAND.



There was also evidence of rapid forgetting for list memory in half and for story memory in one third of the patients with HAND. The memory deficits found in this group of patients with HIV are most likely mixed with deficits in encoding and retrieval and a subgroup of patients demonstrating evidence of rapid forgetting. The memory deficits demonstrate involvement of both fronto-striatal and hippocampal networks. On the RBANS cortical-subcortical deviation score, 48/62 patients with HAND had scores greater than zero suggesting underlying cortical pathology. Apraxia was the most common finding on neurological examination. Apraxia is typically a cortical finding. The findings of cortical deficits on both the neuropsychological batteries and the neurological examination support my hypothesis that the late effects of HIV on the brain are neurodegenerative.

Chapter 4 discussed the MR features of 50 patients who had a positive screen for cognitive impairment and subsequently underwent detailed neuropsychological and neurological assessment. Grey matter atrophy was demonstrated on VBM analysis in this cohort of patients in the anterior cingulate, left hippocampus, left temporal lobe, left central opercular cortex, left post-central gyrus, left supplementary motor area, right lateral occipital cortex, right precuneus, bilateral Heschl's gyrus, bilateral insular cortex, bilateral parietal operculum, bilateral occipital poles and bilateral pre-central gyrus ( $p < 0.05$ ).

There were no significant correlations to clinical or neuropsychological data but there was atrophy in regions consistent with the neuropsychological deficits found in the

patient cohort. Within the subset of the cohort who had MR imaging, 82% (n=41) had abnormalities in delayed memory, 78% (n=39) had abnormalities in immediate memory, 54% had abnormalities in attention (n=27), 42% had abnormalities in language (n=21) and 20% had abnormalities in visuospatial skills (n=13). There was evidence of atrophy in the left hippocampus and left temporal lobe, both of which are involved in learning and memory (460, 461). The anterior cingulate is involved in executive control of attention (459). The precuneus is involved in episodic memory (465). The occipital lobes are involved in visual perception (461). Apraxia was the most common examination abnormality in this group of patients. There was significant atrophy of the pre and post central gyri and atrophy of the left supplementary motor area (SMA). The SMA is involved in motor planning (464). Recent imaging studies have demonstrated a relationship between apraxia and the pre-supplementary area (472). Ideomotor apraxia has also been associated with lesions of the premotor cortex (473). The pattern of atrophy is consistent with the cognitive deficits found in this group of patients suggesting that there is an anatomical basis and correlate for the cognitive profile seen in this cohort.

On DTI analysis of white matter microstructural integrity, there was a statistically significant reduction in FA in the genu of the corpus callosum, bilateral anterior thalamic radiation, bilateral cingulum hippocampus, bilateral corticospinal tracts, bilateral inferior fronto-occipital fasciculi, bilateral superior longitudinal fasciculi, bilateral anterior corona radiata, bilateral forceps minor and forceps major ( $p < 0.0005$ ). There was a statistically significant decrease in axial diffusivity in the left anterior

thalamic radiation, left corticospinal tract and left forceps minor ( $p<0.01$ ). There was a statistically significant increase in axial diffusivity in the left forceps major ( $p<0.05$ ). There was a statistically significant increase in mean diffusivity in the genu of the corpus callosum, body of corpus callosum, bilateral anterior thalamic radiation, bilateral superior longitudinal fasciculi, right anterior corona radiata and right inferior fronto-occipital fasciculus ( $p<0.01$ ). There was increased radial diffusivity in the body of the corpus callosum, bilateral forceps minor, bilateral forceps major, left anterior thalamic radiation, left inferior longitudinal fasciculus, right inferior fronto-occipital fasciculus and right superior longitudinal fasciculus ( $p<0.0005$ ).

With regard to correlation to clinical and neuropsychological data there were correlations between some neuropsychological scores and increased FA, decreased MD and decreased RD suggesting that higher scores correlated with better white matter integrity. These were particularly evident for the list learning, figure copy and recall sub-scores on the RBANS.

FA and MD are sensitive markers of white matter integrity. Abnormalities in AD have been shown to correlate to axonal degeneration (402, 403). Increases in RD measures are associated with myelin pathology (391, 404). There was evidence of widespread disruption of white matter microstructural integrity with both axonal and myelin disruption. The most abnormalities were in FA, MD and RD with fewer abnormalities in AD measures thus possibly suggesting that disruption of myelin may be driving the white matter disruption seen in HAND. Demyelination is usually driven by



inflammation and these findings of abnormal radial diffusivity support the candidate's hypothesis that there are inflammatory changes in HAND in addition to degenerative changes which would be supported by the changes in axial diffusivity measures in HIV positive patients in this cohort.

Chapter 5 discussed the cerebrospinal fluid findings of 13 patients; nine of whom met criteria for ANI, three of whom met criteria for MND and one patient who had neuropsychological abnormalities that did not meet diagnostic criteria for HAND. Flow cytometry, cytokine analysis and analysis of tau and beta-amyloid were performed.

Half of the patients in the study group showed a greater proportion of CD8<sup>+</sup> T cells in PBMCs and more than half in CSF despite all of the patients being virally suppressed. The majority of CD4<sup>+</sup> and CD8<sup>+</sup> T cells bore an effector memory phenotype in the CSF (CD45<sup>-</sup>CD27<sup>+</sup>) suggesting activated T cells migrating from secondary lymphoid tissue to the brain. This suggests that despite viral suppression peripherally and centrally there are still memory cells present in the CSF attempting to control viral replication and possibly also suggesting that there is inflammation ongoing centrally to which CD8<sup>+</sup> T cells are responding or contributing as activated memory cells may release IFN- $\gamma$  and IL-2. There were low frequencies found of NK cells, NK like cells and gamma delta cells both in PBMCs and CSF suggesting that there may be activation of the innate immune response in these HIV positive patients with cognitive impairment. There were higher levels of B cells in the CSF compared to PBMCs. There were low levels detected in the CSF of all the patients of IL-6 and tumour necrosis factor alpha (TNF- $\alpha$ ) both of which

are pro-inflammatory. Tau and amyloid measurements were normal in all thirteen patients.

## **6.2 General Discussion**

The underlying hypothesis of the study was that there are inflammatory and/or immune mediated changes early on in HAND and that later on it becomes primarily a degenerative process. It was also the assertion of the candidate that there would be more cortical features in this group, both on neurocognitive testing and on MRI analysis.

This study has demonstrated that there was a high rate of a positive screen for cognitive impairment in a previously unscreened population attending a specialist HIV clinic in Ireland which was associated with multiple factors. The wide variety of factors that were associated with a positive screen for cognitive impairment highlights both the heterogeneity of the disease process and the patient group. Co-infection with hepatitis C, lack of virological suppression, a low nadir CD4 and a low current CD4 count are all likely to contribute to inflammation and ongoing immune mediated changes with the brain. Alcohol use is likely to be degenerative and is associated with cerebral atrophy and cognitive impairment. The use of methadone, anti-depressants, anti-psychotics and benzodiazepines confound the situation and are not definitively linked to inflammation or degeneration but clearly show a detrimental effect on cognitive function in this cohort of patients.

The amnesic, dysexecutive cognitive profile along with the presence of rapid forgetting and apraxia in smaller subsets of the study cohort support our hypothesis that there are evolving cortical features to HAND. This predominantly cortical profile in patients with HIV with minimal psychomotor speed issues or other deep grey matter symptoms (e.g. Parkinsonism) would support a neurodegenerative process with cortical neuronal loss similar to other cortical degenerative disorders like Alzheimer's disease. The MRI analysis demonstrated focal cortical atrophy in multiple areas and DTI showed evidence of widespread disruption of white matter integrity across all four measures (measures of both myelin loss and axonal injury) suggesting that there is indeed an inflammatory degenerative continuum in HIV related cognitive impairment.

### **6.3 Strengths**

The participants were well characterised and every care was taken to ensure good ascertainment of data with very few missing data points. All of the testing and studies were conducted in a single centre by the candidate. The neuropsychological batteries used are easy to apply and use clinically. The MRI data acquired was of excellent quality and VBM and DTI are accepted and sensitive biomarkers of cortical atrophy and white matter integrity disruption respectively. The VBM and DTI data were compared to a group of HIV negative healthy volunteers.



## **6.4 Limitations**

The study was cross-sectional in nature thus making casual associations difficult. As with all studies there may be a selection bias. There was a lack of culturally appropriate normative data for the neuropsychological batteries for all the participants. While there were 604 patients initially screened only 104 patients underwent neuropsychological assessment, only 50 patients had MRIs and only 13 patients had CSF examined therefore the numbers were smaller in the subsequent studies and make extrapolation more difficult to the entire cohort but the 104 who underwent neuropsychological testing were fairly representative of the cohort as a whole.

## **6.5 Future directions**

Longitudinal follow-up is essential to fully determine the nature and pathophysiology of HAND, in order to determine the rate of progression, if any, of the neurocognitive deficits found and to determine if those patients who had deficits in only one domain on neuropsychological testing progress to having further deficits and thus fulfil diagnostic criteria for HAND. Neurodegenerative disorders by their nature must be progressive and only longitudinal follow-up can confirm the hypothesis that there are neurodegenerative mechanisms to HAND particularly in the later phase of the disease. Further CSF analysis, both cross-sectionally and longitudinally, should be undertaken to further explore the relationship between immune mediated processes and HAND. It would be imperative to have comparison CSF from HIV positive patients with normal

cognitive testing and HIV negative patients to definitively determine the nature of the relationship between these immune markers and HAND.

Another research registrar will take over the project and perform longitudinal neuropsychological characterisation of the patients who underwent detailed neuropsychological testing and will also perform longitudinal follow-up of the patients who had MR imaging performed. This longitudinal study will allow correlation between follow-up clinical and neurocognitive testing and longitudinal MRI patterns and will enable us to learn more about how HIV affects the brain longitudinally. With longitudinal data, we will learn more about the neuropsychological profile of HAND in the post-HAART era and it will enable us to learn more about the natural history of HAND.

A physiotherapy and exercise intervention program will be commenced in the department in conjunction with the Academic Department of Physiotherapy in Trinity College Dublin. No proven pharmacological therapies have yet been shown to be effective in the treatment of HAND. This will be a non-pharmacological intervention with half of the group undergoing a structured exercise program and half receiving routine care. There will also be a group of HIV positive patients without cognitive impairment undergoing the same interventions for comparison purposes. The feasibility of an exercise program in those with dementia and cognitive impairment has been demonstrated (544). Chang et al have demonstrated a dose-response relationship between exercise duration and cognition (545). Aerobic fitness has been

demonstrated to be associated with less cerebral atrophy (546) and increased hippocampal volume and better spatial memory (547). HIV increases cardiovascular risk and the risk of cognitive impairment so a non-pharmacological intervention such as an exercise intervention program would hopefully be beneficial.

**6.6 Recommendations**

Only 604 patients were screened and over 2200 patients attend the GUIDE clinic for management of HIV thus it would be essential to extend the screening program to the rest of the group and to continue screening the entire cohort biennially. The European AIDS Clinical Society currently recommends screening all patients without a highly confounding condition every two years for cognitive impairment if they answer yes to any of the questions outlined in Table 2-1. It would be my recommendation that all patients should be screened for cognitive impairment biennially without any screening questions due to the high prevalence of cognitive impairment in this group. Nurses and doctors could be easily and quickly trained to administer the screening tool. This could be administered at any of their routine clinical attendances.

Patients with positive screens should then be referred to a specialist neurology service for full neurological evaluation. This should involve a complete neurological history, examination and neuropsychological evaluation. If on neuropsychological evaluation they meet diagnostic criteria for HAND they should have MRI and CSF examination as should those who have neuropsychological abnormalities in only one domain.



Neuropathological examination in the future would enable greater conclusions to be drawn with regard to causality of HAND.

Although there are no current treatments for HAND, I believe that it is important for patients to be screened and assessed for cognitive impairment. For patients whose testing is normal but who are concerned about the risk of cognitive impairment in HIV, it is reassuring. For those patients who are symptomatic it enables greater discussion and understanding of their symptoms, both for them and their treating physicians. It also allows for the development of strategies for the patients, their families and their treating physicians in terms of compliance with medications, coping strategies such as “to do” lists, avoiding multi-tasking, maintaining a routine and memory aids such as wearing a watch, keeping a diary, keeping important items such as their phone, wallet/purse and keys in the same place always. Additionally if any treatments become available in the future, patients will already be well characterised in terms of their cognition and this will enable their physicians to make quicker, more informed decisions about any potential new therapies for cognitive impairment.

## **6.7 Conclusion**

This was the first large scale cross-sectional study of cognitive impairment secondary to HIV undertaken in Ireland. A high prevalence rate of a positive screen for cognitive impairment was found. The findings of cortical deficits on both the neuropsychological batteries and the neurological examination in addition to the findings of grey matter atrophy demonstrated that there are neurodegenerative features to HAND. The

presence of abnormalities of RD on DTI analysis and immune activation on CSF examination strongly supported that there are immune mediated and inflammatory changes in HAND also. Overall the study strongly supported that there is an inflammatory degenerative continuum to HAND. This hypothesis can only be reliably confirmed with longitudinal neuropsychological, MRI and CSF follow-up and extensive neuropathological analysis of this well characterised cohort of patients with HIV.

## **7 Appendices**

### **Appendix 1: Patient Information Leaflet for Prevalence Study**

SJH / AMNCH RESEARCH ETHICS COMMITTEE

#### **Information and Consent for Patients**

##### **1. Title of study**

Cognitive impairment in HIV.

##### **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 attending the HIV clinic in St. James's Hospital will be offered screening tests to look for symptoms or signs of cognitive impairment. These screening tests are called the Brief NeuroCognitive Screen and the Hospital Anxiety and Depression Scale. This will involve a short test of memory, mood and some simple tasks. It will take 20-30 minutes of your time.

Patients will then be selected for further more detailed testing if they have evidence of memory problems on the initial screening tests. The more detailed tests will involve being seen by a neurology doctor and having a neurological examination. The neurological examination 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. It will also involve testing of your memory, thinking and concentration.

##### **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. Patricia McNamara who can be telephoned at 014103000 and extension 4146 or on mobile 086 7256193.

If your doctor learns of important new information that might affect your desire to remain in the study, he or she will tell you.

## **Appendix 2: Consent Form for Prevalence Study**

### **SJH / AMNCH RESEARCH ETHICS COMMITTEE**

#### **CONSENT FORM**

##### **Consent for study on Cognitive Impairment in HIV**

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.

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:

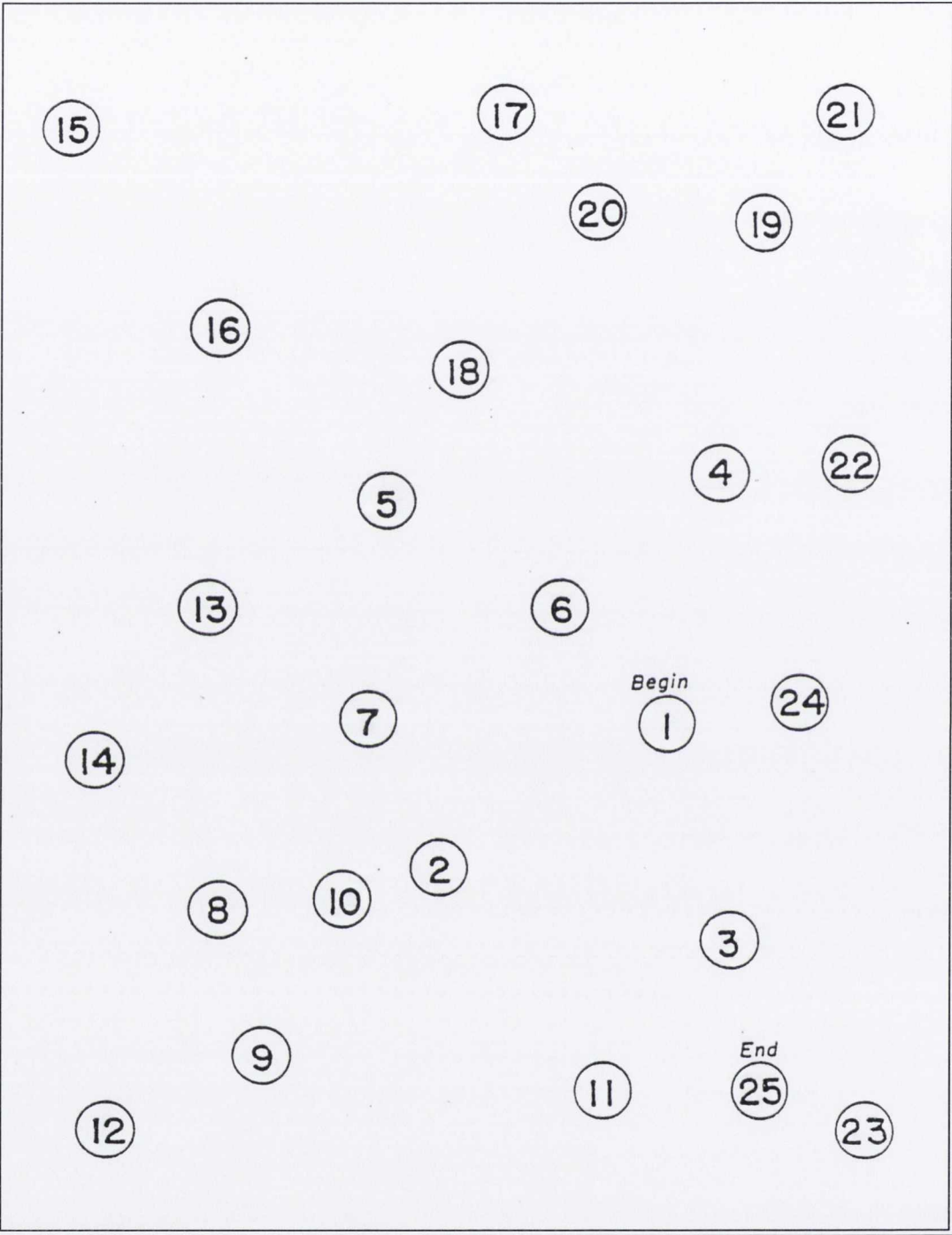
**Statement of investigator’s responsibility:** I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician’s signature:

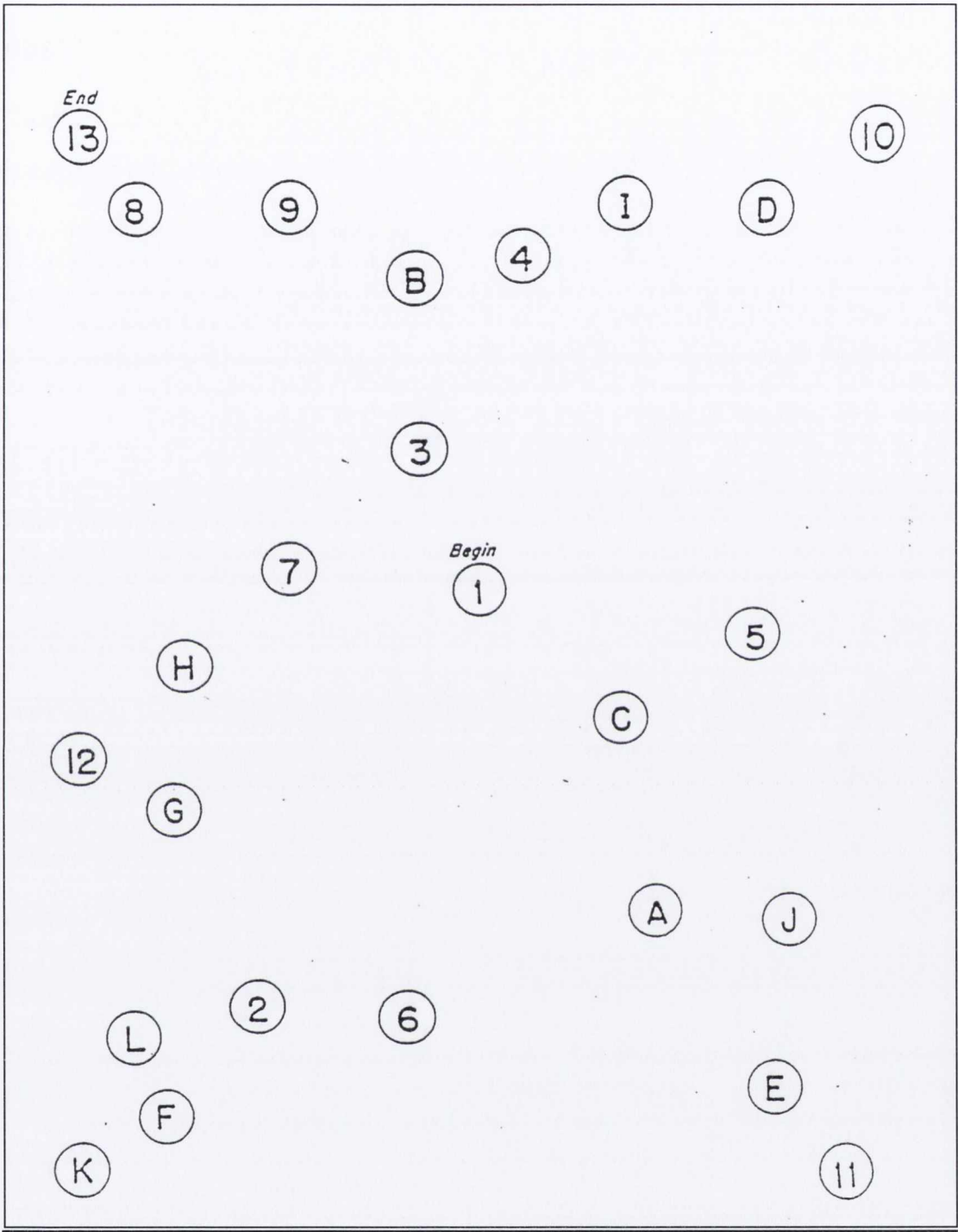
Date:



**Appendix 3: Copy of Tests Used to Assess for Cognitive Impairment**  
**Trail Making A**



**Trail Making B**



Digit Symbol Test

|                 |        |        |        |        |        |        |        |        |        |                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DIGIT<br>SYMBOL | 1<br>— | 2<br>⊥ | 3<br>⊐ | 4<br>L | 5<br>⊏ | 6<br>O | 7<br>^ | 8<br>X | 9<br>= | SCORE<br><div></div> |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| SAMPLES         |        |        |        |        |        |        |        |        |        |                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 2               | 1      | 3      | 7      | 2      | 4      | 8      | 2      | 1      | 3      | 2                    | 1 | 4 | 2 | 3 | 5 | 2 | 3 | 1 | 4 | 5 | 6 | 3 | 1 | 4 |
|                 |        |        |        |        |        |        |        |        |        |                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 1               | 5      | 4      | 2      | 7      | 6      | 3      | 5      | 7      | 2      | 8                    | 5 | 4 | 6 | 3 | 7 | 2 | 8 | 1 | 9 | 5 | 8 | 4 | 7 | 3 |
|                 |        |        |        |        |        |        |        |        |        |                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 6               | 2      | 5      | 1      | 9      | 2      | 8      | 3      | 7      | 4      | 6                    | 5 | 9 | 4 | 8 | 3 | 7 | 2 | 6 | 1 | 5 | 4 | 6 | 3 | 7 |
|                 |        |        |        |        |        |        |        |        |        |                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 9               | 2      | 8      | 1      | 7      | 9      | 4      | 6      | 8      | 5      | 9                    | 7 | 1 | 8 | 5 | 2 | 9 | 4 | 8 | 6 | 3 | 7 | 9 | 8 | 6 |
|                 |        |        |        |        |        |        |        |        |        |                      |   |   |   |   |   |   |   |   |   |   |   |   |   |   |



Appendix 4: Hospital Anxiety and Depression Scale

Read each item and place a firm tick in the box opposite the reply that comes closest to how you have been feeling in the past week.

Don't take too long over your replies; your immediate reaction to each item will probably be more accurate than a long thought-out response.

Tick only one box in each section

|                             |   |  |
|-----------------------------|---|--|
| I feel tense or "wound up": | A |  |
| Most of the time            | 3 |  |
| A lot of time               | 2 |  |
| Time to time, Occasionally  | 1 |  |
| Not at all                  | 0 |  |

|                                           |   |   |
|-------------------------------------------|---|---|
| I still enjoy the things I used to enjoy: | D |   |
| Definitely as much                        |   | 3 |
| Not quite so much                         |   | 2 |
| Only a little                             |   | 1 |
| Hardly at all                             |   | 0 |

|                                                                              |   |  |
|------------------------------------------------------------------------------|---|--|
| I get a sort of frightened feeling as if something awful is about to happen: | A |  |
| Very definitely and quite badly                                              | 3 |  |
| Yes, but not too badly                                                       | 2 |  |
| A little, but it doesn't worry me                                            | 1 |  |
| Not at all                                                                   | 0 |  |

|                                               |   |   |
|-----------------------------------------------|---|---|
| I can laugh and see the funny side of things: | D |   |
| As much as always could                       |   | 3 |
| Not quite so much now                         |   | 2 |
| Definitely not so much now                    |   | 1 |
| Not at all                                    |   | 0 |

|                                       |   |  |
|---------------------------------------|---|--|
| Worrying thoughts go through my mind: | A |  |
| A great deal of the time              | 3 |  |
| A lot of the time                     | 2 |  |

|                                            |          |  |
|--------------------------------------------|----------|--|
| <i>From time to time but not too often</i> | <i>1</i> |  |
| <i>Only occasionally</i>                   | <i>0</i> |  |

|                         |          |          |
|-------------------------|----------|----------|
| <b>I feel cheerful:</b> | <b>D</b> |          |
| <i>Not at all</i>       |          | <i>3</i> |
| <i>Not often</i>        |          | <i>2</i> |
| <i>Sometimes</i>        |          | <i>1</i> |
| <i>Most of the time</i> |          | <i>0</i> |

|                                            |          |  |
|--------------------------------------------|----------|--|
| <b>I can sit at ease and feel relaxed:</b> | <b>A</b> |  |
| <i>Definitely</i>                          | <i>3</i> |  |
| <i>Usually</i>                             | <i>2</i> |  |
| <i>Not often</i>                           | <i>1</i> |  |
| <i>Not at all</i>                          | <i>0</i> |  |

|                                       |          |          |
|---------------------------------------|----------|----------|
| <b>I feel as if I am slowed down:</b> | <b>D</b> |          |
| <i>Nearly all the time</i>            |          | <i>3</i> |
| <i>Very often</i>                     |          | <i>2</i> |
| <i>Sometimes</i>                      |          | <i>1</i> |
| <i>Not at all</i>                     |          | <i>0</i> |

|                                                                              |          |  |
|------------------------------------------------------------------------------|----------|--|
| <b>I get a sort of frightened feeling like “butterflies” in the stomach:</b> | <b>A</b> |  |
| <i>Not at all</i>                                                            | <i>3</i> |  |
| <i>Occasionally</i>                                                          | <i>2</i> |  |
| <i>Quite often</i>                                                           | <i>1</i> |  |
| <i>Very often</i>                                                            | <i>0</i> |  |

|                                               |          |          |
|-----------------------------------------------|----------|----------|
| <b>I have lost interest in my appearance:</b> | <b>D</b> |          |
| <i>Definitely</i>                             |          | <i>3</i> |
| <i>I don’t take so much care as I should</i>  |          | <i>2</i> |
| <i>I may not take quite as much care</i>      |          | <i>1</i> |
| <i>I take just as much care as ever</i>       |          | <i>0</i> |

|                                                        |          |  |
|--------------------------------------------------------|----------|--|
| <b>I feel restless as if I have to be on the move:</b> | <b>A</b> |  |
| <i>Very much indeed</i>                                | <i>3</i> |  |
| <i>Quite a lot</i>                                     | <i>2</i> |  |
| <i>Not very much</i>                                   | <i>1</i> |  |
| <i>Not at all</i>                                      | <i>0</i> |  |

|                                                 |          |          |
|-------------------------------------------------|----------|----------|
| <b>I look forward with enjoyment to things:</b> | <b>D</b> |          |
| <i>As much as ever I did</i>                    |          | <i>3</i> |
| <i>Rather less than I used to</i>               |          | <i>2</i> |
| <i>Definitely less than I used to</i>           |          | <i>1</i> |

|                      |  |   |
|----------------------|--|---|
| <i>Hardly at all</i> |  | 0 |
|----------------------|--|---|

|                                        |          |  |
|----------------------------------------|----------|--|
| <b>I get sudden feelings of panic:</b> | <b>A</b> |  |
| <i>Very often indeed</i>               | 3        |  |
| <i>Quite often</i>                     | 2        |  |
| <i>Not very often</i>                  | 1        |  |
| <i>Not at all</i>                      | 0        |  |

|                                                          |          |   |
|----------------------------------------------------------|----------|---|
| <b>I can enjoy a good book or radio or TV programme:</b> | <b>D</b> |   |
| <i>Often</i>                                             |          | 3 |
| <i>Sometimes</i>                                         |          | 2 |
| <i>Not often</i>                                         |          | 1 |
| <i>Very seldom</i>                                       |          | 0 |



Appendix 5: Case Report Form for Prevalence Study

Case Report Form

Official Use Only

Study Number: \_\_\_\_\_

Date of Study: \_\_\_\_\_

Handedness – Right                      Left \_\_\_\_\_

Symptoms – Yes                      No \_\_\_\_\_

Age: \_\_\_\_\_

Gender:              Male                      Female \_\_\_\_\_

Country of birth: \_\_\_\_\_

Year of diagnosis with HIV: \_\_\_\_\_

Mode of transmission of HIV: \_\_\_\_\_

- IVDU
- MSM
- Heterosexual
- Vertical
- Blood Products
- Occupational

Nadir CD4 count: \_\_\_\_\_

Current CD4 count: \_\_\_\_\_

Current Viral Load: \_\_\_\_\_

Medical History:

| Name                  | Yes | No | Pre-existing | New |
|-----------------------|-----|----|--------------|-----|
| Hepatitis B           |     |    |              |     |
| Hepatitis C           |     |    |              |     |
| Hypertension          |     |    |              |     |
| Hypercholesterolaemia |     |    |              |     |
| Diabetes              |     |    |              |     |
| Syphilis              |     |    |              |     |

Neurological disorders/complications of HIV:

| Name                    | Yes | No | Pre-existing | New |
|-------------------------|-----|----|--------------|-----|
| Cryptococcal meningitis |     |    |              |     |
| CNS TB                  |     |    |              |     |
| Toxoplasmosis           |     |    |              |     |
| Encephalitis            |     |    |              |     |
| PML                     |     |    |              |     |
| IRIS                    |     |    |              |     |
| Epilepsy                |     |    |              |     |
| Neuropathy              |     |    |              |     |
| Stroke                  |     |    |              |     |

Psychiatric History:

| Name          | Yes | No | Pre-existing | New |
|---------------|-----|----|--------------|-----|
| Depression    |     |    |              |     |
| BPAD          |     |    |              |     |
| Anxiety       |     |    |              |     |
| Schizophrenia |     |    |              |     |
| Psychosis     |     |    |              |     |

Medications (other than ART):

| Name               | Yes | No | Details |
|--------------------|-----|----|---------|
| Statins            |     |    |         |
| Anti-hypertensives |     |    |         |
| Anti-epileptics    |     |    |         |
| Methadone          |     |    |         |
| Benzodiazepines    |     |    |         |
| Anti-depressants   |     |    |         |
| Anti-psychotics    |     |    |         |

Highest Level of Education Reached:

- None
- Primary Level
- Secondary Level
- College/University

Employment:

- Student
- Employed
- Unemployed

Smoking history:

- Smoker
- Ex-smoker (> 3 months stopped)
- Never

Alcohol History:

- None
- <10 units per week
- 10-20 units per week
- 20-30 units per week
- 30-40 units per week
- >40 units per week

Illicit Substances:

- Never
- Yes
- IV drug use
- Previous (> 3 months)

Tick all drugs that are being taken currently or have been taken in the past:

Heroin  
Cocaine  
Marijuana/cannabis  
Ecstasy  
Methamphetamines  
Headshop substances

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

ART:            Yes                      No                      Only for MTCT

\_\_\_\_\_

Date of First ART:

\_\_\_\_\_

Date of Commencement of Most recent ART:

\_\_\_\_\_

| <u>Current ART</u>                                                       |  | <u>Past Rx</u>                                                           |  |
|--------------------------------------------------------------------------|--|--------------------------------------------------------------------------|--|
|                                                                          |  |                                                                          |  |
| <b>Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI)</b> |  | <b>Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI)</b> |  |
|                                                                          |  |                                                                          |  |
| Zidovudine                                                               |  | Zidovudine                                                               |  |
| Didanosine                                                               |  | Didanosine                                                               |  |
| Zalcitabine                                                              |  | Zalcitabine                                                              |  |
| Stavudine                                                                |  | Stavudine                                                                |  |
| Lamivudine                                                               |  | Lamivudine                                                               |  |
| Abacavir                                                                 |  | Abacavir                                                                 |  |
| Tenofovir disoproxil                                                     |  | Tenofovir disoproxil                                                     |  |
| Emtricitabine                                                            |  | Emtricitabine                                                            |  |
|                                                                          |  |                                                                          |  |
| <b>Non-nucleoside reverse transcriptase inhibitors (NNRTI)</b>           |  | <b>Non-nucleoside reverse transcriptase inhibitors (NNRTI)</b>           |  |
| Nevirapine                                                               |  | Nevirapine                                                               |  |
| Delavirdine                                                              |  | Delavirdine                                                              |  |
| Efavirenz                                                                |  | Efavirenz                                                                |  |
| Etravirine                                                               |  | Etravirine                                                               |  |
|                                                                          |  |                                                                          |  |
| <b>Protease inhibitors (PI)</b>                                          |  | <b>Protease inhibitors (PI)</b>                                          |  |
| Saquinavir                                                               |  | Saquinavir                                                               |  |
| Indinavir                                                                |  | Indinavir                                                                |  |
| Ritonavir                                                                |  | Ritonavir                                                                |  |
| Nelfinavir                                                               |  | Nelfinavir                                                               |  |
| Amprenavir                                                               |  | Amprenavir                                                               |  |
| Lopinavir                                                                |  | Lopinavir                                                                |  |
| Fosamprenavir                                                            |  | Fosamprenavir                                                            |  |
| Atazanavir                                                               |  | Atazanavir                                                               |  |
| Tipranavir                                                               |  | Tipranavir                                                               |  |
| Darunavir                                                                |  | Darunavir                                                                |  |
|                                                                          |  |                                                                          |  |



|                                  |  |                                  |  |
|----------------------------------|--|----------------------------------|--|
| <b>Integrase inhibitors (II)</b> |  | <b>Integrase inhibitors (II)</b> |  |
| Raltegravir                      |  | Raltegravir                      |  |
|                                  |  |                                  |  |
| <b>Fusion inhibitors (FI)</b>    |  | <b>Fusion inhibitors (FI)</b>    |  |
| Enfuvirtide (T-20)               |  | Enfuvirtide (T-20)               |  |
|                                  |  |                                  |  |
| <b>Anti-CCR5</b>                 |  | <b>Anti-CCR5</b>                 |  |
| Maraviroc                        |  | Maraviroc                        |  |

**Results of Screening tests:**

BNCS

Trails A

Trails B

WAIS-R

HADS

## Appendix 6: Data Dictionary for Prevalence Study

| Abbrev  | Full Name of Variable |                        | Definition of Variable                          |  | Source(s) of Variable |  | Type of Variable |             | Measurement |                                                                                   |
|---------|-----------------------|------------------------|-------------------------------------------------|--|-----------------------|--|------------------|-------------|-------------|-----------------------------------------------------------------------------------|
|         | Study_ID              | Study ID Number        | Number assigned chronologically to participants |  | Assigned              |  | Q/Q              | C/D         | Level       | Coding Option                                                                     |
|         |                       |                        |                                                 |  |                       |  | NA               | NA          | NA          | Enter Number                                                                      |
| DoStudy |                       | Date of Study          | Date of review of patient                       |  | Case Report Form      |  | Date             | Date        | NA          | Enter in date format (dd/mm/yyyy)                                                 |
|         |                       |                        |                                                 |  |                       |  |                  |             |             |                                                                                   |
| Hand    |                       | Handedness             | Handedness of patient                           |  | Case Report Form      |  | Qualitative      | Categorical | Nominal     | 1=Right handed<br>2=Left handed                                                   |
|         |                       |                        |                                                 |  |                       |  |                  |             |             |                                                                                   |
| Symp    |                       | Symptoms               | Endorsement of cognitive symptoms               |  | Case Report Form      |  | Qualitative      | Categorical | Nominal     | 1=Yes<br>2=No                                                                     |
|         |                       |                        |                                                 |  |                       |  |                  |             |             |                                                                                   |
| Age     |                       | Age at Review          | Age of participant at Date of Review            |  | Case Report Form      |  | Quantitative     | Continuous  | Interval    | Enter as number                                                                   |
|         |                       |                        |                                                 |  |                       |  |                  |             |             |                                                                                   |
| Gender  |                       | Gender                 | Gender of patient                               |  | Case Report Form      |  | Qualitative      | Categorical | Nominal     | 1=Male<br>2=Female                                                                |
|         |                       |                        |                                                 |  |                       |  |                  |             |             |                                                                                   |
| CoB     |                       | Country of Birth       | Country of birth of patient                     |  | Case Report Form      |  | Qualitative      | Categorical | Nominal     | 1=Ireland<br>2=Rest of Europe<br>3=Africa<br>4=Asia<br>5=South America<br>6=Other |
|         |                       |                        |                                                 |  |                       |  |                  |             |             |                                                                                   |
| Eng_1st |                       | English First Language | English as a first language                     |  | Case Report Form      |  | Qualitative      | Categorical | Nominal     | 1=Yes<br>2=No                                                                     |

|         |                      |                                                              |                                      |              |             |          |                                                             |
|---------|----------------------|--------------------------------------------------------------|--------------------------------------|--------------|-------------|----------|-------------------------------------------------------------|
| Yr_Dx   | Year of Diagnosis    | Year of diagnosis with HIV                                   | Case Report Form or<br>Medical Chart | Date         | Date        | NA       | Enter in date<br>format<br>(dd/mm/yyyy)                     |
| Len_Dx  | Length of Diagnosis  | Length of time elapsed since diagnosis at time of<br>review  | Compute from dates                   | Quantitative | Continuous  | Interval | (Computed)<br>Enter as number                               |
| MOT     | Mode of Transmission | Mode of transmission of HIV                                  | Case Report Form or<br>Medical Chart | Qualitative  | Categorical | Nominal  | 1=Heterosexual<br>2=MSM<br>3=IV/DU<br>4=Vertical<br>5=Other |
| N_CD4   | Nadir CD4 count      | Lowest CD4 count                                             | Medical chart or EPR                 | Quantitative | Continuous  | Interval | Enter as number                                             |
| C_CD4   | Current CD4 count    | CD4 count at time of review                                  | Electronic Patient<br>Record         | Quantitative | Continuous  | Interval | Enter as number                                             |
| VL      | Current Viral Load   | HIV Viral Load at time of review                             | Electronic Patient<br>Record         | Quantitative | Continuous  | Interval | Enter as number                                             |
|         |                      |                                                              |                                      |              |             |          | Except for not<br>detected - enter<br>as                    |
|         |                      |                                                              |                                      |              |             |          | ND                                                          |
| Vir_Sup | Viral suppression    | Viral suppression at time of review for patients on<br>HAART | Calculated from VL                   | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No<br>3=Not on HAART                             |
| Hep B   | Hepatitis B          | If the patient also has hepatitis B                          | Case Report Form or<br>Medical Chart | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No                                               |
| Hep C   | Hepatitis C          | If the patient also has hepatitis C                          | Case Report Form or                  | Qualitative  | Categorical | Nominal  | 1=Yes                                                       |









|          |                    |                                                 |                                   |              |             |          |                                                      |
|----------|--------------------|-------------------------------------------------|-----------------------------------|--------------|-------------|----------|------------------------------------------------------|
| Anti_Hyp | Anti-hypertensives | If the patient is taking any anti-hypertensives | Case Report Form or Medical Chart | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No                                        |
| AEDs     | Anti-epileptics    | if the patient is taking any anti-epileptics    | Case Report Form or Medical Chart | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No                                        |
| Meth     | Methadone          | If the patient is taking methadone              | Case Report Form or Medical Chart | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No                                        |
| Benzos   | Benzodiazepines    | If the patient is taking any benzodiazepines    | Case Report Form or Medical Chart | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No                                        |
| Anti_Dep | Anti-depressants   | If the patient is taking anti-depressants       | Case Report Form or Medical Chart | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No                                        |
| Anti_Psy | Anti-psychotics    | If the patient is taking anti-psychotics        | Case Report Form or Medical Chart | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No                                        |
| Educ     | Years of education | Numbers of years spent in education             | Case Report Form                  | Quantitative | Continuous  | Interval | Enter as number                                      |
| Employ   | Employment status  | Employment status of patient                    | Case Report Form                  | Qualitative  | Categorical | Nominal  | 1=Employed<br>2=Student<br>3=Unemployed<br>4=Retired |
| Smoking  | Smoking history    | Smoking history of the patient                  | Case Report Form                  | Qualitative  | Categorical | Nominal  | 1=Smoker<br>2=Non Smoker<br>3=Ex-smoker              |
| Alcohol  | Alcohol History    | Alcohol consumption of the patient per week     | Case Report Form                  | Qualitative  | Categorical | Nominal  | 1=None                                               |





|        |               |                                              |                                      |             |             |         |               |
|--------|---------------|----------------------------------------------|--------------------------------------|-------------|-------------|---------|---------------|
| Zid    | Zidovudine    | If the patient is on zidovudine currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Did    | Didanosine    | If the patient is on didanosine currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Stav   | Stavudine     | If the patient is on stavudine currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Lam    | Lamivudine    | If the patient is on lamivudine currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Abac   | Abacavir      | If the patient is on abacavir currently      | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Tenof  | Tenofovir     | If the patient is on tenofovir currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Emtric | Emtricitabine | If the patient is on emtricitabine currently | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Nev    | Nevirapine    | If the patient is on nevirapine currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Efav   | Efavirenz     | If the patient is on efavirenz currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Etrav  | Etravirine    | If the patient is on etravirine currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
| Saq    | Saquinavir    | If the patient is on saquinavir currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |

|        |               |                                              |                                      |             |             |         |               |
|--------|---------------|----------------------------------------------|--------------------------------------|-------------|-------------|---------|---------------|
| Ind    | Indinavir     | If the patient is on indinavir currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Rit    | Ritonavir     | If the patient is on ritonavir currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Nelf   | Nelfinavir    | If the patient is on nelfinavir currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Amp    | Amprenavir    | If the patient is on amprenavir currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Lop    | Lopinavir     | If the patient is on lopinavir currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Fosamp | Fosamprenavir | If the patient is on fosamprenavir currently | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Ataz   | Atazanavir    | If the patient is on atazanavir currently    | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Dar    | Darunavir     | If the patient is on darunavir currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Ralteg | Raltegravir   | If the patient is on raltegravir currently   | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |
| Mara   | Maraviroc     | If the patient is on maraviroc currently     | Medical Chart or<br>Pharmacy Records | Qualitative | Categorical | Nominal | 1=Yes<br>2=No |
|        |               |                                              |                                      |             |             |         |               |
|        |               |                                              |                                      |             |             |         |               |



| CPE      | CPE Score            | CNS Penetration Effectiveness Score of current | Calculated from current     | Quantitative | Continuous  | Interval | Enter as number |
|----------|----------------------|------------------------------------------------|-----------------------------|--------------|-------------|----------|-----------------|
|          |                      | HAART regime                                   | HAART regime                |              |             |          |                 |
| Trails_A | Trails A             | Score from Trails A section of BNCS            | Case Report Form            | Quantitative | Continuous  | Interval | Enter as number |
| Trails_B | Trails B             | Score from Trails B section of BNCS            | Case Report Form            | Quantitative | Continuous  | Interval | Enter as number |
| DST      | Digit Symbol Test    | Score from Digit Symbol section of BNCS        | Case Report Form            | Quantitative | Continuous  | Interval | Enter as number |
| HADS_A   | HADS Anxiety         | Score from anxiety section of HADS             | Case Report Form            | Quantitative | Continuous  | Interval | Enter as number |
| HADS_D   | HADS Depression      | Score from depression section of HADS          | Case Report Form            | Quantitative | Continuous  | Interval | Enter as number |
| CI       | Cognitive Impairment | Positive Screen for Cognitive Impairment       | Calculated from BNCS Scores | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No   |
| Anx_S    | Anxiety Screen       | Positive Screen for Anxiety                    | Calculated from HADS Scores | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No   |
| Dep_S    | Depression Screen    | Positive Screen for Depression                 | Calculated from HADS Scores | Qualitative  | Categorical | Nominal  | 1=Yes<br>2=No   |

## **Appendix 7: Patient Information Leaflet for Prevalence Study**

SJH / AMNCH RESEARCH ETHICS COMMITTEE

### **Information and Consent for Patients**

#### **1. Title of study**

The Inflammatory Neurodegenerative Continuum in HIV Related Cognitive Impairment – Detailed Neuropsychological Testing

#### **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:**

Patients attending the HIV clinic in St. James's Hospital who have evidence of cognitive impairment on screening tests will be offered detailed neuropsychological testing (memory testing). The more detailed tests will involve being seen by a neurology doctor and having a neurological examination and examination of your memory and thinking. The neurological examination 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. It will also involve testing of your memory, thinking and concentration.

#### **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. Patricia McNamara who can be telephoned at 014103000 and extension 4146.

If your doctor learns of important new information that might affect your desire to remain in the study, he or she will tell you.



**Appendix 8: Consent Form for Detailed Neuropsychological Study  
SJH / AMNCH RESEARCH ETHICS COMMITTEE CONSENT FORM**

**Consent for study "The Inflammatory Neurodegenerative Continuum in HIV Related  
Cognitive Impairment – Detailed Neuropsychological Testing"**

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:

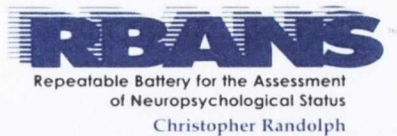
SIGNATURE:

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:

Appendix 9: Neuropsychological Tests Used



UK Adaptation  
Record Form A

Name \_\_\_\_\_ Age \_\_\_\_\_ Sex \_\_\_\_\_ Education Level \_\_\_\_\_

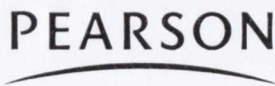
Examiner \_\_\_\_\_ Date of Testing \_\_\_\_\_ Ethnicity \_\_\_\_\_

Observations: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

|                                  | Immediate<br>Memory | Visuospatial/<br>Constructional | Language | Attention | Delayed<br>Memory |                 | Total<br>Scale             |
|----------------------------------|---------------------|---------------------------------|----------|-----------|-------------------|-----------------|----------------------------|
| Index<br>Score                   |                     |                                 |          |           |                   |                 |                            |
| Confidence<br>Interval<br>_____% |                     |                                 |          |           |                   |                 |                            |
| Percentile                       |                     |                                 |          |           |                   |                 |                            |
| Index Score                      |                     |                                 |          |           |                   | Percentile Rank | Total Scale<br>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                         |



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# Information and Orientation (Optional)

Scoring: All responses verbatim. Items 1-14: 0-1 point for each item. Do not score Items 15-18.

| Item                                                                                                                    | Response | Score<br>0 or 1 |
|-------------------------------------------------------------------------------------------------------------------------|----------|-----------------|
| 1. What is your full name?                                                                                              |          |                 |
| 2. How old are you?                                                                                                     |          |                 |
| 3. When were you born?                                                                                                  |          |                 |
| 4. Where were you born?                                                                                                 |          |                 |
| 5. What is your mother's first name? (If examinee does not know, ask, "Then what is your father's name?")               |          |                 |
| 6. Who is the Prime Minister of the United Kingdom?                                                                     |          |                 |
| 7. Who was the Prime Minister before him?                                                                               |          |                 |
| 8. What year is this?                                                                                                   |          |                 |
| 9. What month is this?                                                                                                  |          |                 |
| 10. What day of the month is this?                                                                                      |          |                 |
| 11. What is the name of the place you are in?                                                                           |          |                 |
| 12. In what city/town is it?                                                                                            |          |                 |
| 13. What day of the week is it?                                                                                         |          |                 |
| 14. Without looking at the clock, what time is it now? reponse ____:____ actual time ____:____ difference in mins. ____ |          |                 |
| 15. Are you left-handed or right-handed?                                                                                |          |                 |
| 16. Do you have any difficulty in hearing?                                                                              |          |                 |
| 17. Do you need glasses for reading?                                                                                    |          |                 |
| 18. Are you colour-blind?                                                                                               |          |                 |

Total Score  
Range=0-14

# 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?*

**Trial 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   |         |         |         |         |
| Motorway |         |         |         |         |
| Saddle   |         |         |         |         |
| Powder   |         |         |         |         |

|                |               |               |               |               |                           |
|----------------|---------------|---------------|---------------|---------------|---------------------------|
| Number Correct | +             | +             | +             | =             |                           |
|                | Total Trial 1 | Total Trial 2 | Total Trial 3 | Total Trial 4 | Total Score<br>Range=0-40 |

# 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 colour within parentheses. Record intrusions or variations in the Responses column.

| Story                                                        | Responses | Trial 1 Score<br>(0 or 1) | Trial 2 Score<br>(0 or 1) | Item Score<br>(0-2) |
|--------------------------------------------------------------|-----------|---------------------------|---------------------------|---------------------|
| 1. On <b>Tuesday</b> ,                                       |           |                           |                           |                     |
| 2. <b>Fourth</b>                                             |           |                           |                           |                     |
| 3. of <b>May</b> ,                                           |           |                           |                           |                     |
| 4. in <b>Grasmere</b> , Cumbria                              |           |                           |                           |                     |
| 5. a <b>serious</b>                                          |           |                           |                           |                     |
| 6. <b>fire</b> broke out,                                    |           |                           |                           |                     |
| 7. <b>Two</b>                                                |           |                           |                           |                     |
| 8. <b>hotels</b>                                             |           |                           |                           |                     |
| 9. and a <b>restaurant</b>                                   |           |                           |                           |                     |
| 10. were <b>destroyed</b>                                    |           |                           |                           |                     |
| 11. before the <b>firemen</b> ( <b>firefighters</b> )        |           |                           |                           |                     |
| 12. were able to <b>put it out</b> ( <b>extinguish it</b> ). |           |                           |                           |                     |
| Total Score<br>(Trial 1 + 2)<br>Range =0-24                  |           |                           |                           |                     |

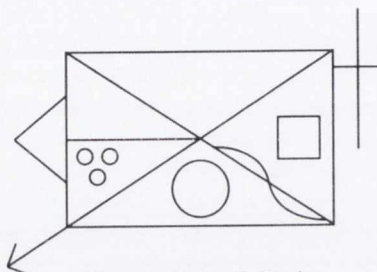
### 3 Figure Copy

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 on the exactness of his or her copy.

**Say:** *In front of you is a figure. I want you to make an exact copy, but, before you begin, I need to tell you I will be timing you. It is important that you make an exact copy of the figure. To let me know you understand can you tell me what I have asked? (Correct as necessary and re-enforce exactness of 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.)

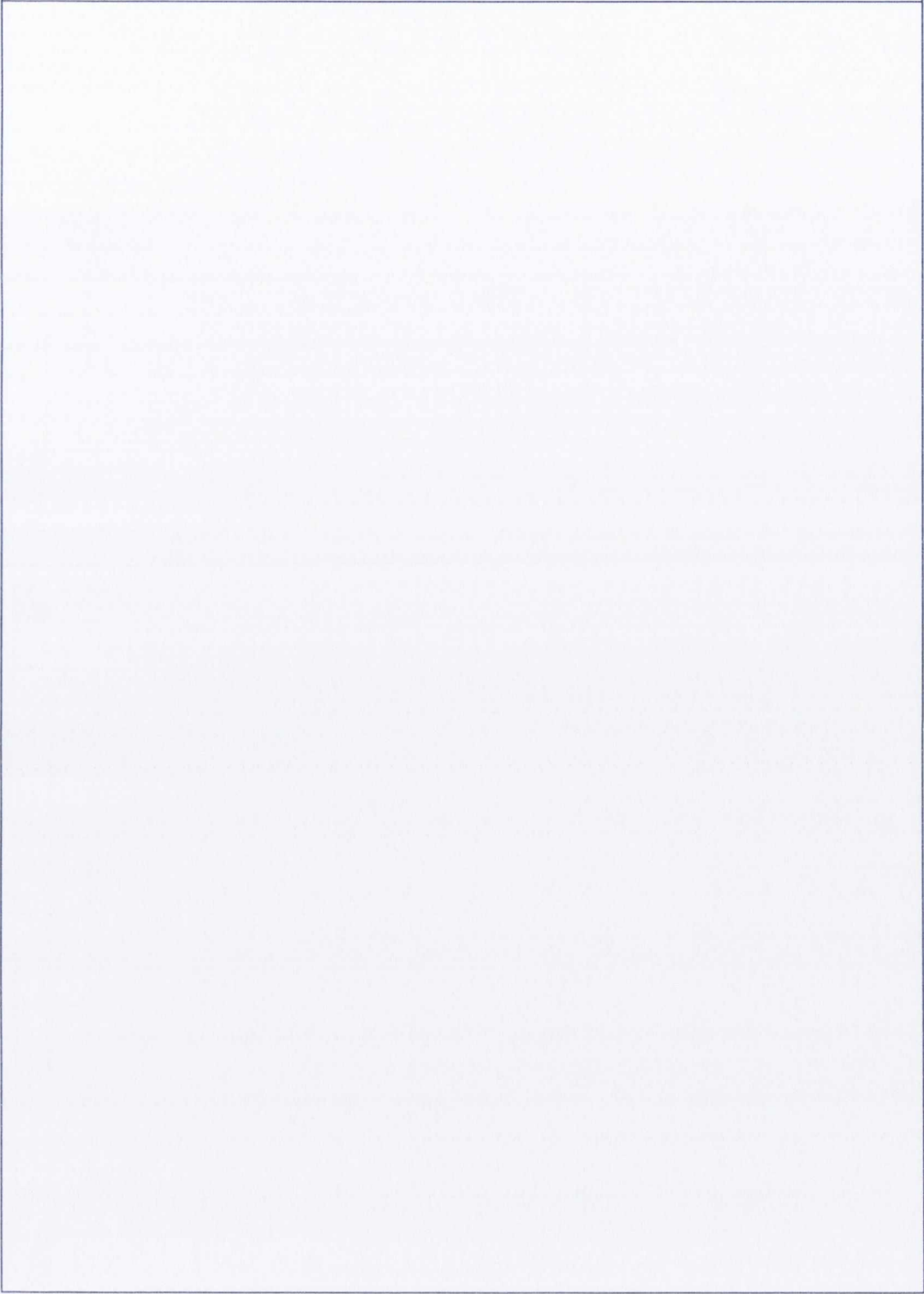
| Item               | Drawing<br>(0 or 1) | Placement<br>(0 or 1) | Score<br>(0, 1, or 2) | Scoring Criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------|---------------------|-----------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. rectangle       |                     |                       |                       | <b>Drawing:</b> lines unbroken and relatively straight; it appears a rectangle.<br><b>Placement:</b> not rotated more than 15 degrees.                                                                                                                                                                                                                                                                                                                               |
| 2. diagonal cross  |                     |                       |                       | <b>Drawing:</b> lines are unbroken, relatively straight and approximately bisect each other.<br><b>Placement:</b> ends of lines should meet corners without significant overlap or significant distance from the corners.                                                                                                                                                                                                                                            |
| 3. horizontal line |                     |                       |                       | <b>Drawing:</b> line is unbroken and relatively straight; should not exceed approximately 1/2 of the width.<br><b>Placement:</b> from approximately the centre of the left side and intersect at approximately the diagonal cross.                                                                                                                                                                                                                                   |
| 4. circle          |                     |                       |                       | <b>Drawing:</b> relatively round, unbroken, and relatively closed; diameter should be approximately 1/4 - 1/3 height of triangle.<br><b>Placement:</b> placed in appropriate segment; not touching any other figure.                                                                                                                                                                                                                                                 |
| 5. 3 small circles |                     |                       |                       | <b>Drawing:</b> relatively round, unbroken and relatively closed; approximately equal in size; approximate triangular arrangement; not touching each other.<br><b>Placement:</b> in appropriate segment; not touching figure; triangle formed not rotated more than approximately 15 degrees.                                                                                                                                                                        |
| 6. square          |                     |                       |                       | <b>Drawing:</b> relatively closed; appears to be a square; lines relatively straight and unbroken; height is approximately 1/4 - 1/3 of triangle.<br><b>Placement:</b> in appropriate segment; not touching any other part of figure; not rotated more than approximately 15 degrees.                                                                                                                                                                                |
| 7. curving line    |                     |                       |                       | <b>Drawing:</b> 2 curved segments; approximately equal in appearance; correct direction of curves.<br><b>Placement:</b> ends of lines approximately touch diagonal; do not touch very corner of rectangle or diagonal intersection.                                                                                                                                                                                                                                  |
| 8. outside cross   |                     |                       |                       | <b>Drawing:</b> vertical line is relatively parallel to side of rectangle; horizontal line crosses the vertical at approximately 90 degrees and is between 20-50% of length of vertical line.<br><b>Placement:</b> horizontal line touches rectangle higher than 2/3 height of rectangle, but below top; it approximately touches the rectangle; vertical line stretches above the height of the rectangle and down to approximately the mid-point of the rectangle. |
| 9. triangle        |                     |                       |                       | <b>Drawing:</b> angle formed by two sides is between approximately 60-100 degrees; sides are relatively straight, unbroken and meet in a point; distance on vertical side of triangle subsumed is approximately 50% of the height of vertical side.<br><b>Placement:</b> approximately at top of rectangle.                                                                                                                                                          |
| 10. arrow          |                     |                       |                       | <b>Drawing:</b> relatively straight and unbroken; lines forming arrow are approximately equal in length.<br><b>Placement:</b> protrudes from appropriate corner of rectangle; it appears an approximate continuation of diagonal staff.                                                                                                                                                                                                                              |

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 *There are a series of lines at the top here* (point to the lines). *There are two lines down here* (point to each one) *and they match two of the lines at the top.* *Can you tell the numbers or point to the lines that they match. This is a practice. Do you understand what you need to do because I cannot give you any more support?* (If the individual is struggling during the task offer help) *Can you show me where the two lines down here might be among these lines up here* (run fingers across all the array of lines at the top). 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<br>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. sailing boat           | used on the water (if "boat," query "what kind") |           |                |
| 6. cannon                 | a weapon, used in war                            |           |                |
| 7. pliers                 | a tool ("nutcrackers" okay)                      |           |                |
| 8. trumpet                | a musical instrument ("cornet" okay)             |           |                |
| 9. peg                    | used to hold laundry/washing on a line           |           |                |
| 10. kite                  | it's flown in the air                            |           |                |
| Total Score<br>Range=0-10 |                                                  |           |                |

6

Semantic Fluency

 Time Limit: 60 seconds

Say

Now I'd like you to tell me the names of all 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<br>(0 or 2) | Second String     | String Score<br>(0 or 1) | Item Score<br>(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<br>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:** Familiarise yourself with these instructions before administering this subtest.

Total Score  
Range=0-89

|          |          |     |         |        |           |     |         |          |
|----------|----------|-----|---------|--------|-----------|-----|---------|----------|
| $\angle$ | $\wedge$ | $=$ | $\perp$ | $\vee$ | $\supset$ | $+$ | $\perp$ | $\vdash$ |
| 1        | 2        | 3   | 4       | 5      | 6         | 7   | 8       | 9        |

SAMPLE \_\_\_\_\_

|     |          |          |          |     |         |           |        |     |          |          |           |     |
|-----|----------|----------|----------|-----|---------|-----------|--------|-----|----------|----------|-----------|-----|
| $=$ | $\vdash$ | $\angle$ | $\wedge$ | $+$ | $\perp$ | $\supset$ | $\vee$ | $=$ | $\vdash$ | $\wedge$ | $\supset$ | $+$ |
|     |          |          |          |     |         |           |        |     |          |          |           |     |

|          |           |        |          |     |          |          |     |         |          |         |          |     |         |
|----------|-----------|--------|----------|-----|----------|----------|-----|---------|----------|---------|----------|-----|---------|
| $\vdash$ | $\supset$ | $\vee$ | $\vdash$ | $=$ | $\wedge$ | $\angle$ | $+$ | $\perp$ | $\wedge$ | $\perp$ | $\angle$ | $+$ | $\perp$ |
|          |           |        |          |     |          |          |     |         |          |         |          |     |         |

|           |          |          |     |        |          |     |         |     |           |          |          |          |
|-----------|----------|----------|-----|--------|----------|-----|---------|-----|-----------|----------|----------|----------|
| $\supset$ | $\vdash$ | $\wedge$ | $=$ | $\vee$ | $\angle$ | $+$ | $\perp$ | $=$ | $\supset$ | $\wedge$ | $\vdash$ | $\angle$ |
|           |          |          |     |        |          |     |         |     |           |          |          |          |

|     |          |          |         |     |          |     |          |           |          |         |     |          |
|-----|----------|----------|---------|-----|----------|-----|----------|-----------|----------|---------|-----|----------|
| $+$ | $\angle$ | $\vdash$ | $\perp$ | $=$ | $\vdash$ | $+$ | $\wedge$ | $\supset$ | $\angle$ | $\perp$ | $+$ | $\vdash$ |
|     |          |          |         |     |          |     |          |           |          |         |     |          |

|          |     |          |           |          |     |         |         |          |     |     |        |         |          |
|----------|-----|----------|-----------|----------|-----|---------|---------|----------|-----|-----|--------|---------|----------|
| $\angle$ | $+$ | $\vdash$ | $\supset$ | $\wedge$ | $=$ | $\perp$ | $\perp$ | $\angle$ | $=$ | $+$ | $\vee$ | $\perp$ | $\wedge$ |
|          |     |          |           |          |     |         |         |          |     |     |        |         |          |

|          |     |         |          |     |        |         |         |          |           |        |         |          |
|----------|-----|---------|----------|-----|--------|---------|---------|----------|-----------|--------|---------|----------|
| $\wedge$ | $=$ | $\perp$ | $\vdash$ | $+$ | $\vee$ | $\perp$ | $\perp$ | $\wedge$ | $\supset$ | $\vee$ | $\perp$ | $\angle$ |
|          |     |         |          |     |        |         |         |          |           |        |         |          |

|     |          |         |           |          |     |          |     |         |        |         |          |           |     |
|-----|----------|---------|-----------|----------|-----|----------|-----|---------|--------|---------|----------|-----------|-----|
| $+$ | $\angle$ | $\perp$ | $\supset$ | $\wedge$ | $=$ | $\angle$ | $+$ | $\perp$ | $\vee$ | $\perp$ | $\wedge$ | $\supset$ | $=$ |
|     |          |         |           |          |     |          |     |         |        |         |          |           |     |

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<br>(Do not read)     | Response | Score<br>(0 or 1) |
|---------------------------|----------|-------------------|
| Market                    |          |                   |
| Package                   |          |                   |
| Elbow                     |          |                   |
| Apple                     |          |                   |
| Story                     |          |                   |
| Carpet                    |          |                   |
| Bubble                    |          |                   |
| Motorway                  |          |                   |
| Saddle                    |          |                   |
| Powder                    |          |                   |
| Total Score<br>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, capitalised (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. grassland | y N        | 17. Powder  | Y n        |
| 3. Market                 | Y n        | 8. Carpet | Y n        | 13. Motorway  | 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<br>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 colour within parentheses. Record intrusions or variations in the Responses column.

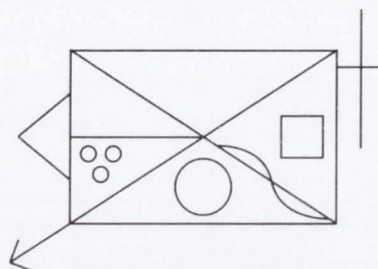
| Story (Do not read.)                                 | Responses | Item Score<br>(0 or 1) |
|------------------------------------------------------|-----------|------------------------|
| 1. On <b>Tuesday</b> ,                               |           |                        |
| 2. <b>Fourth</b>                                     |           |                        |
| 3. of <b>May</b> ,                                   |           |                        |
| 4. in <b>Grasmere</b> , Cumbria                      |           |                        |
| 5. a <b>serious</b>                                  |           |                        |
| 6. <b>fire</b> broke out.                            |           |                        |
| 7. <b>Two</b>                                        |           |                        |
| 8. <b>hotels</b>                                     |           |                        |
| 9. and a <b>restaurant</b>                           |           |                        |
| 10. were <b>destroyed</b>                            |           |                        |
| 11. before the <b>firemen (firefighters)</b>         |           |                        |
| 12. were able to <b>put it out (extinguish it)</b> . |           |                        |
| Total Score<br>Range=0-12                            |           |                        |

## 12 Figure recall

Say *Do you remember that figure that I asked you to 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 Copy Criteria**  
(Fold back for use.)

| Item                      | Drawing<br>(0 or 1) | Placement<br>(0 or 1) | Score<br>(0, 1, or 2) | Scoring Criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|---------------------------|---------------------|-----------------------|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. rectangle              |                     |                       |                       | <b>Drawing:</b> lines unbroken and relatively straight; it appears a rectangle.<br><b>Placement:</b> not rotated more than 15 degrees.                                                                                                                                                                                                                                                                                                                               |
| 2. diagonal cross         |                     |                       |                       | <b>Drawing:</b> lines are unbroken, relatively straight and approximately bisect each other.<br><b>Placement:</b> ends of lines should meet corners without significant overlap or significant distance from the corners.                                                                                                                                                                                                                                            |
| 3. horizontal line        |                     |                       |                       | <b>Drawing:</b> line is unbroken and relatively straight; should not exceed approximately 1/2 of the width.<br><b>Placement:</b> from approximately the centre of the left side and intersect at approximately the diagonal cross.                                                                                                                                                                                                                                   |
| 4. circle                 |                     |                       |                       | <b>Drawing:</b> relatively round, unbroken, and relatively closed; diameter should be approximately 1/4 - 1/3 height of triangle.<br><b>Placement:</b> placed in appropriate segment; not touching any other figure.                                                                                                                                                                                                                                                 |
| 5. 3 small circles        |                     |                       |                       | <b>Drawing:</b> relatively round, unbroken and relatively closed; approximately equal in size; approximate triangular arrangement; not touching each other.<br><b>Placement:</b> in appropriate segment; not touching figure; triangle formed not rotated more than approximately 15 degrees.                                                                                                                                                                        |
| 6. square                 |                     |                       |                       | <b>Drawing:</b> relatively closed; appears to be a square; lines relatively straight and unbroken; height is approximately 1/4 - 1/3 of triangle.<br><b>Placement:</b> in appropriate segment; not touching any other part of figure; not rotated more than approximately 15 degrees.                                                                                                                                                                                |
| 7. curving line           |                     |                       |                       | <b>Drawing:</b> 2 curved segments; approximately equal in appearance; correct direction of curves.<br><b>Placement:</b> ends of lines approximately touch diagonal; do not touch very corner of rectangle or diagonal intersection.                                                                                                                                                                                                                                  |
| 8. outside cross          |                     |                       |                       | <b>Drawing:</b> vertical line is relatively parallel to side of rectangle; horizontal line crosses the vertical at approximately 90 degrees and is between 20-50% of length of vertical line.<br><b>Placement:</b> horizontal line touches rectangle higher than 2/3 height of rectangle, but below top; it approximately touches the rectangle; vertical line stretches above the height of the rectangle and down to approximately the mid-point of the rectangle. |
| 9. triangle               |                     |                       |                       | <b>Drawing:</b> angle formed by two sides is between approximately 60-100 degrees; sides are relatively straight, unbroken and meet in a point; distance on vertical side of triangle subsumed is approximately 50% of the height of vertical side.<br><b>Placement:</b> approximately at top of rectangle.                                                                                                                                                          |
| 10. arrow                 |                     |                       |                       | <b>Drawing:</b> relatively straight and unbroken; lines forming arrow are approximately equal in length.<br><b>Placement:</b> protrudes from appropriate corner of rectangle; it appears an approximate continuation of diagonal staff.                                                                                                                                                                                                                              |
| Total Score<br>Range=0-20 |                     |                       |                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

**Figure Recall Drawing Page**

(Fold back for use.)

A large, empty rectangular box with a thin black border, intended for a drawing. It occupies the majority of the page below the header.



Supplemental Discrepancy Analysis Page

Index Differences

| Score 1 – Score 2                              | Score 1 | Score 2 | Difference | Statistical Significance Level | Frequency of Difference in Standardisation 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                   |         |         |            |                                |                                                   |

## Score Conversion Page

### I. Immediate Memory

1. List Learning Total Score.....

2. Story Memory Total Score.....

### II. Visuospatial/Constructional

3. Figure Copy Total Score.....

4. Line Orientation Total Score.....

### III. Language

5. Picture Naming Total Score.....

6. Semantic Fluency Total Score.....

### IV. Attention

7. Digit Span Total Score.....

8. Coding Total Score.....

### V. Delayed Memory

9. List Recall Total Score.....

10. List Recognition Total Score..

11. Story Recall Total Score.....

12. Figure Recall Total Score.....

Total  
Scores

Index  
Scores




(+)




(+)




(+)




(+)



(=)



Sum of  
Index Scores

Total Scale

Note. Use Appendix 2 in Stimulus Booklet A to convert total scores to index scores and sum of index scores to total scale.

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R  
Final Revised Version A (2005)

Name :  
Date of birth :  
Hospital no. :

Date of testing: ..... / ..... / .....  
Tester's name: .....  
Age at leaving full-time education: .....  
Occupation: .....  
Handedness: .....

Addressograph

|                                                                                                                                                                                                                                                                                                                                                                                                                                   |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-----------------------|-----------------------|--------|---------|--|-------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| <b>ORIENTATION</b>                                                                                                                                                                                                                                                                                                                                                                                                                |                       |                       |                       |        |         |  | [Score 0-5]<br><div></div> <div></div>                                  | O<br>R<br>I<br>E<br>N<br>T<br>A<br>T<br>I<br>O<br>N                                                   |
| ➤ Ask: What is the                                                                                                                                                                                                                                                                                                                                                                                                                | Day                   | Date                  | Month                 | Year   | Season  |  |                                                                         |                                                                                                       |
| ➤ Ask: Which                                                                                                                                                                                                                                                                                                                                                                                                                      | Building              | Floor                 | Town                  | County | Country |  |                                                                         |                                                                                                       |
|                                                                                                                                                                                                                                                                                                                                                                                                                                   |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| <b>REGISTRATION</b>                                                                                                                                                                                                                                                                                                                                                                                                               |                       |                       |                       |        |         |  | [Score 0-3]<br><div></div> <div></div>                                  | A<br>T<br>T<br>E<br>N<br>T<br>I<br>O<br>N<br>&<br>O<br>R<br>I<br>E<br>N<br>T<br>A<br>T<br>I<br>O<br>N |
| ➤ 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).<br>Register number of trials .....                                                                                                                                          |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| <b>ATTENTION &amp; CONCENTRATION</b>                                                                                                                                                                                                                                                                                                                                                                                              |                       |                       |                       |        |         |  | [Score 0-5]<br><div></div> <div></div><br>(for the best performed task) | A<br>T<br>T<br>E<br>N<br>T<br>I<br>O<br>N<br>&<br>O<br>R<br>I<br>E<br>N<br>T<br>A<br>T<br>I<br>O<br>N |
| ➤ 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)<br>Stop after five subtractions (93, 86, 79, 72, 65). .....<br>➤ Ask: 'could you please spell <b>WORLD</b> for me? Then ask him/her to spell it backwards:<br>..... |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| <b>MEMORY - Recall</b>                                                                                                                                                                                                                                                                                                                                                                                                            |                       |                       |                       |        |         |  | [Score 0-3]<br><div></div> <div></div>                                  | Y                                                                                                     |
| ➤ Ask: 'Which 3 words did I ask you to repeat and remember?'<br>.....                                                                                                                                                                                                                                                                                                                                                             |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| <b>MEMORY - Anterograde Memory</b>                                                                                                                                                                                                                                                                                                                                                                                                |                       |                       |                       |        |         |  | [Score 0-7]<br><div></div>                                              | R                                                                                                     |
| ➤ 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'<br>Score only the third trial                                                                                                                                                                                                                    |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
|                                                                                                                                                                                                                                                                                                                                                                                                                                   | 1 <sup>st</sup> Trial | 2 <sup>nd</sup> Trial | 3 <sup>rd</sup> Trial |        |         |  | O<br>M<br>E<br>M<br>O<br>R<br>Y                                         |                                                                                                       |
| Harry Barnes                                                                                                                                                                                                                                                                                                                                                                                                                      |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| 73 Orchard Close                                                                                                                                                                                                                                                                                                                                                                                                                  |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| Kingsbridge                                                                                                                                                                                                                                                                                                                                                                                                                       |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| Devon                                                                                                                                                                                                                                                                                                                                                                                                                             |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |
| <b>MEMORY - Retrograde Memory</b>                                                                                                                                                                                                                                                                                                                                                                                                 |                       |                       |                       |        |         |  | [Score 0 -4]<br><div></div>                                             | M                                                                                                     |
| ➤ Name of current Prime Minister .....<br>➤ Name of the woman who was Prime Minister .....<br>➤ Name of the USA president .....<br>➤ Name of the USA president who was assassinated in the 1960's .....                                                                                                                                                                                                                           |                       |                       |                       |        |         |  |                                                                         |                                                                                                       |



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 |

Y

C

N

E

➤ 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 |

U

L

F

LANGUAGE - Comprehension

➤ Show written instruction:

[Score 0-1]

Close your eyes

E

G

A

U

➤ 3 stage command:

'Take the paper in your right hand. Fold the paper in half. Put the paper on the floor'

[Score 0-3]

G

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]

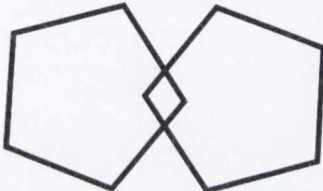
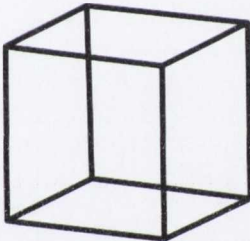
N

A

L

| LANGUAGE - Repetition                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                   |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| <p>➤ Ask the subject to repeat: 'hippopotamus'; 'eccentricity'; 'unintelligible'; 'statistician'</p> <p>Score 2 if all correct; 1 if 3 correct; 0 if 2 or less.</p>                                                                                                                                                                                                                                                                                                                           | <p>[Score 0-2]</p> <div></div>                                                    |
| <p>➤ Ask the subject to repeat: 'Above, beyond and below'</p>                                                                                                                                                                                                                                                                                                                                                                                                                                 | <p>[Score 0-1]</p> <div></div>                                                    |
| <p>➤ Ask the subject to repeat: 'No ifs, ands or buts'</p>                                                                                                                                                                                                                                                                                                                                                                                                                                    | <p>[Score 0-1]</p> <div></div>                                                    |
| LANGUAGE - Naming                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                   |
| <p>➤ Ask the subject to name the following pictures:</p> <div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> <div><div></div><div></div></div> </div> | <p>[Score 0-2]<br/>pencil + watch</p> <div></div> <p>[Score 0-10]</p> <div></div> |
| LANGUAGE - Comprehension                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                   |
| <p>➤ Using the pictures above, ask the subject to:</p> <ul style="list-style-type: none"> <li>• Point to the one which is associated with the monarchy</li> <li>• Point to the one which is a marsupial</li> <li>• Point to the one which is found in the Antarctic</li> <li>• Point to the one which has a nautical connection</li> </ul>                                                                                                                                                    | <p>[Score 0-4]</p> <div></div>                                                    |

E  
G  
A  
U  
G  
N  
A  
L

|                                                                                                                                                                                              |                                            |                                                          |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|----------------------------------------------------------|
| LANGUAGE - Reading                                                                                                                                                                           |                                            | L<br>A<br>N<br>G<br>U<br>A<br>G<br>E                     |
| <p>➤ Ask the subject to read the following words: [Score 1 only if all correct]</p> <p>sew<br/>pint<br/>soot<br/>dough<br/>height</p>                                                        | <p>[Score 0-1]</p> <div></div>             |                                                          |
| VISUOSPATIAL ABILITIES                                                                                                                                                                       |                                            |                                                          |
| <p>➤ Overlapping pentagons: Ask the subject to copy this diagram:</p>                                       | <p>[Score 0-1]</p> <div></div> <div></div> |                                                          |
| <p>➤ Wire cube : Ask the subject to copy this drawing (for scoring, see instructions guide)</p>            | <p>[Score 0-2]</p> <div></div>             |                                                          |
| <p>➤ Clock: Ask the subject to draw a clock face with numbers and the hands at ten past five.<br/>(for scoring see instruction guide: circle = 1, numbers = 2, hands = 2 if all correct)</p> | <p>[Score 0-5]</p> <div></div>             | V<br>I<br>S<br>U<br>O<br>S<br>P<br>A<br>T<br>I<br>A<br>L |

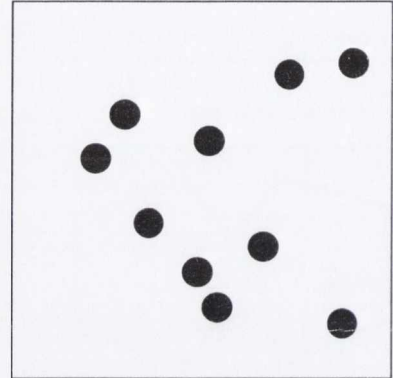
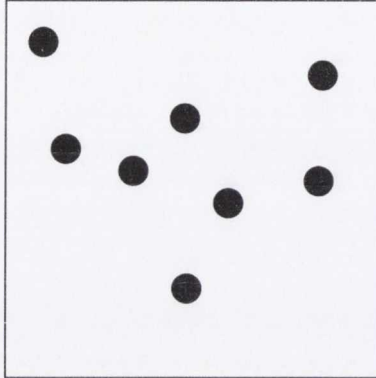


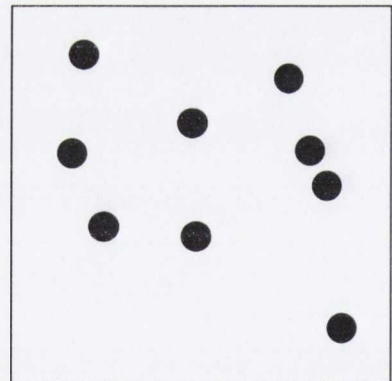
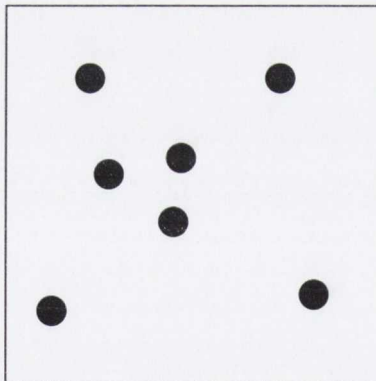
PERCEPTUAL ABILITIES

➤ Ask the subject to count the dots without pointing them

[Score 0-4]





J  
A  
I  
T  
A  
P  
S  
O  
U  
S  
I  
V

## PERCEPTUAL ABILITIES

- Ask the subject to identify the letters

[Score 0-4]

V  
I  
S  
U  
O  
S  
P  
A  
T  
I  
A  
L

## RECALL

- Ask "Now tell me what you remember of that name and address we were repeating at the beginning"

Harry Barnes .....  
73 Orchard Close .....  
Kingsbridge .....  
Devon .....

[Score 0-7]

Y  
R  
O  
M  
E

## 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 |

M

## General Scores

|       |      |
|-------|------|
| MMSE  | /30  |
| ACE-R | /100 |

E  
R  
O  
S

## 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% sensitivity and 89% specificity for dementia

Cut-off <82 gives 84% sensitivity and 100% specificity for dementia

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## FRONTAL ASSESSMENT BATTERY

CIRCLE SCORE

## Stage 1 Similarities – Conceptualisation

|                                                                                               |                                |
|-----------------------------------------------------------------------------------------------|--------------------------------|
| <i>"In what way are they alike ?"</i>                                                         |                                |
| <i>"A banana and an orange"</i>                                                               |                                |
| <i>"A table and a chair"</i>                                                                  |                                |
| <i>"A tulip, a rose and a daisy"</i>                                                          |                                |
| Only category names such as fruit, furniture and flowers are correct                          |                                |
| Three correct:                                                                                | 3 points                       |
| Two correct:                                                                                  | 2 points                       |
| One correct:                                                                                  | 1 points                       |
| None correct:                                                                                 | 0 points                       |
| <b>Stage 2 Lexical Fluency – Mental Flexibility</b>                                           |                                |
| <i>"Say as many words as you can beginning with the letter S, any words except names"</i>     |                                |
| Cue after 5 seconds: <i>"for example: snake"</i>                                              |                                |
| Cue after 10 seconds: <i>"any word beginning with S"</i>                                      |                                |
| Only 60 seconds are allowed                                                                   |                                |
| Exclude repetitions, variations (show, shoemaker) or names                                    |                                |
| More than 9 words:                                                                            | 3 points                       |
| Six to nine words:                                                                            | 2 points                       |
| Three to five words:                                                                          | 1 point                        |
| Less than three words:                                                                        | 0 points                       |
| <b>Stage 3 Motor Series – Prefrontal Motor Programming</b>                                    |                                |
| <i>"Look carefully at what I'm doing:"</i>                                                    |                                |
| Alone the examiner does the Luria sequence three times with their left hand (first edge palm) |                                |
| <i>"Now with your right hand do the same series, first with me and then alone"</i>            |                                |
| Patient performs six correct consecutive series (with and alone) :                            | 3 points                       |
| Patient performs at least three correct consecutive series alone:                             | 2 points                       |
| Patient fails alone but performs three consecutive with examiner:                             | 1 point                        |
| Patient cannot perform any three consecutive series (even with the examiner):                 | 0 points                       |
| <b>Stage 4 Conflicting Instructions – Sensitivity to Interference</b>                         |                                |
| <i>"Tap twice when I tap once"</i>                                                            | three trials are run 1 – 1 – 1 |
| <i>"Tap once when I tap twice"</i>                                                            | three trials are run 2 – 2 – 2 |
| Then : 1 – 1 – 2 – 1 – 2 – 2 – 2 – 1 – 1 – 2                                                  |                                |
| No error:                                                                                     | 3 points                       |
| One to two errors:                                                                            | 2 points                       |





| VISUOSPATIAL / EXECUTIVE                                                                              |               |               |               |              | Copy<br>cube                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Draw CLOCK (Ten past eleven)<br>(3 points)                                                                                                                                                                                                                                      | POINTS |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
|-------------------------------------------------------------------------------------------------------|---------------|---------------|---------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|------------------------------------------------------------------------------------------------------------------------|---------------|--------------------------------------------------------------------|--------------------------------------------------|--------------|------------|-------------------------------------|-----------------------|--|--|--|--|--|---------------------|--|--|--|--|--|
|                                                                                                       |               |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | <div style="display: flex; justify-content: space-around; margin-top: 20px;"> <span>[ ]</span> <span>[ ]</span> <span>[ ]</span> </div> <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <span>[ ]</span> <span>[ ]</span> <span>[ ]</span> </div> |        | <div style="display: flex; justify-content: space-between;"> <span>[ ]</span> <span>[ ]</span> <span>[ ]</span> </div> |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| <b>NAMING</b>                                                                                         |               |               |               |              | <div style="display: flex; justify-content: space-around; align-items: flex-end;"> <div style="text-align: center;"> <br/>[ ]         </div> <div style="text-align: center;"> <br/>[ ]         </div> <div style="text-align: center;"> <br/>[ ]         </div> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| <b>MEMORY</b>                                                                                         |               |               |               |              | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 150px; padding: 5px;">Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.</td> <td style="width: 100px; padding: 5px;">FACE</td> <td style="width: 100px; padding: 5px;">VELVET</td> <td style="width: 100px; padding: 5px;">CHURCH</td> <td style="width: 100px; padding: 5px;">DAISY</td> <td style="width: 100px; padding: 5px;">RED</td> <td rowspan="3" style="width: 50px; text-align: center; vertical-align: middle;">No<br/>points</td> </tr> <tr> <td style="padding: 5px;">1st trial</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td style="padding: 5px;">2nd trial</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>                                |                                                                                                                                                                                                                                                                                 |        | Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.                                | FACE          | VELVET                                                             | CHURCH                                           | DAISY        | RED        | No<br>points                        | 1st trial             |  |  |  |  |  | 2nd trial           |  |  |  |  |  |
| Read list of words, subject must repeat them. Do 2 trials. Do a recall after 5 minutes.               | FACE          | VELVET        | CHURCH        | DAISY        | RED                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | No<br>points                                                                                                                                                                                                                                                                    |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| 1st trial                                                                                             |               |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| 2nd trial                                                                                             |               |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| <b>ATTENTION</b>                                                                                      |               |               |               |              | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 400px; padding: 5px;">Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order</td> <td style="width: 100px; text-align: center;">[ ] 2 1 8 5 4</td> <td rowspan="2" style="width: 50px; text-align: center; vertical-align: middle;">_ / 2</td> </tr> <tr> <td style="padding: 5px;">Subject has to repeat them in the backward order</td> <td style="text-align: center;">[ ] 7 4 2</td> </tr> </table>                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        | Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order                                   | [ ] 2 1 8 5 4 | _ / 2                                                              | Subject has to repeat them in the backward order | [ ] 7 4 2    |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order                  | [ ] 2 1 8 5 4 | _ / 2         |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Subject has to repeat them in the backward order                                                      | [ ] 7 4 2     |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors    |               |               |               |              | <div style="display: flex; justify-content: space-between;"> <span>[ ] FBACMNAAJKLBFAFAKDEAAAAJAMOF AAB</span> <span>_ / 1</span> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Serial 7 subtraction starting at 100                                                                  |               |               |               |              | <div style="display: flex; justify-content: space-between;"> <span>[ ] 93</span> <span>[ ] 86</span> <span>[ ] 79</span> <span>[ ] 72</span> <span>[ ] 65</span> </div> <div style="display: flex; justify-content: space-between; font-size: 0.8em;"> <span>4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 1 correct: 1 pt, 0 correct: 0 pt</span> <span>_ / 3</span> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| <b>LANGUAGE</b>                                                                                       |               |               |               |              | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 400px; padding: 5px;">Repeat : I only know that John is the one to help today. [ ]</td> <td rowspan="2" style="width: 50px; text-align: center; vertical-align: middle;">_ / 2</td> </tr> <tr> <td style="padding: 5px;">The cat always hid under the couch when dogs were in the room. [ ]</td> </tr> </table>                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                 |        | Repeat : I only know that John is the one to help today. [ ]                                                           | _ / 2         | The cat always hid under the couch when dogs were in the room. [ ] |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Repeat : I only know that John is the one to help today. [ ]                                          | _ / 2         |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| The cat always hid under the couch when dogs were in the room. [ ]                                    |               |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Fluency / Name maximum number of words in one minute that begin with the letter F                     |               |               |               |              | <div style="display: flex; justify-content: space-between;"> <span>[ ] _____ (N ≥ 11 words)</span> <span>_ / 1</span> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| <b>ABSTRACTION</b>                                                                                    |               |               |               |              | <div style="display: flex; justify-content: space-between;"> <span>Similarity between e.g. banana - orange = fruit [ ]</span> <span>train - bicycle [ ]</span> <span>watch - ruler [ ]</span> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| <b>DELAYED RECALL</b>                                                                                 |               |               |               |              | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 150px; padding: 5px;">Has to recall words WITH NO CUE</td> <td style="width: 100px; padding: 5px;">FACE<br/>[ ]</td> <td style="width: 100px; padding: 5px;">VELVET<br/>[ ]</td> <td style="width: 100px; padding: 5px;">CHURCH<br/>[ ]</td> <td style="width: 100px; padding: 5px;">DAISY<br/>[ ]</td> <td style="width: 100px; padding: 5px;">RED<br/>[ ]</td> <td rowspan="3" style="width: 100px; text-align: center; vertical-align: middle;">Points for<br/>UNCUED<br/>recall only</td> </tr> <tr> <td style="padding: 5px;">Optional Category cue</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td style="padding: 5px;">Multiple choice cue</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table> |                                                                                                                                                                                                                                                                                 |        | Has to recall words WITH NO CUE                                                                                        | FACE<br>[ ]   | VELVET<br>[ ]                                                      | CHURCH<br>[ ]                                    | DAISY<br>[ ] | RED<br>[ ] | Points for<br>UNCUED<br>recall only | Optional Category cue |  |  |  |  |  | Multiple choice cue |  |  |  |  |  |
| Has to recall words WITH NO CUE                                                                       | FACE<br>[ ]   | VELVET<br>[ ] | CHURCH<br>[ ] | DAISY<br>[ ] | RED<br>[ ]                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Points for<br>UNCUED<br>recall only                                                                                                                                                                                                                                             |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Optional Category cue                                                                                 |               |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| Multiple choice cue                                                                                   |               |               |               |              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| <b>ORIENTATION</b>                                                                                    |               |               |               |              | <div style="display: flex; justify-content: space-between;"> <span>[ ] Date</span> <span>[ ] Month</span> <span>[ ] Year</span> <span>[ ] Day</span> <span>[ ] Place</span> <span>[ ] City</span> </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |
| © Z.Nasreddine MD Version 7.0 <a href="http://www.mocatest.org">www.mocatest.org</a> Normal ≥ 26 / 30 |               |               |               |              | TOTAL _____ / 30<br>Add 1 point if ≤ 12 yr edu                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                 |        |                                                                                                                        |               |                                                                    |                                                  |              |            |                                     |                       |  |  |  |  |  |                     |  |  |  |  |  |

## **Information and Consent for Patients**

### **1. Title of study**

The Inflammatory Neurodegenerative Continuum in HIV Related Cognitive Impairment – MRI and CSF characteristics

### **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 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:**

MRI (Magnetic Resonance Imaging) brain scan will be carried out to look for any changes that HIV can cause in the brain that may be associated with memory difficulties. A new generation of MRI scanner called 3 Tesla MRI is available in St James's Hospital now. It is advanced compared to conventional MRI in detecting changes in the structure of the brain. Those who are selected will undergo MRI scan of brain using 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.

A lumbar puncture will also be carried out. A lumbar puncture involves taking a sample of spinal fluid from the base of the spine. You will be asked to lie on your side and curl up into a ball. You will be given local anaesthetic and a needle will be passed into the space between the bones in your lower back to get the sample of fluid. You will then have to lie down for two hours afterwards.

### **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:**

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.

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 fetus 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.

## Complications of Lumbar Puncture

### *Relatively common*

1) Post Lumbar puncture headache - headache increases when upright and diminishes when lying down, occasionally associated with nausea and vomiting, onset within 1-3 days and usually lasts less than 5 days. Usually resolves with lying flat and drinking 3-4litres of fluid. Rarely it may require intravenous fluid.

2) Post Lumbar puncture back pain (less than one third of patients, due to local soft tissue trauma, resolves within a few days)

*Rare*

3) Infection

4) Bruising at the site of lumbar puncture or very rarely inside the lining of the spinal canal but usually occurs in patients who have a bleeding disorder.

5) Transient nerve root irritation may occur during the procedure. It consists of a brief sensation of pins and needles in the legs and disappears within a few seconds.

#### **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. Patricia McNamara who can be telephoned at 014103000 and extension 4146 or on 086 7256193.

If your doctor learns of important new information that might affect your desire to remain in the study, he or she will tell you.



**Appendix 11: Consent Form for MRI Study**

**SJH / AMNCH RESEARCH ETHICS COMMITTEE.**

**CONSENT FORM FOR MRI BRAIN**

**Consent for study "The Inflammatory Neurodegenerative Continuum in HIV Related Cognitive Impairment – MRI and CSF Characteristics"**

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.

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 12: 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 MPS (mm) =       |                       | 1.00 / 1.00 / 1.00; |
| Act. WFS (pix) / BW (Hz) = |                       | 2.431 / 178.7;      |



### Appendix 13: MRI Protocol for T2-weighted Imaging

| T2 TSE ax               |                     |
|-------------------------|---------------------|
| FOV AP (mm) =           | 230;                |
| RL (mm) =               | 184;                |
| FH (mm) =               | 149;                |
| Voxel size AP (mm) =    | 0.575               |
| RL (mm) =               | 0.72                |
| Slice thickness (mm) =  | 4;                  |
| Recon voxel size (mm) = | 0.45                |
| Reconstruction matrix = | 512;                |
| Stacks =                | 1;                  |
| type =                  | parallel;           |
| slices =                | 30;                 |
| slice gap =             | user defined;       |
| gap (mm) =              | 1;                  |
| slice orientation =     | transverse;         |
| fold-over direction =   | RL;                 |
| fat shift direction =   | P;                  |
| Scan mode =             | MS;                 |
| technique =             | SE;                 |
| Fast Imaging mode =     | TSE;                |
| shot mode =             | multishot;          |
| TSE factor =            | 15;                 |
| TE =                    | user defined;       |
| (ms) =                  | 80;                 |
| Flip angle (deg) =      | 90;                 |
| Refocusing control =    | yes;                |
| angle (deg) =           | 120;                |
| TR =                    | user defined;       |
| (ms) =                  | 3000;               |
| NSA =                   | 1                   |
| Total scan duration =   | 01:48.0             |
| ACQ matrix M x P =      | 400 x 255;          |
| ACQ voxel MPS (mm) =    | 0.57 / 0.72 / 4.00; |
| REC voxel MPS (mm) =    | 0.45 / 0.45 / 4.00; |
| WFS (pix) / BW (Hz) =   | 2.130 / 204.0;      |

## Appendix 14: MRI Protocol for FLAIR Imaging

| FLAIR AX   |                         |                     |
|------------|-------------------------|---------------------|
| FOV        | AP (mm) =               | 230;                |
|            | RL (mm) =               | 183                 |
|            | FH (mm) =               | 149;                |
| Voxel size | AP (mm) =               | 0.65                |
|            | RL (mm) =               | 0.87                |
|            | Slice thickness (mm) =  | 4;                  |
|            | Recon voxel size (mm) = | 0.449               |
|            | Reconstruction matrix = | 512;                |
|            | Stacks =                | 1;                  |
|            | type =                  | parallel;           |
|            | slices =                | 30;                 |
|            | slice gap =             | user defined;       |
|            | gap (mm) =              | 1;                  |
|            | slice orientation =     | transverse;         |
|            | fold-over direction =   | RL;                 |
|            | fat shift direction =   | P;                  |
|            | Scan mode =             | MS;                 |
|            | technique =             | IR;                 |
|            | Fast Imaging mode =     | TSE;                |
|            | shot mode =             | multishot;          |
|            | TSE factor =            | 31;                 |
|            | TE =                    | user defined;       |
|            | (ms) =                  | 125;                |
|            | Refocusing control =    | yes;                |
|            | angle (deg) =           | 120;                |
|            | TR =                    | user defined;       |
|            | (ms) =                  | 11000;              |
|            | Water-fat shift =       | maximum;            |
|            | IR delay (ms) =         | 2800;               |
|            | acquire during delay =  | yes;                |
|            | NSA =                   | 1                   |
|            | Total scan duration =   | 05:08.0;            |
|            | ACQ matrix M x P =      | 352 x 186;          |
|            | ACQ voxel MPS (mm) =    | 0.65 / 0.98 / 4.00; |
|            | REC voxel MPS (mm) =    | 0.45 / 0.45 / 4.00; |
|            | WFS (pix) / BW (Hz) =   | 1.416 / 306.9;      |

### Appendix 15: 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;          |
|            | shot 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 =       | 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 MPS (mm) =  | 2.19 / 2.19 / 2.50; |
| EPI factor =          | 51;                 |
| WFS (pix) / BW (Hz) = | 14.299 / 30.4;      |

Appendix 16: Consent form for CSF Study

**SJH / AMNCH RESEARCH ETHICS COMMITTEE.  
CONSENT FORM FOR CSF**

**Consent for study "The Inflammatory Neurodegenerative Continuum in HIV Related  
Cognitive Impairment – MRI and CSF Characteristics**

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.

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)).



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