A longitudinal clinical and SPECT scan study on HIV-infected patients with New Onset Seizures and No Identifiable Cause

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Declaration

I, Kapila Ranchhodbhai Hari declare that this thesis is my own work. It is being submitted for the degree of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signed _____

At Johannesburg

This day _____ of December 2015

Dedication

I dedicate this thesis to my husband Arvind and all the patients without whose support I would not have completed this thesis.

Presentations arising from this work

Poster Presentation

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Title of Poster: Demography and aetiologies of new onset seizures in an HIV-infected population.

Abstract

New onset seizures associated with HIV infection have been studied. The data investigating new onset seizures in HIV infected individuals have predominantly originated from developed countries and prior to the availability of anti-retroviral therapy. No longitudinal studies have been conducted.

The HIV pandemic has its roots in Africa and South Africa currently bears the brunt of the burden. This study sought to evaluate the current profile of HIV infected individuals presenting with new onset seizures in the South African setting, and to follow up patients in whom no cause is identifiable at baseline.

Two hundred Black African patients were recruited from the three major teaching hospitals affiliated to the University of the Witwatersrand, Johannesburg. They had clinical assessments and were extensively investigated to determine a cause for the seizure. In the present study the majority of HIV infected patients with new onset seizures had an underlying cause, the most common of which were meningitis and focal brain lesions. Infectious aetiologies predominated; specifically in patients with advanced disease i.e. HIV stages 2 and 3. Tuberculosis and cryptococcal infections were the most frequent. This is consistent with the prevalent infections in our region. Our patients had advanced immune-suppression as evidenced by the mean CD4 count of 167 µµL. HAART coverage was sub-optimal with the majority of HAART-eligible patients not accessing therapy. The predominant seizure type was generalized tonic-clonic. Neuroimaging abnormalities were present in most patients.

Fifteen patients with no identifiable cause were enlisted in the long-term study. These patients had repeated clinical assessments; laboratory investigations; MRI and brain SPECT scans. All patients presented with generalized tonic-clonic seizures. Non-specific white matter lesions on MRI; temporal lobe abnormalities on visual SPECT scans and regional cerebral hypo-perfusion on quantitative SPECT scan analysis were common findings. The patients who initiated HAART during the study period improved on laboratory monitoring and on quantitative SPECT analysis. One patient developed HIV associated dementia during follow up.

New Onset Seizures in HIV infected individuals in whom no cause is identifiable may possibly represent:

- Early stages of the spectrum of neurocognitive dysfunction seen in HIV infection
- A precursor for dementia

These patients are likely to benefit from the initiation of anti-retroviral therapy.

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AAN	American Academy of Neurology
ADA	Adenosine Deaminase
ADC	AIDS dementia complex
AED	Anti-epileptic Drug
AFB	Acid-fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ANI	Asymptomatic Neurocognitive Impairment
ANOVA	Analysis of Variance
ARVs	Antiretrovirals
AZT	Azidothymidine
BBB	Blood-brain Barrier
CCR	Chemokine Receptor
CD4	Cluster of Differentiation 4
CDC	Centers for Disease Control
CFT	Complement Fixation Test
CHARTER	Central Nervous System Human Immunodeficiency Virus Anti-Retroviral Therapy Effects Research
CK	Creatine Kinase
CM	Cryptococcal Meningitis
CMP	Calcium, Magnesium and Phosphate
CMV	Cytomegalovirus
CNS	Central Nervous System
CPE	CNS Penetration Effectiveness
CSF	Cerebrospinal fluid
CT	Computed Tomography
CXCL	Cytokine ligands
CXR	Chest X-ray
DNA	Deoxyribonucleic acid

DSP	Distal Sensory Polyneuropathy
EBV	Epstein-Barr Virus
EEG	Electroencephalogram
ELISA	Enzyme-Linked Immunosorbent Assay
ESR	Erythrocyte Sedimentation Rate
FBC	Full Blood Count
FBLs	Focal Brain Lesions
FDA	United States Food and Drug Administration
Gp	Glycoprotein
HAART	Highly Active Antiretroviral Therapy
HAD	HIV-Associated Dementia
HAND	HIV Associated Neurocognitive Disorder
HIV	Human Immunodeficiency Virus
HIVE	HIV encephalopathy
HMPAO	Hexamethylpropyleneamine oxime
HTLV-1	Human T-Cell Lymphotropic Virus-1
HTLV-III	Human T-Cell Lymphotrophic Virus-III
IFNý	Interferon gamma
IgG	Immunoglobulin G
IHDS	International HIV Dementia Scale
IL	Interleukin
IRIS	Immune Reconstitution Inflammatory Syndrome
JCV	John Cunningham Virus
KS	Kaposi's sarcoma
LFT	Liver Function Test
LP	Lumbar puncture
MBq	Megabecquerel
MDR-TB	Multi-drug Resistant TB
MMWR	Morbidity and Mortality Weekly Report

MND	Mild Neurocognitive Disorder
MRI	Magnetic Resonance Image
NAAT	Nucleic Acid Amplification Technology
NCC	Neurocysticercosis
NHLS	National Health Laboratory Service
NIC	No Identifiable Cause
NNRTIs	Nonnucleoside Reverse Transcriptase Inhibitors
NOS	New onset seizures
PBAS	PMOD Base Functionality
PCNSL	Primary CNS Lymphoma
PCP	Pneumocystis carinii pneumonia
PCR	Polymerase Chain Reaction
PIs	Protease inhibitors
PML	Progressive Multifocal leukoencephalopathy
PN	Peripheral Neuropathies
rCBF	Regional cerebral blood flow
RNA	Ribonucleic acid
ROI	Regions of Interest
SD	Standard Deviation
SIV	Simian Immunodeficiency Virus
SPECT	Single-Photon Emission Computed Tomography
SSA	Sub-Saharan Africa
TB	Tuberculosis
TBM	Tuberculous meningitis

^{99m}Tc-HMPAO	Technetium Exametazime hexamethylpropyleneamine oxime
TE	Toxoplasma encephalitis
T. Gondii	*Toxoplasma* Gondii
Toxo	Toxoplasmosis
U&E	Urea and Electrolytes
UN	United Nations
USA	United States of America
VL	Viral Load
VM	Vacuolar Myelopathy
VOI	Volumes of Interest
WHO	World Health Organization
WML	White Matter Lesions
Xpert MTB/RIF	*Mycobacterium tuberculosis* and resistance to Rifampicin assay

CHAPTER ONE

INTRODUCTION

1.1 The Human Immunodeficiency Virus Pandemic

1.1.1 History of the Global Pandemic

There are reports of Human Immunodeficiency Virus (HIV) infection prior to 1970 but the current pandemic likely began in the mid-to late 1970s. The initial reports originated in the United States of America (USA). The Acquired Immunodeficiency Syndrome (AIDS) was recognized a few years later.

In early 1981, Hymes et al identified a group of eight homosexual males with Kaposi's sarcoma (KS), in New York. The disease in these patients was unusual in that it occurred in younger males, was generalized and more aggressive than previously described. [KS is now a well-recognized clinical manifestation of the disease.] Later that year Gottlieb et al described the unusual occurrence of Pneumocystis carinii pneumonia (PCP) in five homosexual men who had no obvious underlying immunodeficiency. Soon numerous reports began to emerge of opportunistic disease in homosexual men and later in intravenous drug abusers. The different presentations meant that there was no uniformity in recording these unusual presentations. In an attempt to address this, the Center Disease Control (CDC) published an update in the weekly Morbidity and Mortality Weekly Report (MMWR), Current Trends Update on AIDS September 24, 1982 recognizing the Acquired Immunodeficiency Syndrome (AIDS) as a specific entity. This name seemed appropriate as the condition was:

- Acquired
- Immune deficiency was a prominent feature
- It was a syndrome rather than a disease, due to the variety of manifestations

The recognition of AIDS as a specific entity occurred eighteen months after the first KS case reports from New York. This initial classification included clinical criteria only.

Barre-Sinoussi et al originally isolated the virus causing AIDS at the Pasteur institute in France in May 1983 and named it the lymphadenopathy-associated virus (LAV). At the stage of identification of the virus the authors postulated that it may play a role in the causation of AIDS. A year later, Gallo and his colleagues published 4 articles (Gallo et al; Popovic et al; Sarngadharan et al and Schüpbach et al; Science. 1984) describing a retrovirus which they named Human T Lymphotrophic Virus-III (HTLVIII). They believed that they had identified the "AIDS virus". It soon became apparent that both the French and American scientists were describing the exact same virus. In 1986 Coffin et al wrote a letter in Nature labelling the virus HIV (Human Immunodeficiency Virus), and officially acknowledging it as the virus responsible for AIDS.

The AIDS pandemic was in its infancy in 1986. The report of the Bureau of Hygiene & Tropical Diseases (1986) 'AIDS newsletter' stated that 38401 patients in 85 countries had acquired the disease. Significantly at this stage it was the Americas which bore the brunt of the disease. There were less than 2500 cases reported in Africa. During the thirty years since AIDS was formally recognized the epidemic has burgeoned. According to the United Nations (UN) AIDS Report on the Global AIDS Epidemic 2013 there have been an estimated 75 million people infected with HIV and 36 million deaths due to AIDS-related illnesses.

The profile of people living with HIV/ AIDS in the developed world is different to that in the developing world. In developed countries the main burden of the disease is amongst marginalized groups e.g. men who have sex with men (MSM), ethnic minorities, refugees and intravenous drug users. In developing countries the epidemic is mainly due to heterosexual transmission in the general population but the marginalized groups mentioned above maintain an increased risk.

There has been some progress in controlling the pandemic in that the annual numbers of new HIV infections and deaths are decreasing. In 2001 new HIV infections peaked at 3.4 million, in 2012 this had reduced to 2.3 million. AIDS-related deaths spiked in 2005 when 2.3 million

were recorded. By 2012 the number had decreased to 1.6 million (UNAIDS Report on the Global AIDS Epidemic 2013).

A significant tool in the management of the AIDS pandemic has been the development and increasing availability of anti-retroviral drugs. In 1987 azidothymidine (AZT) became the first drug approved by the U.S. Food and Drug Administration (FDA). There are currently more than 20 anti-retroviral drugs approved for use in the management of HIV infection. The current recommendations are for the use of Highly Active Antiretroviral Therapy (HAART). Multiple drugs are used in an effort to decrease drug resistance. The estimated number of people accessing therapy was 9.7 million in 2012; a significant increase from the 1.3 million in 2005 which was when it became readily available (UNAIDS Report on the Global AIDS Epidemic 2013).

1.1.2 HIV and AIDS in Africa

There is evidence to confirm that HIV originated in Africa. Keele et al, 2006 proved that a strain of Simian Immunodeficiency Virus (SIV) isolated in chimpanzees in Cameroon is a viral ancestor of HIV-1, the cause of the current AIDS. It is believed that SIV was first transferred to humans in the 1930s and that in the 1960s people in Africa were already infected with HIV (Nahmias et al, 1986). The virus was isolated from stored samples of blood.

The first African patients with AIDS most likely originated in Kinshasa, the capital city of the Democratic Republic of Congo. Medical workers documented increased numbers of patients with opportunistic disease i.e. cryptococcal meningitis; Kaposi's sarcoma and miliary tuberculosis (TB). In Kinshasa the virus spread rapidly within the urban sexual network. This initiated the rapid heterosexual transmission of HIV in Africa. Numerous factors facilitated this spread:

- Widespread labour migration
- High ratio of men to women in the urban environment
- Lack of circumcision
- Prevalence of sexually transmitted diseases (STDs)

Sex workers are believed to have been instrumental in the accelerated spread of HIV in Africa (Faria et al, 2014).

In the 1980s the population in Uganda was severely affected by AIDS, but it had not been recognized as a specific entity. The disease was known locally as "Slim Disease". The first cases had been seen in 1982 and the numbers had increased in the ensuing years. The cardinal features documented in these patients were severe wasting and weight loss, hence the name. There was no evidence to link this disease with the disease seen in white homosexual men in the USA. Clinicians recognized that their patients had some criteria consistent with AIDS but the extreme weight loss and diarrhoea were atypical (Quinn et al, 1986).

In 1984 Clumeck et al in Belgium diagnosed AIDS in 22 African patients. Their most significant finding was that none of their patients had the "traditional" risk factors that were presumed to be associated with the development of AIDS i.e.

- Underlying immunosuppressive disease
- Blood-product transfusion
- Homosexuality
- Intravenous-drug abuse

This was the first report of AIDS in patients outside the well-recognized high risk groups documented in the USA. This study was both prospective and retrospective. The first case consistent with the diagnosis of AIDS had been seen in 1979. The realization that AIDS occurred in African patients led to prospective studies in both Rwanda and Zaire in efforts to identify potential cases. In less than a month, 26 patients were identified in Rwanda (van der Perre et al, 1984) and 38 in Zaire (Piot et al, 1984). During this period it became obvious that the AIDS epidemic in Africa was different in that it was related primarily to heterosexual transmission of the virus.

The epidemic gained momentum during the late 1980s. In 1990 Chin estimated that there were 9 million people living with HIV infection in the world and of these 5.5 million were believed to be in Africa. A prominent feature of the epidemic in Africa is the higher infection rate in women. The infection continued to spread across the continent, especially in sub-Saharan Africa (SSA). SSA remains the most seriously affected region worldwide, with an estimated 70% of the 35 million people living with HIV. The prevalence varies both between regions and within countries in SSA. Southern Africa has a higher prevalence than East and West Africa. In Southern Africa the highest prevalence is in Swaziland where it is presumed to be 27%. SSA remains at the epicentre of the AIDS pandemic. In 2012 approximately 70% of new infections documented worldwide occurred in SSA (UNAIDS Report, 2013).

1.1.3 The HIV epidemic in South Africa

Ras et al, 1983 first documented HIV infection in South Africa. They described two white homosexual males with the clinical manifestations consistent with AIDS. During the early years it was believed to be a disease of predominantly homosexual men and recipients of blood transfusions. During the 1990s however it became obvious that, as in the rest of Africa, South Africa was experiencing an epidemic due to heterosexual transmission of the disease.

- Approximately 18% of adults in South Africa are HIV-positive.
- The rate among Blacks is higher than amongst other race groups.
- The highest rates occur amongst sexually active young women.

The initial response by the health authorities to the potential problem was inadequate and the number of infections rose dramatically, especially amongst women attending antenatal clinics.

- In 1990 the HIV prevalence in women attending antenatal clinics was 0.7%.
- The prevalence peaked in 2005 at 30%.
- The prevalence rate appears to have stabilized and in 2011 and 2012, it was recorded at 29.5%.

(The National Antenatal Sentinel HIV & Herpes Simplex Type-2 Prevalence Survey in South Africa, 2012).

The rollout of HAART to patients in the public sector was delayed and only began in 2004. There has been substantial progress since in making the drugs available. According to Campion, 2015 an estimated 2.4 million adults and 156 000 children are accessing therapy. The effect of the widespread availability of anti-retroviral therapy is seen in the reduction in the number of new infections amongst all age groups. There are no retention figures on the number of people that remain on therapy twelve months after initiating HAART.

South Africa continues, however to be severely affected. It is home to the highest number of people living with HIV (6.1 million) and in 2012 there were 350 000 new infections (UNAIDS Global Report, 2013).

1.2 The Structure and Life Cycle of HIV

Human Immunodeficiency Virus is a retrovirus, and is part of the subclass of lentiviruses. The HIV, like all viruses is unable to grow or reproduce by itself. It has to infect cells in a living organism to multiply.

The virus is 0.1 microns in diameter. The virus consists of a:

- Viral envelope
    - Composed of two lipid layers
- Viral core which contains
    - Three enzymes required for replication i.e. reverse transcriptase; integrase and protease
    - Genetic material, two identical Ribonucleic acid (RNA) strands

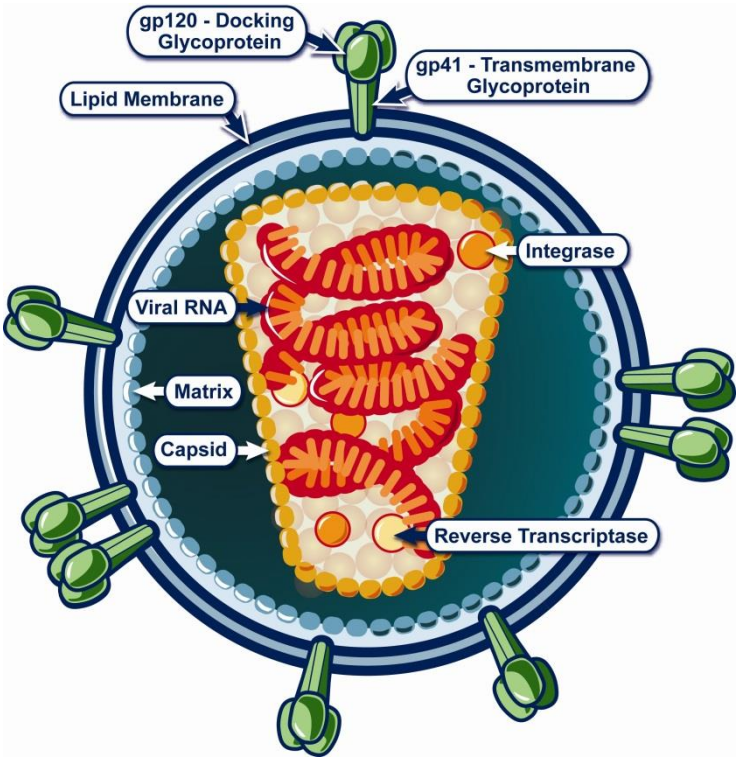


Figure 1.1 Structure of HIV

Source: National Institutes of Health (NIH). Wikimedia Commons (2014)

Legend: gp=glycoprotein

Most organisms store genetic material on Deoxyribonucleic acid (DNA). Retroviruses are unusual in that they utilize RNA. This complicates the replication process. Replication is mainly restricted to human cells that carry the surface protein Cluster of Differentiation 4 (CD4). The spikes on the surface of the HIV particle fuse with the CD4 and the contents are released into the cell. The reverse transcriptase enables the viral RNA to be converted into DNA. Integrase ensures that the DNA is incorporated into human DNA. The virus may remain dormant for a prolonged period before activation. Upon activation it is converted into messenger RNA before being transported outside the nucleus. New HIV enzymes and proteins are produced in this way. Protease cuts the protein strands into smaller pieces. These pieces build new viral core particles which infect other cells and continue the replication process.

1.3 HIV Subtypes

There are two types of HIV:

- HIV-1
- HIV-2

The two subtypes are similar in terms of:

- Genetic composition (30-60%)
- Structure
- Transmission routes
- Cellular targets

Although HIV-1 infection is responsible for most of the global AIDS pandemic, HIV-2 is an important cause of disease in some regions of the world. HIV-2 was initially found in West African nations, but has spread to other parts of Africa, Europe, India, and the United States. HIV-2 is less virulent and the disease progression less aggressive. HIV-1 is the type identified in South Africa.

HIV-1 has been sub-divided into 3 groups: major (M); outlier (O) and non-M non-O (N). See Figure 1.2 below. In 2009 Plantier et al described a single case of a new HIV-1 lineage which they labeled group P. The M group is the most common circulating subtype within the HIV-1 group and has been divided into subtypes (also called clades) denoted with letters and sub-subtypes denoted with numerals. Subtype C is the most prevalent clade accounting for more than half of all infection. HIV-1, subtype C viruses account for the vast majority of the South African epidemic (Ariën , Vanham and Arts , 2007).

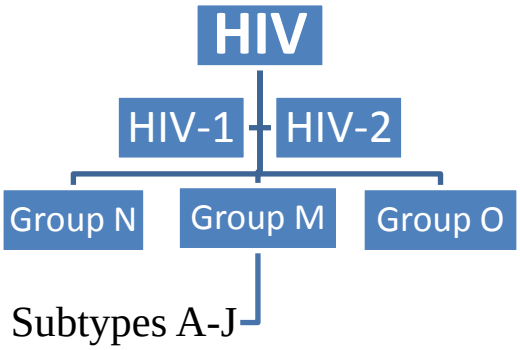


Fig 1.2 HIV Sub-types

1.4 Viral transmission

The foremost modes of transmission are:

- Sexual intercourse
- Exposure to infected blood via transfusions or needle sharing
- Perinatal transmission

The mode of transmission varies between developed and developing nations. In developing countries vaginal sex accounts for the vast majority of infections. In developed countries male to male sex and intravenous drug abuse play a significant role.

Beyrer, in 2007 documented the numerous established risk factors for heterosexual HIV transmission:

- Co-infection with Herpes Simplex Virus-2
- High risk sexual behaviour
    - High level of concurrent partnerships
    - Receptive anal sex
- High viral load
- Host and genetic factors
    - Gender inequalities
    - Young age of initial sexual activity
- Labour migration patterns
- Lack of circumcision
- Low rates of condom use
- Presence of ulcerative sexually transmitted infections

1.5 Pathogenesis of Neurologic Diseases in HIV

Neurologic disease may be due to:

- Direct effects of the HIV on the nervous system
- HIV-related immune dysfunction with CD4 cell depletion

The CD4 depletion renders the individual susceptible to opportunistic infections and neoplasms of the nervous system.

Zayyad and Spudich, 2015 have reviewed this topic. The virus may enter the brain during the viraemia that accompanies the early systemic infection. This early seeding of the virus in the brain may set the scene for long-term low grade inflammation within the brain. The presence of HIV in the central nervous system (CNS) causes activation of the immune system and increased expression of inflammatory cytokines. The cytokines lead to a breakdown of the blood-brain barrier (BBB) and further cause damage to the surrounding glial cells and neurons. The virus may access the CNS at numerous stages during the course of the disease. This access may be provided by distinct events or a sustained vulnerability of the BBB during the course of the infection. The term CNS compartmentalization has been coined. This refers to the localization of the HIV infection within the CNS and the ensuing genetic changes found in the viral strains within the cerebro-spinal fluid (CSF) and brain tissue.

The HIV uses a minimum of two surface receptors to enter any target cell. The primary receptor is the CD4 molecule. This molecule is present on the surface of:

- CD4 lymphocytes
- Dendritic cells
- Macrophages
- Monocytes

The virus initially binds to the CD4 receptor and then binds to a co-receptor which is either chemokine receptor 5 (CCR5) or chemokine receptor 4 (CCR4). During the initial phases of the infection CCR-5 tropic virus (R5) predominates. The virus that has an affinity for CCR4 (X4) is a more pathogenic phenotype. During disease progression the X4 phenotype gains ascendancy.

Bilgrami and O' Keefe, 2014 have described the numerous cell types involved in the pathogenesis:

- Microglia
  - Express the CCR 5 co-receptor
  - Primary target cells of HIV
- Monocytes and Macrophages
  - Express both co-receptors
  - Assist in HIV growth and replication
- Astrocytes
  - May become infected, but are not involved in growth and replication
  - May have a role in neuronal dysfunction
- Endothelial cells
  - They express both co-receptors and may become infected.
  - Precise role is unclear
- Neurons and oligodendrocytes
  - Uninfected by HIV

The virus is transported across the BBB in infected $CD14^+$ and $CD\ 16^+$ monocytes. There has been recent evidence to suggest that the virus also causes a disruption of the BBB. The HIV affects the modifying proteins that maintain the endothelial tight junctions in the BBB. Some of the monocytes differentiate into macrophages and perivascular microglia within the CNS. These macrophages are present in peri-vascular spaces, the meninges and the choroid plexus. The perivascular macrophages infect new cells using matrix metalloproteinase-9. The macrophages function as antigen-presenting cells and in the activated state produce neurotoxic substances. The macrophages exist in three different activation states:

- M1 causes a pro-inflammatory response
  - Activated by Interferon gamma (IFN-ý)
- M2 causes an anti-inflammatory response
  - Activated by Interleukin (IL)-4 and IL-13
- dM which causes immune-suppression
  - Activated by IL-10

In normal conditions during M1 and M2 activation there is an effective immune response to organisms that invade the CNS. HIV infection causes the system to be reversed. The virus uses M1 and M2 tracks to promote the dissemination of the virus. Macrophages can house unintegrated HIV DNA which supports the transcription of the viral genes nef and tat. These genes induce the cytokine ligands (CXCL) 9 and CXCL10 which are known neurotoxins. They can also stimulate macrophages to produce quinolinic acid, a neurotoxin. A further mechanism that macrophages use to cause disease in HIV infection is via the inhibition of long-term signal transmission between neurons. Macrophage activation results in a toxic, inflammatory and deregulated environment within the CNS (Lamers et al, 2014).

It has recently emerged that in addition to the gut and bone marrow, the CNS may be an additional reservoir for HIV. Canestri et al, 2010 have shown that the virus is able to multiply within the CNS in patients that have evidence of systemically effective HAART. This phenomenon has been termed "CSF escape".

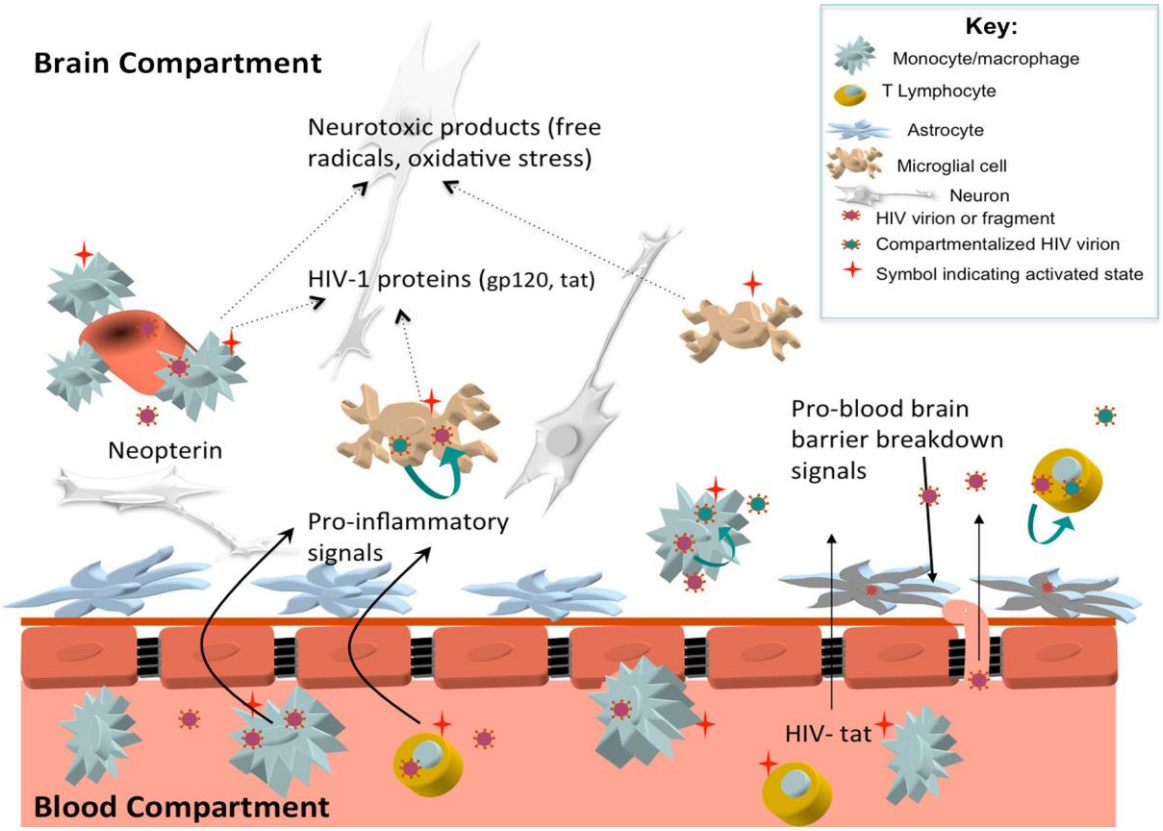


Fig. 1.3 Neuropathogenesis of HIV-related CNS injury

From Zayyad Z, Spudich S Curr HIV/AIDS Rep. 2015 Jan 22. Neuropathogenesis of HIV: From Initial Neuroinvasion to HIV-Associated Neurocognitive Disorder (HAND). Permission obtained, see Appendix A

1.6 Clinical Progression of HIV Infection

- Viral Transmission
- Acute (Primary) HIV Infection
- Chronic HIV Infection
    - Asymptomatic
    - Early Symptomatic HIV Infection
    - AIDS
      (CD4 cell count <200cells/μL or presence of AIDS-defining condition)
    - Advanced HIV Infection
      (CD4 cell count <50cells/μL)

Progression of the disease is highly variable. Patients may develop a chronic symptomatic illness over a period of 6 months to 30 years following seroconversion. Some patients (referred to as Elite Suppressors) are able to naturally control the infection without HAART. In patients not receiving anti-retroviral therapy the mean time to the development of AIDS is between 10 and 11 years with survival thereafter of less than four years.

Neurologic disease can occur at any stage of HIV infection, but occurs more commonly in patients with advanced disease.

1.7 Neurology of HIV Infection

1.7.1 The Early Days

Neurological disease represents a significant portion of the spectrum of both primary and opportunistic illnesses associated with HIV infection.

Disorders of the central and peripheral nervous system were recognized early. In 1983 Snider et al analyzed fifty patients with neurological complications attributed to the recently described AIDS syndrome. HIV itself had not been isolated yet. They identified five categories of neurological complications:

- Infections
- Tumours
- Vascular
- Peripheral neuropathy
- Undiagnosed

Infections were the predominant finding and were present in 31 patients (62%). The most commonly identified "infection" was progressive dementing encephalitis (subacute encephalitis) which was present in 18 patients. The patients presented with subtle cognitive changes, malaise, lethargy and social withdrawal. They were noted to have psychomotor retardation. The affected individuals deteriorated over time and eventually developed features of a severe dementia. The symptoms and signs described in these patients are typical of the disease currently known as HIV Associated Neurocognitive Disorder (HAND). It was believed that Cytomegalovirus (CMV) was the cause of the encephalitis because intracellular inclusions which were suggestive of CMV were present in some patients' post-mortem. The other infections detected were *Toxoplasma gondii*, patients presented with focal brain lesions; Progressive Multifocal leukoencephalopathy (PML), indicative of underlying John Cunningham virus (JCV) infection; cryptococcal meningitis; candida albicans and possible mycobacterium avium intracellulare.

The neoplasms identified in this group were lymphoma (primary neurological and metastatic); sarcoma and plasmacytoma in individual patients. Vascular complications included endocarditis, cerebral haemorrhage and cerebral arteritis. The undiagnosed group comprised patients with focal brain lesions; aseptic meningitis and retinopathy.

The extent of neurological involvement in AIDS patients was well documented during the initial stages of the epidemic. In April 1985 Levy, Bredesen and, Rosenblum described the neurological symptoms in an unselected group of 352 homosexual patients with AIDS. One hundred and twenty-four patients (39%) were affected. The authors conceded that their figure may be an underestimate as mild disease may have been overlooked. This was borne out by their own autopsy series where 30 patients (73%) had neuropathological abnormalities. Their categorization of neurological complications was similar to that discussed above. Viral and non-viral infections accounted for the majority (85%) of patients presenting with neurological disease. The most common complication identified in this cohort was sub-acute encephalitis which was present in 28% of patients. The authors believed that CMV could be responsible for the encephalitis.

In late 1985 the Human T Lymphotropic Virus III (HTLV III) virus, as it was known was isolated in the CSF and neural tissues. Two independent studies (Ho et al and Levy JA et al, 1985) were published describing the presence of the virus in patients with documented clinical neurological syndromes consistent with AIDS. The authors concluded that the virus was neurotropic. It was proposed that the virus itself could cause acute and chronic meningitis; dementia; spinal cord degeneration and peripheral neuropathy.

A seminal article by Berger in 1988 delineated the neurological complications of HIV infection and classified them into two groups:

- Direct HIV Infection
- Indirect HIV Infection

Neurological complications in HIV have increased since then in parallel with the increased number of HIV-infected individuals. The introduction of HAART has led to an improvement in the quality of life in some people with HIV. In the face of this progress, neurologic complications still occur in approximately 70% of patients (Sacktor, 2002). HAART itself may contribute to the burden of neurological disease.

Table 1.1 Neurological Disorders in HIV

- **Primary HIV neurological disease**

(The virus is both necessary and sufficient by itself to cause the disease)

  - Acute HIV Infection
  - HIV Associated Neurocognitive Disorders
    - Asymptomatic Neurocognitive Impairment
    - Minor Neurocognitive Disorder
    - HIV Associated Dementia
  - Neuromuscular Syndromes
  - Peripheral neuropathies
  - HIV-associated Myopathies
  - Vacuolar myelopathy

- **Secondary/ Opportunistic Neurological Syndromes**

  - Opportunistic Infections
    - Bacterial Infections
      - Neurologic Tuberculosis
    - Fungal Infections
      - Cryptococcal meningitis
    - Parasitic Infections
      - Malaria
    - Protozoal Infections
      - Toxoplasmosis
    - Viral Infections
      - Cytomegalovirus
      - JCV, causing PML
  - Opportunistic Malignancies
    - Primary Central Nervous System Lymphoma

- **Treatment Related Neurological Disease**

  - Antiretroviral toxic neuropathy
  - Immune Reconstitution Inflammatory Syndrome
  - Toxic myopathy

1.7.2 Primary HIV-related Neurological Disease

Acute HIV Infection

The virus manifests itself clinically after an incubation period of 1 to 4 weeks. The initial symptoms are similar to that of an influenza-like illness. The majority of HIV-infected patients develop this self-limiting syndrome. The neurological manifestations of the acute illness include:

- Meningitis
    - May be accompanied by cranial neuropathies
    - Usually aseptic
    - CSF abnormalities include a pleocytosis and elevated protein
- Headache
    - Dull, bilateral ache
    - Inconsistent descriptions of site; duration and response to therapy
- Cranial Neuropathies
    - The nerves commonly affected are the trigeminal; facial and vestibulocochlear
        - Facial nerve is the most frequently affected nerve and in rare cases there may be bilateral involvement
        - Precedes seroconversion by 4–6 weeks
        - In the early stages of the infection, acyclovir and a short course of steroids may be given
- Peripheral Neuropathies
    - Inflammatory demyelinating neuropathies predominate at this stage
    - Brachial neuritis; sensory neuropathy and ataxic neuropathy can occur but are rare
- Myalgia
    - Common symptom
    - Can rarely be accompanied by rhabdomyolysis

There may be a transient decrease in the CD4 counts during this phase.

The diagnosis is based on:

- Presence of p24 antigen
    - Limited use
- Identification of viral RNA
    - Viral load assays are widely available

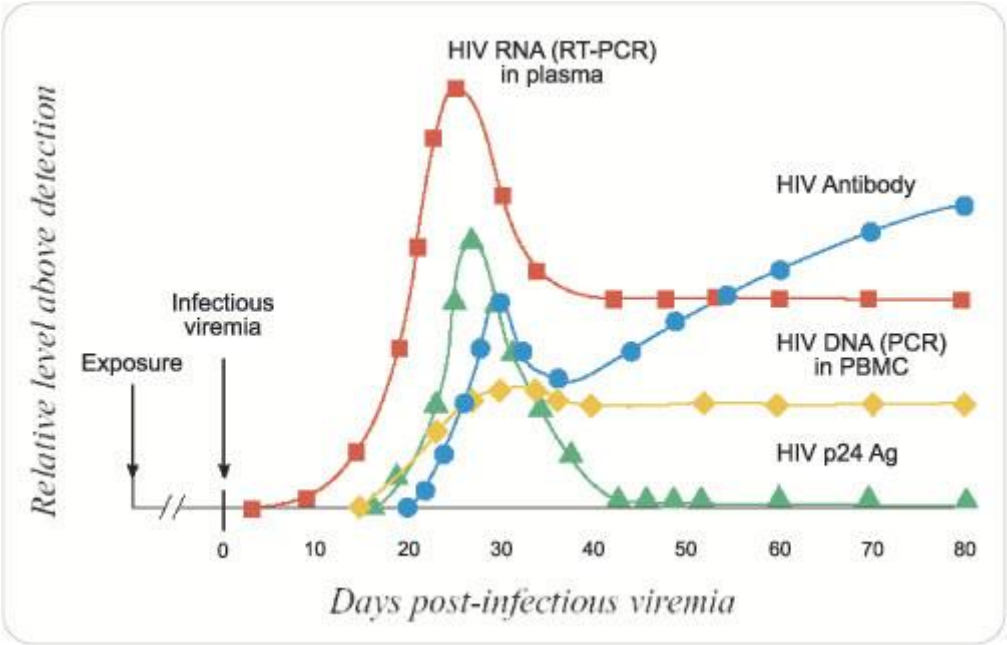


Fig. 1.4 Laboratory Diagnosis of HIV Infection
From https://depts.washington.edu/labweb/Divisions/Viro/HIVTest.htm.
Permission obtained , see Appendix B

The management includes:

- Psychosocial evaluation
- Advice on
    - Heightened infectiousness
    - Effective transmission risk reduction strategies
- Supportive care for the acute symptoms
- The role for HAART is this setting is not yet well established, but does need to be considered

The presence of severe symptoms during acute infection predicts more rapid clinical progression to AIDS

HIV Associated Neurocognitive Disorders (HAND)

During the early stages of the HIV epidemic the entity of subacute encephalitis was recognized in HIV infected patients with features of dementia. The neuropathological hallmark was the presence of multinucleated giant cells on autopsy and the absence of other potential pathogens. The terms AIDS dementia complex (ADC) and HIV encephalopathy (HIVE) were coined later and described the clinical features and neuropathology of this primary HIV infection of the brain. A 1986 autopsy study by Navia, Jordan and Price indicated that two thirds of patients had evidence of this encephalopathy. The syndrome was soon recognized as a spectrum of cognitive deficiency ranging from mild, to moderate, to severe. It was believed that the degree of immunosuppression correlated with the neuropathogenicity of the virus. The early stage described as a disturbance in intellect was characterized by:

- Fatigue and malaise
- Headache
- Increasing social isolation
- Loss of sexual drive

Patients described increasing forgetfulness and a slowing of their thought processes. The clinical features were typical of a “sub-cortical dementia”.

During the course of the syndrome they developed tremors, gait disturbances and imbalance. Patients with advanced disease were unable to care for themselves. The diagnosis of HIVE was based on the exclusion of other diseases and required serology, CSF examination and neuroimaging.

Neurocognitive dysfunction remains the most significant neurological disorder in patients on HAART. The currently advocated terminology for HIV-associated neurocognitive disorders (HAND) are the Frascati criteria, conceived by Antinori et al, 2007. This is based on and includes neuropsychological testing and the assessment of functional status and is not applicable at the bedside. The neuropsychological component of the evaluation requires assessment of the following ability domains:

- Verbal/language
- Attention/working memory
- Abstraction/executive
- Memory (learning; recall)
- Speed of information processing
- Sensory-perceptual
- Motor skills

Functional status is based on activities of daily living and may rely on self-reporting; require corroboration or long-term follow-up.

Three categories are described based on the severity of the impairment.

- Asymptomatic Neurocognitive Impairment (ANI):
    - Neurocognitive test performance 1 standard deviation (SD) below an appropriate normative mean in at least 2 cognitive domains, but
        - No decline in functional status.
    - Patients are asymptomatic and formal neuropsychological evaluation is necessary to confirm the diagnosis.
- Mild Neurocognitive disorder (MND):
    - Neurocognitive test performance 1 SD below mean in at least 2 cognitive domains
    - Mild impairment in activities of daily living.
    - Patients in this stage of the disease have minor cognitive impairment which impacts both on their home and work life.
    - There may be minor abnormalities on clinical examination.

- HIV-associated Dementia (HAD):
    - Severe cognitive impairment in two or more domains by 2 SD below mean
    - Notable impairment in activities of daily living.
    - Neurological dysfunction is evident and impacts on all spheres of the patients' life.
    - Clinical deficits (spasticity, especially of lower limbs, clonus, frontal release signs, tremor and ataxia) develop with progression of the disease.
    - Seizures and myoclonus can occur in advanced disease.
    - Severe dementia may be accompanied by a co-occurring myelopathy and/or peripheral neuropathy.

  This advanced clinical picture is uncommon in regions where HAART usage is widespread, but continues to occur in patients with:
    - Non-adherence
    - Limited access to anti-retroviral therapy
    - Treatment failure

The CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) study (Heaton et al, 2011) a large multi-centre study based in the US estimates current prevalence of HAD at less than 5%. The milder subtypes of HAND, however, continue to be reported in patients despite sustained virological control. In the CHARTER study mild neurocognitive impairment was detected in 45% of patients:

- ANI in 33%
- MND in 12%

About half of HAART –treated patients have milder forms of neurocognitive impairment (Heaton et al, 2007).

Possible reasons for the inadequate response to therapy include:

- The "legacy effect" of HIV infection i.e. the lasting damage that occurs prior to the initiation of HAART
- Persistent CNS disease activity due to a lack of viral suppression, secondary to poor CNS penetration effectiveness (CPE) of HAART
- Potential neurotoxicity of HAART
- Associated comorbidities
    - Psychiatric illness
    - Substance abuse
    - Cerebrovascular disease
    - Ageing

The current epidemiological pattern dominated by milder forms of HAND is applicable in developed countries. In developing regions which carry a much greater disease burden HAART availability has lagged behind. Increasing the access to anti-retroviral therapy is likely to have the same effects as seen in the developed regions of the world with a considerable decrease in the disease burden of HAD and an increase in the milder HAND subtypes.

Neuromuscular Syndromes

- Peripheral Neuropathies (PN)
    - HIV-associated distal sensory neuropathy
        - Increased frequency as the degree of immunosuppression increases
        - Symptoms include numbness and severe neuropathic pain
        - Clinical features include a stocking pattern of impaired sensation and decreased/ absent ankle reflexes
        - This is usually a diagnosis of exclusion
        - Effective therapy has not yet been established but lamotrigine, high dose topical capsaicin and recombinant nerve growth factor may provide symptomatic relief
    - Inflammatory Demyelinating Polyneuropathies
        - Usually occur with mild to moderate immunosuppression
        - Clinical, diagnostic features and management are similar to non-HIV infected patients
    - Autonomic neuropathy
        - Common in patients with advanced disease
    - PN associated with diffuse infiltrative lymphocytosis syndrome (DILS)
        - Uncommon
- HIV-associated myopathies
    - HIV-associated Polymyositis
        - Can present at any stage
        - Clinical features vary from minimal weakness to debilitating weakness
        - Diagnosis and therapy is the same as in non-HIV infected patients
    - HIV wasting Syndrome
        - Significant in patients with advanced immunosuppression
        - Myalgia and proximal weakness are common
        - Initiation of HAART is associated with an improved survival

Vacuolar Myelopathy (VM)

It is the commonest spinal cord pathology identified in the setting of HIV, typically occurring with advanced immunosuppression. Clinical myelopathy is uncommon, but the pathological findings consistent with the diagnosis are frequently described in autopsy studies. The influence of HAART on the occurrence of VM is poorly documented.

The pathological hallmark of the disease is patchy vacuolation of the white matter affecting the dorsal and lateral columns.

The clinical features which develop over weeks to months are:

- Bilateral lower limb weakness and spasticity
- Bladder, bowel and erectile dysfunction
- Hyperactive deep tendon reflexes and an extensor plantar response
- Unsteady gait
- Variable sensory abnormalities

VM and distal sensory polyneuropathy frequently occur in the same patient. This combination can lead to a mixed clinical picture where lower limb hyper-reflexia can be present in conjunction with absent ankle jerks and extensor plantar responses.

The diagnosis is one of exclusion:

- Serology
    - Serum vitamin B12 and copper levels should be normal
    - Syphilis and human T-lymphotrophic virus-1 (HTLV-1) negative
- CSF analysis
    - Helps to exclude other infectious aetiologies
- Magnetic Resonance Image (MRI) spine
    - Atrophy of the thoracic cord
    - Non-specific hyper- intensities on T2 weighted images which do not enhance

There is no specific therapy as HAART has not been proven to delay the progression or improve the underlying deficits in patients with VM. Symptomatic treatment of spasticity, urinary urgency and erectile dysfunction is advocated.

1.7.3 Secondary/ Opportunistic Neurological Syndromes

Opportunistic Infections

The epidemiology of neurological complications of HIV-infection has changed since the introduction of HAART. Opportunistic neurological diseases have decreased in the developed world. In developing nations opportunistic infections continue to be a significant cause of morbidity, even in patients on HAART. Tan et al, in 2012 reviewed HIV-associated opportunistic infections of the CNS:

- Usually occur when the CD4 cell count is less than 200cells/µL
- Diagnosis should be based on:
    - Clinical presentation
    - Temporal evolution
    - CSF features
    - Radiology findings
- Multiple infections may be present and some may only be discovered after HAART is initiated
- HAART should be started/ modified or continued in association with appropriate antimicrobial therapy
- Prophylactic antimicrobial therapy may be required until immune recovery occurs (CD4 cell count greater than 200cells/µL

The incidence of opportunistic infections varies in different regions and is dependent on the underlying prevalence.

Table 1.2 Regional Infections in HIV

- Asia and Pacific regions
  - Cerebral toxoplasmosis
  - Cryptococcal meningitis
  - Japanese encephalitis B
  - Tubercuolous meningitis
- Europe and North America
  - Cerebral toxoplasmosis
  - Cryptococcal meningitis
  - Progressive Multifocal Leukoencephalopathy
- South America
  - Cerebral toxoplasmosis
  - Cryptococcal meningitis
  - Tubercuolous meningitis
  - Chagas Disease
- Sub-Saharan Africa
  - Cryptococcal meningitis
  - Tubercuolous meningitis
  - Cytomegalovirus
  - Malaria

from Tan et al, HIV-associated opportunistic infections of the CNS, 2007

The common opportunistic infections; especially those in SSA will be reviewed.

Tuberculosis (TB) of the Central Nervous System

Nearly one third of the global population is infected with *Mycobacterium tuberculosis.* Disseminated forms of TB, including neurologic disease in tuberculosis occur more commonly in patients co-infected with HIV. The co-infection rates are highest in Sub-Saharan Africa.

The varied presentations include:

- Tuberculous meningitis (TBM)
- Focal Brain Lesions (FBLs)
    - Tuberculoma
    - TB abscess
- Spinal TB
    - TB myeloradiculopathy
    - Tuberculoma

The clinical features depend on the specific presentation.

The features associated with TBM are:

- Initially non-specific i.e. anorexia; headache; malaise; myalgia and pyrexia
- Focal neurological deficits which can progress to an altered level of consciousness and later coma
- Cranial nerve palsies which may be the initial presenting feature

FBLs

- The signs and symptoms depend on the location and include ataxia and movement disorders
- Focal or generalized seizures can occur during any stage, including after therapy has been completed

Spinal TB

- Features of cord compression including bladder and bowel dysfunction
- Spinal or radicular hyperaesthesia; pain or paresthesiae

Early diagnosis of neurological TB is crucial but can be difficult. The clinical features, especially in TBM are non-specific and the currently available diagnostic tests are either non-specific or timeous.

- The CSF features include:
    - Low glucose; lymphocytic pleocytosis and a raised protein
    - Positive Ziehl-Neesen stain, which has variable sensitivity

  Repeated large volume lumbar punctures (LPs) may improve the yield. The *Mycobacterium tuberculosis* and resistance to Rifampicin (Xpert MTB/RIF) assay can assist in both the diagnosis and the detection of drug resistance in CSF samples. The assay utilizes nucleic acid amplification technology (NAAT) which can decrease detection times and improve sensitivities

- Neuro-imaging
    - In patients with TBM there may be basilar enhancement; hydrocephalus and infarcts in the basal ganglia or cortex
    - FBLs are usually associated with basal meningeal enhancement

The therapy is based on standard regimens:

- A four drug regimen is given for 2 months:
  - Ethambutol; Isoniazid; Pyrazinamide and Rifampicin
- A combination of Isoniazid and Rifampicin is given for a further 9-12 months The therapy for neurological multi-drug resistant TB (MDR-TB) is based on guidelines accepted for the treatment of pulmonary MDR-TB
- Adjuvant corticosteroids may have a role but this has not been conclusively established in the setting of HIV
- The optimal timing of HAART in patients with TB is dependent on the immune status of the patient as there is a risk of Immune Reconstitution Inflammatory Syndrome (IRIS).

IRIS is higher in patients with advanced immune-suppression. Initiating HAART and TB medications simultaneously is not recommended.

Cryptococcal Meningitis (CM)

Cryptoccus neoformans is the most common cause of meningitis in HIV-infected patients. In Sub-Saharan Africa it is the commonest cause identified in adult patients. The meningitis typically occurs in patients with advanced disease; (CD4 cell count is below 50cells/µl). The introduction of antiretroviral therapy and use of fluconazole has led to a decrease in the incidence of CM in the developed world.

The clinical presentation is that of sub-acute meningitis and the symptoms include:

- Fever; headache and malaise
- Patients with elevated intracranial pressure can present with:
    - Symptoms of encephalopathy i.e. altered level of consciousness; lethargy; memory loss and personality changes
    - Focal signs including abducens nerve palsy and hearing loss; in patients with basilar meningitis there may be abnormalities of multiple cranial nerves

The diagnosis is based on:

- CSF examination:
    - The cell count; glucose and protein level may be normal
    - Detection of cryptococcal antigen which is both highly sensitive and specific
    - Culture of the organism remains the gold standard but can delay the diagnosis
- Radiology
    - Not routinely available in resource poor settings
    - Either Computed Tomography (CT) or MRI scans of the brain are recommended prior to the performance of a LP in patients with focal deficits or clinical features suggestive of increased intracranial pressure

NB! The serum cryptococcal antigen can be helpful as an initial screening tool, especially in patients initiating HAART.

The therapy of CM is multi-faceted and includes:

- Antifungal therapy
    - Induction Phase
        - Amphotericin B and Flucytosine (if available)
        - Minimum period of two weeks
    - Consolidation Phase
        - Fluconazole 400mg orally for eight weeks
    - Maintenance Phase
        - Fluconazole 200mg orally until the patient is asymptomatic and the CD4 cell count is greater than 200cells/µl for a period of six months, whilst on HAART

- Decreasing intracranial pressure if necessary
    - The opening CSF pressure should be recorded at the time of the diagnostic Lumbar puncture (LP)
    - The pressure should be decreased with daily LPs
    - A lumbar drain; ventricolostomy or ventriculoperitoneal shunt should be considered if the elevated pressure is not resolved with serial LPs

- Improvement of immune function by the initiation or optimization of HAART
    - The timing of initiation should be delayed for 2-10 weeks until the CSF culture becomes negative

- Management of IRIS, if necessary
    - IRIS is more common in HAART-naïve patients with high HIV RNA levels
    - Antifungal therapy and HAART need to be continued
    - Corticosteroids may be of value in severe cases

Malaria

Infection is due to the parasitic protozoan organisms, genus *Plasmosium*. In HIV-infected patients malaria is a leading cause of morbidity especially in geographical regions where the two diseases overlap:

- South America
- Southeast Asia
- Sub-Saharan Africa

HIV infection increases both the risk and the severity of malaria infection. The prevalence of malaria increases as the CD4 count cell decreases. Increased burden of the parasite expedites malaria transmission. The malaria in turn is associated with CD4 cell activation and cytokine up regulation. The resultant milieu creates an ideal environment for the spread and transmission of HIV. Acute malarial infection is accompanied by an increase in the HIV burden and a decrease in the number of CD4 cells. These changes resume normalcy following successful treatment of malaria.

The clinical features are similar to that in non HIV-infected patients but there is an increased prevalence of:

- Anaemia
- Severe malaria
- Treatment failure
- Malaria-attributable mortality

Therapy is similar to that in HIV-uninfected patients but there may be a reactivation of drug-resistant organisms. Follow-up is therefore imperative.

In malaria-endemic areas:

- Bed nets and prophylaxis with trimethoprim-sulfamethoxazole are recommended
- Pregnant patients should receive monthly sulfadoxine-pyrimethamine during the pregnancy
- HAART should be initiated/ optimized as it decreases the incidence of malaria

Cerebral Toxoplasmosis

The incidence of neurological toxoplasmosis has decreased since the introduction of HAART, but it remains the commonest opportunistic neurological infection identified. Toxoplasma encephalitis (TE) is due to reactivation, rather than a primary infection. The incidence rates are dependent on the seroprevalence of *toxoplasma gondii (T. gondii)* in the general population.

The risk factors include:

- Degree of immunosuppression
    - The risk increases as the cell count falls below 100cells/µl
- Use of prophylaxis
    - Bactrim is effective for prevention

The neurological clinical features include:

- Non-specific features
    - Headache; pyrexia; lethargy
- Focal signs and symptoms
    - Ataxia; hemiparesis

The diagnosis is usually based on a combination of:

- Characteristic neuroimaging findings (MRI is more sensitive than CT)
    - Multiple ring-enhancing lesions with variable mass effect and oedama
    - Predilection for the basal ganglia and corticomedullary junction
- Positive serologic test for Immunoglobulin G (IgG) antibodies to *T gondii*
- Clinical and radiologic improvement following empiric therapy.
- Polymerase Chain Reaction (PCR) of the CSF:
    - Highly specific but not very sensitive in detecting the parasite

Toxoplasma therapy requires a combination of antimicrobial therapy and HAART for immune recovery. The antimicrobial therapy is given in two phases:

- Initial phase (6 weeks)
    - Treats the acute symptoms, a combination of sulfadiazine (or clindamycin), pyrimethamine and leukovorin
    - Corticosteroids may be indicated if there are features of severe raised intracranial pressure
- Maintenance phase
    - Goal is to prevent relapse
    - Can be discontinued in asymptomatic patients with immune recovery

Cytomegalovirus (CMV) Encephalitis

The virus is part of the herpes group and is endemic worldwide. The risk of severe neurological disease depends on:

- CD4 cell count (<50 cells/µL) and/ or HIV RNA level (>100 000/mL)
- Host genetic risk factors
- Neurovirulence of the CMV strain
- Previous CMV infection (IgG positive)

The incidence of CMV infection and the associated mortality has decreased in regions where HAART is readily available.

The spectrum of neurological presentations includes:

- Encephalitis
    - Brainstem signs and symptoms
    - Hyponatraemia (adrenal disease)
    - Rapidly progressive encephalopathy
    - Seizures
- Mononeuritis multiplex
    - Asymmetric, progressive, multi-focal
    - Motor symptoms are more prominent than sensory
- Radiculomyelitis
    - Lumbosacral region usually affected
    - Rapidly progressive cauda equine syndrome (resembles Guillain-Barre Syndrome)
- Retinitis
    - Can occur in patients with extra-ocular disease
    - Symptoms include central scotoma; floaters and loss of peripheral vision
    - Visual loss due to retinal necrosis

The diagnostic investigations performed depend on the specific clinical presentation and include:

- CSF analysis
  - Neutrophil predominance and negative bacterial culture
  - PCR assay for CMV is highly sensitive and specific
    - It can be used to monitor the effectiveness of therapy
- Fundoscopy
  - Haemorrhagic infarction
  - Perivascular sheathing
  - Retinal opacification
- Neuroimaging
  - Contrast-enhanced and thickened nerve roots on MRI (Patients with radiculomyelitis)
  - Meningeal enhancement or periventricular inflammation
- Biopsy
  - Useful to confirm the diagnosis but not routinely performed

The response to specific anti-CMV therapy is unpredictable and is usually more effective if administered early during the course of the infection.

- Induction Phase
  - Ganciclovir and Foscarnet should be administered intravenously until there is improvement (may take months)
- Maintenance
  - Foscarnet intravenously and Valganciclovir orally until immune recovery occurs

HAART should be initiated within two weeks of starting anti-CMV therapy.

The risk of the patients developing IRIS is a concern, especially as it can lead to blindness in patients with retinopathy and even death in rare instances

Progressive Multifocal Leukoencephalopathy (PML)

The disorder is a progressive, demyelinating disease. It is caused by the ubiquitous John Cunningham virus (JCV), a human polyoma virus. The incidence has decreased following the introduction of HAART.

The clinical presentation depends on the area of the brain that is affected. The disease should be suspected in patients with:

- Focal signs and symptoms
    - Develop over weeks to months
- Progressive dementia which may lead to coma and death
- Low CD4 counts (less than 100 cells/µL); may be normal

The diagnosis is based on:

- Clinical presentation
- CSF analysis
    - PCR assay for JCV has a high sensitivity and specificity; except in patients on HAART
- Neuroimaging
    - MRI is more sensitive and shows hypo intensities on T1 images and hyper intensities on T2 images
- Brain biopsy
    - The characteristic features are bizarre astrocytes; lipid-laden macrophages and myelin loss

There is no definitive therapy but HAART can improve the clinical deficits and prolong survival and should therefore be initiated or augmented.

1.7.4 Opportunistic Malignancies of the Central Nervous System

Primary CNS Lymphoma (PCNSL)

This is a primary non-Hodgkins lymphoma that is limited to the CNS. The disease normally occurs in patients with advanced immune dysfunction i.e. CD4 cell counts less than 50cells/µL. The incidence has decreased in regions where HAART use is widespread but the decline has not been as expected. The vast majority of cases are linked to infection with Epstein-Barr virus (EBV). B cells that are infected with EBV undergo monoclonal proliferation in patients with severe immune dysfunction.

The clinical presentation includes:

- B symptoms
    - Nightsweats; unexplained pyrexia and weight loss
- Focal and non-focal signs
    - Aphasia; confusion; headache; hemiparesis; memory loss and seizures

The diagnosis is dependent on:

- CSF analysis
    - Cytology
    - PCR assay for EBV has a high sensitivity and specificity
- Neuroimaging
    - MRI is more sensitive
    - Lesions are irregular; single and usually enhance with contrast,
- Brain biopsy
    - Stereotactic biopsy is useful to establish a definitive diagnosis

HAART confers a longer survival and should be initiated upon diagnosis of PCNSL. High dose methotrexate and whole brain radiation have shown promising results in clinical trials.

1.7.5 Treatment Related Neurological Disease

Antiretroviral toxic neuropathy

In the pre-HAART era the distal symmetric polyneuropathy (HIV-DSP) was linked to severe immunosuppression. In developing regions peak HIV viral load and the nadir CD4 cell count are still risk factors for the development of the condition. In areas of widespread HAART availability HIV-DSP continues to be highly prevalent due to:

- Chronic inflammation
- Continued low levels of viral replication
- Possible nerve damage secondary to immune reconstitution
- Risk factors associated with an increased life expectancy
    - Drug-induced neurotoxicity (other medications including illicit drugs)
    - Co-morbid medical disorders e.g. diabetes mellitus

The clinical features are typical of those commonly associated with a small fibre sensory neuropathy and include:

- Absent ankle reflexes
- Painless numbness or painful paresthesiae in the feet
- Progressive symptoms over weeks to months
- Symptoms confined to the lower limbs, below the knees

The diagnosis is based on a combination of:

- Clinical features
- Electromyography and nerve conduction studies
    - May be normal
- Skin biopsy
    - Can provide a definitive diagnosis

The therapy is two-fold:

- Stop reverse transcriptase inhibitors which have been implicated
    - Didanosine; stavudine and zalcitabine

- Symptomatic therapy for the pain
    - Lamotrigine may be effective
    - Topical capsaicin and recombinant human nerve growth factor may be beneficial (limited evidence)
    - Traditional drugs used for neuropathic pain have not been proven to be useful in HIV-DSP e.g. amitriptyline; gabapentin and pregabalin

Immune Reconstitution Inflammatory Syndrome (IRIS)

In this syndrome the patient undergoes clinical deterioration in the face of immune recovery. Neurological IRIS is an uncommon phenomenon in well-resourced regions. In resource-limited regions it is more prevalent. The well identified risk factors are:

- Rapid immune recovery, evidenced by a precipitous decrease in the viral load
- Severe immunosuppression at HAART initiation, reflected by a low nadir CD4 cell count
- Underlying opportunistic infections

Genetic factors may also influence the development of IRIS:

- Cytokine polymorphisms
- Expression of pro-inflammatory cytokines e.g. IL 6

The clinical syndromes associated with the diagnosis of IRIS are not uniform. There are numerous clinical, immunologic and radiologic features which are encompassed by the diagnosis of IRIS. The specific presentation depends on the underlying opportunistic infection. The organisms commonly associated with IRIS include:

- Bacterial pathogens
    - *Mycobacterium tuberculosis*
        - Neurological IRIS occurs later than IRIS in other organs
        - Clinical deterioration may be due to:
            - Development/ recrudescence of TBM
            - Expansion of intracranial tuberculomas
- Fungal pathogens
    - *Cryptococcus neoformans*
        - Most significant fungal pathogen in CNS-IRIS
        - Occurs 3 to 20 months after initiation of HAART
        - Clinical features are non-specific and the condition may be difficult to diagnose
        - Consider if:
            - CSF shows sterile inflammation and organism is not cultured
            - New enhancement becomes evident on neuroimaging

- Parasitic pathogens
    - *Toxoplasma gondii*
        - Commonly presents as an encephalitis
- Viral pathogens
    - Herpes viruses
        - Varicella Zoster Virus
            - Can present as a painful dermatomal rash; encephalitis; transverse myelitis or a vasculitis which can cause cerebral infarcts
        - Cytomegalovirus
            - Typical presentation in IRIS is that of a retinopathy
    - JC Virus
        - Usually develops 4 to 8 weeks after the initiation of HAART
        - Diagnosis is difficult
        - The prognosis is poor and mortality is high

There is no single therapeutic regimen to manage CNS-IRIS. Prevention, which requires early initiation of HAART, is the best option. There are certain guidelines which can assist in the optimum management of IRIS:

- Delaying the initiation of HAART by 1 month in patients with diagnosed opportunistic infections
- Immunomodulatory therapy
    - High dose steroids are indicated when there is a risk of herniation, due to inflammation
    - The use of steroids in other scenarios is controversial

Toxic Myopathy

This condition was initially recognized following the introduction of azidothymidine (AZT). Numerous other anti-retrovirals have subsequently been associated with the disease and include didanosine; etravarine; raltegravir and tenofovir.

The clinical features are non-specific:

- Hip and shoulder girdle weakness
- Mildly elevated creatine kinase (CK) levels

The diagnosis can be confirmed by:

- Blood levels for CK
- Electrophysiology (non-specific)
- Muscle biopsy
    - Abnormal mitochondria
    - Ragged red fibres

The symptoms tend to improve following withdrawal of the offending drug.

1.8 Epilepsy

1.8.1 Definitions

Epileptic seizures are defined as a "transient occurrence of signs and or symptoms due to abnormal, excessive or synchronous neuronal activity in the brain". Seizure activities include:

- Alterations in level of consciousness
- Involuntary autonomic; motor; psychic or sensory events

Seizure activity usually lasts only a few seconds to minutes. Seizures can occur in anyone if the appropriate pathophysiological conditions exist.

Seizures due to acute and transient conditions have been described as provoked or acute symptomatic epileptic seizures.

Epilepsy refers to the condition in which a persistent alteration of brain function leads to a risk of recurrent epileptic seizures. Not everyone who has had a seizure has epilepsy. Epilepsy is not a single disease; it refers to a group of disorders that reflect underlying brain abnormalities secondary to a variety of causes.

1.8.2 New onset versus newly diagnosed

The time from onset to diagnosis may vary significantly and is dependent on many factors including:

- Access to medical facilities
- Seizure type
- Socio-economic background

The extent of the incidence of new onset epilepsy and the incidence of newly diagnosed epilepsy may fluctuate. In new onset epilepsy the numerator comprises patients identified at their second unprovoked seizure. Newly diagnosed epilepsy includes both new onset epilepsy and people with more than two unprovoked seizures (initial diagnosis occurs during the study period, Beghi et al, 2010)

1.8.3 Classification of Seizures

The pathogenesis of seizures and epilepsy are currently better understood due to improved techniques in neuroimaging, genetics and molecular biology. The International League Against Epilepsy (ILAE) Commission on Classification and Terminology has accordingly revised the seizure and epilepsy classification systems (Berg et al, 2010).The clinically significant classifications are based on the mode of seizure onset and underlying aetiology.

- **Generalized**
    - The seizures may originate at a specific locus but rapidly involve bilateral networks. The seizures can be asymmetric.
    - Cortical and subcortical structures may be involved.
    - The onset may appear focal but the origin and lateralization are not consistent with subsequent seizures.
- **Focal**
    - The seizures originate within the networks of one hemisphere.
    - They can begin in subcortical structures.
    - The onset of seizures is consistent in subsequent events.
- **Unclassified**
    - Seizures that cannot be categorised are referred to as unclassified.
    - New information may lead to accurate diagnosis at a later stage.
- **Genetic**
    - In these patients the epilepsy is directly attributable to known or presumed genetic defects with resultant seizures
- **Structural or metabolic**
    - Specific structural and metabolic disorders carry an increased propensity for epilepsy.
    - The disorder may be either congenital (e.g. cortical dysplasia) or acquired (e.g. stroke).
- **Unknown**
    - The underlying cause in these patients is not currently known.
    - All types of epilepsies with normal imaging and no documented genetic, metabolic or immune etiology are included in this category.

CHAPTER TWO

LITERATURE REVIEW

2.1 New onset seizures (NOS) in HIV infected Individuals

2.1.1 Introduction

New onset seizures have been well documented in HIV infected individuals. The first comprehensive description of the neurological complications of AIDS by Levy, Bredesen and Rosenblum in 1985 included two patients with presumed sub-acute encephalitis in who the only clinical feature identified was seizures. Holtzman , Kaku and So in 1989 published the first study looking specifically at new onset seizures in HIV. Multiple studies since then have described the occurrence. The study by Kellinghaus et al, published in 2008 was conducted in the HAART era.

In some individuals seizures may be the initial presenting symptom signalling an underlying neurological disorder. Seizures occur at any stage of the disease. They have been described during the sero-conversion stage, but they occur with greater frequency in patients with advanced disease. In the majority of patients a definitive cause can be found for the seizure occurrence. The most common causes can be divided into five categories:

- Focal Brain Lesions (FBLs)
- HIV-Associated Neurocognitive Disorder (HAND)
- Meningitis
- Metabolic Dysfunction
- No Identifiable Cause (NIC)

The patients in whom no cause has been identified account for up to 46% of patients presenting with NOS (Wong; Suite and Labarl, 1990). The interest in this group lies in the fact that there is no data on their course and outcome. The seizures may be a forerunner of established causes or it may denote a primary manifestation of the HIV itself.

The seizure patterns described include focal; focal with secondary generalization and generalized seizures. In the studies published to date generalized seizures predominate. The presence of focal seizures does not necessarily imply an underlying focal lesion. Status epilepticus has been documented in up to one fifth of patients. The significance of electroencephalographic (EEG) abnormalities is uncertain as non-specific abnormalities have been documented in asymptomatic HIV-infected people.

The use of anti-epileptic drugs (AEDs) for a prolonged period is recommended except when the seizure can be attributed to a reversible metabolic cause. The concurrent use of enzyme inducing AEDs and HAART can lead to significant drug interactions.

2.1.2 Incidence

The published data on seizures in HIV-infected patients is largely derived from retrospective, hospital based studies that were done prior to the availability of HAART (see Table 2.1 page 51). The incidence amongst HIV-infected patients ranged from 2-20%. In the 2005 study by Sinha et al, 20% of patients presented with NOS. This study was conducted at a specialist neurology referral centre with a resultant selection bias. In the most recent study, Kellinghaus et al, 2008 identified new onset seizures in 6% of their HIV infected patients, the majority of who were on HAART. This figure is probably an accurate reflection of the frequency of seizures in developed countries where HAART is widely accessed. The information available from developing countries is limited, especially in the current age of increasing availability of anti-retroviral therapy.

Table 2.1 Summary of Studies on New Onset Seizures in HIV

	Holtzman et al	Wong et al	Van Paeschen et al	Dore et al	Pesola et al	Pascual et al	Chadha et al	Modi et al	Sinhaet al	Kellinghaus et al
Country	**USA**	**USA**	**USA**	**Australia**	**USA**	**SPAIN**	**INDIA**	**South Africa**	**INDIA**	**GERMANY**
Year	1989	1990	1995	1996	1998	1999	2000	2000	2005	2008
Study Design	RS	RS	RS	RS	RS	PS	PS	PS	RS	RS
Sample Size	5604	630	1574	519	N/A	550	455	N/A	500	831
No with NOS (%)	100 (2)	70 (11)	68 (4)	50 (10)	26	17 (3)	23 (5)	60	99 (20)	51 (6)
Seizure Type(%)										
GTC	65	80	63	84	100	71	65	62	63	78
Focal	35	20	19	10	0	11	35	28	34	22
SE		14	18	0	0	19	0	10	8	0
Aetiology(%)										
FBL	32	33	37	30	35	35	48	53	48	40
Toxo	28	16	12	22	19	29	30	2	23	14
TB	0	0	0	0	0	0	13	28	N/A	0
PML	1	0	0	6	8	6	4	0	1	14
PCNSL	4	11	0	2	4	0	0	0	1	0
Other	0	6	25	0	4	0	0	23	0	0
Meningitis	16	10	12	14	12	0	17	22	N/A	N/A
CM	13	10	4	8	4	0	N/A	7	41	N/A
TBM	0	0	1	0	0	0	N/A	8	N/A	N/A
Other	3	0	7	6	8	0	N/A	7	N/A	N/A
HIVE/ HAD	24	7	2	16	31	0	4	N/A	5	22
Metabolic	3	11	N/A	N/A	15	12	0	0	N/A	N/A
NIC	23	46	44	42	27	6	0	25	N/A	49
Px on HAART(%)	0	0	0	0	0	0	0	0	0	84

Legend
RS=Retrospective; PS=Prospective; N/A=Not Available; SE= Status Epilepticus

2.1.3 Aetiology

The causes for seizures in HIV-infected individuals can be categorized into five main groups:

- Focal brain lesions (FBLs)
    - Infections
    - Toxoplasma encephalitis (TE)
    - Tuberculomas
    - Progressive multifocal leukoencehalopathy (PML)
- Neoplasms
    - Primary CNS Lymphoma (PCNSL)
- Meningitis
    - Cryptococcal Meningitis (CM)
    - Tuberculous Meningitis (TBM)
- HIV-associated Neurocognitive Disorder (HAND)
- Metabolic Abnormalities
- No Identifiable Cause (NIC)

There is a significant disparity in aetiologies between cohorts from developed and developing countries. Infectious diseases occur more commonly in developing regions.

Focal Brain Lesions

The characteristics of the four most common causes of FBLs are detailed below.

Toxoplasmosis

Seizures are a well-recognized clinical manifestation in patients with TE and are a common early presentation. In the pre-HAART era toxoplasmosis was the commonest cause of HIV-associated FBLs and responsible for up to 30% of NOS in the pre-HAART era (see Table 2.1 page 51). Cerebral toxoplasmosis has decreased in incidence in developed countries since HAART has become commonplace, it remains however the commonest opportunistic infection of the CNS. In developing countries the decrease has not been as significant, possibly due to low nadir CD4 counts. The incidence of toxoplasmosis in HIV-infected patients is closely aligned to the CD4 cell count. In the recent study by Kellinghaus et al, 2008 toxoplasmosis accounted for 14% of patients with NOS.

Tuberculomas

Tuberculomas as a cause of NOS were only described in studies from developing countries (India and South Africa). In the 2000 study by Modi et al, the FBLs were more commonly due to TB than toxoplasmosis, an indication of the underlying disease prevalence. South Africa has the highest prevalence of TB in the world (Churchyard et al, 2014).

In developing countries HIV infected patients with focal seizures and tuberculous abscesses have been described. The abscesses can present as FBLs. They are usually solitary lesions, which are larger and progress more swiftly than tuberculomas. The abscess cavity contains pus and culture may yield *M. tuberculosis.* Surgical drainage of the pus improves the long term prognosis.

Progressive Multi-focal leukoencephalopathy (PML)

Seizures occur in approximately one tenth of patients and occur early during the course of the disease. Focal and generalized seizures have been described. The pathogenesis of seizures in PML, a white matter disease is complex and includes:

- Presence of lesions adjacent to the cortex, which act as excitatory foci
- Cortical lesions have also been described
- Axonal conduction abnormalities
- Disturbances in neuron-glia balance

PML has been implicated in patients presenting with new onset seizures (NOS) in up to 14% of patients. The Kellinghaus study, 2008 conducted in the HAART-era had the highest proportion of patients with PML. It is possible that PML in some patients was a clinical presentation of IRIS.

Primary CNS Lymphoma (PCNSL)

This is the commonest neurological malignancy in the setting of HIV. The incidence has declined since HAART has become readily available. It occurs in up to 11% of patients (see Table 2.1 page 51) presenting with NOS and is more common in patients with advanced disease. Epstein-Barr virus is implicated in almost all cases of PCNSL. In HIV infected patients with PCNSL seizures occur in approximately one third of patients.

Table 2.2 Characteristics of FBLs

	Toxoplasmosis	TB	PML	PCNSL
CD4 count (cells/ µL)	<200	Variable	<100	<100
Other Findings	IgG antibodies in serum and urine to *T. Gondii*	Evidence of pulmonary or non-neurological TB		
CSF Characteristics				
Glucose	Normal/ decreased	Normal/ decreased	Normal	Normal
Protein	Normal/ increased	Increased	Normal/ increased	Normal
White cell count	Normal/ increased lymphocytes	Increased lymphocytes	Normal	Normal/ increased lymphocytes
Other Findings	*T. gondii* PCR	Microscopy for AFBs Culture for *Mycobacterium Tuberculosis* NAAT	JC Virus PCR	EBV PCR
Neuroimaging				
Lesion numbers	Usually multiple	Solitary/ multiple	Solitary/ multiple	Solitary/ multiple
Common locations	Basal ganglia; frontal and parietal	Basal ganglia; brainstem; cerebellum and cerebral hemispheres	Brainstem; cerebellum and subcortical white matter	Corpus callosum; periepndymal, periventricular and temporal
Enhancement	Ring-enhancing	Basal meningeal and ring-enhancement Bright rim may be seen on MRI pre-contrast	Occurs in 25% (↑ in IRIS)	Common, heterogenous enhancement
Other Findings	Usually 1-2cm Mass effect	Hydrocephalus if lesion causes obstruction Mass effect	Cortical ribbon sparing	Usually >3cm

Adapted from Tan et al, 2012

Legend: AFB = Acid Fast Bacilli

Meningitis

Meningitis is an important cause of NOS in HIV-infected individuals. The incidence is higher in cohorts from developing regions.

Cryptoccal Meningitis (CM)

Cryptococcal neoformans is responsible for most of the cryptococcal infections in HIV infected individuals. The majority of patients with meningitis have an associated systemic cryptococcal infection. The incidence of CM has decreased in the western world following the introduction of HAART. CM remains a significant problem in resource-limited countries where HIV prevalence is high and access to healthcare is limited. Most cryptococcal meningitis cases occur in sub-Saharan Africa where it is one of the leading causes of death. Seizures are more frequent in patients from Africa. This probably reflects delayed presentation in the region (Antinori, 2013). In studies from developing regions it is a notable cause of NOS. In the 2005 study by Sinha et al, 41% of patients with NOS had underlying CM. The occurrence of CM in the study by Kellinghaus et al, 2008 is unfortunately not documented.

Tuberculous Meningitis (TBM)

The organism associated with meningitis is typically *Mycobacterium tuberculosis*. Tuberculous meningitis (TBM) is not a common cause of NOS except in regions of high TB prevalence (see Table 2.1 page 51). In the South African study by Modi et al, 2000 it was slightly more common than CM and accounted for 8% of all patients presenting with NOS.

Table 2.3 Characteristics of CM and TBM

	CM	TBM
CD4 count (cells/ µL)	<100	Variable
Other Findings	Evidence of systemic infection	Evidence of pulmonary or non-neurological TB
CSF Characteristics		
Glucose	Normal/ Decreased	Decreased
Protein	Normal/ Increased	Normal/ Increased
White cell count	Normal/ Increased lymphocytes	Increased lymphocytes
Other Findings	Opening pressures ↑ Cryptococcal Antigen is sensitive and specific	Microscopy for AFBs Culture for *Mycobacterium Tuberculosis* NAAT
Neuroimaging		
Lesion numbers	Usually multiple	Ill-defined exudates
Common locations	Basal ganglia	Not applicable
Enhancement	Leptomeningeal, especially in patients with IRIS	Basilar enhancement
Other Findings	Communicating hydrocephalus with ↑intra-cranial pressure Punched-out cystic lesions	Hydrocephalus possible

HIV-associated Neurocognitive Disorder (HAND)

There are three stages of neurocognitive impairment recognized:

- Asymptomatic Neurocognitive Impairment (ANI)
- Mild Neurocognitive disorder (MND)
- HIV-associated Dementia (HAD)

Seizures are well described in patients with HAD (see Table 2.1 page 51). The nomenclature describing neurocognitive dysfunction has undergone numerous changes. The number of patients presenting with NOS due to this is difficult to fully elucidate. In the first study of NOS by Holtzman , Kaku and So in 1989 almost a quarter of their patients were believed to have neurocognitive dysfunction, accounting for their seizures. Kellinghaus et al, 2008 found neurocognitive dysfunction in one fifth. This was unexpected as the majority of their patients were on anti-retroviral therapy. HAND was not a significant cause in the studies from developing countries (India and South Africa). The condition may have been under-diagnosed in their resource limited settings in the early days.

Metabolic Disorders

The prevalence of metabolic disorders amongst patients with NOS varies from none recorded to 15% (see Table 2.1 page 51). The 1996 study by Dore, Law and Brew was the only case-controlled study and they found that metabolic abnormalities occurred with the same frequency in HIV-infected patients with and without seizures. The study by Van Paesschen**,** Bodian and Maker in 1995 included very ill patients. In their cohort renal failure and hypomagnesemia were found to be risk factors for NOS, especially status epilepticus. Drug toxicity has been implicated in up to half of patients with NOS. In the small study (17 patients) of Pascual-Sedano et al, 1999 8 (47%) patients had a drug-related cause. The drugs implicated include the anti-viral foscarnet sodium and illicit drugs like cocaine and heroin.

No Identifiable Cause (NIC)

See section 2.2 page 63 for details

2.1.4 Seizure Patterns

Focal; generalized; secondary generalized; epilepsy partialis continuum and status epilepticus have been well documented in HIV-infected individuals. Generalized seizures occur most commonly in all the major studies to date (see Table 2.1 page 51). Pesola and Westfal, 1998 only recruited patients with generalized seizures. Secondary generalized seizures may be under-reported as generalization may occur rapidly and eyewitness reports may be inaccurate. Presentation with focal seizures was not necessarily indicative of an underlying focal lesion in the majority of studies. Diffuse neurological disease as in HAD can cause focal seizures. In a small study of seventeen patients Pascual-Sedono et al, 1999 documented status epilepticus in 19% of patients. In 1995 Van Paesschen**,** Bodian and Maker diagnosed status epilepticus in 18% of their patients. The authors postulated that in HIV-infected patients' renal failure and hypomagnesaemia may be risk factors for the development of status epilepticus. Lee, Garcia and Alldredge, 2005 looked specifically at 42 HIV-infected patients with status epilepticus. The majority of their patients had advanced disease. Opportunistic neurological disease (infections and malignancies) were identified in half their patients. There have been isolated reports of epilepsy partialis continua in the setting of HIV.

2.1.5 EEG Changes

Non-specific EEG abnormalities, the most common of which is generalized slowing have been documented in new onset seizures associated with HIV infection (Holtzman et al, 1989; Wong et al, 1990 and Kellinghaus et al, 2008). The significance of these findings is not readily apparent as similar findings have been reported in asymptomatic HIV-infected individuals. Epileptiform discharges are an uncommon finding and in the recent study by Kellinghaus et al, 2008 only one patient had epileptiform discharges. Similar findings were reported by Wong et al, 1990 and Chadha et al, 2000. The only study in which epileptiform discharges were detected in the majority of patients was by Modi et al in 2000. Normal readings have been documented in 20-60% of patients with documented seizures (see table 2.1 page 51). EEGs are an insensitive tool in the diagnosis of new onset seizures associated with HIV infection (Holtzman et al, 1989; Wong et al, 1990 and Kellinghaus et al, 2008).

2.1.6 Anti-Epileptic Drug (AED) Use

The International League Against Epilepsy (ILAE) and American Academy of Neurology (AAN) issued guidelines in 2012 for antiepileptic drug selection in people with HIV/ AIDS (Birbeck et al, 2012).

They concluded that it may be important to avoid enzyme-inducing AEDs in patients on HAART regimens that included protease inhibitors (PIs) or nonnucleoside reverse transcriptase inhibitors (NNRTIs). Drug interactions in these patients could lead to virologic failure. The efficacy of the HAART regimen needs to be closely monitored if patients require enzyme-inducing AEDs for optimum seizure control.

Acute seizure management in HIV-infected individuals is not different to that in non-HIV infected individuals. Normal protocols should be adhered to and the foremost requirement is termination of the seizure.

The drug levetiracetam has been recommended for use in HIV¯infected individuals. Levetiracetam has:

- A broad spectrum of activity
- Favorable side-effect profile
- Minimal drug interactions

Unfortunately the drug is not widely available in resource-limited settings. Valproic acid is recommended in these instances. Valproic acid does not cause enzyme-induced virologic failure but there are other well recognized side effects with this drug. There has been evidence to indicate that dose adjustments may be required in patients on lopinavir/ritonavir and zidovudine.

2.1.7 Conclusion

The studies reviewed indicate that NOS are a significant occurrence in HIV-infected individuals both in South Africa and internationally. The risk is higher in patients with advanced disease. Important etiologies include:

- Focal brain lesions
- Meningitis
- HIV-associated Neurocognitive Disorder
- Metabolic abnormalities and
- No Identifiable Cause

The majority of studies delineating NOS were done in the pre-HAART era. The only significant study done in the HAART-era was published in 2008 by Kellinghaus et al. It was based in Germany, a developed country where HAART is readily available and accessible. Data from developing regions in the current era of increasing HAART availability is deficient.

2.2 New onset seizures in HIV infected Individuals with No Identifiable Cause

2.2.1 Incidence

Up to half of patients presenting with NOS have no identifiable cause for their seizures. In the 1989 study by Holtzman , Kaku and So, 23 patients (23%) had NIC for their seizures, despite extensive investigations being performed. The mean follow-up period for these patients was 6.5 months. During this period none of the patients developed an obvious cause for their seizures. Three patients subsequently developed dementia. It is possible that HIV encephalopathy, as it was known at that stage was underdiagnosed initially. The authors inferred that seizures may be a symptom of primary CNS involvement in HIV. Wong, Suite and Labar did a similar study in 1990 and found NIC in 32 patients (46%). They performed autopsies in 7 of their patients in whom there was no readily apparent cause. The histological findings in these patients were typical of HIV encephalitis i.e. microglial nodules; multinucleated cells and inclusion-bearing cells. A significant finding in their study was that their CT scans were both sensitive (90%) and specific (100%) in the detection of FBLs. They reached a similar conclusion to Holtzman et al i.e. HIV infection of the brain was the most likely cause for seizures in the group with NIC. In the 1996 study by Dore, Law and Brew 42% of the patients had NIC. This may be an inflated figure as 18% were on the anti-viral Foscarnet, which has been reported to cause seizures (AIDS Research Group, 1995). Pascual-Sedano et al, 1999 had only 1 patient (6%) in whom they did not identify a cause for the seizures. A significant proportion of their patients (47%) had toxic-metabolic causes identified but as mentioned above the significance of metabolic abnormalities is uncertain.

The 2002 study by Modi et al looked specifically at a cohort of 15 patients in whom there was NIC. The majority of their patients had advanced disease. None of their patients were on HAART. A significant finding in their patients was an absence of white matter lesions (WML) on MRI scans, a common finding in patients with HAD.

Their patients had focal hypoperfusion defects on Technetium Exametazime hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO) Single-photon emission computed tomography (SPECT) scans. The authors postulated that the SPECT abnormalities were indicative of either a focal metabolic encephalopathy or an encephalopathy due to HIV infection.

Kellinghaus et al, 2008 found no apparent cause in almost half their patients. The significant drawbacks in this study were the lack of CSF analysis as a routine in the patients evaluated and the lack of imaging in some individuals. The unavailability of these investigations may account for the high frequency of patients in who no cause was readily identifiable.

2.2.2 Pathogenesis

Primary HIV infection probably has a role in the pathogenesis of epilepsy in this sub-group An autopsy study in 1986 by Navia, Jordan and Price described nine patients with new-onset seizures in whom the autopsies did not reveal any opportunistic disease. The authors concluded that direct infection of the brain by HIV was responsible for the seizures. Their findings are supported by the autopsy findings of findings of Wong, Suite and Labar,1990 who performed autopsies on seventeen HIV-infected patients with NOS in whom no cause had been identified. In six of their patients the pathology was consistent with primary HIV infection. There are many processes believed to contribute to the development of seizures:

- HIV-mediated neuronal cell death
- Neurotoxic substances
    - Cytokines
    - Eicosanoids
    - Free radicals

These substances enhance glutamate availability and activate voltage-gated calcium channels and N-methyl-D-aspartate (NMDA) receptors.

It is likely that the underlying process causing seizures is similar to that causing HIV-associated Dementia.

2.2.3 SPECT scans in HIV Neurology

SPECT scans qualitatively estimate regional cerebral blood flow (rCBF). The technique of brain perfusion imaging with SPECT has contributed to the understanding of the pathophysiology of disorders in both neurology and psychiatry (Nicola's et al 1993; Catafau et al 1994; Parellada et al 1998 and Catafau et al 1999). The SPECT scan offers the ability to detect regional cerebral blood flow in both the normal and the abnormal brain. The normal perfusion in the brain of an adult individual shows bilaterally symmetrical tracer distribution. Usually there is higher activity in temporal, parietal, and occipital regions/ cortices, basal ganglia, thalami, and cingulate gyrus (Camargo, 2001). Abnormal findings include focal or regional areas of either decreased or increased tracer uptake. The tracer HMPAO has few side-effects (Henderson, 2012).

Brain SPECT scans have long been used to study HIV infected individuals. Perfusion defects have been well described in patients with HIV-related neurocognitive dysfunction (Holman et al, 1992 and Sacktor et al 1995{a}). It has been suggested that the number of perfusion defects may correspond to the severity of the dysfunction (Pohl et al, 1988 and Ajmani et al, 1991). Pohl et al studied 12 HIV infected individuals with varying degrees of neurocognitive dysfunction. The SPECT scans in most of their patients were abnormal. They did follow-up scans in some patients which revealed increased numbers of defects, equivalent to the increase in clinical symptoms. The sites most commonly affected are the frontal; parietal; temporal and thalamic regions *(*Maini et al, in 1990; Schwartz et al, 1994 and Kramer and Sanger, 1990).

There has been some evidence to suggest that perfusion abnormalities on SPECT may precede the clinical features of dementia in HIV-infected individuals (Schielke et al, 1990 and Sacktor et al 1995{b}). A technical sub-committee of the AAN concluded, that SPECT scans could be used in the early diagnosis of HIV encephalopathy (Assessment of Brain SPECT. Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology, 1996). In 2009 the European Association of Nuclear Medicine Neuroimaging Committee also suggested that SPECT scans were useful in the diagnosis of HIV encephalopathy (Kapucu et al, 2009).

The study by Modi et al, in 2002 specifically studied the SPECT scans in HIV infected individuals presenting with NOS. They performed SPECT scans on 15 HAART-naïve patients with NOS. None of their patients had evidence of any other neurological disease or clinical features suggestive of a dementia. The patients were extensively investigated including CSF evaluations and MRI scans all of which were normal. The only consistent abnormality in their patients was the presence of temporal lobe perfusion defects on SPECT scans. On the basis of their SPECT results, the authors postulated that there was a causal relationship between cerebral infection and the HIV itself. They believed that the infection could be accounting for the abnormalities documented on the SPECT scans and therefore the epilepsy itself.

2.2.4 Quantification of SPECT scans

The use of visual evaluation of SPECT images on its own may be limited in the assessment of subtle variations in regional perfusion. This is the main indication for the use of quantification in clinical research (Catafau, 2001).The quantification of the ratios of tracer uptake in different regions of the brain assists in the estimation of the relative rCBF distribution within the brain. Regional counts can be obtained from the average counts per pixel of regions of interest (ROIs) drawn and placed on different cerebral areas. Ratios are obtained by comparing the regional count to a specific region which is presumed to be uninvolved in the pathological process e.g. the cerebellum. The left-to-right ratio of the same cerebral region or asymmetry indices are used to enhance left/right rCBF differences (Catafau et al, 1996). The process is simple but labour-intensive and lengthy if a high number of ROIs and ratios is to be generated. There can be complications relating to ROI design and selection of an appropriate reference region. Geometric or regular ROIs can be generated and placed on specific cerebral territories or they can be created to fit anatomic structures (irregular ROIs). They can also be created to fit particular cerebrovascular territories. However, drawing the same ROIs and placing them in exactly the same cerebral areas is a time-consuming process and requires special dedication. Results may not be accurate if the process is not carefully adhered to. The selection of the reference region is decisive in the determination of region-to-reference uptake ratios. The differences in ratios and the generation of the ROIs usually vary. In the search for more standardized and objective alternatives for brain perfusion SPECT quantification, circumferential profiles and polar

maps have been proposed with limited success (Spreafico et al, 1988; Ichise et al, 1995). Software programs have been developed to allow statistical comparison of different groups of subjects. These automated assessments of the perfusion defects improve the consistency of image interpretation (Van Laere et al, 2002). Quantification has been shown to be accurate with minimal errors in the clinical setting (Ritt et al, 2011). Bailey and Willowson in 2013 reported that quantification was accurate within 5% of the true radionuclide concentration.

The study by Tran Dinh et al, 1990 assessed the disturbances in the cerebral perfusion of 18 asymptomatic HIV-positive individuals compared to 9 control subjects. They used quantitative SPECT measurements to detect changes in cerebral blood flow. The majority of their patients (16) had areas of hypo perfusion compared to the controls. The regions affected were:

- Frontal 77%
- Cerebellum, Thalamus, Occipital 61%
- Temporal 55%
- Parietal 44%

They concluded that quantitative measurement of cerebral blood flow can be used to detect early changes in neurologically asymptomatic HIV infected individuals.

2.3 Study Objectives

The aims of the current study were to document the current Profile of South African Patients with New Onset Seizures and to complete a longitudinal evaluation of the group of HIV-infected patients with NOS where at the outset and at initial evaluation no cause is found. The profile of NOS in South Africa has not been documented in the current era of an anti-retroviral treatment program. There are no longitudinal studies and no published data evaluating patients with NIC. All studies to date are cross-sectional.

The study will:

- Profile HIV-infected patients presenting with NOS in South Africa.
- Attempt to address the important question of the significance of the presence of NIC in some HIV-infected individuals

  The patients with NOS who have NIC at the outset will presumably follow one of the following courses:
  - Remain without a cause i.e. The epilepsy is a primary HIV-related manifestation
  - Develop an HIV-related neurological illness e.g. intra-cranial mass lesion; meningitis
  - Develop HIV-associated dementia

CHAPTER THREE

New Onset Seizures in HIV Infected Individuals;

Current Profile Of South African Patients

3.1 Methods

3.1.1 Study Design

Cross-sectional; descriptive-analytic study.

The protocol was granted approval by the Human Research Ethics Committee (Medical), University of the Witwatersrand, Johannesburg. (Appendix C).

Patients were recruited over an eighteen month period i.e. January 2013 to June 2014.

3.1.2 Study Population

All individuals presenting with new onset seizures at the following sites:

- Charlotte Maxeke Johannesburg Academic Hospital, Parktown, Johannesburg, Gauteng
- Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg, Gauteng
- Helen Joseph Hospital, Auckland Park, Johannesburg, Gauteng

The three hospitals are tertiary-level public sector referral hospitals, affiliated to the University of the Witwatersrand.

3.1.3 Study Criteria

Inclusion

- New onset seizures diagnosed clinically at admission; at the neurology out-patient department or via the neurology consult service.
- Documented HIV positivity, either at the time of the NOS or within 12 months of the NOS
- Age greater than 18 years

Exclusion

- Patients unwilling to sign consent for participation in the study
- Previously diagnosed epilepsy (more than one month prior to recruitment into the study)

We screened 246 patients consecutively over the eighteen month period. 200 patients were evaluated for the cross-sectional component of the study.

The reasons for exclusions from the cross-sectional study were as follows:

- Patients refused consent 18
- Incorrect diagnosis 12
- Patients absconded 16

3.1.4 Measurements

Semi-structured interview and clinical examination (see Appendix D)

The following information was obtained:

Demographic data:	Age; gender; sexual orientation and level of education
Seizure data:	Date of initial seizure; type of seizure and previous history of seizures
Risks and possible triggers:	Family history; alcohol intake; drug use (herbal; illicit and prescription)
Previous medical history:	Neurological and non-neurological illnesses were documented
HAART:	Date of initiation; compliance and specific regimen being used was obtained
General Information:	An open-ended question requesting any information that the patient wished to share was posed at the end of the interview

A clinical examination was performed on all patients.

General examination:	Assessment of stigmata of chronic disease and immune suppression; cardiovascular system examination; respiratory and abdominal examinations.
Neurological examination:	Assessment of the level of consciousness; higher functions; the presence of meningism; cranial nerve examination; evaluation for the presence of primitive reflexes; cranial nerve examination; gait evaluation; cerebellar examination and assessment of the motor and sensory systems.

Seizure characteristics

The classification of the predominant seizure type was documented for each patient. The classification used was elucidated by Berg et al (2010). See table below

Table 3.1. Classification of Seizures

- Generalized seizures
    - Tonic-clonic (in any combination)
    - Absence
        - Typical
        - Atypical
        - Absence with special features
        - Myoclonic
    - Clonic
    - Tonic
    - Atonic
- Focal seizures
- Unknown*

*Seizures that cannot be clearly diagnosed should be considered unclassified until further information allows accurate diagnosis. This is not a specific category.

HIV Stage

The HIV staging was determined for each patient. The staging was adapted from the Centers for Disease Control (CDC) revised classification as described by Schneider et al in 2008. The staging was based on CD4 T-lymphocyte counts only. A summary of the staging system used is tabulated below.

Table 3.2 Stages of HIV Infection

- Stage 1
  - $CD4^+$ T-lymphocyte count $\geq$ 500 cells/µL
- Stage 2
  - $CD4^+$ T-lymphocyte count 200-499 cells/µL
- Stage 3
  - $CD4^+$ T-lymphocyte count <200 cells/µL

Laboratory Investigations

Routine investigations were performed on all patients. These were submitted to the National Health Laboratory Service (NHLS) branches at each of the hospitals. The investigations included: Full Blood Count (FBC); Urea and Electrolytes (U&E); Erythrocyte Sedimentation Rate (ESR); Blood Glucose; Liver Function Test (LFT) and Calcium, Magnesium and Phosphate (CMP) levels.

In all patients the HIV serology was confirmed via an enzyme-linked immunosorbent assay (ELISA) test. The CD4 count was determined via flow cytometry on serum samples.

Further blood laboratory tests investigations were performed to specifically identify recognized causes of seizures. These investigations included syphilis serology; complement fixation tests (CFTs) for cysticercosis and toxoplasmosis; IgM and IgG tests for CMV and polymerase chain reaction for HTLV-1.

Lumbar punctures were performed in patients unless contra-indicated (based on brain scan findings). The CSF samples were sent for routine investigations i.e. chemistry, microscopy and cell count; and specific studies including Veneral Disease Research Laboratory (VDRL) studies for syphilis; cryptococcal antigen and india ink stain; PCR studies for JC Virus; EBV and herpes viruses and adenosine deaminase levels (ADA). The samples were sent for bacterial culture and culture for mycobacterium tuberculosis.

Electroencephalogram (EEG)

Standard 12-lead EEGs recordings were performed when possible. The length of the recording was 20 minutes. The recording machine used was a Nihon Kohden Model EEG-9100K

The EEGs were reported on by registrars in the Neurology departments at the respective hospitals and reviewed by consultant neurologists. The EEGs were reviewed by the researcher to ensure consistency in reporting. The results were recorded as follows:

- Normal
- Focal slowing
- Generalized slowing
- Epileptiform discharges present
- Not done

Radiology

- Chest X-ray (CXR)
  This was done routinely on all patients.
- Computer Tomography Brain (CTB)
  Scans were done on all patients. The scans were reported on by registrars in the Radiology departments at the respective hospitals and reviewed by consultant radiologists. The scans were reviewed by the researcher to ensure consistency in reporting. The recording machines used were:
    - Charlotte Maxeke Johannesburg Academic Hospital
        - Philips Brilliance 64 slice
        - Philips Brilliance 128 slice
    - Chris Hani Baragwanath Academic Hospital
        - Toshiba Aquiliom 64 slice
    - Helen Joseph Hospital
        - Philips Brilliance 64 slice

- Magnetic Resonance Image (MRI) Scans Brain

  These were done on patients in whom laboratory investigations and CTBs were normal. The scans were reviewed by the researcher and a senior radiologist to ensure consistency in reporting. The machines used were:

  - Charlotte Maxeke Johannesburg Academic Hospital
    - Siemens 1.5 Tesla
  - Chris Hani Baragwanath Academic Hospital
    - General Electric 1.5 Tesla

3.1.5 Criteria for Diagnosis

Metabolic Disorders

They were the presumed cause of the seizures if

- Serum sodium was less than 115mmol/l
- Serum calcium was less than 1.2mmo/l
- Serum magnesium was less than 0.8mmol/l
- Urea nitrogen was greater than 35.7mmol/l
- Creatinine was greater than 884µmol/l

These values are based on the criteria outlined by Beghi et al, 2010 for the identification of acute symptomatic seizures.

Meningitis

The diagnosis of meningitis was based on a combination of clinical features and CSF analysis.

The clinical features included:

- Altered mental status
- Fever
- Neck stiffness
- Photophobia
- Vomiting

The CSF results were interpreted as follows:

- Bacterial meningitis:
    - Abnormal cell count (elevated white cells with >50% neutrophils) and
    - Positive Gram stain or
    - Positive bacterial culture
- Cryptococcal meningitis (described by Antinori, 2013):
    - Direct identification of the organism by India ink stain or
    - Positive cryptococcal antigen in CSF (latex agglutination method)
    - Positive culture for *Cryptococcus neoformans*
- Tuberculous meningitis (described by Marx and Chan, 2011):
    - Acid-fast bacilli on stain
    - Positive culture for *mycobacterium tuberculosis*
    - Lymohocyte-predominant pleocytosis
        - Total white cell counts usually between 100-500 cells/µL
        - In the very early stage there may be a neutrophil predominance and lower cell count
    - Elevated protein levels

      Typically between 1 and 5g/L
    - Low glucose

      Usually < 0.45g/L or CSF: plasma ratio <0.5
    - Adenosine deaminase level >10U/L

  Neuroimaging features suggestive of TBM included
    - Basal meningeal enhancement
    - Hydrocephalus

Focal Brain Lesions (FBLs)

We were unable to obtain histopathological diagnoses. We therefore presumed the aetiology of FBLs by collating information obtained from clinical, biochemical and radiological findings in conjunction with non-neurological illness and response to therapy. The algorithm used to identify presumed diagnoses was based on the approach outlined by Modi M, Mochan and Modi G, 2004. This was used in conjunction with a response to therapy.

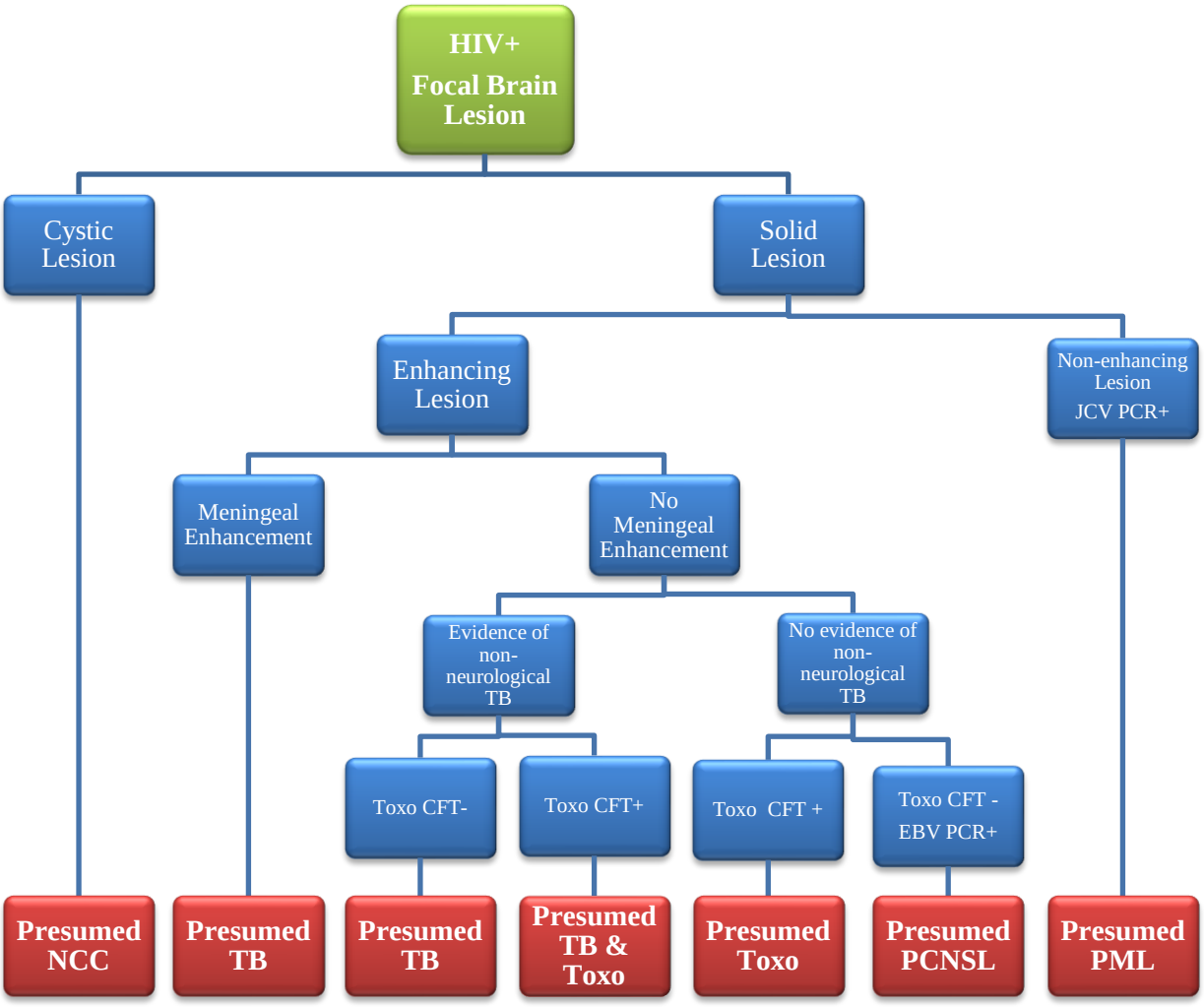


Figure 3.1 Approach to FBLs in HIV+ individuals

(Adapted from Modi M, Mochan and Modi G, 2004)

Legend

NCC=Neurocysticercosis; Toxo=Toxoplasmosis

The additional information utilized to make a presumptive diagnosis was based on the table below

Table 3.3 Characteristics of FBLs

	Toxoplasmosis	TB	PML	PCNSL	NCC
CD4 count (cells/ µL)	<200	Variable	<100	<100	Variable
Other Findings	IgG antibodies in serum and urine to *T. Gondii*	Evidence of pulmonary or non-neurological TB			Concomitant neurological infections
CSF Features					
Glucose	Normal/ decreased	Decreased	Normal	Normal	Normal/ decreased
Protein	Normal/ increased	Increased	Normal/ increased	Normal	Increased
White cell count	Normal/ increased lymphocytes	Increased lymphocytes	Normal	Normal/ increased lymphocytes	Increased lymphocytes
Other Findings	*T. gondii* PCR	Microscopy for AFBs Culture for *Mycobacterium Tuberculosis*	JC Virus PCR	EBV PCR	CSF and serum ELISA for NCC
Neuro-imaging					
Lesion numbers	Usually multiple	Solitary/ multiple	Solitary/ multiple	Solitary/ multiple	Multiple
Common locations	Basal ganglia; frontal and parietal	Basal ganglia; brainstem; cerebellum and cerebral hemispheres	Brainstem; cerebellum and subcortical white matter	Corpus callosum; periepndymal, periventricular and temporal	

(Adapted from Tan et al, 2012 and Serpa et al, 2007)

Legend:
NCC= Neurocysticercosis

HIV-associated Dementia (HAD)

The International HIV Dementia Scale (IHDS) described by Sacktor et al, 2005 was used as a screening tool to test for the presence of HIV-related cognitive abnormalities. They showed that the IHDS has good sensitivity and adequate specificity in screening for HIV dementia in both the industrialized and developing world. See table 3.4 on page 83.

Patients with a score of < 10 on the IHDS were evaluated further for possible dementia.

The diagnosis of HAD was based on a combination of:

- Clinical features outlined above
- CSF analysis
    - Normal or non-specific abnormalities
- Radiology (CT and or MRI Brain)
    - Normal or
    - Age-inappropriate cerebral atrophy with corresponding ventricular enlargement
    - Patchy confluent high-intensity white matter signal changes on T2-weighted MRI scans

Table 3.4 International HIV Dementia Scale (IHDS)

Memory Registration

Give the patient four words to recall. Give the patient 1 second to say each word. Tell the patient that you will ask for recall of the words later.

Motor Speed

Have the patient tap the first two fingers of the non-dominant hand as widely and as quickly as possible

- 4 = 15 in 5 seconds
- 3 = 11-14 in 5 seconds
- 2 = 7-10 in 5 seconds
- 1 = 3-6 in 5 seconds
- 0 = 0-2 in 5 seconds

Psychomotor Speed

Have the patient perform the following movements with the non-dominant hand as quickly as possible

- Clench hand in fist on flat surface
- Put hand flat on surface with palm down
- Put hand perpendicular to flat surface on the side of the 5^{th} digit
  - 4 = 4 sequences in 10 seconds
  - 3 = 3 sequences in 10 seconds
  - 2 = 2 sequences in 10 seconds
  - 1 = 1 sequences in 10 seconds
  - 0 = unable to perform

Memory-Recall

Ask the patient to recall the four words. If the words are not recalled, then you can prompt with a clue

- Give 1 point for each word recalled spontaneously
- Give 0.5 point for each correct answer after prompting
- Maximum = 4 points

The maximum possible score is 12 points.

A patient with a score of < 10 should be evaluated further for possible dementia.

3.1.6 Data Management and Analysis

The patients clinical and laboratory data was captured on an Excel spreadsheet by the researcher Kapila Hari.

Some of the detailed data was analysed with the assistance of Lusendo Matondo (Quantitative analyst). The analyses were carried out using Statistical Packages for Social Sciences (SPSS) version 13.

The specific analyses performed include:

- The Kolmogotov Smirnov test to demonstrate the skewed nature of the CD4 count data (Figure 3.2 page 86)
- The Mann Whitney test was used to calculate the difference in the mean CD4 count between the CM and TBM groups (Table 3.6 page 92)
- The Chi square test of independence was used to determine whether there was a significant relationship between FBLs and:
    - Seizure type (Table 3.7 page 93)
    - EEG abnormalities (Table 3.9 page 94)

3.2 Results

3.2.1 Demography of Study Sample

Two hundred patients were analysed.

In the study sample 115 patients (57.5%) were male and 85 (42.5%) were female. The ratio of males to females was 1.4: 1.

The mean age of the patients was 37.9 years. The patients ranged in age from 19 to 66 years.

All the patients were Black African.

All the patients were heterosexual.

3.2.2 HAART

Eighty-five patients (42.5%) were receiving HAART at the time their first seizure occurred. The mean duration of HAART was 20 months.

In the group there were 47 (55.3%) males and 38 (44.7%) females. The ratio of males to females was 1.2 to 1.

The degree of coverage amongst our group was 43.4%, based on current WHO guidelines i.e. HAART to be prescribed if the patients' CD4 count is <500 cells/μL (World Health Organisation consolidated Guidelines on the use of Antiretroviral drugs, June 2013).

3.2.3 CD4 Count

All study participants had at least one CD4 count recorded. The CD4 count at the time of baseline was used for analysis purposes.

The mean CD4 count for the entire group was 167cells/ µL. The mean CD4 count in the HAART sub-group was 191.5cells/µL, and in the HAART-naïve group it was 149cells/µL. The CD4 counts ranged from <1 to 1090 cells/ µL. The CD4 count distribution is displayed in a histogram below. The data was skewed.

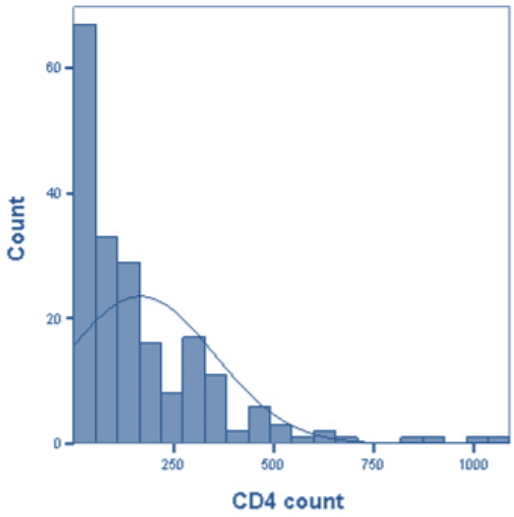


Figure 3.2 The Kolmogotov Smirnov test of the CD4 count

3.2.4 HIV Stage

The HIV stage was determined for each patient. The classification as outlined previously (table 3.2 page 74) was used.

Table 3.5 HIV Stages in Study Population

Stage	No. of patients	Percentage
1	11	5.5
2	49	24.5
3	140	70

The majority of patients (70%) were in stage 3.

3.2.5 Aetiology

The aetiologies identified were

- Meningitis 58 patients
- FBLs 55 patients
- Strokes 30 patients
- HAD 25 patients
- NIC 22 patients
- Trauma 9 patients
- Metabolic dysfunction 5 patients

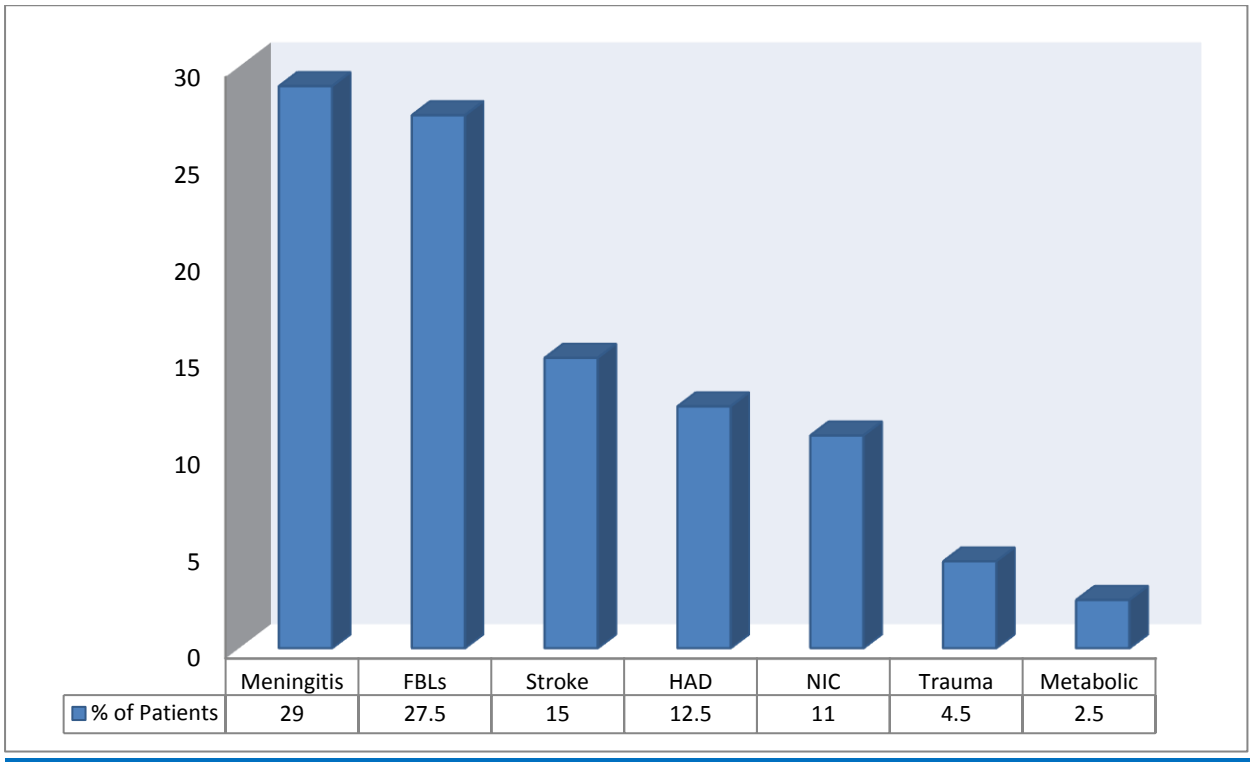
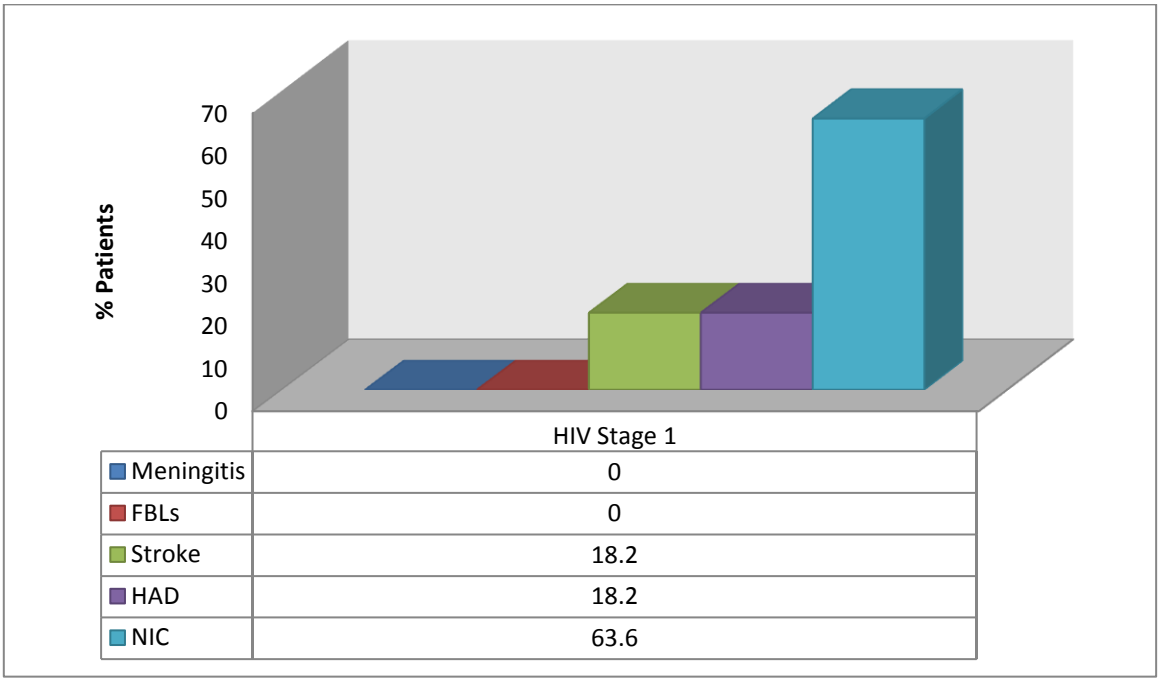


Figure 3.3 Seizure Aetiologies

3.2.6 Aetiology within HIV Stages

Patients in stages 1, 2 and 3 were analysed to determine the relative frequency of the aetiologies within each stage.



	HIV Stage 1
Meningitis	0
FBLs	0
Stroke	18.2
HAD	18.2
NIC	63.6

Figure 3.4 Aetiologies in HIV Stage 1

There were 11 patients in stage 1.

- NIC 7 patients
- Stroke 2 patients
- HAD 2 patients

There were no patients with meningitis, FBLs or metabolic dysfunction.

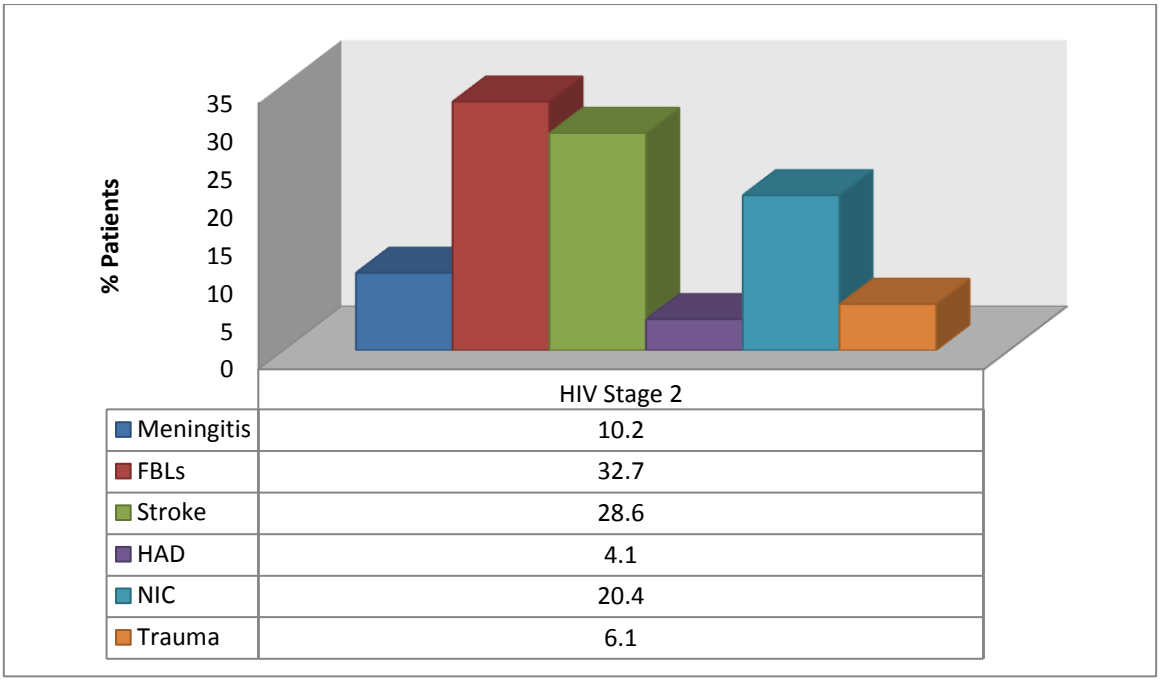


Figure 3.5 Aetiologies in HIV Stage 2

There were 49 patients in stage 2.

- FBLs 16 patients
- Strokes 14 patients
- NIC 10 patients
- Meningitis 5 patients
- Trauma 3 patients
- HAD 2 patients

There was1 patient with dual pathology (stroke and meningitis).

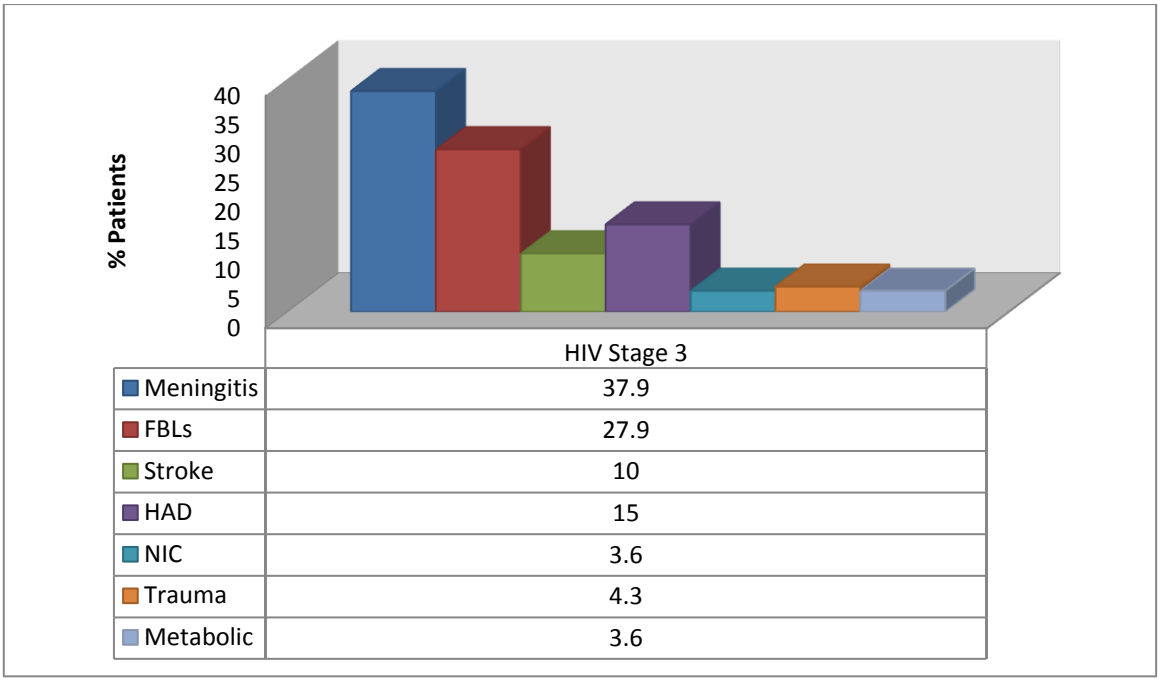


Figure 3.6 Aetiologies in HIV Stage 3

There were 140 patients in stage 3.

- Meningitis 53 patients
- FBLs 39 patients
- HAD 21 patients
- Stroke 14 patients
- Trauma 6 patients
- NIC 5 patients
- Metabolic dysfunction 5 patients

Three patients had dual pathology (2 had meningitis and FBLs and 1 patient had meningitis and stroke).

The meningitis, FBLs and metabolic sub-groups were further analysed to determine the frequency of individual conditions.

- **Meningitis**

  There were 58 patients

  - Cryptococcal Meningitis 30
  - Tuberculous Meningitis 21
    (Culture positive in CSF: 16)
  - Bacterial meningitis 3
  - Viral meningo-encephalitis 4

- **FBLs**

  There were 55 patients

  - Tuberculomas 31
    (Culture positive: 22)
  - Toxoplasmosis 9
  - Neurocysticercosis 8
  - Aneurysms 3
  - PML 2
  - Pituitary adenoma 1
  - Meningioma 1

- **Metabolic**

  There were 5 patients

  - Renal failure 5

Profile of patients with CM versus TBM

The sub-group of patients with meningitis was analysed further to distinguish between patients with CM and TBM.

There were 30 patients with CM, all of whom were in HIV stage 3; the majority of patients with TBM (81%) were in HIV stage 3.

The mean CD4 count in patients with CM (60.5) was lower than in the TBM group (140). The mean CD4 count difference between the two groups was statistically significant as the *P*-value was <0.001. The difference in the mean CD4 count between the CM and TBM groups was analysed using the nonparametric version the t test i.e. the Mann Whitney test as the CD4 data was not normally distributed.

Table 3.6 Mean CD4 count (CM versus TBM)

		No.	Minimum	Maximum	Mean	Standard Deviation	Median	P value
CD4 count	TBM	21	9	416	140.04	107.34	111	0.000
	CM	30	5	165	60.53	50.14	46	

3.2.7 Seizure Classification

One hundred and seventy patients (85%) had generalized tonic-clonic seizures.

Thirty patients (15%) had focal seizures.

We did not identify any patients with status epilepticus.

FBLs and Seizure Type

The sub-group of patients' with FBLs was analysed determine whether there was a correlation with focal seizures. The chi square test of independence was used to test the two categorical variables.The relationship is statistically significant (*p*-value = 0.003). This evidence suggests that FBLs are associated with focal seizures (see Table 3.7 below).

Table 3.7 FBLs and Seizure Type

Chi-square; p-value: 8.962; 0.003		Focal seizures		Total
		Yes	No	
Focal brain lesion	Yes	15	40	55
	No	15	130	145
Total		30	170	200

3.2.8 EEG Results

Standard EEG recordings were performed on 161 patients (80.5%).

Table 3.8 EEG Findings

EEG Finding	No. of Patients	Percentage (%)
Generalized slowing	84	52.2
Normal recording	37	23
Focal slowing	30	18.6
Epileptiform discharges	10	6.2

Focal Brain Lesions and EEG Abnormalities

The sub-group of patients' with FBLs was analysed to assess whether there was an association with focal slowing on EEG. The chi square test of independence was used to test the two categorical variables. The relationship between FBLs and focal abnormalities was significant (*p* value = 0.000).

Table 3.9 FBLs and EEG Abnormalities

Chi-square; p-value: 25.536; 0.000		Focal brain lesion		Total
		Yes	No	
EEG	EEG Normal	7	32	39
	EEG Focal Abnormality	20	11	31
	EEG Generalised Abnormality	18	73	91
Total		45	116	161

3.2.9 CTB Results

Eight patients (4%) with cryptococcal meningitis and no focal deficits did not have brain scans. One hundred and ninety-two patients (96%) had CT scans of their brains done.

The scans were abnormal in 169 patients (85%). The specific findings are listed below.

Table 3.10 CTB Findings

CTB Finding	No. of Patients	Percentage (%)
FBL	55	28.6
Atrophy	54	28.1
Basal enhancement	34	17.7
Infarct	28	14.6
Normal	24	12.5
Haemorrhage	5	2.6

3.2.10 Summary of Results

In this study there were 200 HIV-infected patients with NOS

- All were Black African
- 115 males; 85 females
- Mean age was approximately 38 years
- HAART coverage was inadequate at 42.5%
- Mean CD4 count was 167 cells/µL
- Majority (70%) were in HIV stage 3
- The most common underlying aetiologies were meningitis and FBLs
    - Tuberculosis and cryptococcal infection predominated
- Generalized tonic-clonic seizures (85%) occurred more often than focal seizures
- More than half (52%) of the patients had generalized slowing on their EEG
- The majority (88%) had abnormal CTB results

3.2.11 Limitations

- HIV viral load measurements were not obtained (cost constraints)
- There were no biopsies performed on patients with focal brain lesions (this is not routine clinical practice at our institutions)

3.3 Descriptive Analysis of New Onset Seizures in HIV in South African patients

3.3.1 Demography

In this study sample the majority (57.5%) of patients were male. The ratio of males to females was 1.4 to 1. This is an unexpected finding as the prevalence of HIV in South Africa is higher amongst females than males. Recent figures show that the overall prevalence was 9.9% in males and 14.4% in females (South African National HIV Prevalence, Incidence and Behaviour Survey, 2012). The higher proportion of males in this study subjects could be explained by the Gauteng provincial demographics:

- In Gauteng, the site of this study the male to female ratio is higher in the age group 20-54 years. (Mid-year population estimates, 2013, Statistics South Africa).
- The majority of our cohort (90%) was between the ages of 20 and 54 years.

The mean age of 37.9 years is consistent with the national HIV prevalence data.

See table 3.11 below (South African National HIV Prevalence, Incidence and Behaviour Survey, 2012).

Table 3.11 HIV Prevalence by Age, South Africa

Age Group (years)	%
0-14	2.4
15-24	7.1
25-49	25.2
50+	7.6

All patients were Black African, which is not unexpected. In South Africa the vast majority of the population (79.8%) are Black African. The teaching hospitals at which the studies were conducted serve a predominantly Black African population. The prevalence of HIV is highest amongst this race group.

See table 3.12 below (South African National HIV Prevalence, Incidence and Behaviour Survey, 2012).

Table 3.12 HIV Prevalence by Race, South Africa

Race	%
Black African	15.0
White	0.3
Coloured	3.1
Indian/ Asian	0.8

3.3.2 HAART

In our cohort there were 85 patients (42.5%) receiving HAART.

One hundred and ten patients (95.7%) of the 115 patients in the HAART-naïve group were eligible for therapy based on their CD4 count alone i.e. <500 cells/µL (WHO Consolidated guidelines on the use of Antiretroviral drugs, June 2013). The guidelines further recommend that priority should be given to patients with CD4 counts <350 cells/µL. In our cohort of HAART-naïve patients there were 101 (87.8%) with CD4 counts <350 cells/µL.

Our figures indicate sub-optimal HAART coverage amongst our patients. The coverage in our group was 43.4% based on the current WHO guidelines. Johnson, 2012 estimated that adult HAART coverage in South Africa was 52% if the criterion of CD4 counts <350 cells/µL was used. If we used this criterion then our coverage was 45.7%. Our lower than expected figure may be due to:

- The high proportion of males in our cohort.
  Johnson, 2012 showed that there is a significant difference in coverage between males (41%) and females (60%). This is borne out in the study. The overall ratio of males to females was 1.4:1 but in the HAART group it was 1.2:1.
- The inherent bias of this study population (Urban, hospital-based).
  It has been hypothesized that patients with advanced HIV migrate to urban regions in Gauteng and the Western Cape in the belief that the health-care services may be superior (Johnson, 2012). Cleary et al, 2012 showed that there were numerous factors in rural regions which contributed to the urban migration of individuals seeking HAART:
    - High healthcare costs in rural regions
    - Stigma associated with accessing HAART in a local setting
    - Perceptions about quality of services

3.3.3 CD4 count

Seizures in HIV-infected individuals are more common in patients with advanced disease. This was well documented in the pre-HAART era and also in recent studies conducted in the HAART-era.

- The overall mean CD4 count in this study was 167cells/μL.
  This is consistent with the findings of Potchen et al, 2014. Their study was similar in that it was conducted at a teaching hospital in Lusaka, Zambia. They evaluated 43 HIV-positive individuals with first onset seizures. The study was done in the HAART era but the coverage was low at 37%. The mean CD4 count in their cohort was 187cells/μL. In the study by Kellinghaus et al, 2008 the mean CD4 count was not calculated but 18/ 33 patients (54.5%) had CD4 counts below 200cells/μL. This was despite the majority of patients (84.1%) being on HAART.

- In our patients on HAART the mean CD4 count (194.5cells/μL) was low.
  This value reflects the low counts at initiation of anti-retroviral therapy in this country. A recent meta-analysis by Siedner et al, 2015 reviewed the CD4 counts of >96 000 South Africans prior to commencement of anti-retroviral therapy. The mean count in their group was 123cells/μL.
  This low figure is consistent with the findings of Olajumoke et al, 2013. They studied 20 Nigerian patients presenting with NOS. All their patients were on HAART for a mean period of 19.5 months. Despite this the mean CD4 count was low at 165cells/μL.

- In the HAART-naïve group the mean count was 149cells/μL.
  The low mean count is to be expected. Numerous hospital-based studies conducted prior to the widespread availability of HAART (Pesola and Wesrfal, 1998; Pascual-Sedono et al, 1999 and Modi et al, 2000) showed similar low mean CD4 counts amongst their patients. The study by Modi et al was done at one of the sites of the current study. They studied 37 patients who were HAART-naïve and the mean CD4 count in their group was 239.

3.3.4 Seizure Classification and EEG Results

- The majority of our patients (85%) had GTC seizures.
  This is consistent with the published studies (see table 2.1, page 51 for details) where GTC seizures account for between 62 and 100% of seizures. A review of seizures in HIV-seropositive individuals documented that generalized seizures were more common (Satishchandra and Sinha, 2008).
  The reporting of generalized seizures may be falsely elevated:
    - There may be rapid secondary generalization
    - History is inadequate.

- Non-specific EEG abnormalities occurred commonly and were present in the majority of patients (52%). Epileptiform discharges were rare (10 patients i.e. 5%). This is consistent with previous studies where EEGs were found to be an insensitive tool in the diagnosis of seizures in HIV-infected populations (Wong, Suite and Labar 1990; Chadha et al, 2000 and Kellinghaus et al, 2007).
  An interesting finding in this study was the significant correlation between focal EEG abnormalities and focal brain lesions. Siddiqui et al, 2015 concluded that EEG abnormalities were common in patients with imaging abnormalities and suggested that EEGs may assist in prioritising patients for neuroimaging in resource-limited settings.

3.3.5 CT Brain Results

- The majority of brain scans (88%) were abnormal in this cohort. Neuroimaging abnormalities were documented in 70% of patients in a Zambian cohort of HIV infected patients with first seizure (Potchen et al, 2014) whilst Kellinghaus et al found abnormalities in only 45.5% of the patients in their German study. The high proportion of neuroimaging abnormalities in studies from the Sub-Saharan region are likely due to:
    - Increased presence of opportunistic infections
      In developed countries HIV-related opportunistic neurological infections have decreased following the widespread use of HAART. Two prospective multi-centre trials reviewing more than thirty thousand patients have confirmed this finding (Monforte et al, 2004 and Garvery et al, 2011).
      In South Africa the experience has not been similar. Asselman et al, 2010 evaluated 75 patients on HAART presenting with neurological disorders. Their study was based at a public sector referral hospital in Cape Town, South Africa. The majority (60%) of their patients had either tuberculosis or cryptococcal infection.

    - Low mean CD4 count
      Almost one third of patients in this study had cerebral atrophy documented on imaging. Kim et al, 1996 reviewed the neuroimaging findings in AIDS Dementia and concluded that atrophy is more common in patients with advanced HIV disease. In the current HAART era low nadir CD4 count has been identified as the greatest risk factor for the development of cerebral atrophy (Cohen et al, 2010).

3.3.6 Aetiology

The majority of our patients (89%) had an identifiable cause for their seizures. This is consistent with the published literature (see table 2.1, page 51 for details). The aetiologies in the specific HIV stages are discussed below.

HIV Stage 1

There were 11 patients in stage 1.

- NIC 7 patients
- Stroke 2 patients
- HAD 2 patients

There were no patients with meningitis, FBLs or metabolic dysfunction.

The category of patients with NIC had extensive investigations to exclude opportunistic infections and is therefore better represented in this stage i.e. CD4 count >500 cells/µL. This sub-group was part of the longitudinal study and the results are analysed further in section 4.3 page 156.

The diagnosis of HAD in patients in stage 1 is uncommon. Both patients with HAD were on HAART at baseline. Joska et al, 2010 reviewed 15 studies in an attempt to determine whether HAART improved neurocognitive function. They concluded that although HAART improved cognition it did not completely resolve neurocognitive deficits.

Several reasons for this apparent treatment failure have been postulated and include:

- The "legacy effect" of HIV infection: permanent damage predating the initiation of HAART
- Ongoing CNS disease activity resulting from lack of viral suppression due to poor CNS penetration effectiveness (CPE) of antiretrovirals (ARVs)
- Potential neurotoxicity of HAART
- Confounding comorbidities like psychiatric illness, substance abuse, cerebrovascular disease
- Ageing

The nadir CD4 counts of the patients with HAD were not available; but it is likely that the counts were low, thus predisposing them to the development of HAD (Heaton et al, 2011). Siedner et al, 2015 showed that the mean CD4 count in South Africans prior to commencement of anti-retroviral therapy was 123cells/µL.

The occurrence of stroke amongst patients with high CD4 counts is not uncommon. Mochan, Modi M. and Modi Gl, 2003 studied 35 Black South African patients at the Chris Hani Baragwanath hospital (a site of the current study). Their patients were all HAART-naïve. Almost one quarter (23%) of the patients in their study was in CDC stage 1. Chow, 2014 reviewed the association between stroke and HIV. She concluded that there was sufficient evidence to support an increased risk of ischaemic stroke in individuals with HIV infection. The factors which increased the stroke risk include:

- Traditional Risk Factors
  - Hypertension
  - Dyslipidaemia
  - Diabetes Mellitus
  - Smoking
- Impaired fibrinolysis with consequent hyper-coagulable state

The absence of patients with meningitis in stage 1 is consistent with the findings of Jarvis et al, 2010. They identified 492 HIV-infected individuals with meningitis in South Africa. None of their patients were in CDC stage 1. The highest CD4 count documented amongst their patients was 467cells/µL. See table 3.13 below for details.

Table 3.13 Meningitis in HIV+ individuals, South Africa

Diagnosis	No. of Patients	Mean CD4 (cells/µL)	CD4 Range (cells/µL)
CM	337	39	18-85
TBM	126	126	61-201
Bacterial Meningitis	29	287	155-467

Adapted from Jarvis et al, 2010

In developing countries focal brain lesions are associated with infections. Three studies have evaluated FBLs in South Africa (Bhigjee et al, 1998; Modi M., Mochan and Modi G., 2004 and Smego, Orlovic and Wadula, 2006). Bhigjee et al evaluated 38 patients with FBLs. They measured the CD4 count in 27 patients, none of who had counts >500cells/µL. Modi et al reviewed 32 patients with FBLs. In their study there were only 4 patients in CDC stage 1. Smego et al, 2006 evaluated 26 patients with FBLs. None of their patients were in CDC stage 1. The studies by Modi et al and Smego et al studies were performed at sites included in the current study in the pre-HAART era. In our setting, FBLs are unusual in patients in CDC stage 1.

All the patients in this study with metabolic dysfunction had renal failure. The absence of patients with renal failure amongst the stage 1 sub-group is unsurprising as it has been shown that a CD4 count < 200cells/µL is a major risk factor for the development of renal disease (Naicker and Fabian, 2010).

CDC Stage 2

There were 49 patients in stage 2. One patient had dual pathology (stroke and meningitis).

- FBLs 16 patients
- Strokes 14 patients
- NIC 10 patients
- Meningitis 5 patients
- Trauma 3 patients
- HAD 2 patients

Almost one third (16) of the patients in stage 2 had FBLs. Amongst the patients with FBLs most had either TB (8) or NCC (3). Modi M., Mochan and Modi G., 2004 studied 22 patients with presumed tuberculomas. Their patients were HAART-naive and even though the mean CD4 count was 188, the range of CD4 counts extended to 610cells/µL.

Neurocysticercosis is well described in the context of HIV infection. Serpa et al, 2007 reviewed the literature on the co-infection and identified 27 patients. Unfortunately individual CD4 counts were only available for 6 patients. Four of the patients were in HIV stage 2. Modi M., Mochan and Modi G., 2004 identified 9 patients with NCC, in their patients the mean CD4 count was 509cells/µL. It has been postulated that symptomatic neurocysticercosis is more likely at higher CD4 counts. A possible reason for this is that lower CD4 counts are associated with decreased host inflammatory responses (Prasad et al, 2006).

Ba-Diop et al, 2014 reviewed the epidemiology, causes and treatment of epilepsy in sub-Saharan Africa. Traumatic brain injury was identified as a risk factor in the region. The causes include:

- Road accidents
- Assaults
- Violent sports

Amongst our patients 2 had been involved in road accidents and one had been assaulted.

There were 5 patients with meningitis; 4 of who had TBM and 1 had a bacterial meningitis. TBM is more common in patients with advanced disease but can present at higher CD4 counts, especially in regions of high TB prevalence (Tan et al, 2012).

HIV Stage 3

There were 140 patients in stage 3.

- Meningitis 53 patients
- FBLs 39 patients
- HAD 21 patients
- Stroke 14 patients
- Trauma 6 patients
- NIC 5 patients
- Metabolic dysfunction 5 patients

Meningitis

This was the most common aetiology identified and was present in 53 patients. Meningitis is a prominent cause of NOS and been described in >40% of patients (Sinha et al, 2005). The common causes of meningitis in this stage were cryptococcal infection and tuberculosis.

Cryptococcal meningitis was diagnosed in 30 patients. Jarvis and Harrison, 2007 have shown that in developing countries cryptococcal neoformans remains an important opportunistic pathogen and that in sub-Saharan Africa it is the leading cause of meningitis in adults. The HAART era has led to a decrease in incidence of CM in the developed world. This effect has not been replicated in the developing world. The burden of cryptococcal disease has remained high in this country even in the current HAART era. The most likely reasons for this are:

- The large number of patients with low CD4 counts that are not accessing HAART
- The low mean CD4 count of patients at initiation of HAART (Jarvis et al, 2007).

The relatively high incidence of CM in our cohort could also be explained by the findings of Antinori, 2013 who showed that seizures are more common in HIV-infected individuals from Africa, compared to the rest of the world. In African patients with CM seizures occur in 9.1% of patients whilst in developed countries they occur in 6.1% of patients. This increase could be a consequence of late presentation.

The risk of CM in HIV-infected patients is highest amongst patients with advanced disease (CD4 count below 100cells/µL). All the patients with CM (even those on HAART) had a CD4 count below 200cells/µL and the majority (76.7%) had counts below 100 cells/µL.

Tuberculous meningitis was identified in 17 patients. TBM has not been documented as a frequent cause of seizures in other studies to date. The relatively high incidence in this cohort is probably due to:

- The high TB prevalence in South Africa.
- The high proportion of TBM in South African patients with meningitis
- The low mean CD4 count

The WHO has identified 22 countries which are high burden countries and account for 81% of the global cases of TB (see Figure 3.7 below). South Africa is one of these countries and has the highest incidence and prevalence of TB within the group (WHO Report on Global Tuberculosis Control, 2012).

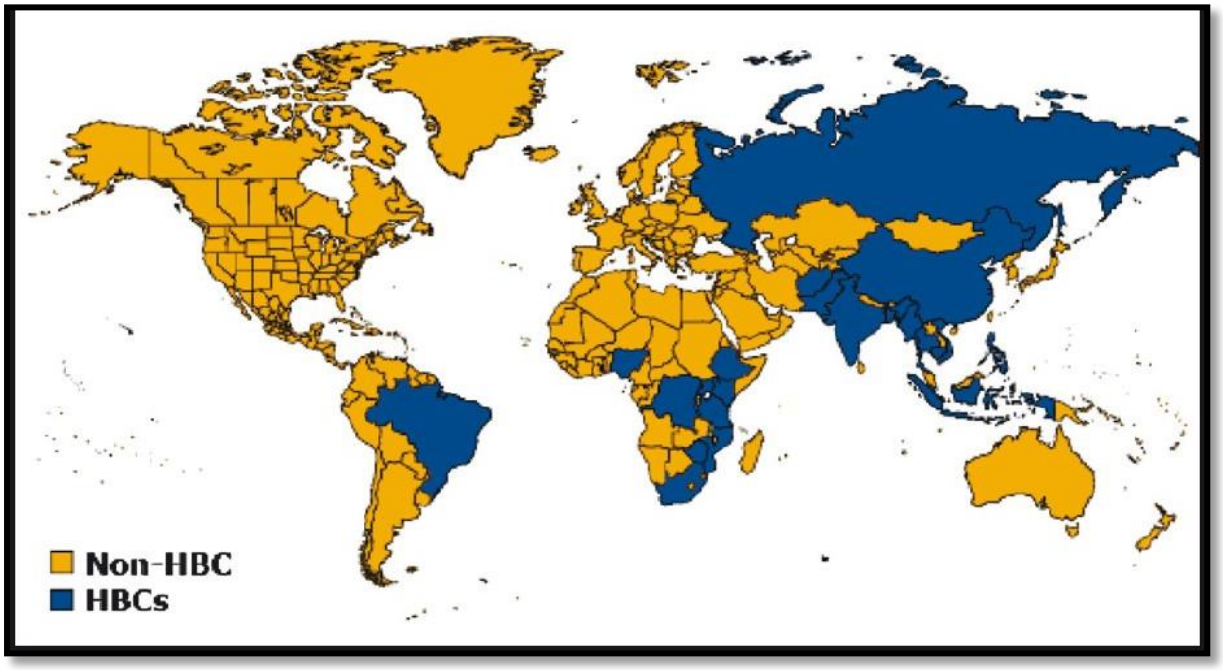


Figure 3.7 High Burden TB countries

(From Kaiser Family Foundation, *www.GlobalHealthFacts.org*, based on WHO, *Global tuberculosis control* 2010, reprinted with permission from the Henry J. Kaiser Family Foundation, California, USA).

TBM has been identified as a significant cause of meningitis in HIV-infected individuals in South Africa (Jarvis et al, 2010). In their patients the mean CD4 count was 126 cells/ µL. Pettit et al, 2011 have also shown that the risk of TB is higher among HIV-infected patients with low CD4 counts.

There were only 3 patients with bacterial meningitis. This is consistent with the findings of Jarvis et al, 2010 who found low numbers of bacterial meningitis. All our patients had Pneumococcus which was also the dominant organism identified in their patients with bacterial meningitis.

Focal Brain Lesions

Focal brain lesions occur frequently in HIV-infected individuals and have been described in up to 53% of patients presenting with NOS (see table 2.1, page 51 for details). In stage 3 we identified FBLs in 39 patients. FBLs in our setting have been shown to occur in patients with advanced disease. In the 2009 study by Modi M., Mochan and Modi G patients with FBLs had a mean CD4 count of 181cells/µL.

The majority of patients (23) had tuberculomas. The high proportion of tuberculomas in this study is consistent with the findings of Modi M., Mochan and Modi G, 2004. Their case series on HIV-associated FBLs was conducted at the Chris Hani Baragwanath Hospital, one of the sites of the current study. TB was the presumed cause in >50% of their patients with FBLs. Their patients were all HAART-naïve. In the Cape Town-based study by Asselman et al, 2010 there were 25 patients with focal brain lesions, 6 of whom had tuberculomas and a further 8 received antitubercular therapy despite no definitive diagnosis.

Seizures are a characteristic finding in adult patients with tuberculomas and occur in 11% of patients (DeLance et al, 2013). They are however not well documented in HIV positive patients with NOS except in the studies by Chadha et al, 2000 (India) and Modi et al, 2000 (South Africa). Both these studies are from high TB burden countries.

Toxoplasmosis has been identified as the commonest cause of FBLs in most of the published studies on NOS in HIV (see table 2.1, page 51 for details) and in the 1999 study on FBLs by Bhigjee et al. The study by Bhigjee et al was done in KwaZulu Natal, South Africa. In the current study only 9 patients, all of who were in stage 3 had FBLs that were attributed to toxoplasmosis. This comparatively low proportion is probably due to:

- Low sero-prevalence of *T. gondii* in this study population
  The sero-prevalence of *T. gondii* in immunocompromised patients reflects that of the background population. A study in Gauteng, South Africa found prevalence in the HIV-positive population to be 8% (Hari et al, 2007). In the studies by Pascual-Sedono et al, 1999 (Spain), and Chadha et al, 2000 (India) almost a third of patients had toxoplasmosis implicated as the cause for their seizures. In Spain the seroprevalence of *T. gondii* has been shown to be 43.8% and in India it was 41.7% (Pappas, Roussos and Falagas 2009). The seroprevalence in Kwazulu Natal, the site of the study by Bhigjee et al was 31.3% (Schneider, Schutte and Bromner 1992).

We identified 8 patients (4%) with neurocysticercosis. The only other study in which NCC featured as a cause of seizures amongst HIV-positive individuals was the 2009 study by Modi M., Mochan and Modi G. In their study there were 3 patients (14%) with NCC. The occurrence of NCC in the South African setting is to be expected. Cysticercosis is endemic in South Africa (WHO report on neglected tropical diseases, 2010). Thirty per cent of epilepsy in endemic regions is due to NCC (Ndimubanzi et al, 2010). In the setting of HIV Modi M., Mochan and Modi G, 2004 have shown that NCC is responsible for almost one third of FBLs.

There were 3 patients with fusiform intra-cranial aneurysms, suggestive of HIV vasculopathy. Intra-cranial aneurysms in HIV-positive adults are rare. There have been only two cases previously reported in the literature with the combination of HIV infection, intracranial aneurysms and seizures, one of whom was an adult South African (Modi et al, 2008).

Metabolic Dysfunction

In this study there were 5 patients (2.5%) with seizures due to metabolic dysfunction. This figure is consistent with published data (see table 2.1, page 51 for details). The 5 patients all had renal failure. Renal failure has been identified as a potential risk factor for NOS (Van Paesschen et al, 1995). Unfortunately CD4 counts were not available for their patients but of their group of 68 patients, 91% had AIDS, suggesting advanced disease (Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. 1987). NOS were described as a presenting feature in 2 patients with end-stage renal failure (Olatinwo and Hewitt, 2002).

3.3.7 Conclusion

In the current era of an anti-retroviral program the majority of HIV infected South African Patients with New Onset Seizures

- Have an underlying cause
    - The most common causes are
        - Meningitis and
        - Focal Brain Lesions
    - Infectious aetiologies predominate; especially in patients with advanced disease i.e. HIV stages 2 and 3.
        - The most commonly identified infections are tuberculosis and cryptococcal infection; which is consistent with the prevalent infections in our region.
- Have advanced immune-suppression
    - Low mean CD4 count (167 cells/µL)
- Are not on HAART
    - The majority of HAART-eligible patients are not accessing therapy
- Present with generalized tonic-clonic seizures
- Have neuroimaging abnormalities

CHAPTER FOUR

New Onset Seizures In HIV Infected Individuals with No Identifiable Cause

4.1 Methods

4.1.1 Study Design

A longitudinal study (clinical and SPECT scan).

The protocol was granted approval by the Human Research Ethics Committee (Medical), University of the Witwatersrand, Johannesburg. (Appendix C).

Patients were recruited over an eighteen month period i.e. January 2013 to June 2014.

4.1.2 Study Population

All patients recruited for the cross-sectional study (see 3.1.2page 70)

4.1.3 Study Criteria

Inclusion

Patients with new onset seizures in who there was no identifiable cause i.e.

- Blood laboratory tests investigations were normal
- CSF analysis was normal
- CT Brain was normal
- MRI brain normal (no specific abnormalities)

Exclusion

- Patients unwilling to sign a request for participation in the longitudinal study which included a separate consent sheet for the performance of SPECT scans (Appendix F)

4.1.4 Measurements

Semi-structured interview and clinical examination (see Appendix E)

The procedure outlined earlier (3.1.4 page 77) was followed for each patient.

This was repeated at 6-monthly intervals for the duration of the patients' inclusion in the study.

Seizure characteristics

The classification of the predominant seizure type was documented for each patient. The classification used was elucidated by Berg et al (2010). See table 3.1 page 73 for details.

HIV Staging

This information was determined for all patients at baseline. The methods utilized have been discussed previously. See table 3.2 page 74.

The International HIV Dementia Scale (IHDS)

This information was determined for all patients at baseline and each visit. The details of this tool have been discussed previously. See table 3.4 page 86.

Laboratory Investigations

The investigations listed previously (page 75), including the CSF analysis were performed on each patient and repeated at 6-monthly intervals for the duration of their inclusion in the study.

- Blood HIV-1 RNA viral loads were requested on all patients and repeated at 6-monthly intervals for the duration of the patients' inclusion in the study.

Electroencephalogram (EEG)

Standard 12-lead EEGs recordings were performed on all patients at baseline.

The EEGs were reviewed by the researcher to ensure consistency in reporting. The results were recorded as detailed previously (page 76).

Radiology

- Chest X-ray (CXR)

  This was done routinely on all patients.
- Computer Tomography Brain (CTB)

  Scans were done on all patients. The scans were reviewed by the researcher and Professor. M. Modi (MBBCH MMed {WITS} FC Rad) to ensure consistency in reporting.

- Magnetic Resonance Image (MRI) Scans Brain

  Scans were done on all patients. The scans were reviewed by the researcher and Professor. M. Modi to ensure consistency in reporting. They were repeated at 6-monthly intervals for the duration of the patients' inclusion in the study.

- The unexplained white matter abnormalities on scans were documented using the well-established Fazekas Scale (Fazekas et al, 1987). See table 4.1 below for details

Table 4.1 Fazekas Scale of White Matter Hyperintensities

- Periventricular Hyperintensities
  - 0 Absent
  - 1 "Caps" or pencil-thin lining
  - 2 Smooth "halo
  - 3 Irregular; extending into deep white matter

- Deep White Matter Hyperintensities
  - 0 Absent
  - 1 Punctate foci
  - 2 Beginning confluence of foci
  - 3 Large confluent areas

Nuclear Medicine

Radionuclide SPECT scans using 99mTc-HMPAO were done in all of the patients in whom no identifiable cause was found. Several radiopharmaceuticals have been used to image the brain for brain perfusion SPECT scans (Bonte , Devous and Holman 1996). HMPAO has been one of the most widely used and successful radiopharmaceutical in brain perfusion imaging. Once administered, its peak activity in the brain is reached within 2 minutes of injection but more importantly there is no redistribution. Consequently, both the initial tracer uptake and distribution are proportional to rCBF at the time of injection and also remain unaltered for a relatively long time (at least 2 hours) to allow imaging (Catafau, 2001). They scans were repeated at 6-monthly intervals for the duration of the patients' inclusion in the study.

- The patients were in a quiet and dimmed room with their eyes open and ears unplugged for approximately 30 min before the administration of the 99mTc-HMPAO injection. Patient interaction was minimized during this stage.
- The patients received an intravenous injection of 740 Megabecquerel (MBq) of 99mTc-HMPAO through a line already placed in the cubital fossa. The room conditions during the injection and tracer uptake were similar.
- They were placed in a supine position on the Gamma camera bed, with the head immobilized on a head rest and secured with Velcro (front and/or chin) straps to minimize head movement.
- Imaging was done with a dual head Gamma camera (Infinia Haweye, GE Medical). A low energy high-resolution collimator was used to scan all the patients.
- SPECT imaging was recorded on step and shoot mode using a 128 X 128 matrix; one projection for each 3° in a 360° orbit for a total of 120 projections. We processed the data after normalization and back projection using a butterworth (0.5 frequencies) and power 10.

Visual Assessments

The scans were analysed by 2 experienced nuclear medicine physicians. The senior physician has vast experience (>20 years) with a special interest in neuroimaging.
The appropriate brain perfusion SPECT interpretation was made using a step-by-step approach.

- Firstly, a set of slices was selected as a basis for identification of the SPECT perfusion patterns. The transaxial slices were the starting point and then the other sets of slices were used for confirmation, localization, and to assess the extension of findings.
- For standardisation and reproducibility, the same regional order of perfusion assessment was followed and included all cerebral regions, such as assessing at first the cortical regions from bottom to top which are: cerebellum, temporal lobes, frontal lobes (orbitofrontal, prefrontal, and superior frontal areas), occipital lobes, and parietal lobes.
- The mesial and lateral aspects of the above-mentioned cerebral regions were also evaluated separately.
- The global and regional tracer uptake and distribution were assessed to identify areas of normal distribution and those with clear perfusion abnormalities.
- Whenever perfusion was considered to be abnormal, symmetry between the two cerebral hemispheres was checked.

Quantification

In this study we used the software program PMOD Base Functionality version 3.6 (PBAS, version 3.6). A well- established way of obtaining brain volumes of interest automatically is by leveraging the most likely localization of brain areas as encoded in the maximum probability atlas constructed by Hammers et al, 2003. This software package offers much improved batch processing which encompasses the automatic generation of brain volume of interest/ regions of interest (VOI/ROI). As previously stated, many types of quantitative analyses are based on the average signal within tissue structures. The PBAS includes a large and flexible set of tools for defining VOI. The definition types include contour outlines, regular geometric objects, object maps, linear VOIs and VOIs with holes. The VOIs can be created using many versatile tools and the calculation of their common pixels can be done. SPECT brain data were transferred as DICOM for recognition and we used automatic VOIs from the adapted brain atlas. Regional counts per pixel were automatically generated for analysis. The brain atlas allows for the selection of 63 brain ROI. We however selected 30 regions; left and right) based on previous literature describing SPECT scan abnormalities in HIV infected individuals (Tran Dinh et al, 1990; Tatsch et al 1990; Modi et al 2002). The ROI/ VOI were grouped as dominant cerebral regions to facilitate analysis. The VOIs used are listed in table 4.2 page 120.

Table 4.2 Volumes/ Regions of Interest

Frontal Superior
Frontal Middle
Frontal Inferior
Cingulate Anterior
Cingulate Middle
Cingulate Posterior
Hippocampus
Occipital
Caudate
Putamen
Pallidum
Thalamus
Parietal
Temporal
Cerebellum

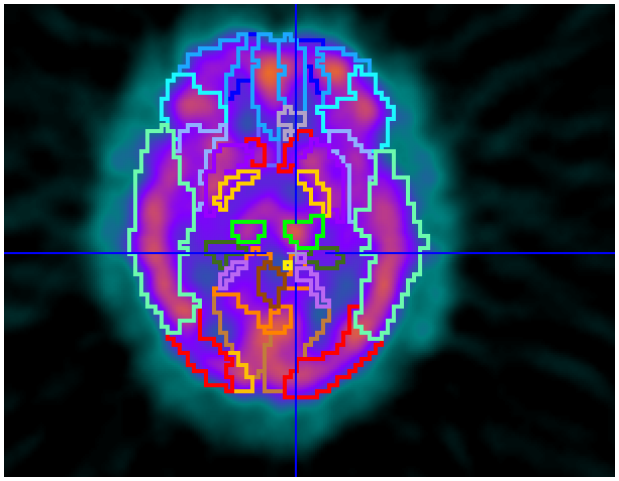


Figure 4.1

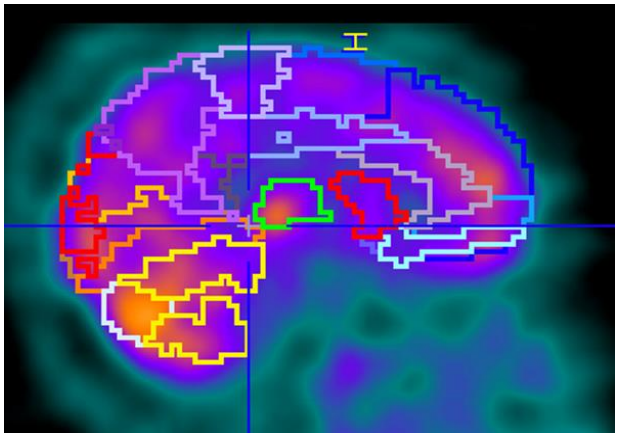


Figure 4.2

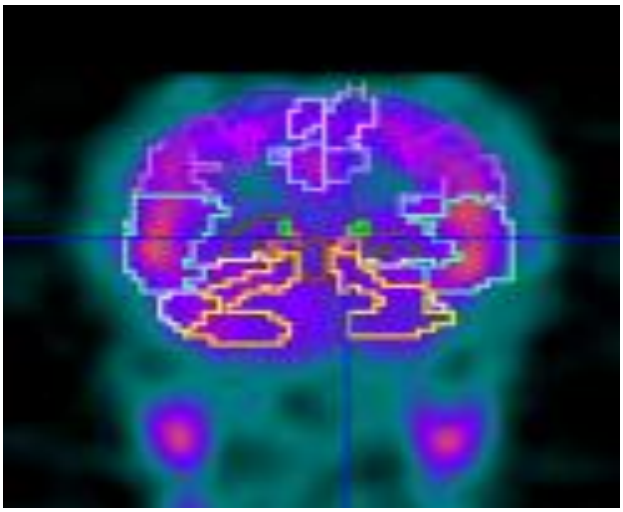


Figure 4.3

ROI/ VOI Selection

Figures 4.1- 4.3 show the automatic creation of VOI. These regions were created using the probability atlas available on the PBAS software. The contour VOIs were interactively adjusted using the VOI features of PBAS, which are described in the User's Guide, 2014. A reduced set of VOI tools was utilized, based on the configuration. (User's Guide)

4.1.5 Data Management and Analysis

The quantification generated copious amounts of data. These data were analysed by the researcher in conjunction with Professor Mboyo-Di-Tamba Vangu and the assistance of Lusendo Matondo. The analyses were carried out using Statistical Packages for Social Sciences (SPSS) version 13 and Excel. The specific analyses included:

- The Kolmogorov-Smirnov test of normality to assess the distribution of the data
- Analysis of Variance (ANOVA) tests to test the null hypothesis that:
    - The means of the dominant cerebral regions for all patients were equal at
        - Baseline
        - Follow-up
    - The means of patients in the different HIV stages at recruitment are equal
- Paired sample t tests to compare baseline data:
    - The left and right cerebral hemispheres in individual patients
    - The dominant cerebral regions in the left and right cerebral hemispheres for all patients
    - The differences between individual cerebral regions for all patients
    - The differences between HIV stages
- Paired sample t tests to compare the differences between individual cerebral regions at follow-up
- Paired sample t tests to compare baseline and follow-up data
    - All regions grouped together for individual patients
    - The left and right cerebral hemispheres in individual patients
    - The dominant cerebral regions for all patients
    - The dominant cerebral regions for patient 1
- Paired sample t tests to compare the pre and post-HAART data for patients 1; 3; 7 and 8
    - The left and right cerebral hemispheres
    - The dominant cerebral regions

4.2 Results

4.2.1 Study Population

There were 22 patients eligible for the study i.e.

- HIV Infection
- New onset Seizures
- No Identifiable Cause
    - Normal laboratory investigations
    - CT and MRI scans of the brain did not reveal any specific abnormality that could account for the seizures

Fifteen patients were enrolled, 7 patients were unwilling to participate in a longitudinal study.

4.2.2 Demography of Study Sample

- There were 10 females and 5 males in our sample at baseline.
- The average age was 36.9 years.
- The ages ranged from 20 to 55 years.

4.2.3 HAART

There were 5 patients on HAART at baseline. Amongst the remaining 10 patients there were 9 patients eligible for HAART.

4.2.4 CD4 Count and HIV-1 RNA Viral loads

- The mean CD4 count was 414.4cells/μL.
- The range was from 49 to 1090 cells/μL.

There were 4 patients in whom the viral load was undetectable

- All were on HAART.

4.2.5 HIV Stage

- Stage 1 6 patients
- Stage 2 5 patients
- Stage 3 4 patients

4.2.6 Seizure Classification

All the patients had generalized tonic-clonic seizures.

4.2.7 EEG Results

- Normal recordings 13 patients
- Generalized slowing 2 patients

4.2.8 MRI Brain Results

The most common abnormality detected on the scans was unexplained white matter lesions. The white matter abnormalities were graded according to the Fazekas Scale (Fazekas et al, 1987), an established and validated method of reporting white matter hyper intensities (Gouw et al, 2008). This visual scale has been shown to correlate closely with quantitative assessments of white matter lesions (Valdés Hernández Mdel et al, 2013) and has previously been used to assess unexplained white matter lesions in HIV Positive individuals (Haddow et al, 2014). A summary of the grading scale is detailed below (Table 4.3).

Table 4.3 Fazekas Scale of Scoring WML

Fazekas Score	MRI Finding
0	None or a single punctate WML
1	Multiple punctate lesions
2	Beginning confluence of lesions (bridging)
3	Large confluent lesions

Table 4.4 Summary of Laboratory Investigations and MRI Results (Baseline)

Patient Number	Age	Sex	HAART (Duration In months)	CD4	HIV-1 RNA VL	Hepatitis C	MRI Score (Fazekas)
1	33	F	Nil	340	356	Positive	2
2	20	F	12	606	Undetectable	Negative	1
3	50	M	Nil	156	113743	Negative	1
4	37	F	18	272	13488	Negative	1
5	46	F	60	1090	Undetectable	Negative	1
6	55	F	180	997	Undetectable	Negative	1
7	41	M	Nil	182	11358	Negative	0
8	30	F	Nil	99	29110	Negative	1
9	30	F	Nil	523	17927	N/D	1
10	40	F	Nil	920	2430	N/D	1
11	32	F	Nil	442	22698	N/D	0
12	50	M	Nil	314	2537	N/D	1
13	33	M	Nil	49	75635	N/D	1
14	28	M	Nil	310	7405	Positive	1
15	29	F	14	172	Undetectable	Negative	0

N/D= Not Done

There were 3 patients in whom the MRI scans were normal i.e. the Fazekas score was 0.

4.2.9 SPECT Scans at Baseline

The SPECT scans were performed in the inter-ictal phase.

- The SPECT scans were initially reported on visually
- The perfusion in the brain images was then quantified.
- We used the software program PBAS, version 3.6 (Referenced as Users' Guide).

Quantitative Assessment of the SPECT scans

- "Quantitative SPECT" refers to SPECT images reconstructed in units of radioactivity concentration per pixel. The count/ pixel is calculated and then averaged out for each ROI. We used thirty ROI (see details in the Methods section page 119) and the initial data for each ROI is documented in Table 4.7 (page 130). These regions are based on the probabilistic atlas of the PBAS program.
- The quantification data generated was then analysed to compare differences between:
    - Left and right cerebral hemispheres in terms of:
        - Individual patients
        - Corresponding dominant cerebral regions
    - The individual dominant cerebral regions
    - Patients at baseline and follow-up in terms of:
        - Individual patients
        - Corresponding dominant cerebral regions
        - CDC Stages
    - Patients pre and post-HAART

Table 4.5 SPECT scans at Baseline (Visual Assessment)

Patient Number	Visual Assessment
1	Perfusion defects in inferior parts of temporal lobes. R>L.
2	Perfusion defects in anterior and mesial parts of temporal lobes. L>R.
3	Perfusion defects in temporal lobes; R>L. and L. basal ganglia
4	Perfusion defects in anterior and inferior parts of temporal lobes
5	Perfusion defects in temporal lobes
6	Normal
7	Normal
8	Perfusion defects in anterior and mesial parts of both temporal lobes and R. basal ganglia
9	Perfusion defects in anterior temporal lobes (L>R) and L. parietal lobe.
10	Perfusion defects both temporal lobes and basal ganglia.
11	Perfusion defects in mesial parts of both temporal lobes and L. parietal lobe.
12	Perfusion defects in anterior and mesial parts of both temporal lobes (R>L).
13	Perfusion defects in anterior and mesial parts of both temporal lobes and parietal lobes.
14	Normal
15	Perfusion defects in inferior parts of both temporal lobes (L>R) and L. parietal lobe.

Table 4.6 SPECT scans at Baseline (Visual Assessment)

Patient Number	Temporal		Parietal		Basal Ganglia		Frontal	
	Left	Right	Left	Right	Left	Right	Left	Right
1	+	++						
2	++	+						
3		++			+			
4	++	++						
5	++	++						
6	Normal							
7	Normal							
8	++	++				+		
9	++	+	+					
10	+	+			+	+		
11	+	+	+					
12	+	++						
13	+	+	+	+				
14	Normal							
15	++	+	+					

Key

+: <2 Deficits present

++: >2 Deficits present

Table 4.7 Quantitative SPECT Data at Baseline (30 ROI)

Patients	1	2	3	4	5	6	7	8	11	12	13	14	15	Mean
Region														
FS L	51.07	48.16	27.70	56.06	105.23	60.13	41.24	35.73	119.57	63.44	55.47	75.92	32.36	65.75
FS R	51.47	49.26	26.84	55.69	106.53	59.34	47.32	35.50	120.25	65.46	56.12	73.86	38.12	66.61
FM L	54.59	46.82	29.29	55.84	102.41	59.57	37.42	36.89	117.29	62.49	56.99	75.09	20.62	65.64
FM R	55.49	51.42	29.26	60.39	108.21	62.89	51.93	39.01	125.19	66.83	58.92	72.96	28.81	69.94
FI L	52.31	48.45	30.37	55.48	109.00	61.57	35.70	37.03	108.43	64.02	55.73	78.59	22.10	67.27
FI R	55.13	48.63	29.66	55.26	113.57	64.27	50.40	37.59	117.71	65.96	56.05	75.54	25.45	69.14
CiA L	56.23	49.24	31.08	55.59	114.69	62.94	50.01	37.55	113.09	68.83	62.31	76.13	35.70	70.42
CiA R	58.28	52.96	30.55	58.23	118.94	62.83	49.61	40.15	120.48	67.45	61.45	75.46	39.89	72.18
CiM L	53.64	51.60	31.81	53.80	123.02	60.91	53.31	36.19	115.33	60.74	56.96	75.23	40.56	69.06
CiM R	55.64	51.74	30.72	56.45	126.30	60.31	52.55	36.47	115.22	64.55	58.61	72.95	43.29	70.42
CiP L	50.59	47.35	27.83	46.19	115.10	51.74	49.50	32.61	103.55	52.38	52.43	75.33	40.28	62.83
CiP R	45.50	46.24	26.40	41.08	109.11	50.60	44.67	32.48	98.84	49.08	54.38	62.87	43.30	60.44
HP L	47.88	42.85	27.02	49.47	116.58	57.79	44.73	30.75	98.82	59.35	51.09	60.39	32.01	61.88
HP R	45.28	42.38	26.75	50.01	114.96	56.32	46.05	32.78	98.96	56.97	51.81	68.70	28.39	61.52
O L	57.53	51.91	34.03	60.29	114.18	67.66	53.56	36.01	131.46	71.73	62.12	68.58	46.89	73.96
O R	54.87	52.23	34.21	58.01	120.70	70.31	35.28	37.08	125.79	68.70	59.21	79.81	45.82	72.63
Ca L	45.98	43.32	26.60	42.81	101.63	50.74	40.56	32.11	96.79	51.21	53.87	81.81	30.47	57.49
Ca R	47.08	42.61	25.97	40.26	111.19	48.19	46.67	32.38	92.06	45.52	49.82	58.96	33.98	58.09
Pu L	54.61	53.76	31.69	61.26	126.53	69.45	50.48	39.22	121.82	69.23	72.08	54.11	24.09	74.53
Pu R	54.43	52.58	32.00	57.52	137.23	68.25	55.76	38.69	123.64	69.84	67.00	77.62	29.36	75.05
Pa L	54.10	52.51	31.35	60.06	135.47	69.33	54.32	38.52	122.84	68.02	74.70	79.40	24.46	75.26
Pa R	54.76	52.67	31.39	58.29	138.62	65.43	52.42	36.76	116.65	69.75	63.11	81.08	31.27	73.17
Th L	49.34	47.95	31.36	53.99	126.97	61.56	51.96	36.36	114.91	63.68	61.72	77.57	29.86	68.98
Th R	54.09	50.32	32.64	52.80	126.37	58.59	48.88	33.05	121.91	62.81	56.16	68.15	31.08	69.40
Par L	54.29	51.73	32.23	54.91	108.42	60.70	45.75	35.98	119.94	65.21	57.70	71.61	39.12	69.41
Par R	50.67	52.28	30.15	52.76	108.09	60.11	35.05	33.78	120.29	61.27	54.73	76.30	34.32	66.83
Tem L	52.40	47.83	29.99	55.66	110.16	61.44	40.60	34.60	107.50	63.66	57.21	75.56	42.03	67.04
Tem R	53.98	49.51	29.81	55.92	116.33	64.52	37.58	34.82	115.67	64.85	58.03	74.06	42.29	68.91
Cb L	59.35	55.93	32.78	59.33	132.88	68.70	51.14	38.52	128.83	70.84	66.02	76.72	44.38	77.25
Cb R	58.61	55.51	33.00	60.61	135.06	68.95	46.24	38.69	132.86	69.44	66.19	81.64	47.43	76.72

NB! There were no quantitative SPECT data for patients 9 and 10 due to technical difficulties

Legend

L=Left; R=Right; FS= Frontal Superior; F M= Frontal Middle; FI= Frontal Inferior; CiA= Cingulate Anterior; CiM= Cingulate Middle; CiP= Cingulate Posterior; HP= Hippocampus; O= Occipital; Ca= Caudate; Pu= Putamen; Pa= Pallidum; Th= Thalamus; Par= Parietal; Tem= Temporal; Cb= Cerebellum

SPECT images reconstructed in units of radioactivity concentration per pixel and averaged per region

The individual ROI were grouped together to represent the dominant cerebral regions. These groupings are based on the brain probabilistic atlas of the PMOD software program (Referenced as Users guide).

- Frontal Superior; Middle and Inferior — Frontal region
- Cingulate Anterior; Middle and Posterior — Cingulate
- Caudate; Thalamus; Putamen and Pallidum — Central region
- Temporal and Hippocampus — Temporal region
- Parietal — Parietal region
- Occipital — Occipital region
- Cerebellum — Cerebellum

The quantitative data, at baseline according to dominant cerebral regions is documented below.

Table 4.8 Cerebral Regions

Patient Number	Frontal	Cingulate	Central Region	Temporal Region	Parietal Region	Occipital Region	Cerebellum
1	320.06	319.88	414.39	199.54	104.96	112.4	117.96
2	292.74	299.13	395.72	182.57	104.01	104.14	111.44
3	173.12	178.39	243	113.57	62.38	68.24	65.78
4	338.72	311.34	426.99	211.06	107.67	118.3	119.94
5	644.95	707.16	1004.01	458.03	216.51	234.88	267.94
6	367.77	349.33	491.54	240.07	120.81	137.97	137.65
7	264.01	299.65	401.05	168.96	80.8	88.84	97.38
8	221.75	215.45	287.09	132.95	69.76	73.09	77.21
11	708.44	666.51	910.62	420.95	240.23	257.25	261.69
12	388.2	363.03	500.06	244.83	126.48	140.43	140.28
13	339.28	346.14	498.46	218.14	112.43	121.33	132.21
14	451.96	437.97	578.7	278.71	147.91	148.39	158.36
15	167.46	243.02	234.57	144.72	73.44	92.71	91.81

SPECT images reconstructed in units of radioactivity concentration per pixel and averaged per region for each patient

4.2.10 Statistical Analysis of Quantitative SPECT Data at Baseline

The details of the statistical analyses are documented in Appendix G.

A significance level of 5% was assumed.

The data of the 30 ROI was normally distributed, enabling parametric tests.

The following findings were relevant:

- There was symmetrical perfusion between the left and right cerebral hemispheres for:
    - Individual patients
    - Dominant cerebral regions (all patients grouped together)

- There were significant regional differences between the cerebellum (reference region) and the:
    - Frontal region
    - Cingulate
    - Central region
    - Temporal region

- There were significant regional differences between the patients in HIV stage 3 and:
    - Stage 1
    - Stage 2

4.2.11 Follow-up Data

Follow-up data is available for 8 patients (patients 1-8 according to table 4.4 page 126).

The data is recorded in tables 4.11 to 4.18 (pages 136-152)

- One patient was pregnant during the study and did not have some of the investigations done at her 6 month visit. She did however continue to follow up and her investigations were completed later (Patient 1).
- One patient did not attend her 6-month follow-up but did come to the 12 month follow-up (Patient 3).

Demography

The average follow-up period for females was 10.2 months and males were 6.6 months.

HAART

Four patients initiated HAART during the study. All patients were on HAART at their last visit. NB! Patient 1 was eligible for HAART from her initial visit but declined therapy on numerous occasions. She only initiated HAART after discovering that she was pregnant.

CD4 Count and HIV-1 RNA Viral Loads

There were 8 patients who had repeat CD4 counts and HIV-1 RNA Viral Loads measured.

A significant change in the CD4 count is defined as a 30% change in the absolute count (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2015). Four patients had significant changes in their CD4 counts during the study period. In 3 of the patients there was an improvement. Three of these patients initiated HAART during the study.

HIV Stages

At final visits:

- There were 4 patients in stage 1
- There were 4 patients in stage 2
- There were no patients in stage 3

Table 4.9 Summary of Laboratory Investigations and MRI Results (6 Months)

Patient Number	HAART (Duration In months)	CD4	HIV-1 RNA VL	MRI Score (Fazekas)	SPECT (Visual)
1	Nil	255	295	2	Perfusion defects in temporal lobes. R>L.
2	18	383	35030	1	Perfusion defects temporal lobes. L>R. Additional perfusion defects in L. parietal lobe
3	6	338	70237	1	Perfusion defects in L. temporal; parietal and frontal lobes
4	Did not attend				
5	66	1066	Undetectable	1	Perfusion defects in temporal lobes L>R
6	186	572	200	2	Perfusion defects in temporal and parietal lobes. L>R.
7	3	251	4878	0	Normal
8	5	280	7430	1	Perfusion defects both temporal lobes

NB! The lumbar punctures at this stage did not reveal any significant abnormalities

Table 4.10 Summary of Laboratory Investigations and MRI Results (12 Months)

Patient Number	HAART (Duration In months)	CD4	HIV-1 RNA VL	MRI Score (Fazekas)	SPECT (Visual)
1	7	299	Undetectable	2	Normal
2	18	598	14465	0	Perfusion defects in both temporal lobes
3	12	546	10658	1	Perfusion defects in both temporal lobes
4	30	539	13488	1	Perfusion defects in inferior parts of temporal lobes

NB!! The data for patient 1 was done at 18 months; she was pregnant at the 12 month visit

Follow-up Data for Individual Patients

The clinical; laboratory; radiology and nuclear medicine data for each of the 8 patients that followed up for a minimum period of 6 months is detailed in the following 8 tables.

Table 4.11 Baseline and Follow-up Data Patient 1

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)	Visit 4 (18 Months)
Clinical Examination	Normal	Normal	Normal (Pregnant)	Normal
Seizure Control	2 GTC seizures prior to her visit	Inadequate (2 events)	Good (0 events)	Good (0 events)
AED	None Sodium Valproate started	Sub-therapeutic Dose adjusted	No adjustment Patient already in 2nd trimester	No adjustment
IHDS	12	12	11	12
CD4 count	340	255	198	299
Viral Load	356	295	423	Undetectable
HAART	No	No	Yes	Yes
HAART Duration	Nil	Nil	1 Month	7 Months
MRI Scan (Fazekas Score)	2	2	Not done (Patient pregnant)	2
Qualitative SPECT	Perfusion defects in inferior parts of temporal lobes. R>L.	No change		Normal
Quantitative SPECT comparisons	P=0.88			P=0.012 (between visit 2 and 3) p=0.005 (between visit 1 and 3)
Frontal	320.06	314.53		510.27
Cingulate	319.88	310.88		487.52
Central Region	414.39	409.23		660.03
Temporal Region	199.54	102.15		301.87
Parietal Region	104.96	105.11		166.54
Occipital Region	112.4	198.92		168.76
Cerebellum	117.96	111.36		172.65

Patient 1 Baseline and Follow-up Data

Baseline

She was clinically stable.

She had 2 generalized tonic-clonic seizures prior to her presentation. She was not on anti-epileptic medication. Sodium valproate was started at an appropriate dose.

She was not on HAART; despite having being referred previously.

Her CD4 count was within the range at which therapy would have been recommended i.e. her CD4 count was < 350 cells/μL (South African antiretroviral therapy guidelines, 2013).

Her MRI scan revealed some unexplained WML.

The visual assessment of her SPECT scan showed asymmetrical temporal lobe defects.

6 Month Visit

She was clinically well but reported 2 seizures during the intervening period. Laboratory investigations revealed that her sodium valproate level was sub-therapeutic and her dose was adjusted accordingly.

She remained reluctant to initiate HAART; despite the decrease in her CD4 count.

Her MRI and visual assessments of her SPECT scan remained unchanged.

The quantitative data for her first and second SPECT scans were compared and the difference was insignificant (p=0.88).

12 Month Visit

She was stable; she had not experienced any seizures since her previous visit.

She was pregnant (4 months). Her anti-epileptic medication was not changed as she was in the second trimester already. She did not have any radiological investigations during this period

She initiated HAART 1 month prior to her visit.

18 Month Visit

She was stable; she had not experienced any seizures since her previous visit.

There was a significant increase in her CD4 count (> 50%) and her serum viral load was undetectable.

The visual assessment of her SPECT scans also improved.

The quantitative SPECT data were compared (see appendix G for statistical analysis)

- No improvement between scans 1 and 2 (p=0.94).
- Improvement between scans
  - 1 and 3 (p=0.005)
  - 2 and 3 (p=0.012)

In this patient the initiation of HAART corresponded with:

- Adequate seizure control
- Significant increase in her CD4 count
- Decrease in her serum viral load
- Improvement in the visual and quantitative assessments of her SPECT scans

Table 4.12 Baseline and Follow-up Data Patient 2

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)
Clinical Examination	Normal	Lower respiratory tract infection	Normal
Seizure Control	Good (0 events)	Good (0 events)	Good (0 events)
AED	Sodium Valproate	Sodium Valproate	Sodium Valproate
IHDS Score	12	11	12
CD4 count	606	383	598
Viral Load	Undetectable	35030	14465
HAART	Yes	Yes	Yes
HAART Duration	12 Months	18 Months	24 Months
MRI Scan (Fazekas Score)	1	1	0
Qualitative SPECT	Perfusion defects in anterior and mesial parts of temporal lobes. L>R.	Additional perfusion defects in L. parietal lobe	Perfusion defects in both temporal lobes
Quantitative SPECT comparisons	P=0.44		P=0.04
Frontal Region	292.74	364.81	754.21
Cingulate	299.13	379.77	796.3
Central Region	395.72	507.56	1079.72
Temporal Region	182.57	125.16	492.73
Parietal	104.01	237.63	269.96
Occipital	104.14	129.85	286.85
Cerebellum	111.44	147.92	309.73

Patient 2 Baseline and Follow-up Data

Baseline

She was clinically stable.

She had 1 generalized tonic-clonic seizure prior to her presentation. She was on sodium valproate which had been started at her local clinic.

She was stable on HAART.

Her MRI scan revealed some unexplained WML.

The visual assessment of her SPECT scan showed asymmetrical temporal lobe defects.

6 Month Visit

She was unwell and had symptoms and signs of a lower respiratory tract infection. She received antibiotics and had improved when seen a week later. Her CXR did not reveal evidence of TB and her sputum samples were negative for AFBs.

Her laboratory investigations revealed that her immune control had deteriorated.

There were no significant changes in her MRI scan.

The visual assessment of her SPECT scan revealed additional defects in the left parietal lobe.

The difference in the quantitative assessments between her initial and repeat SPECT scans was insignificant ($p=0.44$).

12 Month Visit

She was stable and her seizure control remained good.

Her immune markers improved.

Her MRI scan and the visual assessment of her SPECT scan also improved.

The quantitative assessment of her SPECT scans revealed a significant improvement in comparison to her previous scan ($p=0.04$).

Table 4.13 Baseline and Follow-up Data Patient 3

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)
Clinical Examination	Normal	Normal	Normal
Seizure Control	Good (0 events)	Good (0 events)	Good (0 events)
AED	Sodium Valproate	Sodium Valproate	Sodium Valproate
IHDS Score	10	10	12
CD4 count	156	338	546
Viral Load	113743	70237	10658
HAART	No	Yes	Yes
HAART Duration	Nil	6 Months	12 Months
MRI Scan (Fazekas Score)	2	1	1
Qualitative SPECT	Perfusion defects in temporal lobes; R>L. and L. basal ganglia	Perfusion defects in L. temporal; parietal and frontal lobes	Perfusion defects in both temporal lobes
Quantitative SPECT comparisons	P=0.03 (between visit 1 and 3)		
Frontal Region	173.12	Not Available	693.18
Cingulate	178.39		645.75
Central Region	243		863.6
Temporal Region	113.57		400.75
Parietal	62.38		235.57
Occipital	68.24		245.67
Cerebellum	65.78		245.26

Patient 3 Baseline and Follow-up Data

Baseline

He was clinically stable.

He had 3 generalized tonic-clonic seizures prior to his presentation. He was on sodium valproate which had been started during his admission to the medical ward one week previously.

He had begun counselling at his local clinic and was due to begin anti-retroviral therapy a week later.

His MRI scan revealed WML.

The visual assessment of his SPECT scan showed asymmetrical temporal lobe and basal ganglia defects.

6 Month Visit

He was clinically stable and had initiated HAART 6 months previously.

His immune control had improved as evidenced by the significant increase in his CD4 count and a decrease in his serum viral load.

His MRI scan and the visual assessment of his SPECT scan also improved.

Unfortunately due to technical difficulties there were no quantitative data for the SPECT.

12 Month Visit

He had clinical improvement and continued his immune recovery.

The visual assessment of his SPECT revealed improvements and his quantitative data was significantly improved when compared to the initial scan ($p=0.03$).

In this patient the initiation of HAART corresponded with:

- Significant increase in his CD4 count over 12 months
- Decrease in his serum viral load
- Improvement in his MRI scan
- Improvement in the quantitative assessments of his SPECT scans ($p=0.03$)

Table 4.14 Baseline and Follow-up Data Patient 4

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)
Clinical Examination	Normal	Patient did not attend	Normal
Seizure Control	Good (0 events)		Good (0 events)
AED	Sodium Valproate		Sodium Valproate
IHDS Score	12		11
CD4 count	272		539
Viral Load	35396		13488
HAART	Yes		Yes
HAART Duration	18 Months		30 Months
MRI Scan (Fazekas Score)	1		1
Qualitative SPECT	Perfusion defects in anterior and inferior parts of temporal lobes		Perfusion defects in inferior parts of temporal lobes
Quantitative SPECT	P=0.002		
Frontal Region	338.72		452.17
Cingulate	311.34		422.23
Central Region	426.99		568.51
Temporal Region	211.06		288.06
Parietal	107.67		151.85
Occipital	118.3		161.62
Cerebellum	119.94		164.31

Patient 4 Baseline and Follow-up Data

Baseline

She was clinically stable.

She had 1 generalized tonic-clonic seizure prior to her presentation. She was on sodium valproate which had been started by her general practitioner.

She was on HAART for 18 months but her virologic control was inadequate and she was referred for review of her therapy.

Her MRI scan revealed WML.

The visual assessment of her SPECT scan showed symmetrical temporal lobe defects.

6 Month Visit

She did not attend as she was away taking care of her ill mother.

12 Month Visit

She was clinically stable and had not experienced any further seizures.

Her immune control had improved following a change in her antiretroviral therapy.

The visual assessment of her SPECT scan also improved.

The quantitative assessment of her SPECT scans revealed a significant improvement in comparison to her previous scan ($p=0.002$).

Table 4.15 Baseline and Follow-up Data Patient 5

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)
Clinical Examination	Normal	Normal	Patient did not return
Seizure Control	2 GTC seizures prior to her visit	Good (0 events)	
AED	None Sodium Valproate started	Sodium Valproate	
IHDS Score	11	11	
CD4 count	1090	1066	
Viral Load	Undetectable	Undetectable	
HAART	Yes	Yes	
HAART Duration	60 Months	66 Months	
MRI Scan (Fazekas Score)	1	1	
Qualitative SPECT	Perfusion defects in temporal lobes	Perfusion defects in temporal lobes. L>R	
Quantitative SPECT	P=0.32		
Frontal Region	367.77	406.78	
Cingulate	349.33	399.28	
Central Region	491.54	577.3	
Temporal Region	240.07	271.07	
Parietal	120.81	136.68	
Occipital	137.97	164.82	
Cerebellum	137.65	160.52	

Patient 5 Baseline and Follow-up Data

Baseline

She was clinically stable.

She had 2 generalized tonic-clonic seizure prior to her presentation. She was started on sodium valproate.

She was on HAART for 60 months and her virologic control was good.

Her MRI scan revealed WML.

The visual assessment of her SPECT scan showed asymmetrical temporal lobe and basal ganglia defects.

6 Month Visit

She remained stable and her seizure control was good.

She maintained her virologic suppression.

The visual and quantitative assessment of her SPECT scans improved, but the improvement in the quantitative data was not significant (p=0.32).

In this patient her clinical stability coincided with:

- An insignificant decrease in her CD4 count
- Maintenance of virologic suppression
- An insignificant increase in cerebral perfusion on quantitative SPECT measurements (p=0.32)

Table 4.16 Baseline and Follow-up Data Patient 6

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)
Clinical Examination	Normal	Features of HAD	Patient did not return
Seizure Control	Good	Fair (1 event)	
AED	Sodium Valproate	Sodium Valproate	
IHDS Score	11	8	
CD4 count	997	572	
Viral Load	<40	200	
HAART	Yes	Yes	
HAART Duration	180 Months	186 Months	
MRI Scan (Fazekas Score)	1	2	
Qualitative SPECT	Normal	Perfusion defects in temporal and parietal lobes. L>R.	
Quantitative SPECT	P=0.007		
Frontal Region	644.95	456.5	
Cingulate	707.16	445.85	
Central Region	1004.01	610	
Temporal Region	458.03	294.26	
Parietal	216.51	152.35	
Occipital	234.88	160.87	
Cerebellum	267.94	170.49	

Patient 6 Baseline and Follow-up Data

Baseline

She was clinically stable.

She had 1 generalized tonic-clonic seizure prior to her presentation. She was on sodium valproate which had been started by her general practitioner.

She was on HAART for 18 months but her virologic control was inadequate and she was referred for review of her therapy. She did not follow up on this referral.

Her MRI scan revealed WML.

The visual assessment of her SPECT scan showed symmetrical temporal lobe defects.

6 Month Visit

She had clinical features of HAD; and her IHDS score deteriorated.

She had 1 seizure; despite her AED level being normal.

Her immunological control deteriorated and she was referred for review of her antiretroviral regimen. The patient claimed that she was compliant on her anti-retrovirals and had proof of regular clinic visits to collect her medication.

Her MRI scan and visual assessments of her SPECT scans were worse.

The quantitative data revealed a significant deterioration ($p=0.007$) between her initial and repeat scans.

In this patient her neurocognitive deterioration coincided with:

- A decrease in her CD4 count
- An increase in her viral load
- An increase in the quantity of WML on her MRI scan
- An increase in perfusion defects
- A significant decrease in cerebral perfusion on quantitative SPECT measurements ($p=0.007$).

Table 4.17 Baseline and Follow-up Data Patient 7

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)
Clinical Examination	Normal	Normal	Patient did not return
Seizure Control	Good (0 events)	Good (0 events)	
AED	Sodium Valproate	Sodium Valproate	
IHDS Score	11	12	
CD4 count	182	251	
Viral Load	11358	4878	
HAART	No	Yes	
HAART Duration	Nil	3 Months	
MRI Scan (Fazekas Score)	0	0	
Qualitative SPECT	Normal	Normal	
Quantitative SPECT	P=0.001		
Frontal Region	264.01	535.64	
Cingulate	299.65	503.91	
Central Region	401.05	679.38	
Temporal Region	168.96	325.8	
Parietal	80.8	177.22	
Occipital	88.84	197.83	
Cerebellum	97.38	196.37	

Patient 7 Baseline and Follow-up Data

Baseline

He was clinically stable.

He had 2 generalized tonic-clonic seizures prior to his presentation. He was on sodium valproate which had been started during his admission to the medical ward three weeks previously.

His MRI scan and the visual assessment of his SPECT scan were normal.

He was referred to the hospital clinic for counselling and initiation of HAART.

6 Month Visit

He was clinically stable and had initiated HAART 3 months previously.

His immune control had improved as evidenced by the significant increase in his CD4 count and a decrease in his serum viral load.

His MRI scan and the visual assessment of his SPECT scan remained normal.

The quantitative SPECT data showed a significant improvement ($p=0.001$).

In this patient the initiation of HAART corresponded with:

- Significant increase in his CD4 count
- Decrease in his serum viral load
- Improvement in the quantitative assessments of his SPECT scans ($p=0.001$).

Table 4.18 Baseline and Follow-up Data Patient 8

Investigation	Visit 1	Visit 2 (6 Months)	Visit 3 (12 Months)
Clinical Examination	Stable	Stable	Patient did not return
Seizure Control	Fair (1 event)	Good	
AED	None	None	
IHDS Score	11	12	
CD4 count	99	280	
Viral Load	29110	7430	
HAART	No	Yes	
HAART Duration	Nil	5 months	
MRI Scan (Fazekas Score)	1	1	
Qualitative SPECT	Perfusion defects in anterior and mesial parts of both temporal lobes and R. basal ganglia	Perfusion defects in anterior, mesial and inferior parts of both temporal lobes	
Quantitative SPECT	P=0.004		
Frontal Region	221.75	698.01	
Cingulate	215.45	700.28	
Central Region	287.09	909.22	
Temporal Region	132.95	415.51	
Parietal	69.76	221.73	
Occipital	73.09	228.92	
Cerebellum	77.21	246.81	

Patient 8 Baseline and Follow-up Data

Baseline

She was clinically stable.

She had 1 generalized tonic-clonic seizure prior to her presentation. She was not on anti-epileptic therapy and unwilling to start.

She was referred to her local clinic for counselling and to initiate HAART.

Her MRI scan revealed WML.

The visual assessment of her SPECT scan showed symmetrical temporal lobe defects and defects in the right basal ganglia.

6 Month Visit

She remained stable and did not experience any further seizures.

She was on HAART and did not experience any significant side-effects.

The visual assessment of her SPECT scans revealed a marginal improvement.

The quantitative SPECT data showed a significant improvement ($p=0.004$).

In this patient the initiation of HAART corresponded with:

- Good seizure control
- Significant increase in her CD4 count
- Decrease in her serum viral load
- Improvement in the visual and quantitative assessments of her SPECT scans

Pre- and Post-HAART Sub-group

Four patients (patients 1; 3; 7 and 8) initiated HAART during the study. Their data was analysed separately.

Table 4.19 Pre and Post-HAART Clinical Data

Patient Number	HAART Duration (months)	CD4 Count		Viral Load		MRI Fazekas Score	
		Pre-HAART	Post-HAART	Pre-HAART	Post-HAART	Pre-HAART	Post-HAART
1	7	255	299	295	Undetectable	2	2
3	12	156	546*	113743	10658	2	1
7	3	182	251*	11358	4878	0	0
8	5	99	280*	29110	7430	1	1

*These patients had a significant increase in their CD4 counts

The SPECT Quantitative data for each of the 4 patients' pre and post-HAART were analysed (see Appendix G for details):

- There was a significant increase in both left and right cerebral perfusion for each patient
- There was a significant increase in the overall perfusion for each patient as indicated by the assessments of the dominant cerebral regions

4.2.12 Summary of Results

There were twenty-two patients were eligible for the study but only 15 patients consented to inclusion in the longitudinal study (10 females and 5 males).

At baseline:

- All patients presented with generalized tonic clonic seizures.
- Most patients (14) were either on sodium valproate or initiated it at that visit
- Only 5 patients were on HAART
- Six patients were in HIV stage 1 and 5 in stage 2
- The majority (13) had normal EEG recordings.
- The most common MRI finding was unexplained WML (12 patients).
- The most common abnormality on visual SPECT assessment was in the temporal region (12 patients).
- Quantitative SPECT revealed regional abnormalities between the cerebellum and Frontal; cingulate; central and temporal regions

Follow-up:

- There was a high rate of attrition i.e. there were 7 patients at the 6 month visit and 4 patients at the 12 month visit.
- Seizure control was adequate except in 1 patient who required a dose adjustment.
- HAART was initiated in 4 patients.
- Unexplained WML persisted; except in :
    - Patient 3 the lesions ↓
    - Patient 6 the lesions ↑
- Visual SPECT assessments:
    - Improved in patients 1; 2; 3; 4 and 7
    - Deteriorated in patient 6
- There was an improvement in the cerebral perfusion as measured by the quantitative SPECT in all patients except patient 6

Pre and post-HAART

- Four patients initiated HAART during the study
- All had improvements in their
    - CD4 counts
    - Viral loads
    - Quantitative SPECT data

4.3 Descriptive Analysis at Baseline and Follow-up

4.3.1 Attrition

There was a high rate of attrition in the longitudinal study. Numerous factors have previously been recognized which contribute to decreased recruitment and high attrition in longitudinal studies (Patel, Doku and Rennakoon 2003 and Booker, Harding and Benzeval 2011).

The following were important factors in this study:

- Male gender
- "Non-White" race
- Underprivileged socio-economic individuals i.e. unemployed or low occupational status
- Low educational status
- Low family income
- People at increased risk of ill-health/ recent illness/ poor present health
- Misgiving about researchers and studies
- Apprehensions about the research and consent processes
- Differences in perceptions between lay belief and medical teaching
- Additional demands of this trial
    - Length of time to complete investigations
      This was crucial in this context as patients had investigations (CTB; MRI and SPECT scans) which are subject to long waiting lists at the research sites
    - Stigma and reluctance to seek care amongst HIV-positive individuals

4.3.2 Demography at baseline and follow-up

The higher proportion of females (66.6%) in this cohort was unexpected because in the larger study population there was a preponderance of males (57.5%). A possible explanation for this is that of the 7 patients at baseline who refused to be included in the study 6 were males, thus skewing the proportions. It has been shown that females are more likely than males to participate in longitudinal studies (Post et al, 2012).

The average follow-up period for females was 10.2 months and males were 6.6 months. Mein et al, 2012 analysed factors which contributed to attrition in longitudinal health studies. They showed that "men are considerably more likely to drop out, all other factors being equal."

4.3.3 HAART at baseline and follow-up

HAART penetration at baseline was 33.3%%, which is lower than that of the study population (42.5%). This finding is consistent with that of Siedner et al, 2015 who showed that despite the availability of HAART in South Africa the number of patients accessing therapy was sub-optimal due in part to:

- Poverty
- Transportation-related barriers
- HIV-associated stigma

All patients were on HAART at the end of the study. Possible explanations include:

- Provision of transport money
- Decreased stigma as patients were dealing with the same doctor and staff at each visit

4.3.4 CD4 Count and HIV-1 RNA Viral loads at baseline and during follow-up

The mean CD4 count at baseline (414.4cells/µL) was much higher than that of the broader study (167cells/µL). This is most likely due to the exclusion of patients with identifiable causes. The majority of our patients with identifiable causes had opportunistic infections, suggesting immune-suppression. Our findings are consistent with those of Modi et al, 2009. In their study patients with NIC for their seizures were analysed separately. The mean CD4 count in their patients with NIC, all of whom were HAART-naive was 323cells/µL. This was higher than their overall mean CD4 count which was 239cells/µL.

During the follow-up period 3 patients registered significant improvements (>30%) in their CD4 counts. All of these patients initiated HAART during the study period. The CD4 count increase in the patients initiating HAART is expected. Smith et al, 2004 studied the long term effects of HAART in 397 patients. The median CD4 count increased by:

- 114cells/µL at 6 months
- 181cells/µL at 12 months.

The mean RNA viral load in our group was 26971.5copies/mL. This was much lower than the mean value in the 2009 study by Modi et al (>410678.9 copies/mL). The use of HAART in our patients was the most likely reason for the lower mean RNA viral loads.

4.3.5 HIV Stages at baseline

- There were 6 patients in stage 1; the majority (4) were on HAART.
- There were 4 patients in stage 3; all were HAART-naive

The use of HAART in some patients was associated with the obvious benefits of lower RNA viral loads and higher CDC stages. Moore and Bartlett, 2011 showed that HAART usage linked to:

- Increased CD4 counts
- Decline in RNA viral loads

Their study population was similar to ours i.e. an urban HIV-infected population, some of whom were receiving HAART.

4.3.6 Seizure Classification and EEG Results at baseline

All 15 patients had generalized tonic-clonic seizures. This finding is consistent with other studies in which patients with NIC for their seizures have been analysed separately. In the 2002 study by Modi et al, thirteen of their 15 patients with NIC had GTC seizures. In the 1990 study by Wong, Suite and Labar they identified 32 patients in whom there was no presumed cause for the seizures except HIV. In the sub-group 22 patients had GTC seizures only and 14 had a combination of GTC and focal seizures.

The EEG recordings were normal in 13 patients and in 2 patients there was generalized slowing. No patients had specific abnormalities of epileptic dysfunction. This correlates with the findings of Siddiqi et al, 2015 who showed that abnormal EEG findings in HIV-infected adults with NOS were associated with abnormal imaging findings. They found a link between white matter abnormalities on MRI and EEG slowing.

4.3.7 MRI scans at baseline and during follow-up

Two thirds (10) of patients at baseline had white matter lesions on their MRI scans.

The significance of these lesions in the clinical setting of HIV infection is unclear. It is likely that the white matter abnormalities observed in our patients are multi-factorial in origin. These lesions have been associated with numerous clinical variables:

- Neurocognitive dysfunction

  None of the patients in this component of the study had evidence of neurocognitive dysfunction at baseline. The presence of abnormal white matter in asymptomatic HIV-infected individuals has been documented previously. Gray et al, 1996 showed pathological evidence of white matter disease in asymptomatic HIV infected individuals. A South African study (Hoare et al, 2011) used diffusion tensor imaging to study the relationship between white matter damage and cognitive impairment. They compared the findings in 46 Clade C HIV-positive subjects to healthy non-HIV controls. They detected white matter abnormalities in HIV+ cognitively normal individuals as well as those with HAND. The changes were more extensive in patients with dementia.

- A low nadir CD4 count

  Cohen et al, 2010 examined the patterns of brain volume loss in HIV-infected neuroasymtomatic individuals. They found lower nadir CD4 counts were associated with white matter abnormalities. This has been confirmed by Jernigan et al, 2011. As part of the CHARTER study they reviewed the clinical factors which contributed to abnormal brain structure in HIV. They found a low nadir CD4 count was the most consistent predictor of white matter abnormalities on MRI scans. In this study four patients had CD4 counts <200cells/µL and six were on HAART at baseline. We did not have the nadir CD4 counts of the patients on HAART. As discussed previously, a recent meta-analysis by Siedner et al, 2015 showed that mean CD4 counts of >96 000 South Africans prior to commencement of anti-retroviral therapy was 123cells/µL.

- Black race

  Haddow et al, 2013 retrospectively reviewed the brain MRI studies of 254 HIV-positive patients. The images were independently reviewed for white matter lesions. They identified black race as the only risk factor for the development of diffuse white matter abnormalities. In this study all patients were Black African. The significance of race in the occurrence of white matter lesions has also been shown in the setting of subclinical cerebrovascular disease associated with cardiovascular disease (Nyquist et al, 2014).

- Older age

  McMurtray et al, 2007 assessed the relationship between age and white matter lesions in HIV-infected individuals. This study was part of the ongoing Hawaii aging with HIV cohort study. They concluded that in the HAART-era the extent of white matter lesions correlated with age and systolic blood pressure. Gongvatana et al, 2011 examined 85 HIV-infected individuals to determine the clinical factors which contribute to white matter abnormalities. Older age was significantly associated with white matter injury in their HIV-infected patients, most of who were on HAART. In our group of 15 patients there were 3 patients who were 50years and older.

- Hepatitis C co-infection

  Co-infection with Hepatitis C virus was a notable factor in patients HIV-infected individuals with white matter injury (Gongvatana et al, 2011). In this study 2 of the 10 people tested were positive for Hepatitis C. Co-infection rates in South Africa are <5% as reported by Rao et al, 2015.

4.3.8 SPECT scans

Visual Data

The perfusion defects documented visually at baseline in our patients were:

- Temporal lobe abnormalities 11 patients
- Parietal lobe abnormalities 4 patients
- Basal ganglia abnormalities 3 patients
- Normal 3 patients

Temporal lobe abnormalities were detected in all our patients with abnormal scans. The defects were bilateral but asymmetrical. The presence of temporal lobe abnormalities in HIV-infected patients with no discernible cause for their NOS has been documented previously (Modi et al, 2002). In their cohort of 15 patients (none of whom were on HAART) all had temporal lobe perfusion defects. They surmised that the defects were indicative of a focal HIV-related encephalopathy.

Ajmani et al, 1991 performed SPECT scans on a small number (5) of patients with varying stages of HIV-related dementia. They documented temporal defects in all their patients; and found that the extent of the defects corresponded to the severity of the dementia. Their findings were confirmed by Schwartz et al in 1994. They detected temporal and frontal lobe abnormalities in their HIV infected individuals with dementia. None of our patients had dementia (as assessed by their IHDS score and clinical examination) at baseline; one patient did develop an HIV-associated dementia during the study period.

Quantitative Data

In epilepsy, brain perfusion SPECT is used to localize the seizure. It is preferable that the patient be imaged both during the inter-ictal period as well as in the ictal state. At present brain SPECT is the only imaging modality able to capture the rCBF changes associated with seizures. Cerebral blood flow abnormalities in patients with idiopathic generalized epilepsy have been documented (Joo, Tae and Hong 2008):

- Anterior and posterior cingulate gyri
- Anterior nucleus of the thalamus Left = Right
- Dorsolateral nucleus of the thalamus Right
- Superior colliculus of the midbrain Right
- Cerebellum

Regional Abnormalities

The regional abnormalities in our patients, based on the quantitative data did not correspond to the regions described above in patients with epilepsy. We detected multiple regions which had significant hypo perfusion when compared to the cerebellum (reference region):

- Frontal Region
- Cingulate
- Central Region
- Temporal Region

[The cerebellum has previously been suggested as an optimum reference region in studies using ^{99m}Tc-HMPAO (Syed et al, 1992)]

The regional abnormalities which we have documented may indicate that the patients with NOS in who no cause has been identified:

- May represent an early phase in the spectrum of neurocognitive dysfunction seen in HIV infection
- May be a precursor for dementia in some patients
  In this study one patient (patient 11) developed features consistent with a diagnosis of HIV-associated dementia.

Pohl et al, 1988 studied 12 HIV infected individuals with varying degrees of neurocognitive dysfunction. The SPECT scans in most of their patients were abnormal. They did follow-up scans in some patients which revealed increased numbers of defects, equivalent to the increase in clinical symptoms.

Schielke et al, 1990 studied 20 patients with HIV infection and no significant neurological dysfunction. Their findings suggested:

- Perfusion abnormalities on SPECT may occur prior to clinical features of dementia

Holman et al, 1992 compared the brain SPECT scans of 20 HIV-infected individuals with dementia and 20 normal control subjects. They found increased cortical defects in patients with dementia. The regions most affected were similar to the regions affected in our patients:

- Frontal
- Temporal
- Parietal

In 1995 (b) Sacktor et al compared SPECT abnormalities in HIV-infected individuals with and without cognitive impairment. They found no difference in the number of abnormalities observed between the two groups. They postulated:

- SPECT abnormalities may precede the development of cognitive impairment

The regional abnormalities in our patients with early dementia were analogous to the findings of Maini et al, 1990. They performed SPECT scans on 26 patients with either early or late AIDS dementia. In their 21 patients with early dementia the following areas were affected (in decreasing frequency):

- Frontal region
- Anterior temporal and posterior parietal lobes
- Anterior parietal lobe

Schwartz et al, 1994 compared the SPECT findings in 27 patients with HIV related dementia to 38 healthy controls. The abnormalities detected in their patients were similar to those in ours. The patients with dementia had significantly increased defects in:

- Medial and lateral frontal region
- Medial and lateral temporal regions

Chang et al, 2000 used perfusion MRI to document decreased regional cerebral blood flow in 19 HIV infected patients with HIV-minor cognitive motor disorder and HIV associated dementia. They found significantly decreased blood flow in:

- Inferior, lateral frontal cortex Right > Left
- Inferior medial parietal region Left = Right

Individual patients at baseline: Left versus Right analysis

In our patients there were no significant differences

- Between the left and right hemispheres overall
- Between the left and right corresponding dominant cerebral regions

This is consistent with the findings of Harris et al, 1994. They attempted to quantitatively demonstrate cerebral perfusion abnormalities in HIV-1 infected individuals. They compared 3 groups of patients:

- 9 HIV infected individuals with mild dementia
- 10 neurologically asymptomatic HIV infected individuals
- 8 HIV uninfected individuals

They did not identify any region that had significantly increased asymmetry compared to controls and concluded that

- HIV-positive individuals have diffuse and variable abnormalities which could be regional; hemispheric or global.

SPECT scans Pre- and Post-HAART

The four patients who initiated HAART during the study showed significant improvements in cerebral perfusion in all regions between the scans done pre- and post-HAART (see Appendix G for calculation).

- Patient 15 had 2 SPECT scans prior to HAART initiation and there was no significant difference between the two scans.
- She had a significant improvement in cerebral perfusion in the third scan; this was done 6 months after she initiated HAART.
- The results for this patient suggest that the significant changes seen were linked in these 4 patients to the initiation of HAART.

In 2013 Bailey and Willowson conducted an evidence based review of quantitative SPECT imaging and potential clinical applications. Amongst the potential uses listed was to:

- Assess disease progression or response to treatment

Several studies have shown the benefit of SPECT scans in the monitoring of response to therapy in neurological disorders:

- Cerebral blood flow during long-term treatment of elderly patients with nootropic drugs (Dormehl et al, 1999)
- Brain Perfusion and Cognitive Function changes in Hypertensive Patients (Efimova et al, 2007)
- Chronic Lyme disease (Donta, Noto and Vento, 2012)
- Traumatic brain injury (Raji et al, 2014)
- Cerebral autoimmune vasculitis (Mauro et al, 2015)

There have been reports of improvements (documented on brain SPECT scans) in patients receiving anti-retroviral therapy. All the reports have been in patients with cognitive dysfunction.

- In 1990 Tatsch et al documented randomly distributed focal or regional areas of hypo perfusion on brain SPECT scans of 40 HIV infected individuals. They concluded that perfusion abnormalities may precede the symptoms of dementia and correlate better with cognitive improvement after therapy than other structural imaging modalities like CT and MRI.
- In 1991 Masdeu et al evaluated HIV-infected patients with dementia. Their investigations included SPECT scans. Four of their patients received AZT, and in these patients there were documented improvements noted on their SPECT scans.
- Tran Dinh et al, 1990 advocated the use of quantitative cerebral SPECT scan imaging as a suitable method for monitoring the effects of therapy in early HIV infection.
- In 1998, Szeto et al studied 10 patients with early AIDS dementia. Their patients had brain SPECT scans done at entry into the study and 12 weeks later. In the interim the patients were given Atevirdine mesylate, a non-nucleoside reverse transcriptase inhibitor. Four patients completed the study and had repeat SPECT scans at week 12. Two patients responded clinically to the therapy and in these patients the SPECT images improved on both qualitative and quantitative analyses.
- Tozzi et al, 1999 performed SPECT scans on13 HIV infected patients with neuropsychological abnormalities. The scans were repeated 6 months later (they received HAART in the interim). Ten patients showed improvement in the deficits, with half of them showing complete reversal of their defects.
- More recently Sprinz et al, 2008 described improvements in the brain SPECT images of a HIV positive patient with cognitive dysfunction. Her initial images showed multiple areas of hypo perfusion on quantitative analysis; the most prominent of which was in the frontal region. She received HAART for 6 months. Her repeat SPECT images showed improvement in areas of hypo perfusion. The authors suggested that the technique could be used as a marker of clinical improvement.

4.3.9 Summary

In our patients with HIV and NOS:

- All had generalized tonic-clonic seizures
- EEG recordings were often normal
- Unexplained WML on MRI were common
- Temporal lobe abnormalities on visual SPECT assessment were common
- Regional cerebral hypo perfusion as documented on quantitative SPECT analysis was present

During the longitudinal follow-up:

- Seizure control was not difficult to achieve with sodium valproate

- One patient developed a HIV-associated dementia
    - Patient 6

      The patient developed clinical features consistent with a diagnosis of HIV-associated dementia

      Her CD4 count decreased and her serum viral load increased

      The visual and quantitative SPECT scan assessments deteriorated.

- The improvements in patients initiating HAART was significant in terms of:
    - CD4 count
    - Serum viral load
    - Cerebral perfusion as evidenced by the quantitative SPECT data

4.3.9 Conclusion

New Onset Seizures in HIV infected individuals in who no cause is identifiable may represent:

- Early stages of the spectrum of neurocognitive dysfunction seen in HIV infection
- May be a precursor for dementia in some patients
  In this study only one patient developed features consistent with a diagnosis of HIV-associated dementia.

These patients are likely to benefit from the initiation of anti-retroviral therapy.

Quantitative SPECT imaging of the brain may play a role in the assessment of HAART related cognitive improvement.

4.3.11 Study Limitations

- The small number of patients recruited for the longitudinal component of the study. The patients were recruited at 3 sites over an eighteen month period. Two hundred patients were recruited in to the first section of the study, making this the largest study of NOS in HIV infected individuals but:
    - Only 22 patients were eligible for the longitudinal component
    - 7 patients refused permission to be included in the longitudinal study
- High rate of attrition in the longitudinal component of the study.
  The possible reasons for this are discussed in the data analysis section
- The duration of follow-up. It would have been preferable to assess the patients over a minimum period of 2 years. This was impractical as the study needed to be completed within the stipulated 2 years.
- The lack of extensive neuropsychological tests on patients in the longitudinal study. The patients in this study, although all South African did not share a common language. Harvey et al, 2003 showed that there are performance differences across English and other languages in executive functions, vigilance, and psychomotor speed.
- Discrepancy between visual assessment and quantitative analysis
    - The presence of deficits in the temporal regions may have been over-estimated. Visual assessment of the temporal region is difficult because of its intrinsically low uptake (Tanaka et al, 2000).

CHAPTER FIVE

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Appendix A Approval for use of Figure 1.3 Neuropathogenesis of HIV

RMS AND CONDITIONS

Apr 22, 2015

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Appendix B Approval for use of Figure 1.4 Diagnosis of HIV infection

Hello Dr. Hari,

I spoke with Dr. Robert Coombs (http://depts.washington.edu/labweb/Faculty/coombs_robert.htm), and he gave you his permission to use the slide on the Virology HIV page.

I'm glad that I could help.

Best regards,

LARA WILLIAMSON | UWMC, LABORATORY MEDICINE

Program Coordinator: Grand Rounds | Research Conference

Medical Laboratory Science Program | Master of Science, Laboratory Medicine

T: 206.598.6078 | F: 206.598.6189 | E: lwilliam@uw.edu

Appendix C Ethics Approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Kapila R Hari

CLEARANCE CERTIFICATE	M121054
PROJECT	A Longitudinal Clinical and Single Photon Emission Computed Tomography (SPECT) Scan Study on Human Immunodeficiency Virus (HIV) Infected Patients with New Onset Epilepsy and no identifiable Cause
INVESTIGATORS	Dr Kapila R Hari.
DEPARTMENT	Dept of Neurosciences/Div of Neurology
DATE CONSIDERED	26/10/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 09/11/2012 CHAIRPERSON .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable.
cc: Supervisor : Prof G Modi

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Appendix D Patient Data Sheet Page 1

Demographic Data	
Name	
Hospital Number	
Age	
Sex	
Sexual Orientation	
Telephone Number	
Seizure History	
Date of 1st seizure	
Previous Epilepsy	
Seizure Type	
Risks/Triggers	
Family History	
Trauma	
Alcohol	
Drugs	
Herbal	
Medicinal	
Recreational	
Previous Medical History	
Medication	
Current	
HAART	
Clinical Examination	
Stigmata of Immune-suppression	
Vitals (PR/BP/T°)	
General Medical Examination	
CNS Examination	
Level of Consciousness	
HIVDS Score	
Meningism	
Cranial Nerves	
Primitive Reflexes	
Motor	
Sensory	
Reflexes	
Cerebellum	
Other	

Appendix D Patient Data Sheet Page 2

Investigations	
Blood laboratory tests	
FBC	
ESR	
U&E & Glucose	
CPM	
LFT	
T Cell Subsets	
Syphilis Serology	
Cysticercosis CFT	
Toxoplasma CFT	
CMV	
HTLV-1	
CSF	
Cell count	
Chemistry	
ADA	
EBV	
HSV	
JCV	
Syphilis	
Other	
EEG	
CT Brain	
MRI Brain	

Appendix E Patient Data Sheet (Longitudinal Study) Page 1

Demographic Data	**Visit 1**	**Visit 2**	**Visit 3**
Name			
Hospital Number			
Age			
Sex			
Sexual Orientation			
Telephone Number			
Seizure History			
Date of 1st seizure			
Previous Epilepsy			
Seizure Type			
Risks/Triggers			
Family History			
Trauma			
Alcohol			
Drugs			
Herbal			
Medicinal			
Recreational			
Previous Medical History			
Medication			
Current			
HAART			
Clinical Examination			
Stigmata of Immune-suppression			
Vitals (PR/BP/T°)			
General Medical Examination			
CNS Examination			
Level of Consciousness			
HIVDS Score			
Meningism			
Cranial Nerves			
Primitive Reflexes			
Motor			
Sensory			
Reflexes			
Cerebellum			
Other			

Appendix E Patient Data Sheet Page 2

Investigations	Visit 1	Visit 2	Visit 3
Blood laboratory tests			
FBC			
ESR			
U&E & Glucose			
CPM			
LFT			
T Cell Subsets			
Syphilis Serology			
Cysticercosis CFT			
Toxoplasma CFT			
CMV			
HTLV-1			
CSF			
Cell count			
Chemistry			
ADA			
EBV			
HSV			
JCV			
Syphilis			
Other			
EEG			
CT Brain			
MRI Brain			
Brain SPECT Scan			

Appendix F Patient Consent Form

CONSENT TO ACT AS A SUBJECT IN RESEARCH

I,_____ being 18 years or older,

consent to participating in a research project entitled:

A LONGITUDINAL CLINICAL AND SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY (SPECT) SCAN STUDY ON HUMAN IMMUNO-DEFICIENCY VIRUS (HIV) INFECTED PATIENTS WITH NEW ONSET EPILEPSY AND NO IDENTIFIABLE CAUSE

The procedures / questionnaires have been explained to me and I understand and appreciate their purpose, any risks involved, and the extent of my involvement. I have read and understand the information.

I understand that radioactive investigations will be carried out on myself, and that they are not necessary for the diagnosis/ treatment of my condition.

I understand that the procedures form part of a research project, and may not provide any direct benefit to me.

I understand that all experimental procedures have been reviewed and approved by the Human Research Ethics Committee, University of the Witwatersrand, Johannesburg. If you have any questions regarding your rights as a participant in this study or any complaints please contact Ms Anisa Keshav from the Ethics Committee on 011 717 1234.

I understand that my participation is voluntary, and that I am free to withdraw from the project at any time without prejudice.

_____ _____

Subject name and signature Date

_____ _____

Dr. Kapila R. Hari and signature Date

0832593480

Appendix G 1

Statistical Analysis of Quantitative SPECT Data for ROI at Baseline

The data was analysed to determine normalcy of the variables. A significance level of 5% was assumed. The results detailed below show a normal distribution, enabling parametric tests.

Kolmogorov-Smirnov Test of Normality

	N	Normal Parameters		Kolmogorov-Smirnov Z	P-value
		Mean	Std. Deviation		
FS L	13	58,01	26,71	0,958	,318
FS R	13	59,32	26,42	0,970	,304
FM L	13	56,68	26,96	0,941	,339
FM R	13	61,52	27,566	0,973	,300
FI L	13	56,68	26,29	0,852	,462
FI R	13	59,97	27,61	0,939	,342
CiA L	12	60,76	26,96	1,043	,226
CiA R	13	63,40	27,21	1,035	,234
CiM L	13	61,48	27,31	1,248	,089
CiM R	13	62,65	27,73	1,150	,142
CiP L	13	55,79	25,21	1,248	,089
CiP R	13	53,47	23,81	1,193	,116
HP L	13	54,86	25,79	0,999	,271
HP R	13	54,22	25,64	1,094	,182
O L	13	65,61	27,98	0,936	,344
O R	13	63,51	29,06	0,915	,372
Ca L	13	51,34	22,91	1,090	,186
Ca R	13	51,31	23,85	1,248	,089
Pu L	13	64,51	30,28	0,892	,403
Pu R	13	65,52	31,81	1,054	,217
Pa L	13	65,47	32,04	0,839	,482
Pa R	13	64,26	31,13	0,996	,275
Th L	13	60,80	29,09	1,106	,173
Th R	13	60,72	30,09	1,149	,143
Par L	13	60,49	25,95	0,989	,283
Par R	13	57,79	27,35	1,065	,206
Tem L	13	58,59	24,56	0,953	,323
Tem R	13	60,27	27,14	1,007	,263
Cb L	13	67,39	30,50	1,086	,189
Cb R	13	67,71	31,60	1,169	,130

Legend

L=Left; R=Right; FS= Frontal Superior; F M= Frontal Middle; FI= Frontal Inferior; CiA= Cingulate Anterior; CiM= Cingulate Middle; CiP= Cingulate Posterior; HP= Hippocampus; O= Occipital; Ca= Caudate; Pu= Putamen; Pa= Pallidum; Th= Thalamus; Par= Parietal; Tem= Temporal; Cb= Cerebellum

SPECT images reconstructed in units of radioactivity concentration per pixel and averaged per region

Appendix G 2

Statistical Analysis of Quantitative SPECT Data for cerebral regions at Baseline

The quantitative data, at baseline according to dominant cerebral regions is documented below.

Cerebral Regions

Patient Number	Frontal	Cingulate	Central Region	Temporal Region	Parietal Region	Occipital Region	Cerebellum
1	320.06	319.88	414.39	199.54	104.96	112.4	117.96
2	292.74	299.13	395.72	182.57	104.01	104.14	111.44
3	173.12	178.39	243	113.57	62.38	68.24	65.78
4	338.72	311.34	426.99	211.06	107.67	118.3	119.94
5	644.95	707.16	1004.01	458.03	216.51	234.88	267.94
6	367.77	349.33	491.54	240.07	120.81	137.97	137.65
7	264.01	299.65	401.05	168.96	80.8	88.84	97.38
8	221.75	215.45	287.09	132.95	69.76	73.09	77.21
11	708.44	666.51	910.62	420.95	240.23	257.25	261.69
12	388.2	363.03	500.06	244.83	126.48	140.43	140.28
13	339.28	346.14	498.46	218.14	112.43	121.33	132.21
14	451.96	437.97	578.7	278.71	147.91	148.39	158.36
15	167.46	243.02	234.57	144.72	73.44	92.71	91.81

SPECT images reconstructed in units of radioactivity concentration per pixel and averaged per region for each patient

Appendix G 3

A paired sample T test was done to compare the left and right hemispheres for individual patients.

Left versus Right Cerebral Hemispheres

(individual patients at baseline)

Patient Number	*p* Value
1	0.99
2	0.96
3	0.96
4	0.97
5	0.92
6	0.99
7	0.10
8	0.99
11	0.47
12	0.10
13	0.98
14	0.90
15	0.89

There were no significant differences detected, indicating symmetrical perfusion for individual patients.

Appendix G 4

Paired sample T tests were used to determine the difference between the left and right hemispheres for the dominant cerebral regions (all patients) at baseline.

Cerebral Regions (Left vs Right)

Region	Left	Right	P Value
Frontal Region	2484.83	2597.98	0.80
Cingulate	2573.68	2568.67	0.99
Central Region	3474.8	3463.37	0.99
Temporal Region	1626.29	1647.16	0.94
Parietal Region	865.7	866.76	0.99
Occipital Region	1840.44	1761.06	0.30
Cerebellum	1803.62	1860.04	0.55

There were no significant differences between the left and right cerebral regions.

Appendix G 5

A single factor Analysis of Variance (ANOVA) test was done to test the null hypothesis that the means of the dominant cerebral regions are equal.

Table 4.15 Cerebral Regions

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Frontal Region	25	4333.17	173.3268	5512.666		
Cingulate	25	4405.03	176.2012	5276.164		
Central Region	25	2807.78	112.3112	2331.844		
Temporal Region	25	1447.45	57.898	575.7495		
Parietal Region	25	1566.51	62.6604	635.3733		
Occipital Region	25	1650.82	66.0328	817.6745		
Cerebellum	25	5929.84	237.1936	11511.8		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	738884.1	6	123147.3	32.33272	1.06E-25	2.152911
Within Groups	639870.4	168	3808.753			
Total	1378755	174				

Conclusion: the F > F crit, we therefore rejected the null hypothesis.

- The means of the different regions are not all equal. At least one of the means is different.

Paired sample T-tests were performed to test each pair of means to determine the source of the difference.

Appendix G 6

Paired sample T tests were used to determine the difference between individual regions. The *p*-values in red, in the table below indicate regions between which there are significant differences.

Individual regions

Region	Frontal Region	Cingulate	Central Region	Temporal Region	Parietal Region	Occipital
Cingulate	0.92					
Central Region	0.02	0.02				
Temporal Region	0.00	0.00	0.00			
Parietal Region	0.00	0.00	0.00	4.1E-05		
Occipital Region	0.00	0.00	0.00	4.9E-05	0.86	
Cerebellum	0.00	0.00	0.00	0.00	0.59	0.70

There were significant differences between most regions. The notable exceptions were between:

- The frontal region and the cingulate
- The occipital and parietal regions
- The cerebellum and
    - The parietal region
    - The occipital region

Appendix G 7

A single factor Analysis of Variance (ANOVA) test was done to test the null hypothesis that the means of patients in the HIV stages are equal.

HIV Stages (Baseline)

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Stage 1	28	8502.39	303.6568	44914.42		
Stage 2	35	10560.88	301.7394	38542.75		
Stage3	27	4624.38	171.2733	11452.64		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	325977.9	2	162989	5.026758	0.008592	3.101295757
Within Groups	2820911	87	32424.27			
Total	3146889	89				

Conclusion: the F > F crit, we therefore rejected the null hypothesis. The means of the patients in the different HIV stages are not all equal. At least one of the means is different. Paired sample T-tests were performed to test each pair of means to determine the source of the difference.

Paired sample T tests were used to determine the difference amongst patients in the different stages. The *p*-values in red, in table 4.18 below indicate HIV stages between which there are significant differences.

Appendix G 8

Difference between HIV Stages (Baseline)

Stage	2	3
1	0.97	0.005
2		0.003

The cerebral perfusion was significantly different between patients in:

- Stages 1 and 3
- Stages 2 and 3

A paired sample T test was done to compare the left and right hemispheres for each patient at follow-up.

Appendix G 9

Cerebral Hemispheres: Left versus Right (individual patients at Follow-up)

Patient Number	*p* Value
1	0.99
2	0.99
3	0.93
4	0.97
5	0.94
6	0.96
7	0.96
8	0.99

There were no significant differences between left and right hemispheres for individual patients. The cerebral perfusion in all patients was symmetrical.

Appendix G 10

The quantitative data, at follow-up according to dominant cerebral regions is documented below. The data is from the last scan done for each patient.

Cerebral regions Follow-up Data

Patient Number	Frontal	Cingulate	Central Region	Temporal Region	Parietal Region	Occipital Region	Cerebellum
1	510.27	487.52	660.03	301.87	166.54	168.76	172.65
2	754.21	796.30	1079.72	492.73	269.96	286.85	309.73
3	693.18	645.75	863.60	400.75	235.57	245.67	245.26
4	452.17	422.23	568.51	288.06	151.85	161.62	164.31
5	456.50	445.85	610.00	294.26	152.35	160.87	170.49
6	406.78	399.28	577.30	271.07	136.68	164.82	160.52
7	535.64	503.91	679.38	325.80	177.22	197.83	196.37
8	698.01	700.28	909.22	415.51	221.73	228.92	246.81

Appendix G 11

A paired sample T test was done to compare the corresponding regions for all patients at baseline and follow-up. The *p*-values in red below indicate regions for which there are significant differences.

Cerebral regions at baseline versus follow-up

Cerebral Region	*p* Value
Frontal	0.004
Cingulate	0.014
Central	0.017
Temporal	0.012
Parietal	0.005
Occipital	0.005
Cerebellum	0.012

There were significant improvements noted in each cerebral region.

Appendix G 12

An ANOVA test was done to test the null hypothesis that the means of the dominant cerebral regions are equal (at follow-up).

Cerebral Regions at Follow-up

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Frontal	8	92	11.5	6		
Cingulate	8	4506.76	563.345	17613.44		
Central	8	4401.12	550.14	21190.38		
Temporal	8	5947.76	743.47	34582.26		
Parietal	8	2790.05	348.7563	6186.028		
Occipital	8	1511.9	188.9875	2271.212		
Cerebellum	8	1615.34	201.9175	2236.869		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3383060	7	483294.3	44.45536	1.32E-20	2.178156
Within Groups	608801.3	56	10871.45			
Total	3991862	63				

Conclusion: the F > F crit, we therefore rejected the null hypothesis. The means of the different regions were not all equal. At least one of the means is different. Paired sample T-tests were performed to test each pair of means to determine individual differences.

Appendix G 13

A paired sample T test was used to determine the difference between individual regions. The *p*-values in red, in the table below indicate regions for which there are significant differences.

Individual regions

Region	Frontal Region	Cingulate	Central Region	Temporal Region	Parietal Region	Occipital
Cingulate	0.85					
Central Region	0.04	0.04				
Temporal Region	0.00	0.04	7.4E-05			
Parietal Region	0.00	1.1E-05	0.00	0.00		
Occipital Region	0.00	1.6E05	0.00	0.00	0.59	
Cerebellum	0.00	2.2E-05	0.00	0.00	0.46	0.81

There were significant differences between most regions. The notable exceptions were between:

- The frontal region and the cingulate
- The occipital and parietal regions
- The cerebellum and
    - The parietal region
    - The occipital region

NB! The regional differences at follow-up are the same as the differences noted at baseline.

Appendix G 14

A paired sample t-test (2 dependent means) was used to calculate the difference between each individual patient at baseline and at last follow-up. The *p*-values in red, in the table below indicate patients for who there are significant differences.

Comparison of Individual patients (all regions)

Baseline versus Follow-up

Patient Number	*p* Value
1	0.005
2	0.003
3	0.003
4	0.002
5	0.005
6	0.007*
7	0.001
8	0.004

The comparative data indicate significant differences between individual patients at baseline and last follow-up. The majority of patients (7) showed significant improvements in their cerebral perfusion.

*NB! The only patient that showed a reduction in cerebral perfusion was patient 6.

Appendix G 15

A paired sample T test was used to compare the left cerebral hemisphere for each patient pre and post-HAART and the right cerebral hemisphere for each patient pre and post-HAART. The *p*-values in red, in the table below indicate the significant differences.

Pre and Post-HAART data (Hemispheres)

Patient Number	Left			Right		
	Pre-HAART	Post-HAART	P Value	Pre-HAART	Post-HAART	P Value
1	538.06	1706.08	0.001	539.22	1714.40	0.001
3	542.75	1315.65	0.001	700.41	1300.49	0.000
7	455.13	1683.64	0.000	449.36	1646.15	0.000
8	779.93	1236.27	0.001	786.14	1231.37	0.001

All patients showed improved cerebral perfusion for both the left and right hemispheres.

Appendix G16

A paired sample T test was used to compare the dominant cerebral regions for the patients' pre and post-HAART. The *p*-values in red, in the table below indicate regions for which there are significant differences

Pre and Post-HAART Quantification Data

(Patients 1; 3; 7 and 8)

Patient Number	Frontal		Cingulate		Central Region		Temporal Region		Parietal Region		Occipital Region		Cerebellum		p
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
1	320.06	510.27	319.88	487.52	414.39	660.03	199.54	301.87	104.96	166.54	112.4	168.76	117.96	172.65	**0.002**
3	173.12	693.18	178.39	645.75	243	863.60	113.57	400.75	62.38	235.57	68.24	245.67	65.78	245.26	**0.001**
7	264.01	535.64	299.65	503.91	401.05	679.38	168.96	325.80	80.8	177.22	88.84	197.83	97.38	196.37	**0.001**
8	221.75	698.01	215.45	700.28	287.09	909.22	132.95	415.51	69.76	221.73	73.09	228.92	77.21	246.81	**0.004**

All patients had improved overall improved perfusion.

Appendix G 17

A paired sample T test was used to compare the dominant cerebral regions for patient 1. Scans 1 and 2 were done pre-HAART. Scan 3 was done post-HAART. The *p*-values in red, in the table below indicate the significant differences between the scans.

Comparison of Patient 1 (all regions)

Baseline versus Follow-up Scans

Frontal		Cingulate		Central Region		Temporal Region		Parietal Region		Occipital Region		Cerebellum		P Value
Scan 1	**Scan 2**	**Scan 1**	**Scan 2**	**Scan 1**	**Scan 2**	**Scan 1**	**Scan 2**	**Scan 1**	**Scan 2**	**Scan 1**	**Scan 2**	**Scan 1**	**Scan 2**	0.878
320.06	314.53	319.88	310.88	414.39	409.23	199.54	105.11	104.96	198.92	112.4	111.36	117.96	116.03	
Scan 1	**Scan 3**	**Scan 1**	**Scan 3**	**Scan 1**	**Scan 3**	**Scan 1**	**Scan 3**	**Scan 1**	**Scan 3**	**Scan 1**	**Scan 3**	**Scan 1**	**Scan 3**	0.005
320.06	510.27	319.88	487.52	414.39	660.03	199.54	301.87	104.96	166.54	112.4	168.76	117.96	172.65	
Scan 2	**Scan 3**	**Scan 2**	**Scan 3**	**Scan 2**	**Scan 3**	**Scan 2**	**Scan 3**	**Scan 2**	**Scan 3**	**Scan 2**	**Scan 3**	**Scan 2**	**Scan 3**	0.012
314.53	510.27	310.88	487.52	409.23	660.03	105.11	301.87	198.92	166.54	111.36	168.76	116.03	172.65	

Patient 1:

- No improvement between scans 1 and 2.
- Improvement between scans
  - 1 and 3
  - 2 and 3

She initiated HAART in the period between scans 2 and 3.