HIV VIRAL LOAD COUNT AS MARKER FOR NEUROPSYCHOLOGICAL IMPAIRMENT

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Submitted in partial fulfilment of the requirements for the degree of

MAGISTER ARTIUM IN CLINICAL PSYCHOLOGY

in the Faculty of Arts
at the
UNIVERSITY OF THE NORTH

November 2000

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DECLARATION

I declare that the dissertation hereby submitted to the University of the North for the degree of Master of Arts in Clinical Psychology has not previously been submitted by me for a degree at any other University, that it is my own work in design and execution, and that all material contained therein has been duly acknowledged.

Signed: _____

ACKNOWLEDGEMENTS

The completion of this work, as well as my training as psychologist, would not have been possible without God Almighty. To Him all the praise. I wish to express my sincere thanks and appreciation for the support and encouragement from a number of sources. These are:

My supervisor, Doctor Anneke Meyer, and co-supervisor, Doctor Marianne Alberts, for their support and enthusiasm during the past two years and their ready willingness to share their knowledge.

The staff of Ampath Pathology laboratory for their support and help.

Drs. J.D. van Wyk, H.J. van Rensburg, H.W. Alberts, E.D. Fourie and J.D. Nel for their support and the opportunity they afforded me to complete this course.

My friends and specially my family Esther, Barend and Annelize, throughout the years of study, for their support and encouragement.

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SUMMARY

When this research project was conceptualised, it seemed like a rather straightforward research study into the correlation between the viral-load count of HIV-positive patients and neurocognitive impairment. It was quickly realised that the topic was of a most complex nature.

The reason for this might be found in the fact that two of the most puzzling and widely researched topics were selected. The one being the much-dreaded HIV and the other, the little understood mystic world of the brain. This research attempted to explain as boldly as possible the physiology of both the virus and the brain, and then went on to discuss the impact the virus has on the brain, both physiologically and psychologically.

To achieve this, neuropsychological assessment tools as well as laboratory blood tests were administered on an HIV-positive group and an HIV-negative control group. The neuropsychological test results were analysed for significant differences between the groups to demonstrate neurocognitive impairment. Correlative analyses were done between the neuropsychological test results and the HIV Viral Load Count to investigate a possible relationship between them. Although the outcome of many of the neuropsychological tests showed no correlation between the Viral Load Count of the HIV-positive group, others proved to be valuable in predicting the patient's neuropsychological functioning.

The conclusion was reached that neurocognitive impairment does occur in the early stages of the infection and that the Viral Load Count could be used as a marker for neurocognitive impairment.

CHAPTER 1

INTRODUCTION

Much research has been done during the past twenty years concerning neuropsychological impairment and HIV positive patients (Anders, Verity, Cancilla & Vinters 1984; Britton, Marquardt, Koppel, Garvey, & Miller, 1982; Grant & Marcotte, 1999). Up to now it is still a controversial issue whether HIV causes neurological impairment in the "early stages" of HIV infection (Goethe, et al., 1989; Grant & Marcotte, 1999). As in the medical model, most of these studies used the immune system (CD4 lymphocyte count) as a marker for the progression of the HIV infection (Grant & Marcotte, 1999). However, research has clearly indicated that the immune system is also greatly influenced by factors other than HIV infection (Dreher, 1995; Goldberg, 1998). Thus, one reason for the discrepancy in research findings of early neurological complications (Gibbs, Andrews, Szmukler, Mulhall, & Bowden, 1990; Skoraszewski, Ball & Mikulka, 1991; Wilkie, Eisdorfer, Morgan, Loewenstein & Szapocznik, 1990), might be the lack of a proper marker for the progression of the infection in an individual at a particular time.

Although the specific path of entrance to the brain is not known for sure (Grant & Marcotte, 1999; Swanson, Cronin-Stubbs, Zeller, Kessler & Bieliauskas, 1993), post-mortem studies have shown clearly that 90% of AIDS patients have some form of brain damage (Friedlander, 1998). It seems that brain invasion also occurs early in the infection and even before involvement of other organs (Davis et al, 1992).

Mason (1997) found that bereavement support groups for HIV-positive patients served as a buffer against a decrement in CD4-cells following the loss of HIV-infected

friends. Furthermore, she found that CD4-cells increased in HIV-negative individuals' post intervention. These findings show that stress may alter cell populations that provide cytotoxic defence against infection in HIV positive individuals.

Not only does mood effect the immune system, but also diet. According to Visser (1998), a number of studies have implicated that ß-carotene increased CD4 lymphocyte count.

It is therefore clear from the above that there are various other factors besides the HIV-infection per se, which affect the immune system. The immune system (CD4) might therefore not be a stable marker for HIV progression and a move towards the Viral Load Count might be indicated (Friedlander, 1998; Grant & Marcotte, 1999).

Most people who contracted HIV, develop brain infection which can be severe and persistent, and may cause loss of memory, inability to concentrate, apathy, and eventually dementia (Friedlander, 1998).

Levy and Bredesen (1988) suggested that 40% of AIDS patients developed neurological complications in the course of their illness and about 10% experienced neuropsychological symptoms as the initial manifestation of AIDS. Van Gorp, Mitrushina, Cummings, Satz and Modesitt, (1989) suggested that AIDS dementia (or "HIV encephalopathy" according to Kaplan, Sadock & Grebb, (1994)) affects primarily subcortical structures. Damage to these subcortical structures results in psychomotor slowing, a pattern of memory disturbances and diminished performance of executive functions.

A study by Skoraszewski et al., (1991) indicated that subjects with AIDS performed worse than AIDS-negative HIV-positive patients in neuropsychological functioning and 80% showed clinical impairment. This study also supported the theory that as HIV progresses, neuropsychological functioning decreases. Gibbs et al. (1990) found that there was no significant difference between HIV-positive asymptomatic and HIV-negative persons in neuropsychological functioning. They concluded that disturbances occur within the context of immuno-suppression.

Goethe et al. (1989) confirmed these findings and concluded that immune compromised patients with abnormal CSF values performed significantly poorer on verbal memory and therefore that cognitive functioning decline with immunological impairment. Miller et al. (1990) and Friedlander (1998) confirmed that there is an insignificant difference in neuropsychological functioning between asymptomatic HIV-positive and HIV-negative patients. The authors further found that irrespective of the CD4-count, the asymptomatic HIV-positive and HIV-negative patients showed no significant differences on neuropsychological functions.

The problem arises that CD4-counts only start to play a role as a predictor of neuropsychological functioning in the symptomatic HIV-positive patient. Therefore, the CD4-count has little predictive value in the early stages of the disease. Because the development of HIV and AIDS varies substantially among individuals, it is very important, for various personal and socio-economic reasons, to be able to predict the development of the particular infection.

In a study by Ioannides, Cappeleri, Lau, Sacks and Skonik, (1996), it was shown that the progression of HIV depends heavily on the rate at which the Viral Load Count increases over time. In their formulae, they can predict, according to a certain multiplication rate and a certain viral load count, the estimated time for a patient to develop full-blown AIDS. (PAE – Progression to Aids Equivalent). It is important for the patient, as well as for his social environment, to plan in advance how to cope with problems experienced during such development. According to Kaplan et al. (1994), patients with HIV encephalitis usually notice subtle mood and personality changes, problems with memory and concentration, and psychomotor slowing.

The aim of this study is to determine whether there is a correlation between neuropsychological decline and the Viral Load Count (VLC) as an alternative to the CD4 lymphocyte count.

DELINEATION OF STUDY

The current study deals with two of the most puzzling and most widely researched phenomena. The one phenomenon belongs to the family of retroviruses known as the Human Immunodeficiency Virus (HIV) and the other phenomenon is the Cognition of the Human Brain. The attribution of cognition to the brain most probably started in the fifth decade when Alcmaeon of Croton claimed that the brain is the locality of the cognition and that the heart only controls the senses (Meyer, 1989). In spite of intensive research, the knowledge regarding the functioning and capabilities of both these two phenomena is far from being exhausted. Both these topics currently return more than a million topics when

searched on the World Wide Web. HIV is seen as one of the most devastating and contradicting topics (Lanka, 1995).

Chapter 2 will focus on the medical aspects of the HIV and the immune system. The virus, its pathogenesis, the diagnosis, as well as its epidemiology will be described. Chapter 3 will deal with the neuropsychological functioning of the brain. The brain will be discussed at a cellular level as well as on an integrated structural level. A cellular framework will be used to conceptualise the relationship between HIV infection and neuropsychological impairment. Luria's (1980) conceptualisation regarding cortical functions of the brain will briefly be discussed. Chapter 4 will focus on neuropsychological complications associated with HIV infection, as well as the classification of HIV progression. Psychiatric sequelae of the infection will be described, while Chapter 5 deals with neuropsychological assessment and a description of tests used as a measure of neuropsychological impairment. Chapter 6 defines the problem statement as well as the aim of the current study. It further deals with the various research hypotheses around this study. Chapter 7 describes the methodology with specific reference to the research design, the study population, and sampling method as well as the psychological measurements and analytical determinants. In chapter 8, the results of the study are provided. A critical overview and statistical discussion of the study, with special reference to the limitations, what was learnt, and what implications may follow for future studies will be discussed in Chapter 9.

CHAPTER 2

THE HIV

EPIDEMIOLOGY

Since the onset of the HIV/AIDS epidemic 15 years ago, the virus has infected more than 47 million people in the world. With more than 2.2 million deaths in 1998, HIV/AIDS has now become the fourth leading cause of mortality and its impact probably will increase. Over 95% of all cases and 95% of deaths because of AIDS occur in the developing world, mostly among young adults and increasingly among women (World Health Organisation, http://www.who.int/emc/diseases/hiv/index.html, 2000).

In the developing world, HIV is spread mainly by heterosexual intercourse and the male to female ratio is virtually 1:1. It is to be noted that female partners of HIV infected males have a 25% chance of acquiring the infection, compared to only 10% of male partners of HIV infected females. Traditional family structures and extended families are breaking down under the strain of HIV and in Africa there is now a marked reduction in life expectancy due to AIDS. This will have a considerable impact on the Continent's economic output (Ebrahim, 1999). According to the Labour Organisation of South Africa, AIDS is likely to devastate the labour market of sub-Saharan Africa, with a projected workforce decline of up to a fifth or more in the worst-hit countries by 2020 (Medscape News http://www.medscape.com/Medscape/features/newsbeat/2000/0700/HIV-developing.html, 2000). The South African government announced in April 2000 that nearly 10% of its population, about 4.2 million people, are infected with HIV (Medscape News http://www.medscape.com/Medscape/features/newsbeat/2000/0700/HIV- developing.html, 2000).

Figure 2.1 A computerised representation of the HIV

From "The Big Picture Book of Viruses, by Sander (2000)

http://www.virology.net/Big_Virology/BVHomePage.html .

THE HIV

HIV is the acronym for the human immunodeficiency virus (Figure 2.1). This is a human retrovirus of the Lentivirus group. There are four of these human T lymphotropic (or leukemia) producing retroviruses, (a) HTLV-I and (b) HTLV-II, and the human

immunodeficiency viruses, (c) HIV-1 and (d) HIV-2. AIDS is caused by the two human immunodeficiency viruses: HIV-1 and HIV-2 (Greenwood, Slack & Peutherer, 1992).

The family Retroviridae is characterised by:

1. Two identical strands of RNA and an enzyme reverse transcriptase that can transcribe RNA to DNA from the RNA template inside a protein coat.
2. Integration of the DNA so formed into the host cell chromosome where it is termed a "provirus".

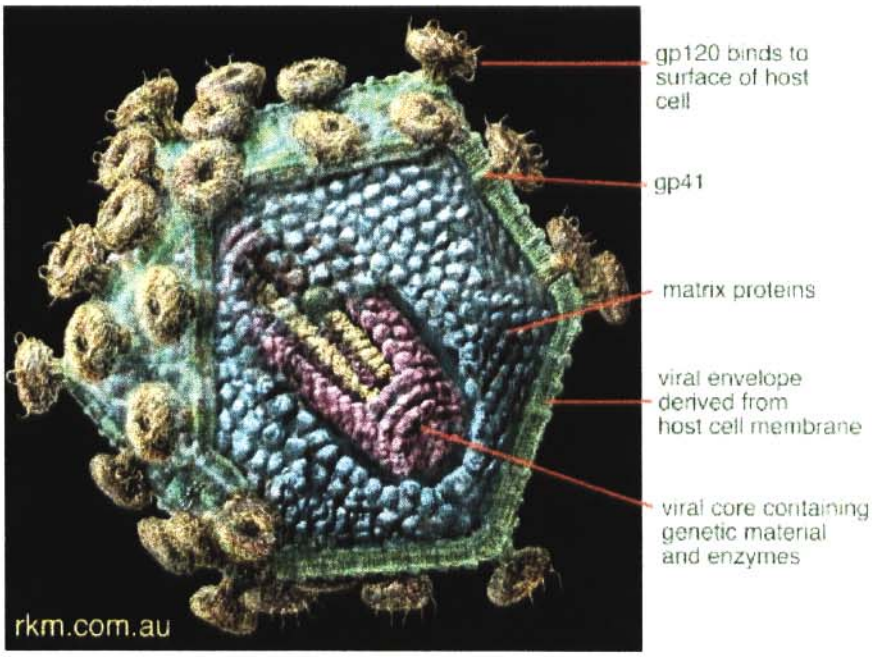


Figure 2.2 A computerised representation of the major antigenic properties of the HIV

From "The Big Picture Book of Viruses, by Sander (2000)

http://www.virology.net/Big_Virology/BVHomePage.html

Figure 2.2 shows the structural composition of the HIV. The outer shell of the virus is known as the viral envelope. Embedded in the viral envelope is a complex protein known as "env", which consists of an outer protruding cap glycoprotein (gp120), and a stem glycoprotein (gp41). Within the viral envelope is an HIV protein called "matrix", and within this is the viral core or capsid, which is made of another viral protein p24 (core antigen). The major elements contained within the viral core are two single strands of HIV RNA, a protein p7 (nucleocapsid), and three enzyme proteins, namely p51 (reverse transcriptase), p15 (protease) and p32 (integrase) (Ebrahim, 1999; Greenwood et al., 1992).

HIV-1 is the most common of the HIVes around the world. HIV-2 is found mainly in West Africa, and may be less virulent and progresses much slower than HIV-1. Generally, when people refer to HIV without specifying the type of virus, they will be referring to HIV-1 (Ebrahim, 1999; Greenwood et al., 1992). Both HIV-1 and HIV-2 are transmitted by sexual contact, through blood, and from mother to child, and they appear to cause clinically indistinguishable AIDS. HIV-1 is a highly variable virus, which mutates very readily. Thus, many different strains of HIV-1 are to be found (Ebrahim, 1999).

These strains can be classified according to groups and subtypes. There are two groups, group M and group O. Group O is only found in Cameroon. Within group M there are currently known to be at least 10 genetically distinct subtypes of HIV-1. These are subtypes A to J. In addition, Group O contains another distinct group of very heterogeneous viruses. The subtypes of group M may differ as much between subtypes as group M differs from group O. The subtypes are very unevenly distributed throughout the world. For instance, subtype B is mostly found in the Americas, Japan, Australia, the Caribbean and Europe; subtypes A and D predominate in sub-Saharan Africa; subtype C in

South Africa and India; and subtype E in Central African Republic, Thailand and other countries of Southeast Asia. Subtypes F (Brazil and Romania), G and H (Russia and Central Africa), I (Cyprus) and group O (Cameroon) are of very low prevalence. In Africa, most subtypes are found, although subtype B is less prevalent (Ebrahim, 1999).

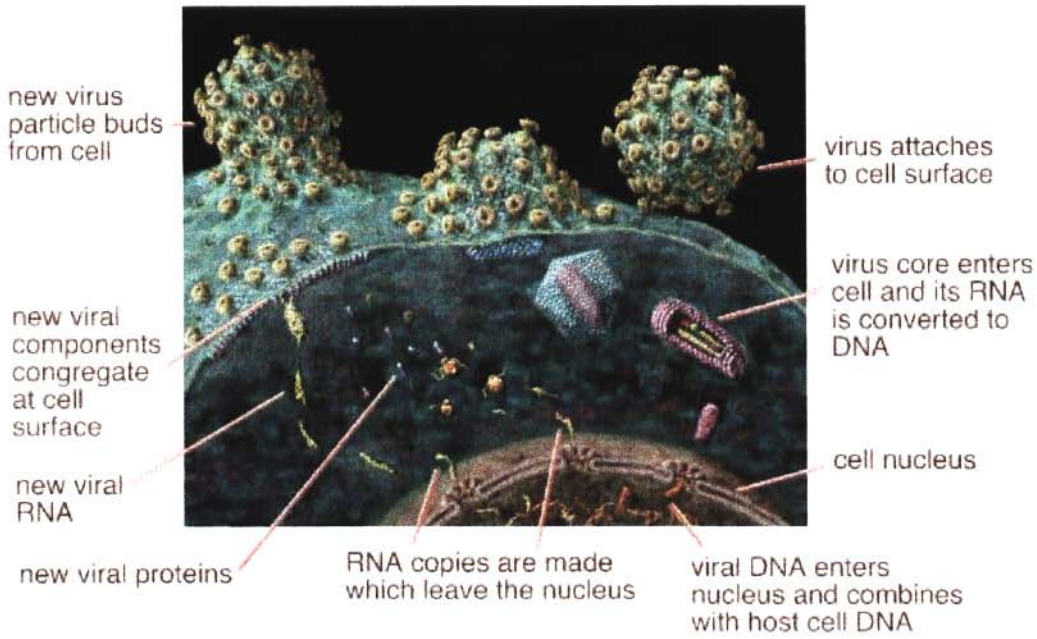


Figure 2.3 A computerised representation of a HIV producing cell

From "The Big Picture Book of Viruses, by Sander (2000)

http://www.virology.net/Big_Virology/BVHomePage.html

Retroviruses have genes composed of ribonucleic acid (RNA) molecules. Like all viruses, HIV can only replicate within a living host cell because they contain only RNA and do not contain DNA. In addition, retroviruses use RNA as a template to make DNA. As illustrated in figure 2.3 the infection begins when an HIV particle encounters a cell with

a surface molecule called CD4. The virus particle uses the viral envelope protein gp120 to attach itself to the cell membrane and then enters the cell. Within the cell, the virus particle releases its RNA, and the enzyme, reverse transcriptase, then converts the viral RNA into DNA. This new HIV DNA then moves into the nucleus of the cell where, with the help of the enzyme integrase, the HIV's DNA is then inserted into the DNA of the host cell. Once it is in the cell's genes, HIV DNA is called a provirus. The HIV provirus is then replicated by the host cell, which can then release new infectious virus particles (Ebrahim, 1999; Greenwood et al., 1992).

PATHOGENESIS OF HIV INFECTION

The most common mode of HIV infection is sexual transmission via the genital route. According to Ebrahim (1999), the principal target of the virus is the macrophages and not the T lymphocytes as suggested by Rosenberg, Anthony, and Fauci (1991). Both these cells express the CD4 molecule on their surface that is critical for binding and direct fusion of the HIV envelope with the cell membrane (Rosenberg et al., 1991). In women the Langerhans' cells and tissue dendritic cells (or macrophages) found in the lamina propria next to the cervicovaginal epithelium are targeted. These cells then fuse with the CD4 lymphocytes and spread to the deeper tissues (Ebrahim, 1999). The time it takes from mucosal infection to the initial viraemia can vary from 4 to 11 days. Breaks in the mucosal barrier and increased inflammation due to concomitant sexually transmitted diseases (STDs) increase the risk of acquiring HIV infection. Although the infection is transmitted most frequently across the genital mucosa, numerous reports demonstrate that infection can also be transmitted across the oral mucosa as a result of genital-oral sex. Nasopharyngeal tonsils and adenoid tissues are rich in macrophages, which are the initial target cells, and

facilitate the transmission of the virus to the CD4 lymphocytes (Ebrahim, 1999). HIV replicates at a rate of up to several billion new virus particles per day with many variants being generated due to mistakes while making DNA copies. Millions of CD4 cells may be destroyed every day (Ebrahim, 1999).

A number of hypotheses have been postulated as to how HIV destroys or causes dysfunction in these cells. Most investigators believe that numerous mechanisms may be occurring simultaneously. Hypotheses include (Grant & Marcotte, 1999; Rosenberg et al., 1991):

1. The CD4 cell is killed when large amounts of virus disrupt the cell membrane, or when viral proteins and nucleic acids collect in the cell and affect its function.
2. Infected cells fuse with uninfected cells and create syncytia (giant cells), associated with rapid disease progression.
3. The disruption of cellular functioning results in programmed cell death (apoptosis) (gp 120 itself, without the presence of HIV, may send a signal for the cells to die).
4. Killer T cells (another type of lymphocyte important in protection against viruses and cancer) may destroy uninfected CD4 cells that have HIV fragments inside them or on their surface.
5. HIV sends a signal that turns off CD4 cells leaving them unresponsive to immune stimulation (anergy).
6. Superantigens (made of HIV or another unrelated agent) may stimulate numerous CD4 cells at once and make them susceptible to HIV.
7. HIV destroys precursors to cells that have immune functions, thus diminishing the ability to regenerate.

Neurobehavioral complications, among others, occur in 30 to 50% of HIV-1 infected individuals, and can range from subtle, "subsyndromic" deficits with little impact on the

person's daily life to severe and debilitating dementia (Heaton et al., 1995; McArthur & Grant, 1998; White, Heaton, & Monsch, 1995).

A number of mechanisms for neuronal damage (and the subsequent development of neurological complications) are hypothesised to occur within the central nervous system (CNS) (Masliah, Achim, DeTeresa, Ge & Wiley, 1994; Masliah, Ge, Achim, & Wiley, 1996). One postulate is an excitotoxic mechanism, in which the envelope protein gpl2O is neurotoxic due to the activation of glutamate receptors which results in the release of calcium with subsequent fluid and electrolyte imbalance, and finally calcium toxicity and neuronal death (Lipton, 1992).

It has also been postulated that macrophages and microglia may mediate damage to neurons. The presence of HIV may lead to chronic activation of macrophages / microglia, which can result in increased production of cytokines, interleukin, and tumour necrosis factor alpha by these cells. These cytokines could then damage neuronal structures directly, or set off disturbances in other cells (e.g., astroglia) that are important in maintaining the viability of neurons (see discussion on secretion of microglia and astroglia, Chapter 3) (Grant & Marcotte, 1999).

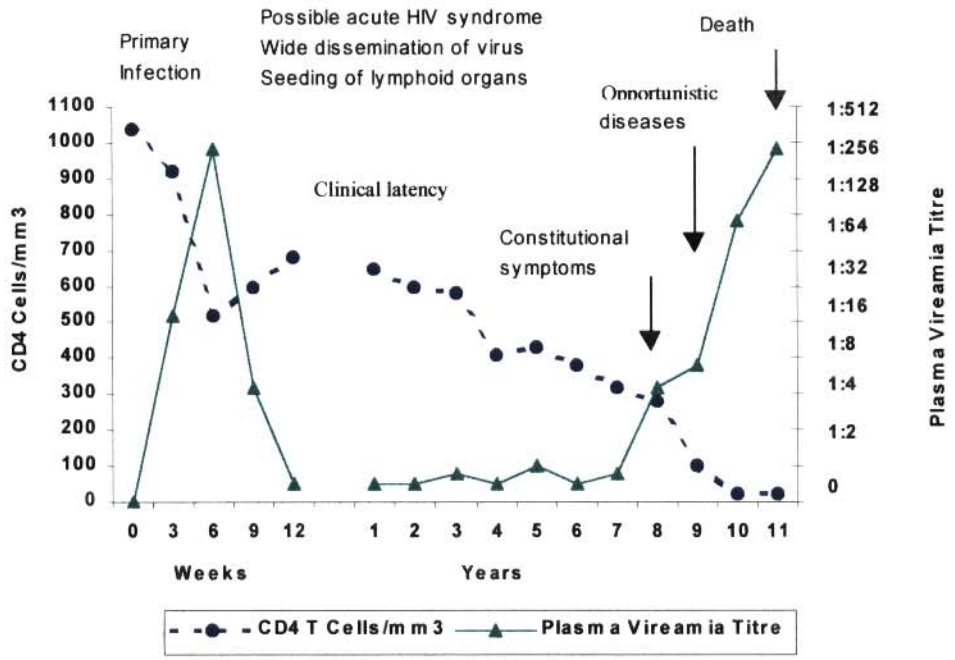


Figure 2.4 HIV Progression

From "The Immunopathogenesis of Human Immunodeficiency Virus Infection", by Pantaleo, Graziosi and Feud (1993) p. 328.

After the initial infection, there is a rapid rise in plasma viraemia and dissemination to the other organs and tissues of the body. An important reservoir for the virus is the central nervous system and tissue macrophages. The initial viraemia can exceed 1 000 000 copies/ml in plasma, but the immune system is capable of reducing this to a steady-state level of viral replication. This decrease is due to virus-specific immune responses that limits viral replication (see Figure 2.4). After the initial drop in viraemia, a viral set point is established. This can take up to six months in an adult or up to nine months in a child. Persons with the highest viral loads are likely to progress rapidly to AIDS and death.

Lowering the viral load during the primary infection with drugs is important in preventing progression of the disease (Ebrahim, 1999).

HIV Diagnosis

Antibody Testing

The following general principles are important regarding HIV antibody testing (Urassa, Matunda, Bredberg-Raden, Mhalu & Biberfeld, 1994).

1. A screening test must be followed by a confirmatory (supplemental) test.
2. Tests must diagnose HIV-l and HIV-2 infection.
3. Tests must be able to diagnose subtype 'O' infections.
4. Screening tests should be highly sensitive.
5. Confirmatory tests should be highly specific.
6. A second specimen should be used to confirm the reactivity and identity of the specimen.

Screening Assays

ELISA tests. In the mid-1980s, enzyme-linked immunosorbent assays (ELISA's) were the first HIV tests to be introduced. With progress in research, first- second- and third-generation assays were developing. The third-generation assays are currently in use and they measure immunoglobulin sub-classes, mainly IgM and IgA, in an effort to reduce the 'window period' during which time antibody tests are negative (Martin & Sim, 2000).

Simple rapid tests. The rapid test is designed to be a field test and reactive samples detected with rapid tests must be confirmed with ELISA testing (Martin & Sim, 2000).

Confirmatory testing. The Western blot (WB) is currently the most commonly used confirmatory test. It permits the detection of antibodies directed at specific viral proteins. In this test, the virus is split into its constituent proteins; these are then passed through a

polyacrylamide gel, which separates out the individual proteins according to size. These proteins are transferred to nitrocellulose paper strips, which are then exposed to the patient's serum. Antigen / antibody complexes can then be identified as individual bands.

Standardisation and interpretation are however a problem. Because of this, the WHO has endorsed a confirmatory testing strategy based on the use of a combination of ELISA's based on sensitivity and specificity (Martin & Sim, 2000).

Antigen Testing

Serological techniques have been developed for the detection of viral antigen in serum and plasma samples. These tests are based on the ELISA principle and can be applied to quantify viral replication in tissue culture supernatants. The techniques most frequently measure the p24 antigen (major core antigen). However, antigen testing is hampered by a lack of sensitivity (Martin & Sim, 2000).

Polymerase Chain Reaction (PCR)

For the prognostic staging or clinical monitoring of patients, the quantitative PCR reaction, also referred to as the viral load, is utilised. The diagnostic PCR is utilised:

1. To assist in the diagnosis of HIV infection during the window period
2. For diagnosis of HIV in the infant
3. To assist in resolving discordant serological results (Martin & Sim, 2000).

There are currently three types of tests in use that measure viral load (VLC) (Ebrahim, 1999):

1. The Amplicor HIV-1 Monitor Test
2. The Quantiplex HIV-1 RNA 2.0 Assay is a branched DNA assay (bDNA)
3. The NucliSens HIV-1 OT Test is a Nucleic Acid Sequence Based Amplification (NAS BA) test.

All three tests measure VLC from the blood sample. The amount of virus in the blood represents only 2% of the total virus in the body. However, recent studies have shown that VLC can be used to accurately predict HIV disease progression (Ebrahim, 1999; Ioannidis et al., 1996).

The Amplicor (PCR technology) can monitor the VLC in the blood through all stages of HIV infection. In this test, the single stranded HIV RNA present in the sample is converted to DNA. This process is called reverse transcription. The DNA strands are then amplified by Polymerase Chain Reaction (PCR), detected, and measured. This test measures from 400 to 750 000 copies of virus by the standard method and down to 50 copies/ml by the ultra sensitive method (Ebrahim, 1999).

With the bDNA assay, numerous probes bind to the HIV RNA. The quantity of HIV-1 RNA is measured at the level found in the sample without amplification of the material. The bDNA Version 3.0 test can detect from 50 to 500 000 copies of HIV per sample (Ebrahim, 1999).

During a NASBA assay, a primer is added and annealing, extension and amplification of HIV RNA takes place. After amplification, chemicals are added to the solution, which cause a chemical reaction resulting in the emission of light (luminescence). The intensity of the luminescence is measured to calculate the viral load (Ebrahim, 1999).

Virus Isolation

Virus isolation remains an option to diagnose HIV infection but is cumbersome, time-consuming, labour-intensive, and expensive and requires specialised laboratory

facilities. Therefore, the culturing of the virus is essentially a research tool, but is occasionally utilised in the diagnostic setting (Martin & Sim, 2000).

CHAPTER 3

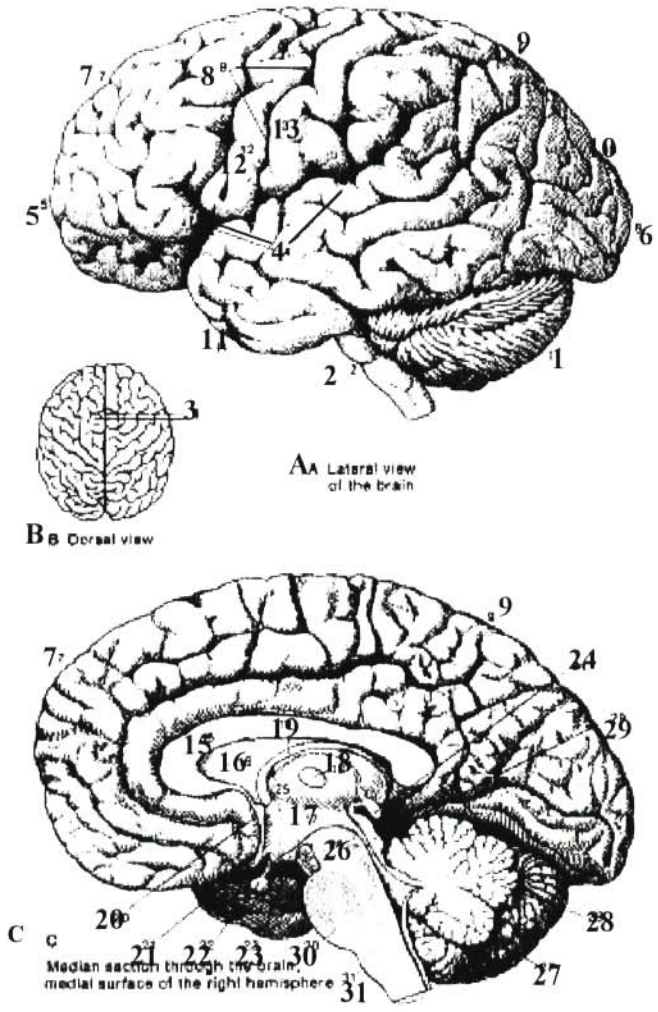
NEUROPSYCHOLOGICAL FUNCTIONING OF THE BRAIN

THE CELLULAR AND STRUCTURAL ARCHITECTURE OF THE BRAIN

Brain Organisation

Bloom and Kupfer (1995) describe the brain as "an assembly of interrelated neural systems that regulate their own and each other's activity in a dynamic, complex fashion" (Bloom & Kupfer, 1995, p.4).

According to Bloom and Kupfer (1995), the brain can be distinguished in macroscopic regions. These visible regions can further be superficially linked to coarse definitions of brain functions. The authors noted that "within and between these visible macroscopic regions lie interconnected cellular elements, which provide their detailed and interdependent operations" (p. 4).



Lateral Aspect
A1 Cerebellum
A2 Brain stem
B3 Longitudinal cerebral fissure
A4 Lateral sulcus (sylvian)
A5 Frontal pole
A6 Occipital pole
A7 Frontal lobe
A8 Central sulcus
A9 Parietal lobe
A10 Occipital lobe
A11Temporal lobe
A12 Precentral gyrus (region of voluntary motor control)
A13 Postcentral gyrus (region of sensibility)

Median Section
C14 Interbrain diencephalon
C15 Corpus callosum
C16 Septum pellucidum
C17 Third ventricle
C18 Interthalamic adhesion
C19 Fornix
C20 Rostral commissure
C21 Optic chiasm
C22 Hypophysis
C23 Mamillary bodies
C24 Epiphysis
C25 Foramen interventriculare (of Monro)
C26 Cerebral aqueduct (of Sylvius)
C27 Fourth ventricle
C28 Cerebellum
C29 Lamina tecti of the midbrain (a relay station for the optic and acoustic tracts)
C30 Pons
C31 Medulla oblongata

Figure 3.1 A lateral, dorsal and a median section of the brain

From " Nervous System and Sensory Organs, by Kahle, Leonhardt and Platzer (1993) p. 11.

The forebrain (cerebral cortex, thalamus, and hypothalamus), the midbrain, the hindbrain (pons, medulla, and cerebellum), and the spinal cord make up the subdivisions of the central nervous system (Figure 3.1) (Bloom & Kupfer, 1995). The largest mass

consists of the cerebral hemispheres, comprised of the outer cellular zone or cortex, and a number of well-defined subcortical regions, named on the basis of their appearance or location: the hippocampal formation, the basal ganglia, the amygdaloid complex, the thalamus, and the hypothalamus.

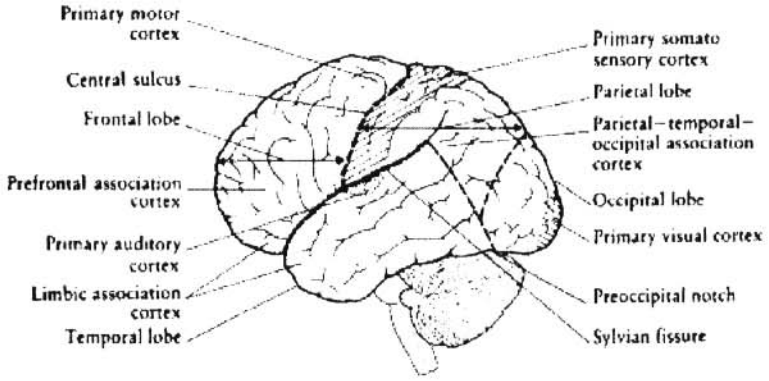
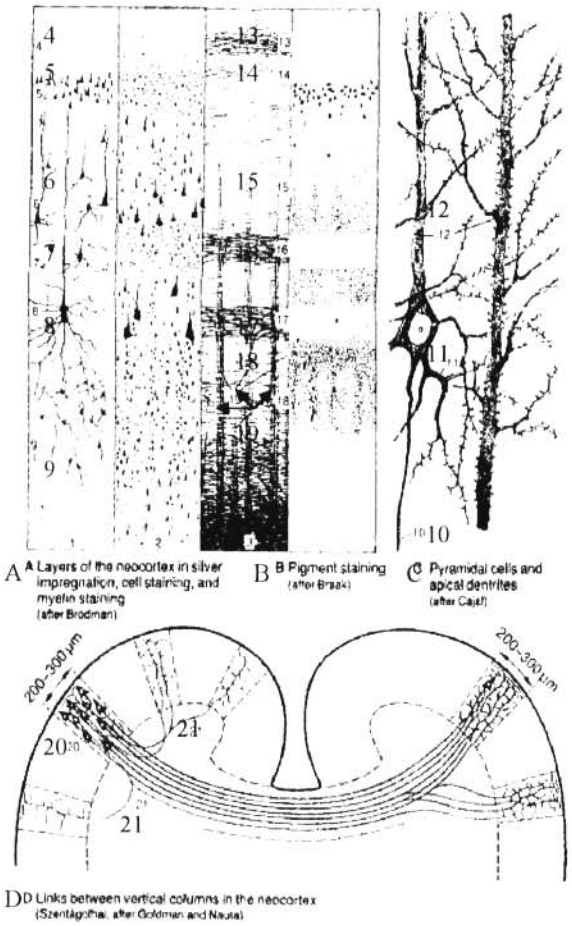


Figure 3.2 The lobe divisions of the human brain and their functional anatomy

From " Neuropsychological Assessment," Lezak (1995) p. 70.

Cerebral hemispheres are classified into cortical regions (figure 3.2) on the basis of one of several characteristics such as the sensory modality (e.g., somatosensory, visual, auditory, or olfactory), while other regions are concerned with motor operations and others are "associational" to imply integrations between sensory modalities and motor performance. It can also be classified on the anatomical location (e.g., frontal, temporal, parietal, or occipital). Bloom and Kupfer (1995) also describe an alternative scheme, the so-called cytoarchitectonic classification, whereby geometrical relationships between cell types are used on a microscopical level for classification. This is done according to their cell type size, shape, and packing density across the major cortical layers.



Cortical Layers according to staining techniques
A4 Molecular layer (1)
A5 External granular layer (II)
A6 Pyramidal layer (III)
A7 Internal granular layer (IV)
A8 Inner pyramidal layer (V)
A9 Multiform layer (VI)
Pyramidal cells and apical dentrites
C10 axon
C11 branch off
C12 apical dendrite
Cortical Layers
A13 Tangential layer
A14 Dysfibrous layer
A15 Suprastriate layer
A16 Fiber-dense external Bail-larger band
A17 Internal Baillarger band
A18 Substriate layer
A19 Vertical divisions by bundles of radial fibers
Vertical links in neocortex
D20 Fiber connections to columns
D21 Fiber connections from columns

Figure 3.3 The Cortical layers and the links between the vertical columns in the neocortex

From "Nervous System and Sensory Organs", Kahle, Leonhardt and Platzer (1993) p. 227.

Within any given region of the cerebral cortex, the six layers of which it is composed will appear essentially uniform microscopically (Bloom et al., 1995; Kahle et al., 1993). The layers (figure 3.3) can be displayed by different histological staining procedures.

Ensembles of vertically connected neurons (figure 3.3D) which span the layers, comprise the elemental processing modules (Kahle et al., 1993). Bloom and Kupfer (1995)

noted that "the specialised functions of a cortical region arise from the connections to and from both other regions of the cortex (corticocortical systems) and noncortical areas of the brain (subcortical systems)" (p. 4). The columnar modules are linked with each other as well as with larger information processing ensembles (Bloom & Kupfer, 1995; Kahle et al., 1993).

Cells of the Brain

The cell types found in the nervous system can be divided into non-neuronal cells (figure 3.4) and neurons (figure 3.5) (Kahle et al., 1993; Kettenmann & Ransom, 1995). The majority of cells belong to the non-neuronal group of cells.

The non-neuronal cells of the central nervous system consist of the macroglia, the microglia, and the cells of the vascular elements, including the intracerebral vessels and the vasculature of the cerebrospinal fluid forming tissues found within the cerebral ventricles, the choroid plexus (Kahle et al., 1993; Leeson and Leeson, 1976). The macroglia and neurons arise from the neuroectoderm (Bloom & Kupfer, 1995). The macroglia can be further divided into (a) the astrocytes and (b) the oligodendroglia (Leeson & Leeson, 1976). The astrocytes are located between the vasculature and the neurons and are regarded as serving supportive metabolic roles for the neurons especially within the grey matter of the brain and spinal cord (Bloom & Kupfer, 1995). The oligodendroglia produce the myelin coating that insulates the axons (the efferent process of neurons) to conduct bioelectric signals rapidly over long distances. The microglia are central nervous system supportive cells; these cells are believed to stem from mesodermal origin and are related to the macrophage monocyte cell lineage (Leeson & Leeson, 1976). This cell class has some members that are permanently resident in the brain, but the general class may be

augmented from the peripheral circulation during injury or acute inflammatory responses like in HIV infection (Bloom & Kupfer, 1995; Kettenmann & Ransom, 1995; Leeson & Leeson, 1976).

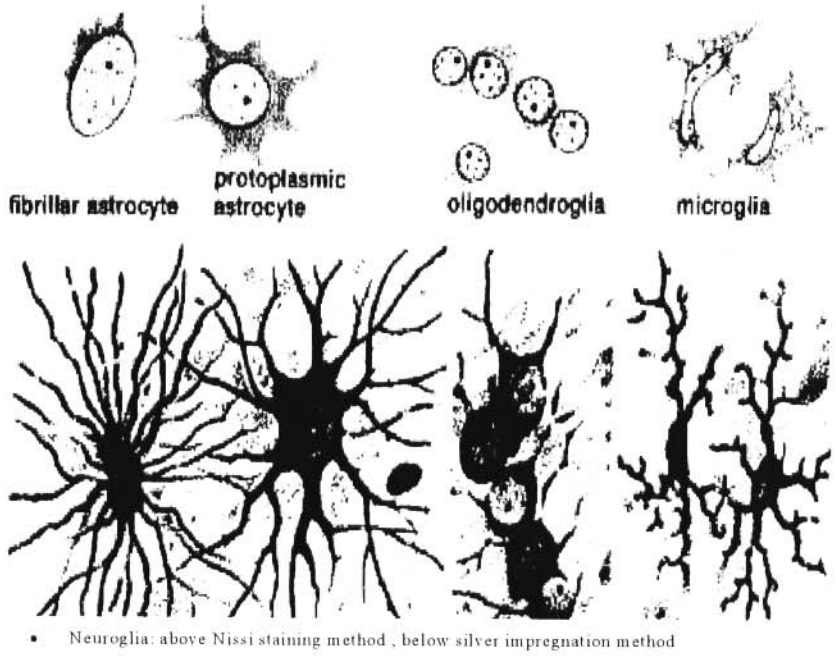


Figure 3.4 Non-neuronal cells in the brain

From " Nervous System and Sensory Organs", Kahle, Leonhardt and Platzer (1993) p. 39.

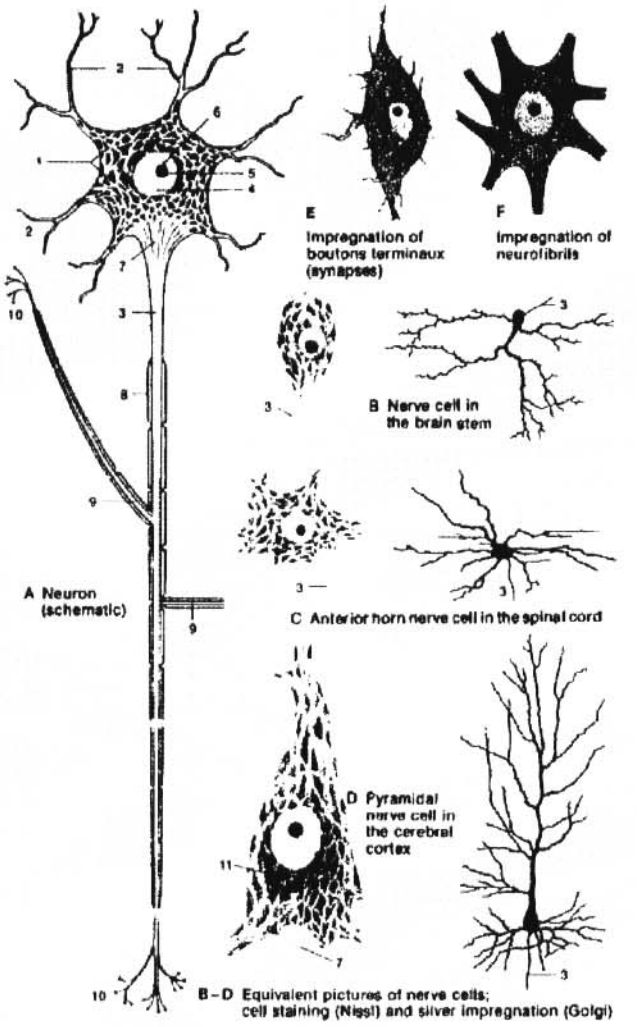


Figure 3.5 Neuronal cells in the brain

From " Nervous System and Sensory Organs, Kahle, Leonhardt and Platzer (1993) p. 17.

The neurons are responsible for the circuitry of the brain, spinal cord, and the peripheral nervous system and give rise to the multicellular nervous system (Bloom & Kupfer, 1995; Kahle et al., 1993; Leeson & Leeson, 1976). The neurons are interconnected on various levels for the formation of functional and processing units (Bloom & Kupfer, 1995; Kahle et al., 1993). Neurons are thus regarded as the information-processing

elements. Neurons differ widely in their size, shape, location, and other intrinsic properties. Neurons communicate chemically by releasing (or secreting) and responding to a wide range of chemical substances, referred to in whole as neurotransmitters (Bloom & Kupfer, 1995; Carlson, 1998). The chemical substances that neurons can secrete when they are active can also influence the non-neuronal cells (Kettenmann & Ransom, 1995). The functional activity of a neuron can also be modified by a different range of chemicals released from non-neuronal cells of the central or peripheral nervous system, and the latter substances are often referred to as neuromodulators (Bloom & Kupfer, 1995; Carlson, 1998). Products released by the non-neuronal cells of the immune system of the brain during acute infections might play an important role in neural-immune interactions and cell damage (Bloom & Kupfer, 1995; Grant & Marcotte, 1999; Kettenmann & Ransom, 1995).

Neuronal Circuitry

According to Bloom and Kupfer, (1995) there are three main patterns of neuronal circuitry:

1. Long hierarchical circuits, such as those characterising the interconnected major pathways of the sensory, motor, and intracortical relay systems in which excitatory amino acids are generally the transmitter.
2. Local circuit neurons such as the short axon neurons, both excitatory and inhibitory, that regulate the extent to which afferent signals can spread.
3. Single-source, differing neurons such as those neurons of the brainstem's reticular core nuclei, whose axons diverge to target cells in many parts of the neuraxis (p. 5).

Bloom & Kupfer, (1995) describe the major chemical classes of neurotransmitters in a similar triadic fashion:

1. Amino acid transmitters, of which glutamate and aspartate are recognised as the major excitatory transmitting signals, and gamma aminobutyrate (GABA) and glycine as the major inhibitory transmitters.
2. The aminergic transmitters (acetylcholine, epinephrine, norepinephrine, dopamine, serotonin, and histamine).
3. The various peptides

(Bloom & Kupfer, 1995, p. 5).

The above authors also noted that it is also likely, but not yet definitively established, that neurons can also make other kinds of molecules, (purines, lipids and prostaglandins and steroids) similar to those made and released by the adrenal cortex. Research has revealed that under some conditions, active neurons may synthesise gaseous signals, such as nitric oxide and carbon monoxide (Bloom & Kupfer, 1995). These gases can carry rapidly disappearing signals over short distances. Nitric oxide also plays a role in the activation of microglia and contributes to the development and progression of the clinical syndrome of the HIV infection (Rostasy et al., 1999).

INTERACTIONS BETWEEN THE NERVOUS SYSTEM AND THE IMMUNE SYSTEM

There is substantial evidence that the nervous system can indeed modulate immune function and vice versa (Kettenmann & Ransom, 1995; Mason, 1997). Dunn, in Bloom and Kupfer, (1995) noted that anomalies of the immune system can certainly cause diseases of the nervous system, and this may be manifested in psychiatric disease. It is clear that effective defence against infections requires a complex co-ordination of the activities of the

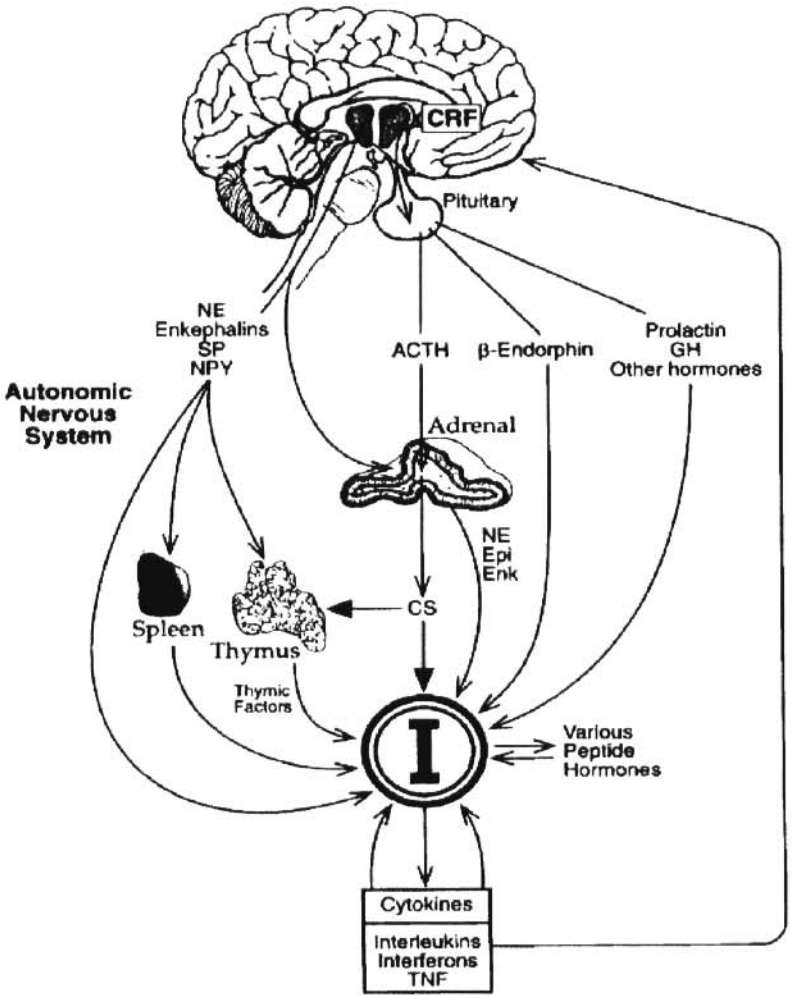
nervous and immune systems, and that abnormalities in the relationships between the two systems can cause disease (Kettenmann & Ransom, 1995).

The experimental evidence for nervous system and immune system interactions can be summarised as follows (Kettenmann & Ransom, 1995):

1. Alterations in immune responses can be conditioned.
2. Electrical stimulation or lesions of specific brain sites can alter immune function.
3. Stress alters immune responses and the growth of tumours and infections in experimental animals.
4. Activation of the immune system correlates with altered neurophysiological, neurochemical, and neuroendocrine activities of brain cells.

A number of specific mechanisms can be postulated by which the nervous system might affect the immune function (Bloom & Kupfer, 1995; Kettenmann & Ransom, 1995). These include glucocorticoids secreted from the adrenal cortex, catecholamines secreted from sympathetic nerve terminals and the adrenal medulla, other hormones secreted by the pituitary and other endocrine organs, and peptides (including endorphins) secreted by the adrenal medulla and autonomic nerve terminals. The network includes not only the autonomic nervous system and classical neuroendocrine mechanisms, but involves an endocrine function of the immune system (Bloom & Kupfer, 1995). A variety of immune system products (e.g., cytokines, peptides, and other factors) that function to co-ordinate the immune response may also provide important signals for the nervous system (Kettenmann & Ransom, 1995). Thus, chemical messengers can account for a variety of interactions between the nervous system and the immune system (Bloom & Kupfer, 1995). Figure 3.6 from Bloom and Kupfer, (1995) provides a schematic diagram of the most well-

known interactions between the nervous system and components of the endocrine and immune systems.



Abbreviations: GRE, corticotropine releasing factor; CS, corticosteroids; Enk, enkephalins; Epi, epinephrine; GH, growthhormone; I, immunocytes; NE, norepinephrine; NPY, neuropeptide Y; SP, substance P; TNF, tumornecrosis factor.

Figure 3.6 A schematic diagram of the interactions between the brain and components of the endocrine and immune systems

From " Psychopharmacology", by Bloom & Kupfer, (1995) p. 720.

The cytokines that are important in HIV-associated cell damage, are a large group of proteins that were originally identified as products of immune cells that functioned as chemical messengers between cells of the immune system. They are now known to be

synthesised by a variety of different cell types in the body and can have a wide range of actions on many organ systems (Grant & Marcotte, 1999; Kettenmann & Ransom, 1995). The cytokines include the interleukins (of which there are at least 13), the interferon tumour necrosis factors, and a variety of cell growth-stimulating factors (Bloom & Kupfer, 1995).

CYTOTOXIC SECRETIONS OF MICROGLIA AND ASTROGLIA

According to various authors, it is possible for mononuclear phagocytes to secrete cytotoxic factors, which can be classified as short-lived (i.e., nitric oxide, hydrogen peroxide, or superoxide anion) or long-lived cell poisons (cytokines and enzymes) (Kettenmann & Ransom, 1995; McArthur & Grant, 1993; Pulliam, Clarke, McGrath, Moore & McGuire, 1996; Pulliam, Zhou, Stubblebine, & Bitler, 1998). Because cytotoxins have been implicated in various neurologic disorders, it was likely that brain inflammatory cells were important sources of these neuron-killing factors (Kettenmann & Ransom, 1995).

Properties of secretions from microglia and astroglia can be summarised as in table 3.1 (Kettenmann & Ransom, 1995).

Table 3.1 A Comparison between the cytotoxic secretions of microglia and astroglia

Cytotoxic secretions	**Microglia**	**Astroglia**
Neuron-killing factors	+	-
Neuronal growth factors	-	+
Molecules size	Small	Large
Heat sensitive	+	-
Molecules type	Toxin	Protein

From " Neuroglia", by Kettenmann & Ransom, (1995) p. 720.

Giulian (1995), gave the following examples (Table 3.2) of molecules secreted by mononuclear phagocytes that have cytotoxic properties (Kettenmann & Ransom, 1995).

Table 3.2 Cytotoxic Molecules Secreted by Mononuclear Phagocytes

Large molecules (>10 kD)	**Small molecules (<1 kD)**
Tumor necrosis factor	Superoxide anion
Complement	Hydrogen peroxide
Interferons	Nitric oxide
Interleukins	Eicosanoids
Proteases	Lipoxins
Lipases	Uric acid

From "Neuroglia", by Kettenmann & Ransom, (1995) p. 720.

MICROGLIA AND AIDS

Acquired immunodeficiency syndrome (AIDS) has a devastating effect upon the brain and spinal cord, with more than 70% of all patients showing loss of memory, paralysis, seizures, sensory deficits, or global dementia (Kettenmann & Ransom, 1995). Human immunodeficiency virus-I (HIV-1) has been isolated from the central nervous system of AIDS patients and identified in several classes of central nervous system mononuclear phagocytes, for example, microglia, macrophages, and multinucleated macrophage-like cells (Kettenmann & Ransom, 1995). At autopsy, the brains of AIDS

patients with dementia showed cortical atrophy and loss of cortical neurons. Although direct viral infection of neurons in brain tissue has not been demonstrated, studies have shown extensive neuronal damage (Friedlander, 1998; Kettenmann & Ransom, 1995). Thus, neurologic disorders of AIDS appear to be an indirect effect of retrovirus infection and might be the result of neurotoxic factors (Grant & Marcotte, 1999; Kettenmann & Ransom, 1995).

According to Giulian in Kettenmann and Ransom (1995), there are five lines of evidence that support the idea that microglia-derived neurotoxins have an important role during neural tissue damage:

1. Neurotoxins were only detected in tissues heavily infiltrated with reactive microglia or macrophages. The levels of neurotoxic activity found in the central nervous system injured by either trauma or ischaemia correlated with the number of mononuclear phagocytes at the lesion sites. Importantly, no toxic activity was detected in neighbouring non-inflamed tissues or in normal animals.
2. Drugs that reduced inflammatory cell numbers also reduced the amount of neuron-killing activity released by damaged tissues.
3. Active mononuclear phagocytes appeared at a time of a delayed loss of neurons and deterioration in neurological function.
4. Suppression of central nervous system inflammation both improved motor neuron survival and preserved motor function.
5. Isolation of specific cell populations confirmed that reactive mononuclear phagocytes were the principal source of neuron-killing factors found in the damaged central nervous system (p. 682).

Reactive microglia is the major source of central nervous system-derived cytokines and appear in almost every type of central nervous system disorder, including infection, trauma, stroke, degeneration, and demyelination (Kettenmann & Ransom, 1995). These inflammatory cells help to regulate neural tissue healing and serve as a link between systemic immune responses and the central nervous system (Kettenmann & Ransom, 1995). Moreover, the cytotoxic agents produced by microglia, demonstrate potent neurotoxic effects (Grant & Marcotte, 1999; Kettenmann & Ransom, 1995; Rostasy et al., 1999). The release of long-lived inflammatory cytotoxins is a delayed process and not detected until a peak of cellular reactivity was reached, often by the second day after injury (Kettenmann & Ransom, 1995). This neuron-killing action could, in turn, be balanced by growth factors released from astroglia at a later phase of wound repair so that recovery of neuronal function would depend to a degree upon the location and numbers of reactive glia (Kettenmann & Ransom, 1995). Giulian (1995) noted that "destruction of neurons adjacent to the sites of injury might be an important mechanism by which reactive microglia suppress the spread of neurologic dysfunction" (Kettenmann & Ransom, 1995, p. 674). Giulian (1995) further noted that tissue debris might continue to stimulate reactive microgliosis, resulting in the production of neuron-killing factors that influence neuronal survival well beyond the period of initial tissue insult. It thus seems that brain damage as a result of HIV, is caused by the exact mechanisms that try to protect it.

THE HIERARCHICAL ORGANISATION OF THE BRAIN ACCORDING TO LURIA

Whenever the central nervous system is compromised, it effects the higher cognitive functions (Mapou & Spector, 1995). In order to understand how neurological disorders

affect the functional systems of the brain, a brief discussion on the hierarchical organisation of the brain according to Luria (1973, 1980) will follow.

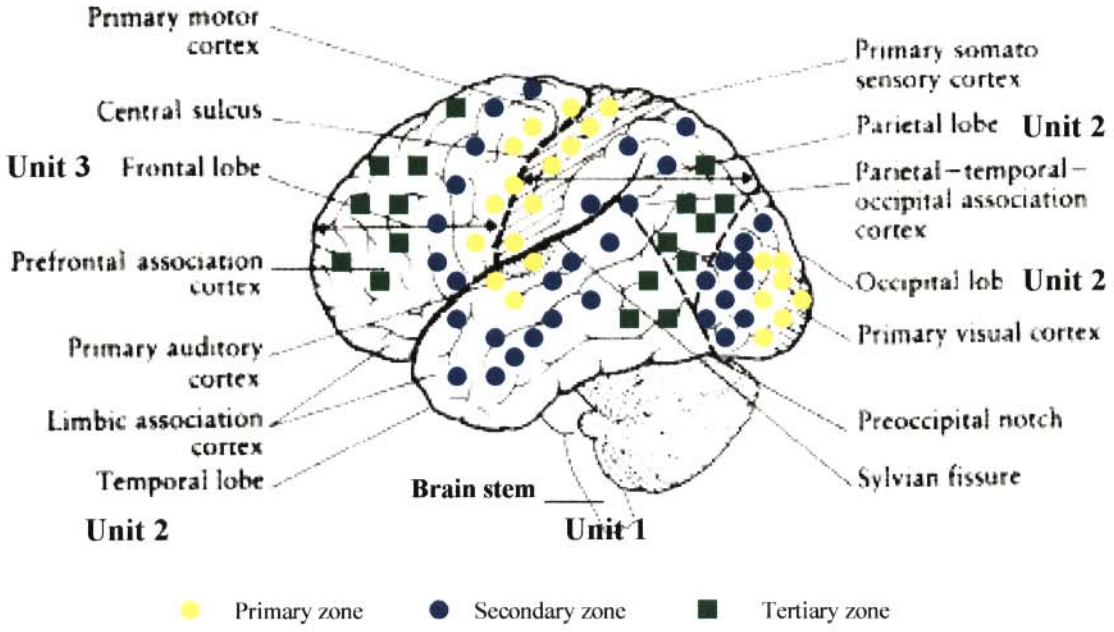


Figure 3.7 A schematic diagram to demonstrate the functional units according to Luria

Considering the concept that the organisation of the brain is hierarchical, Luria hypothesised about the cerebral organisation of mental processes, and advanced further to distinguish among three functional cerebral units, viz. (Figure 3.7),

Unit One (metencephalon, mesencephalon, diencephalon, and the medial zones of the hemispheres). This unit regulates arousal (cortical tone) or alertness for maintaining functions, like orientation, learning and memory.

Unit Two (cortex posterior to the central sulcus) is specialised for receiving, processing and sorting of input from the sensory organs.

Unit Three (cortex anterior to the central sulcus) is responsible for planning, initiating, regulating and monitoring behaviour and mental activity.

Thus, primarily the types of mental activity taking place within these functional units determine this division. However, Luria emphasised that this division does not mean that each unit is an autonomous module, but that any complex mental process, such as perception and voluntary movement, is the result of the activity in a functional system. This functional system is the result of a network of co-operating cortical and subcortical structures in all three functional units.

The hierarchical view of the organisation of the brain is manifested partly in the reciprocal relationship among the functional units and partly in the internal organisation of each unit. Unit Three, which also includes the prefrontal cortex among other areas, is regarded as possessing a regulating influence on the other two units. The prefrontal cortex (unit three) has vast reciprocal connections both with the cortex posterior to the central sulcus (particularly with the association areas or tertiary zones of unit two), and with the subcortical structures included in unit one.

These three functional units of the brain are internally organised in a hierarchical fashion. Luria distinguished three zones (figure 3.11) in units Two and Three, being primary, secondary and tertiary zones, and proposed a hierarchical relationship among these three zones (Luria, 1973).

The primary zones (projection) correspond to the projection areas of the cortex. The posterior part of the cortex is specialised for reception of afferent impulses from sensory

organs (Luria, 1973). In the anterior part of the cortex the primary zones correspond to the motor cortex that control the motor system through pyramidal tracts (Luria, 1973).

The secondary zones of unit two are adjacent to the primary zones. These zones are specialised for analysis and synthesis of the afferent impulses received by the primary zones from the sensory organs. They are sometimes referred to as "gnostic" cortical zones, in order to emphasise that these zones form an important neuronal basis for the recognition of sensory impressions (Luria, 1973).

The function of the secondary zones (projection-association) in unit three is to generate and prepare motor programs and sequences, for which the motor cortex serves as an output channel (Luria, 1973).

The primary and secondary zones are together labelled "analysers", as they are specific functional modules for receiving and processing information.

The tertiary zones (zones of overlapping) are the association areas in both the posterior part of the cortex and in the prefrontal cortex.

The tertiary zones of unit two manage the inter- or polymodal integration of information from the modally specific analysers (Luria, 1973). The tertiary zones in unit three are thought to be involved in the direction and control of behaviour on a superior level, and correspond to the prefrontal cortex (Luria, 1973).

Luria introduced the concept of the "executive functions" of the brain as characteristic for the prefrontal areas. Among others, this concept refers to activities such as planning, initiation, and monitoring of behaviour and inhibition of irrelevant impulses

for actions. He also saw a parallel between the hierarchical organisation of these cortical areas and therefore posed a dual application of the concepts of primary, secondary and tertiary zones in both the anterior and the posterior part of the cortex. In both units two and three, there is a controlling and regulating influence from the tertiary zones through the secondary and primary zones. The tertiary zones mainly regulate the process of perception and gnosis in unit two. In unit three, the tertiary zones regulate behaviour.

Vygotsky and Luria posed a hypothesis or "Law of the hierarchical organisation of the cortex" in their earlier work in developmental psychology (Mapou & Spector, 1995). With this, they presumed that adequate functioning of the primary zones in the posterior part of the cortex of a child is essential for the normal development of the secondary and tertiary zones. Vygotsky's point of view proposed that the main direction of interaction between the cortical zones of a child runs from "bottom, up", while the main direction of interaction between the cortical zones of an adult runs "from top, down" (Mapou & Spector, 1995, p. 220). To support this viewpoint, he proposed that during infancy the development of normal spatial concepts depend on the integrity of the visual and somatosensory analysers. In the same manner, the development of verbal abilities seems to be dependent on intact functioning of the auditory analyser. Then, as adulthood is reached, the association areas of unit two develop, and they are thought to become increasingly important for the perceptual processing by the analysers. In the fully developed adult individual, perception seems very much controlled by the complex cognitive processes connected to the tertiary zones. Sensory impressions are organised according to previously internalised concepts, experiences, and expectations.

The above perspective forms the basis for a differentiated understanding of the relationship between the time of onset of a brain injury and the consequences of the injury for cognitive functioning. Thus, according to Luria (1973), lesions or defective development of one of the analysers in infancy will cause more serious disturbances than a similar lesion occurring after the brain is fully developed, and intact functioning of the tertiary zones is ensured. Processes in the tertiary zones can, to some extent, compensate for dysfunctioning of the analysers occurring late in development (Luria, 1973).

Luria hypothesised that one can regulate the influence of the tertiary zones on the analysers, and that there is a potential for compensation in case of limited dysfunction at a hierarchically lower level.

From the above, it can be concluded that the functional brain with its equilibrated interconnected biological subsystems, somehow is translated into behaviour. Certain changes in its functioning will result in observable changes in behaviour. However, Luria (1973) himself stated that there is no simple, unequivocal relation between symptom or behaviour and localisation. According to Lezak (1995), behaviour can be divided into three functional systems, namely cognition, which is the information handling aspect of behaviour, emotion, which concerns feelings and motivations, and lastly, executive functions, as the way in which behaviour is being expressed. In the case of brain damage, usually more than one system is affected (Lezak, 1995). Furthermore, because of the interconnected subsystems, it is possible that the same symptom will be a manifestation of a number of different localised brain lesions. Therefore, in a study where brain damage is expected due to the direct or indirect effect of a virus infection like the HIV, neurological assessment must be constructed to encompass damage in the broadest sense possible.

CHAPTER 4

NEUROPSYCHOLOGICAL IMPAIRMENT AND HIV INFECTION

CLASSIFICATION OF HIV COMPLICATIONS

The Centre for Disease Control (CDC) located in Atlanta, Georgia, USA developed the most widely used classification for HIV disease (Ebrahim, 1999). It is based on the presence of clinical symptoms and signs, the presence of certain conditions and on investigative findings, the availability of HIV screening, and the degree of immunosuppression as measured by the CD4 lymphocyte count (see Table 4.1).

Table 4.1 A summary of the CDC 1993 classification system for HIV disease

CD4 Lymphocyte count x 10^6/L	**(A) Asymptomatic including Groups I, II, III**	**(B) Symptomatic not A or C Groups IV**	**(C) AIDS defining conditions**
[1] >500	A1	B1	C1
[2] 200-499	A2	B2	C2
[3] <199	A3	B3	C3

From " A Basic Guide to the Management of Patients with the Disease," Ebrahim, (1999) p. 9.

Infection with HIV causes a spectrum of clinical problems beginning at the time of seroconversion and terminating with AIDS and death.

The infection is divided into four groups (Ebrahim, 1999):

Group I: Seroconversion

This represents the acute infection with HIV. It usually occurs around 1-2 weeks after exposure to the virus but may be delayed. Up to 70-80% of people present with symptoms at the time of seroconversion. The clinical manifestation range from mild glandular fever-like illness to encephalopathy. The differential diagnosis varies and the diagnosis of HIV is often missed at this stage.

Group II: Asymptomatic Phase

After seroconversion, HIV antibodies continue to be detectable in the blood. The rate of replication of the virus is slow although it does not stop. CD4 and CD8 lymphocyte counts are usually in the normal range and the patient is clinically well. This phase may persist for as long as 10 years.

Group III: Persistent generalised lymphadenopathy

This may be the presenting feature of IV infection in a patient ho is other ise ell. IV related lymphadenopathy persists for ± 3 months or longer, in at least two sites other than the groin and is not due to any other cause.

Group IV: Symptomatic Infection

This stage correlates with the decline in immune competence and the appearance of symptoms, signs, and infection at various sites. The type of infection that develops is dependent on the CD4 count. At this stage, viral replication increases and more CD4 and CD8 are destroyed. The CD4 count may drop to around 200 $x10^6$/L. The spectrum of disease includes constitutional symptoms (fever, weight loss, diarrhoea), opportunistic

infections, neurological disease and secondary neoplasms. Prophylactics for various infections and antiretroviral therapy should be discussed with the patient at this stage.

NEUROCOGNITIVE COMPLICATIONS OF HIV INFECTION

Neurocognitive complications of HIV infection can be classified as being either primary or secondary (see table 4.2) (Grant & Marcotte, 1999). Primary complications are those directly linked to HIV infection of the brain. Secondary complications are linked to immunodeficiency, or other adverse events associated with HIV disease or its treatment.

1. PRIMARY NEUROCOGNITIVE COMPLICATIONS

A. HIV-1 Neurocognitive Complications

1. Neuropsychological impairment
2. HIV-1 Mild Neurocognitive Disorder (MND / MCMD)
3. HIV-1 Associated Dementia (HAD)

B. Other HIV-Neurobiological Complications

1. HIV-1 meningitis
2. HIV-1 vacuolar myelopathy
3. HIV-1 neuropathies
   a. Acute demyelinating (Guillain-Barre)
   b. Relapsing or progressive demyelinating (e.g., mononeuritis multiplex)
   c. Predominantly sensory neuropathy
4. HIV-1 myopathy

2. SECONDARY NEUROBIOLOGICAL COMPLICATIONS (GENERALLY CAUSING DELIRIUM)

A. Infections

1. Toxoplasma encephalitis/abscess
2. Cryptococcus meningitis
3. Cytomegalovirus (CMV) encephalitis
4. Progressive multifocal leukoencephalopathy (PML)
5. Other infections of the CNS

B. Neoplasia

1. Primary or secondary CNS lymphoma
2. Kaposi's sarcoma of the CNS
3. Other neoplasia

C. Cerebrovascular disease related to HIV infection

D. Other delirium

1. Adverse effects of drugs
2. Hypoxemia, hypercapnia (e.g., with PCP pneumonia)
3. Other metabolic and nutritional disorders

From " HIV Infection: Medical, Neuropsychological, and Neuropsychiatric Aspects", Grant and Marcotte (1999) p. 13.

Grant and Atkinson, as cited in Kaplan and Sadock (1995), proposed an improved taxonomy classification of HIV-related neurocognitive disturbances namely:

1. Neurocognitive deficit (NCF): The individual has scored in the impaired range on tests comprising a single ability area. For example, if the impairment was in learning, the individual would be classified as having a learning deficit.

2. Neurocognitive Impairment (NCI): If an individual has a deficit in at least two cognitive domains, he/she is considered Neurocognitively Impaired. It is only when a person reaches the level of Neurocognitive Impairment that the individual is considered to have a true neurocognitive abnormality. Since HIV is unlikely to produce a singular cognitive deficit, the meaning of such a deficit would be questionable. However, a more generalised impairment, using the above definition, is associated with implications for real life functioning.

3. Neurocognitive Disorder (NCD). The term Neurocognitive Disorder is reserved for the case in which impairment is considered "clinically meaningful." Clinically meaningful implies that the patient is experiencing clinically definite symptoms, such as problems at work, or difficulties with other aspects of social or day-to-day functioning (p. 13).

In this study, the attention is specifically towards NCF and NCI. The prevalence of mild NCI, particularly during the early stages of the disease, has remained a point of controversy (Grant et al.,1987; McArthur & Grant, 1989; Selnes et al., 1990; Sonnerborg, Ehrnst, Bergdahl, Pehrson, Skoldenberg & Strannegard, 1988). However, there is growing evidence that a subset of medically asymptomatic individuals do indeed show evidence of at least mild NCI (Grant et al., 1987; Heaton et al., 1995; Stern et al., 1991). Although the incidence and prevalence of mild NCI are not yet fully reported, it seems from studies (Heaton, Ryan, Grant, & Matthews, 1996) that overall rates of dysfunction increase with disease progression based on clinical symptoms and CD4 counts (Grant & Marcotte, 1999).

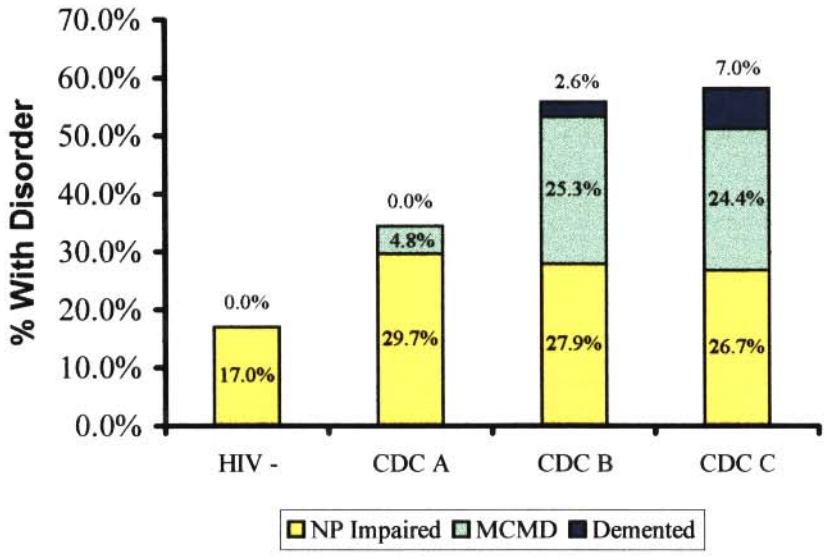


Figure 4.1 Percent of subjects with neurocognitive disorders

From " HIV Infection: Medical, Neuropsychological, and Neuropsychiatric Aspects", Grant and Marcotte (1999) p. 15.

According to the findings of Grant & Marcotte, (1999) (Figure 4.1) NCI was seen in 17% of seronegative controls and in 30% of CDC A, 28% of CDC B and in 27% of CDC C subjects. NCI is diagnosed when there is evidence of neuropsychological impairment based on testing, but the individual does not report any difficulties in every day functioning. Early studies also demonstrated abnormal neuropsychological test results in seropositive non-Aids subjects (Skoraszewski, et al., 1991). Minor cognitive motor disorders (MCMD) were relatively rare in medically asymptomatic individuals (CDC A - 5%), but increased to 25% in the symptomatic stages. Dementia was seen in only 0.8% of CDC A subjects, 2.6% of CDC B subjects, and 7.0% of those with an AIDS defining illness (Grant & Marcotte, 1999).

Studies using large neurological test batteries (> 14 tests) were significantly more likely to find impairment in the HIV positive asymptomatic group (Grant & Marcotte, 1999; Skoraszewski et al., 1991). The median rate of NCI for HIV positive subjects was approximately 35% (compared to 12% in HIV negative subjects) (Grant & Marcotte, 1999).

PSYCHIATRIC SEQUELAE OF HIV INFECTION

It is being accepted that emotion and behaviour have biologic underpinnings, and are influenced by the social context (Bloom & Kupfer, 1995; Dreher, 1995; Grant & Marcotte, 1999; Mason, 1997). According to Grant & Marcotte, (1999) it can be expected that HIV sufferers will present with both premorbid psychiatric disorders as well as new onset neuropsychiatric disorders which may complicate a patient's response to infection, disease, and treatment.

Three models currently explain the origin of psychiatric conditions observed in the course of infection and illness from HIV and can be described as follows (Grant & Atkinson, 1995):

The first is the Transitional Model. According to this model it can be expected that adverse psychological disorders (e.g., adjustment disorders, mood disorders, anxiety disorders) might be anticipated during the progression of HIV. As the infection progresses, there will be a breakdown in coping capacities that lead to psychological disorders. Specific psychological key areas during progression might be: The discovery of seroconversion, initiation of antiretroviral treatment, onset of physical symptoms, advance in HIV illness stage, and the breakdown in coping mechanisms.

Secondly is the Biological Model. This model suggests that HIV involvement of the CNS itself may be associated directly with neurocognitive disorders, depression syndromes or psychotic phenomena. Alternatively, individuals may respond to awareness of their neurocognitive decline with psychological distress, or neurocognitive decline itself may increase the likelihood of such symptoms.

Thirdly is the Background Model. This model emphasises that psychiatric disorders, which preceded the HIV epidemic, may also emerge during the course of an individual's illness. There is no direct association with the HIV progression, it only acknowledges emotional distress during illness-related events.

It must be emphasised that from a study of high risk populations, psychological background seems to play a more important role in psychological disorders than HIV infection per se (Grant & Atkinson, 1995). It has been found that in terms of the injection drug using risk group, studies preceding the HIV epidemic have indicated high lifetime rates of personality disorders, anxiety disorders, and mood disorders. Elevated lifetime rates of antisocial personality disorders (ranging from 25 to over 50%), phobias (10%), and major depression (20 to 25%) are reported (Grant & Atkinson, 1995).

Grant and Atkinson (1995) do note however, that HIV is known for its social stigmatisation and political interchange. Fear of HIV and AIDS is widespread in the general community and the practical consequences of the infection still include loss of employment, denial of medical benefits or life insurance, derailment of career aspirations and leaning heavily on the individual's immediate social support (Grant & Atkinson, 1995). Thus, regardless of the CDC stage, HIV seropositive individuals with satisfying and

stable social support, will tend to be significantly less distressed than individuals with unsatisfying or inconsistent support (Grant & Atkinson, 1995; Mason, 1997).

PSYCHIATRIC ISSUES SURROUNDING TRANSITION POINTS DURING HIV PROGRESSION

Grant and Atkinson (1995) describe the following transition points based on the progression of the disease:

The first is the seroconversion. The first personal encounter that the individual has with HIV is when they know their own serostatus. Grant and Atkinson (1995) note that many of the psychological issues surrounding seroconversion may re-emerge throughout the entire course of the infection. The stages, as with other life-threatening illnesses, are shock, anger, anxiety, guilt, and denial. A difference to other illnesses is the fear of disclosure of serostatus. Seroconverted individuals also fear the loss of control, of abandonment by family and friends, of being unable to work, of medical expenses and of pain (Grant & Marcotte, 1999). The anger that individuals experience in this stage might be directed at friends, family, lovers, physicians, and other caregivers.

This is a time when loss of self-esteem and self-blame is common, and regret or guilt over their lifestyle is usual (Grant & Marcotte, 1999). Individuals become sceptic and conflicts around trust may emerge. Grant and Marcotte (1999) also describe a tendency towards dependency that may develop on physicians and others. During this phase, it is important for pre- and post-test counselling (Grant & Marcotte, 1999). Counselling involves the explanation of HIV test results, education for risk reduction, risks of transmission of infection and the monitoring of various markers like the VLC and CD4

count (Grant & Marcotte, 1999). Studies using standardised questionnaires and rating scales suggest that symptoms of depression and anxiety are usually only moderately elevated from baseline in groups of individuals examined before and immediately after HIV antibody test results are announced. These ratings return to slightly below immediate pre-testing levels within three months (Grant & Marcotte, 1999).

No evidence of widespread suicidal ideation or suicide attempt following testing positive for HIV has been found. The most prevalent psychiatric disorder in this phase is that of an adjustment disorder with depressed or anxious features, the prevalence of which has been as high as 20% in some samples (Grant & Marcotte, 1999).

The following phase that Grant and Marcotte, (1999) describe is, when the individual transforms from an asymptomatic to a medically symptomatic stage of AIDS.

Despite the struggles with adjusting to the threats and uncertainties posed by infection, most studies according to Grant and Marcotte, (1999) indicate that levels of anxious and depressed moods are comparable for physically asymptomatic seropositive men and seronegative controls selected from similar HIV risk categories. Both groups still have more psychiatric symptoms than general community controls (Grant & Marcotte, 1999).

As individuals become medically more symptomatic, measurable levels of distress may increase, but this issue is somewhat complicated since physical symptoms of HIV disease (e.g., fatigue, loss of appetite, autonomic arousal) may overlap with emotional symptoms used to rate depressed and anxious moods (Grant & Marcotte, 1999). Individuals, who are younger, socio-economically disadvantaged, or with reduced levels of

social support, are most likely to experience greater psychological distress (Grant & Marcotte, 1999).

Neurocognitive disorders consequent to HIV infection of the brain, include disorders like those mentioned in table 4.4. Psychosis is uncommon in HIV illness, but syndromes with paranoid, manic, schizophreniform and catatonic features can occur, usually in the context of an organic mental disorder complicating AIDS (Grant & Marcotte, 1999). These conditions may be associated with early mortality, but occasionally also appear in the absence of marked physical symptoms of the illness (Grant & Marcotte, 1999).

As mentioned earlier, the major neuropsychiatric disorders that can accompany the HIV disease, are adjustment disorders, anxiety disorders, mood disorders and psychotic disorders (Grant & Marcotte, 1999). Following, is a brief description of the disorders:

Adjustment Disorders are defined as emotional or behavioural disturbances of sufficient intensity to interfere with life functioning, occurring after an acute or persisting life stress (American Psychiatric Association, 1994). The common emotional reactions consist of anxiety, depression, or combinations of these. There may also be behavioural disturbance, e.g., an increase in illegal activity and failure in meeting responsibility. Adjustment symptoms develop within three months of experiencing the stressor, and usually resolve spontaneously within six months. The course can be more protracted if the stressor is chronic, or is perceived as having lasting threatening consequences (diagnosis of AIDS, or being told one must retire from work because of a drop in CD4 count are examples) (Grant & Atkinson, 1995).

Anxiety Disorder is the difficulty to control apprehension and worry coupled with muscular tension and autonomic arousal (American Psychiatric Association, 1994). It is common in normal populations from time to time. A relatively small proportion experience more severe disabling anxiety disorders (American Psychiatric Association, 1994). With the syndrome of generalised anxiety disorder, people experience worry, tension (including muscle tension and pain), restlessness, irritability, difficulty concentrating, sleeplessness, and autonomic arousal on a chronic basis. With panic disorders, there are brief episodes of intense fear and discomfort accompanied by fear of dying, loss of control, or some other dreaded happening, plus intense physical symptoms e.g., choking, palpitations, dizziness, breathlessness, etc. Between attacks, there is worry about a future attack, and often avoidance behaviours, e.g., avoidance of elevators (American Psychiatric Association, 1994).

With *Obsessive Compulsive Disorder* there are recurrent unwanted thoughts, or impulses which one unsuccessfully tries to ignore or suppress, and which cause worry and distress (American Psychiatric Association, 1994). Studies have shown that HIV positive patients do not suffer more from each of the three severe anxiety disorders than HIV negatives at risk or community controls (Grant & Atkinson, 1995).

Mood Disorders include Major Depressive disorder and Mania. The essential feature of major depression is persistent depressed mood (i.e., sad, blue, empty, dejected, despondent, tearful, hopeless) (American Psychiatric Association, 1994). This is typically accompanied by some combination of other features, such as loss of interest (including in living), feelings of worthlessness, difficulty concentrating, fatigue, agitation, middle of the night or early morning awakening (occasionally hypersomnia), and weight loss

(occasionally gain) (American Psychiatric Association, 1994). It is not yet determined whether HIV itself increases the likelihood of major depression beyond the expected increases found in other chronic diseases. Preliminary work seems to indicate that a previous mood disorder is the strongest predictor of subsequent depressive illness (Grant & Atkinson, 1995). The diagnosis of major depressive disorders can be complicated as individuals become more ill, because then somatic symptoms of disease (e.g., fatigue, weight loss, insomnia) or of neurocognitive impairment (e.g., slow thinking, diminished powers of concentration, or forgetfulness) may resemble the general symptoms of depression. Cognitive complaints of mental slowing and lack of concentration can be part of a depressive picture or be evidence of mild neurocognitive disorders or dementia (Grant & Atkinson, 1995). According to Grant and Atkinson, (1995) studies indicated that depression will have little effect on detailed neuropsychological assessment of attention, language, visual spatial function, memory, or speeded information processing, although they may be somewhat slower in motor functioning.

Mania refers to a persistently elevated, expansive, and/or irritable mood (American Psychiatric Association, 1994). Manic syndromes without hallucinations, delusions, or a disorder of thought process occur rarely, but can complicate any stage of the HIV infection. According to Grant & Atkinson (1995) examples are when individuals who were previously described as stable, over a period of days or weeks undergo a change in personality (e.g., become pompous, belittling and sexually inappropriate), and then progress into a fuller manic picture. The mood is generally elevated, and there is associated grandiosity. Diminished need for sleep, teeming thoughts, and unrealistic personal or financial plans may appear (Grant & Marcotte, 1999).

Psychotic Disorders are usually later stage complications of the HIV infection (Grant & Marcotte, 1999). The most prevalent symptom seems to be delusions and hallucinations (occurring in almost 90% of cases) with persecutory, grandiose, or somatic components (American Psychiatric Association, 1994).

CHAPTER 5

NEUROPSYCHOLOGICAL ASSESSMENT AND DIAGNOSTIC ASSAYS

In order to assess cognitive deficits or impairment as a result of central nervous system damage, hypothetical constructs are needed that will most likely be influenced by the condition under investigation. Cognitive functions are normally described by hypothetical constructs such as memory, language, and attention (Mapou & Spector, 1995). It must be pointed out that the precise details of what damage is, can vary from case to case. The aim of this study is not to establish the exact underlying area of damage in the brain, but rather to search for the most sensitive neuropsychological test that demonstrates diffuse neurological damage and thus can be used to correlate with markers (CD4 and VLC) of the HIV.

NEUROPSYCHOLOGICAL ASSESSMENT ASSOCIATED WITH HIV AND AIDS

As seen from the anatomy of infection in the previous sections, it is clear that neural damage as a result of HIV is not localised to a specific neuronal region. Impairment may be subtle and any single or combination of ability areas may be affected (Friedlander, 1998; Grant & Atkinson, 1995). The fact that deficits are "spotty", has a diverse implication on neuropsychological testing (Heaton et al., 1995). White et al., (1995) found that the likelihood of detecting impairment in asymptomatic subjects was in part dependent upon the comprehensiveness of the neuropsychological battery. This finding stretches the diverse assault that the HIV has on the brain.

Both the National Institute of Mental Health (Butters et al., 1990) and the HIV Neurobehavioral Research Centre in San Diego (HNRC) (Grant & Marcotte, 1999)

developed neuropsychological test batteries that can be used to measure neuropsychological impairment. The duration of the batteries used varied between nine hours to forty-five minutes. The HNRC found that for practical purposes, a step-down battery was still sensitive enough to demonstrate HIV-related deficits as long as multiple ability areas are tapped (Grant & Marcotte, 1999). The battery was composed of tests to evaluate functioning in the areas of fluency, flexible thinking, speed / information processing and attention and motor speed. As seen from figure 5.1, the cognitive areas most vulnerable to be affected by HIV are: attention (28%), learning (25%), motor functions (21)% and memory and verbal ability (both 19%) (White et al., 1995). The neuropsychological patterns observed in HIV infected persons correlated with neuropsychological patterns seen in patients with "subcortical" dementias studied by Becker, Caldararo, Lopez, Dew, Dorst & Banks (1995) and Peavy et al. (1994).

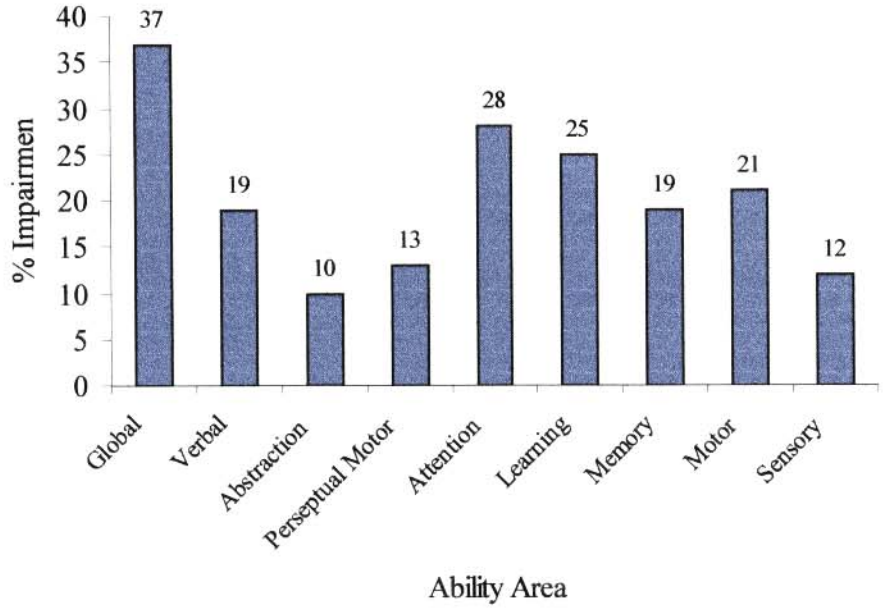


Table 5.1 An overall HIV + impairment rate and impairment rates for each neuropsychological domain

From " HIV Infection: Medical, Neuropsychological and Neuropsychiatric Aspects", Grant and Marcotte (1999) p. 30.

Rationale and Description of Tests Used

As seen from the graph in figure 5.1, the most likely domains to encounter deficits are in the area of attention / speed of information processing, motor skills, learning and memory. The variables that will affect neuropsychological performance include age and education. Motor tasks are most frequently affected by increasing age while education has little effect on motor performance. Education on the other hand, will affect performance if tests utilise an individual's knowledge base or acquired skills while age will have little effect (Heaton et al., 1996).

In HIV studies, education may also be of greater importance in neuropsychological performance as seen from the study done by Satz et al. (1993). They found that, if education was ignored, little difference was found between HIV negative men and asymptomatic HIV positive men. Most studies could not show an interaction between age and serostatus (Grant & Marcotte, 1999; Van Gorp et al., 1994).

While HIV infected individuals in the advanced stage experienced fatigue and constitutional symptoms, the HNRC could not find a systematic association between neuropsychological and constitutional symptoms (Heaton et al., 1995).

Difference in neuropsychological performance and race has received little attention when testing HIV positive individuals. Although race and neuropsychological performance is well documented (Adams, Boake & Crain, 1982; Inouye, Albert, Mohs, Sun & Berkman, 1993; Kaufman, McClean & Reynolds, 1988; Miller, Bing, Selnes, Wesch & Becker, 1993) only 11 out of 57 HIV-positive studies evaluated by White et al. (1995) commented on race. Grant and Marcotte (1999) found no racial difference in their study when using new African-American norms. Unfortunately, no data could be found on African or South African norms for the tests being used in this study.

No evidence could be found for individuals belonging to a high-risk group for HIV and the development of cognitive impairment (Grant & Marcotte, 1999). However, studies from the HNRC did find that disease progression is being significantly associated with an increased probability of developing neuropsychological dysfunction (Grant & Marcotte, 1999). Earlier studies support this finding (Gibbs et al., 1990).

Although many HIV positive individuals suffer from major depression, the role that major depression has on neuropsychological dysfunction does not seem to be significant (Grant et al., 1993; Hinkin, Van Gorp, Satz, Weisman, Thommes & Buckingham, 1992).

In the light of previous findings, the tests selected by the researcher to include in this study were the Digit Span, Digit Symbol Substitution, and the computerised Wisconsin Card Sorting Test. Selection was based on tests that will be the most sensitive to cognitive deficits in the widest possible sense. It is beyond the scope of this study to differentiate between specific domains of brain damage, or to demonstrate the anatomic location of such damage in HIV positive individuals. The reason for this is that brain damage because of an infective condition is normally diffused in nature (Mann, Neary, & Testa, 1994).

Digit Span

Digit Span is a subtest of the Wechsler Intelligent Scale-Revised (Wechsler, 1981). It includes two tests, namely digits forward and digits backward, which involve different mental activities and are affected differently by brain damage (Lezak, 1995). The test is considered to be a test of short-term memory and attention or as an "auditory vocal sequencing memory test" as described by Bannatyne (1997). Mentally the test involves two phases. In the first phase attention and encoding is necessary for the correct reception of information. In the second phase the testee must recall, sequence and vocalise the information. Testees with distractibility tendencies will have problems with the first phase while those with short-term memory difficulties will struggle in the second phase (Groth-Marnat, 1997). The digit forward is the simpler of the two tests and involves a straightforward rote memory. The digit backward is more complex. With the digit backward test, the testee has to hold memory longer and need to transform it prior to

verbalising the response. The Digit Span performance is considered to be the most susceptible to the effects of anxiety. Low scores will indicate difficulty with concentrating as a result of anxiety or unusual thought processes (Groth-Marnat, 1997).

Both tests consist of seven pairs of random number sequences (appendix A) that the examiner reads aloud at the rate of one per second, and both thus involve auditory attention (Lezak, 1995). The test allows two trials for each span length. With the Digits Backward, the subject's task is to say them in an exactly reversed order on hearing them. The normal raw score difference between digits forward and digits reversed tends to range a little above 1.0, with a spread of 0.59 and 2.00 (Lezak, 1995). In rare protocols, the digit backward is longer than digit forward and is a good indication of excellent numerical abilities (Groth-Marnat, 1997). Limits for Digits Backward performance to be normal should be 4 to 5, with 3 as borderline defective, and 2 as defective (Lezak, 1995). Age only start to play a role after the seventh decade when it drops one point. Normally the scores of digit forward and digit backward are added, but in this study the researcher interpreted them separately because of the risk of losing information as pointed out by previous studies in Lezak, (1995).

Neuropsychological findings show that digit repetition tasks tend to be more vulnerable to left hemisphere involvement than to either right hemisphere or diffuse damage. Glucose metabolism increases bilaterally, however, mostly in anterior dorsal regions during digit repetition (Lezak, 1995). Swierchinsky, (1995), found that the digits forward is most likely to be lowered with left hemisphere involvement, while digits backward is more consistent with diffuse or right frontal involvement.

Digits forward becomes noticeably reduced in length during the later stages of dementia, but are not affected in Korsakoff's psychosis, while patients with significant frontal lobe involvement may substitute bits of over-learned sequence strings (e.g., 3-5-6-7 instead of 3-5-9) or perseverate from the previous series (Lezak, 1995).

Digits backwards involves mental double-tracking in that both the memory and the reversing operations must proceed simultaneously. That means divided and/or shifting of attention. It is thus more of a memory test than digits forward (Lezak, 1995). Bender suggested that the ability to reverse digits, is probably characteristic of normal cognitive function and language processes related to the brain's normal function of temporal ordering. Factor analysis has indicated that both visual and verbal processes contribute to the reversed digit span performance (Lezak, 1995). The role of visual scanning in conceptual tracking has become apparent in studies of such conceptual tracking tasks as digit span reversed, or spelling a long word or name in reverse (Lezak, 1995). The capacity for double or multiple tracking is one of the first most likely to break down with many forms of brain damage (Lezak, 1995).

Testees with high scores in the digit backward test reflect an individual who is flexible with good concentration and a high stress tolerance (Groth-Marnat, 1997). They have the ability to form, maintain, and scan visual mental images formed from auditory stimulus (Lezak, 1995).

Wisconsin Card Sorting Test

The computerised Wisconsin Card Sorting Test (WCST) is a psychological tool used to measure abstract reasoning ability (Ormond Software Enterprises, 1998). It is a variant

of the so-called Weigl (1941) sorting procedures and requires the subject to sort out a deck of cards (Mapou & Spector, 1995). The cards may be sorted according to three criteria, namely colour, form or the number of symbols on each card. After every sort, the subject receives accuracy feedback that he/she must use to figure out what the correct sorting rule is. The sorting rule changes after every 10 successive correct responses, cycling through colour, form and number. The testee must be able to disengage from the previous response set and shift attention to finding and following the new criterion. Neurological impaired individuals may exhibit deficits in benefiting from response feedback, in order to modify their behaviour in response to current task demands (Mapou & Spector, 1995).

The test has a high sensitivity to frontal lobe damage, although the specificity has proved to be relatively low (Lezak, 1995).

The WCST yields a number of indices for isolating the features of an executive processing deficit, and is sensitive to problems in the ability to reason and to shift response sets. Lezak et al. (1995) described the indices as follows:

Perseveration consists of staying with an incorrect response despite being continuously told after each trial that the sorting principle is wrong. The number of perseverative errors and perseverative responses is increased in patients with frontal lobe damage (Lezak, 1995; Ormond Software Enterprises, 1998) and in seropositive asymptotic patients with HIV related attention deficits (Mapou & Spector, 1995).

Failure to maintain set, which occurs whenever the patient switches to a new rule after at least five consecutive trials of using a correct sorting strategy. This behaviour may indicate an attentional problem as opposed to an executive processing deficit.

The number of categories achieved provides information about the patient's ability to recognise the sorting principles.

Non-perseverative errors involve sorting to the wrong principle and may indicate guessing, forgetting the principle, or developing an elaborate hypothesis that is not part of the test.

Digit Symbol Substitution

The Digit Symbol Substitution test is a subtest of the Wechsler Intelligent Scale-Revised (Wechsler, 1981). Rapid visual-motor integration and psychomotor speed is necessary for high scores (Groth-Marnat, 1997). Processes at work involve the learning of unfamiliar tasks, accurate eye-hand co-ordination, attention, short-term memory and the combination of new learned memory of the digit with the symbol to finally execute the drawing of the symbol (Groth-Marnat, 1997). The Digit Symbol Substitution is extremely sensitive to brain damage (Groth-Marnat, 1997; Lezak, 1995).

The substitution task consists of three rows containing, in all, 75 small blank squares, each paired with a randomly assigned number from one to nine. Above these rows is a printed key that pairs each of nine numbers with a different nonsense symbol. Following a practice trial on the first eight squares, the task is to fill in the blank spaces with the symbol that is paired to the number above the blank space as quickly as possible for 90 seconds. The score is the number of squares filled in correctly (Appendix A).

According to Lezak, (1995) the Digit Symbol has the following neurological characteristics:

It is a test of psychomotor performance that is relatively unaffected by intellectual powers, memory, or learning. The copying speed accounts for 72% of the total score value variance. Motor persistence, sustained attention, response speed, and visuo-motor co-ordination play important roles in a normal person's performance, but visual acuity does not (Lezak, 1995).

It is highly sensitive to age (Groth-Marnat, 1997). There is a drastic decrease in response time from the age of 60 and there seems to be no practice effects when this test was given four times with intervals of one week to three months. Women consistently outperform men. There was no relationship found between cognitive ability as measured by WAIS-R and the digit substitution task although the WAIS-R Vocabulary test was related to the Digit Symbol (Lezak, 1995).

The test is sensitive to minimal brain damage, regardless of the location of the lesion and might be influenced by different factors or their interplay (Groth-Marnat, 1997; Lezak, 1995; Reitan & Wolfson, 1993; Swiercinsky, 1978.) This test is extremely sensitive to dementia and, being one of the first tests to decline, it can be used as a measure of the rate at which dementia progresses (Lezak, 1995). Glucose metabolism studies show a bilateral increase in posterior areas, with the right side higher than the left side (Lezak, 1995).

Rapid visual, spatial, and motor co-ordination is necessary for high scores as well as an intact executive action to draw the symbol (Groth-Marnat, 1997). The test is sensitive to perfectionism, anxiety and depression (Groth-Marnat, 1997). High scores indicate excellent visual-motor ability, mental efficiency, rote learning of new material and quick psychomotor reaction. Low scores may have a reduced capacity for visual associative

learning, impaired visuo–motor functioning, and poor mental alertness (Groth-Marnat, 1997).

DIAGNOSTIC ASSAYS

Apart from the above neurocognitive tests, two analytical tests will also be performed. They are the CD4 count and the VLC. A short methodology abstract used by the performing laboratory will follow:

Viral Load Count

Viral Load Counts were done with the Quantiplex Ch Iron Diagnostics test kit. The Quantiplex HIV-1 RNA 3.0 Assay (bDNA) is a signal amplification nucleic acid probe assay for the direct quantitation of human immunodeficiency virus Group M type 1 (HIV-1) RNA in plasma of HIV-1 infected individuals using the Chiron Diagnostic Quantiplex bDNA System 340 (Chiron Diagnostics, 1999). The ability to measure viral load is a valuable tool in the management of HIV-1 infected patients. An increase in viral load has been shown to correlate with progression of HIV-1 disease, as characterised by decreasing CD4+ cell counts and increasing symptoms (Killian, 1997; Ho, Neuman, Perelson, Chen, Leonard & Markowitz, 1995; Saag, 1997). Studies have shown the value of the viral load testing in predicting outcome or survival time, in determining the initiation and effect of an antiretroviral regimen, and in determining viral transmission in the pregnant mother (De la Maza, 1997; Kilian, 1997, Saag, 1997; Swanson, 1997). Highly active antiretroviral therapies often decrease viral load levels below the detection limits of many currently available tests.

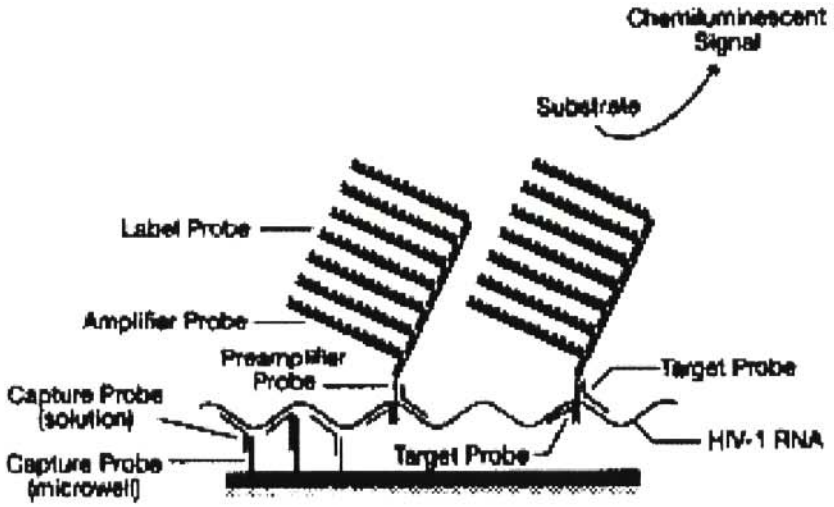


Figure 5.2 A Schematic presentation of the Quantiplex HIV-1 RNA 3.0 Assay

From " Quantiplex HIV-1 RNA 3.0 Assay (bDNA)", by Chiron Diagnostics Corporation (1999) p. 1.

Chiron Diagnostics, (1999) describe the assay principle as follows:

> The Quantiplex HIV-1 RNA 3.0 Assay (bDNA) is a sandwich nucleic acid hybridization procedure (Figure 5.2) for the direct quantitation of HIV-1 RNA in human plasma. HIV-1 is first concentrated from plasma by centrifugation. After HIV-1 genomic RNA is released from the virions, the RNA is captured to a microwell by a set of specific, synthetic oligonucleotide capture probes. A set of target probes hybridises to both the viral RNA and the pre-amplifier probes. The capture probes, comprised of 17 individual capture extenders, and the target probes, comprised of 81 individual target extenders, bind to different regions of the pol gene of the viral RNA. The amplifier probe hybridises to the pre-amplifier forming a branched DNA (bDNA) complex. Multiple copies of an alkaline phosphatase (AP) labeled probe are then hybridised to this immobilised complex. Detection is achieved by incubating the complex with a chemiluminescent substrate. Light emission is directly related to the amount of HIV-1 RNA present in each sample, and results are recorded as relative light units (RLUs) by the analyser. A standard curve is defined by light emission from standards containing known concentrations of beta propiolactone (BPL)-treated virus. Concentrations of HIV-1 RNA in specimens are determined from this standard curve (p.1).

Results are reported as relative light units (RLUs). The light emitted is directly related to the number of HIV-1 RNA copies/ml. A 4-parameter logistic curve is used to model the logarithm of the RLUs as a function of HIV-1 RNA concentration (Chiron Diagnostics, 1999).The lowest value reported by the assay is 50 copies/ml and samples with values greater than 500 000 copies/ml are above the upper limit of quantitation and must be diluted to obtain a quantitative value.

CD4 Lymphocyte Count

Human lymphocytes can be divided into three major populations based on their biologic function and cell-surface antigen expression: T lymphocytes, B lymphocytes, and natural killer (NK) lymphocytes (Nicholson, Hubbard & Jones, 1996; Nicholson, Jones & Hubbard, 1993).

Helper/inducer lymphocytes are a subset of T lymphocytes (CD3+) that are CD4+. The CD3+ CD4+ counts are used to characterise and monitor some forms of immunodeficiency and autoimmune diseases (Cohen and Weetman, 1988; Smolen, Chused, Leiserson, Reeves, Alling & Steinberg, 1982). Determining counts of helper/inducer T lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals (Giorgi & Hultin, 1990). Individuals with HIV typically exhibit a steady decrease of helper/inducer T lymphocyte counts as the infection progresses (Landay, Ohlsson-Wilhelm & Giorgi, 1990).

Suppressor/cytotoxic lymphocytes are a subset of T lymphocytes (CD3+) that are CD8+. CD3+CD8+ counts are also used to characterise and monitor some forms of immunodeficiency and autoimmune diseases (Antel, Bania, Noronha & Neely, 1986). The CD8+ subset is elevated in many patients with either congenital or acquired immune deficiencies, such as severe combined immunodeficiency (SCID) and acquired immune deficiency syndrome (AIDS) (Schmidt, 1989; Giorgi & Hultin, 1990). The CD8+ cell population is often decreased in active systemic lupus erythematosus (SLE), but can also be increased in SLE undergoing steroid therapy.

According to Becton Dickinson (1998), the TriTest used to determine CD4 lymphocytes is based on the following principle:

> When whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leukocyte surface antigens. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scattered and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. TriTEST reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate. A known volume of sample is stained directly in a TRUCOUNT Tube. The lyophilised pellet in the tube dissolves, releasing a known number of fluorescent beads. The absolute number (cells/uL) of positive cells in the sample can be determined by dividing the number of positive cellular events by the number of fluorescent bead events, then multiplying this by the bead concentration.

The reference ranges for CD4+, CD8+, and CD3+ T lymphocytes came from haematological normal adults between the ages of 18 and 65 years and are shown in Table 5.1.

Table 5.1 Representative Reference Ranges in Haematological Normal Adults

Subset	**N**	**Mean**	**Lower 2.5 Percentile**	**Upper 97.5 Percentile**
Helper/Inducer T lymphocytes (cells/uL)	523	880	410	1590
Suppressor/cytotoxic T lymphocytes (cells/uL)	523	490	190	1140
Total T lymphocytes (cells/uL)	516	1410	690	2540

From " TriTEST™ CD4 FITC/CD8 PE/CD3 PerCP Reagent", by Becton Dickinson (1998) p. 1.

CHAPTER 6

PROBLEM DELINEATION

INTRODUCTION

Individuals infected with HIV usually ask questions like, "how long will I live?", "how long will I be able to work?", "will my employer lay me off?" , etc. (International newsgroup, 1995). Uncertainty is a key complaint with these patients and very little individualised information is available for the patient to plan his/her future. As seen previously from the WHO's classification, progression is marked by CD4 counts and physical symptoms. In an attempt to decrease uncertainty and to plan therapy, markers such as CD4 and lately VLC are done as routine investigations for those who can afford it.

However, no screenings are done to predict cognitive deterioration, which happens in at least 30% of cases (Gibbs et al., 1990; Grant & Marcotte, 1999; Wilkie et al., 1990). What is more, these markers are not always satisfactory, because the patient is already experiencing his deterioration and therefore the CD4 count is merely stating a fact and contributes little towards future planning. It is also seen from previous work that the decrease of CD4-counts and, therefore, the replenishment of the immune system, do not always reflect the rate of the development in a particular patient (Mason, 1997; Phair, 1997). Because of the neuropsychological damage associated with the HIV RNA virus, patients infected with the HIV may start to experience slight functional problems in the asymptomatic stage of the disease with normal CD4-counts (Grant & Marcotte, 1999).

It also follows that, if neuropsychological impairment can be predicted, infected employees can be accommodated in less complex tasks. This will grant them the

opportunity to lead an economically viable and meaningful life that should have a positive effect on their mood and immune system and the community as a whole.

Furthermore, it will also provide an opportunity for the patient and employers to familiarise themselves with the possible progression of the disease and future accommodation of the infected employee without unsound fears and stigmas.

PROBLEM STATEMENT

Because the progression of HIV and AIDS varies substantially among individuals as discussed earlier, it is important, for personal and socio-economic reasons, to be able to predict the development of the particular infection. The problem is that CD4-counts only start to play a role as a predictor of disease progression in the symptomatic HIV-positive patient. This means that in most cases, therapy will only start if the CD4 value is below a certain level. From the previous discussion, it has been noted that for at least 30% of individuals, neurocognitive damage would already have started. Therefore, the CD4-count has no predictive or protective value in the early stages of the disease when needed. Thus, there seems to be a need for a reliable quantitative marker that can be used as a prognostic determinant for the development of neuropsychological impairment in HIV positive asymptomatic patients.

AIMS OF THE STUDY

The main aim of the study was to determine whether the Viral Load Count (VLC) is a more reliable predictor for neuropsychological impairment than the CD4-count. If this is the case, the VLC can not only be used as a trigger to start chemotherapy, but can also serve as a marker to commence screening for neurocognitive deficits. The implications of

this study will add to theory building. It will also contribute to the socio-economic well-being of the community as well as the overall protection of the particular individual.

HYPOTHESES

The hypotheses for this study were as follows:

Research Hypothesis I:

Cognitive impairment is a result of HIV infection, therefore persons who are HIV-positive perform poorer on neuropsychological tests than persons who are HIV-negative.

Specific hypotheses derived from the Research hypothesis I

1. Null hypothesis 1:

There is no significant difference in the performance on the WAIS-R Digit Symbol Substitution test between HIV+ and HIV- subjects

2. Null hypothesis 2:

There is no significant difference in the performance on the WAIS-R Digits Forward test between HIV+ and HIV- subjects

3. Null hypothesis 3:

There is no significant difference in the performance on the WAIS-R Digits Backward test between HIV+ and HIV- subjects

4. Null hypothesis 4

There is no significant difference in the performance on the Correct sorts test between HIV+ and HIV- subjects

5. Null hypothesis 5:

There is no significant difference in the performance on the Incorrect sorts test between HIV+ and HIV- subjects

6. Null hypothesis 6:

There is no significant difference in the performance on the Categories achieved test between HIV+ and HIV- subjects

7. Null hypothesis 7:

There is no significant difference in the performance on the Perseverative responses test between HIV+ and HIV- subjects

8. Null hypothesis 8:

There is no significant difference in the performance on the Perseverative errors test between HIV+ and HIV- subjects

9. Null hypothesis 9:

There is no significant difference in the performance on the Non-perseverative errors test between HIV+ and HIV- subjects

10. Null hypothesis 10:

There is no significant difference in the performance on the Move to first category shift test between HIV+ and HIV- subjects

11. Null hypothesis 11:

There is no significant difference in the performance on the Conceptual level response test between HIV+ and HIV- subjects

12. Null hypothesis 12:

There is no significant difference in the performance on the Failure to maintain set test between HIV+ and HIV- subjects

Research Hypothesis II

Viral Load Count (VLC) can be used as an indicator for cognitive impairment and therefore will correlate negatively with performance on neuropsychological tests.

Specific hypotheses derived from the Research hypothesis II

1. Null hypothesis 1:

There is no significant correlation between VLC and performance on the WAIS-R Digit Symbol Substitution test.

2. Null hypothesis 2:

There is no significant correlation between VLC and performance on the WAIS-R Digits Forward test.

3. Null hypothesis 3:

There is no significant correlation between VLC and performance on the WAIS-R Digits Backward test.

4. Null hypothesis 4:

There is no significant correlation between VLC and performance on the Correct sorts test.

5. Null hypothesis 5:

There is no significant correlation between VLC and performance on the Incorrect sorts test.

6. Null hypothesis 6:

There is no significant correlation between VLC and performance on the Categories achieved test.

7. Null hypothesis 7:

There is no significant correlation between VLC and performance on the Perseverative responses test.

8. Null hypothesis 8:

There is no significant correlation between VLC and performance on the Perseverative errors test.

9. Null hypothesis 9:

There is no significant correlation between VLC and performance on the Non-perseverative errors test.

10. Null hypothesis 10:

There is no significant correlation between VLC and performance on the Move to first category shift test.

11. Null hypothesis 11:

There is no significant correlation between VLC and performance on the Conceptual level responses test

12. Null hypothesis 12:

There is no significant correlation between VLC and performance on the Failure to maintain set test

A description of the statistical tests employed to accept or reject the hypotheses formulated here will be supplied in the next chapter.

CHAPTER 7

METHODOLOGY

INTRODUCTION

The purpose of this investigation was to determine whether the VLC is a marker for early neurocognitive deficits, and whether it is able to detect those deficits at an earlier stage than the CD4 marker. The following variables were measured using different measuring instruments: neurocognitive functioning, the immune response of HIV infected individuals and the serum HIV viral load in the infected individual.

RESEARCH DESIGN

A correlational and comparative study was undertaken. The performances on various neuropsychological tests were correlated with progression markers of HIV infection, while the performances on the various neuropsychological tasks were also compared between groups. The HIV negative group served as a control group for the measured constructs of cognitive functioning. In the comparative study, the research design employed is a quasi-experimental design that approximates a true experimental design in that the effects of a non-manipulated variable on a dependent variable are investigated.

SAMPLE

HIV positive individuals, who were referred by their physicians for routine VLC and CD4 counts, were screened by a phlebotomist at the pathology laboratory according to selection criteria. The selection criteria were:

1. To be included in the HIV positive group, individuals had to have a confirmed positive HIV ELISA test.

2. To be included in the HIV negative group, an individual had to have a proven HIV negative ELISA test.
3. A minimum of 12 years academic training (grade 12) was required.
4. Participants had to have a comprehension of the English or Afrikaans Language.
5. There had to be no history of neurological or psychiatric conditions, including colour blindness.
6. They should be on no medication (excluding antiviral medication e.g. AZT). If they were using antiviral medication, the name and duration were noted.
7. The required age group was between twenty and sixty years of age.

Individuals who met the criteria were invited by the researcher to be included in the study. The testing was done during the same appointment as the venesection. After an informed consent, the researcher conducted a structured interview (Appendix C) that was followed by a battery of neurological tests (Appendix A & B). During the structured interview, information was obtained through questioning and covered the following areas:

1. Age
2. Handedness
3. Medical and mental history
4. Information around the estimate time of infection
5. HIV (AZT) treatment status
6. Computer literacy
7. Educational qualifications
8. Occupational status
9. Substance abuse

The researcher did the neuropsychological testing and scoring. Data were recorded on an Excel spreadsheet.

Table 7.1 The Demographic Characteristics of the Sample

	Subject group	
Variable	**HIV Negative**	**HIV Positive**
Sample size (N)	30	20
Age mean	29	31
Minimum years education	12	12
Handedness:		
right handed	30	20
left-handed	0	0
Male	10	5
Female	20	15
HIV medication	0	2
HIV + for <1y	0	5
HIV + for >1y	0	9
Infected period unknown	0	6
Currently employed	30	20

Members of the control group were recruited from local advertisements and treated in the same way as the HIV positive group, with the exception that no venesection was performed, because of their confirmed negative HIV status. The sample size was HIV positive (N=20) and HIV negative (N=30), a total sample of fifty (N = 50).

The tests were done at the only private pathology laboratory in the Northern Province. Patients, who are referred to this laboratory, usually have a medical aid or are financially in a higher income group.

MEASUREMENTS

A schematic operational plan for measuring constructs as described earlier can be seen in Figure 7.1

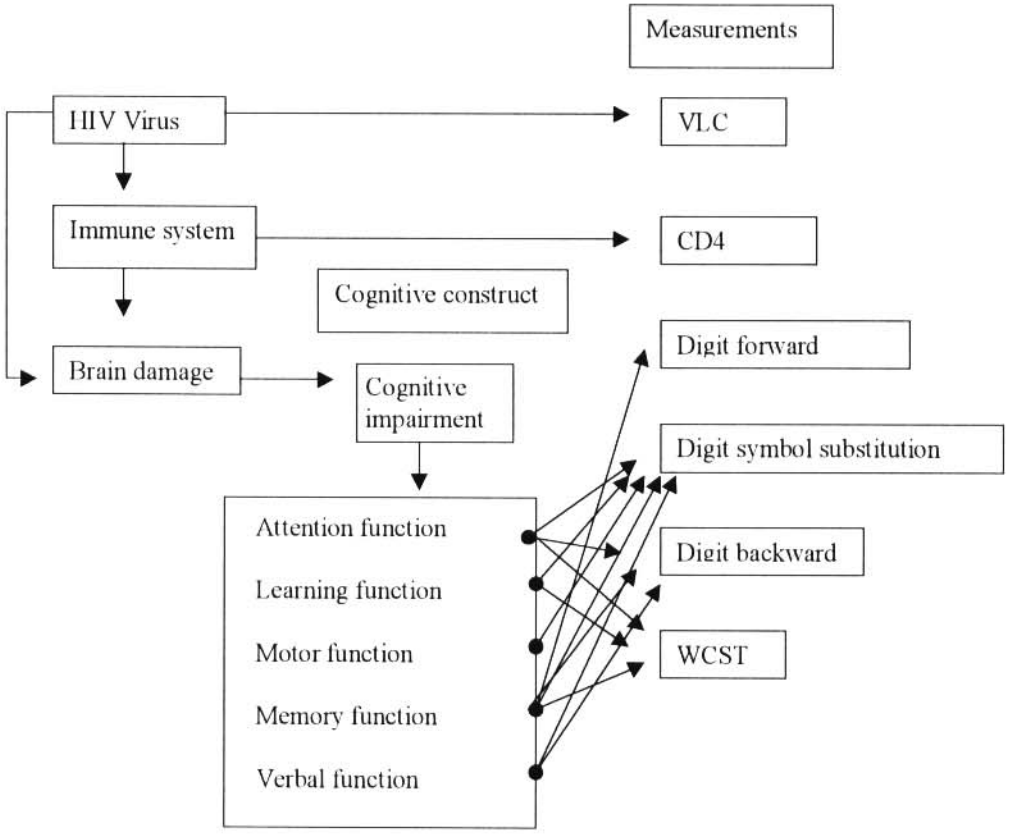


Figure 7.2 A schematic operational plan for measuring cognitive constructs

Neuropsychological assessment

Neuropsychological tests were selected on the basis of their sensitivity towards early detection of brain dysfunction as described earlier (Grant & Marcotte, 1999; Lezak, 1995; Mapou & Spector, 1995). The tests should also be able to detect as diverse as possible damage. Tests selected were:

1. Digit Symbol Substitution test
2. Digits Forward test
3. Digits Backward test
4. Wisconsin Card Sorting test

1. The Digit Symbol Substitution as discussed in a previous chapter measures psychomotor performance. Motor persistence, sustained attention, response speed, and visuo-motor co-ordination play important roles. It is relatively unaffected by intellectual powers, memory, or learning. The test-retest reliability is high and has correlation coefficients of .82 to .88 (Lezak, 1995). The testee simply copies the symbol from the key into the empty square below its corresponding number. The testee is handed the test and is told that this test was to be completed within ninety seconds.

2. The Digits tests as discussed in a previous chapter measure the span of immediate verbal recall. These involve different mental activities and are affected differently by brain damage. Both tests consist of seven pairs of random numbers that the tester reads out aloud at the rate of one per second. Digits Forward measures efficiency of attention. It is sensitive to education, and anxiety tends to affect it negatively. It has a test-retest reliability coefficient of .89 (Lezak, 1995). The testee has to repeat a sequence of numbers in exactly the same order. The test starts with three digits and a second string, if the testee struggles with the first string. If the testee answers correctly, the string becomes one digit longer. The score is the highest string with no errors. The Digits Backwards is a mental double-tracking operation. It measures memory as well as a temporal ordering function. Both visual and verbal processes are involved. The testee has to repeat a sequence of numbers in a reverse order. The test starts with two digits as an example. If the testee answers correctly, the string becomes one digit longer. The score is the highest string with no errors.

3. The Wisconsin Card Sorting test as discussed in a previous chapter is a measure of abstract behaviour and shift of set. The computerised version of Ormond Software Enterprises (1998) was used for testing and scoring. The test has a high sensitivity but low specificity to frontal lobe damage. The number of perseverative errors and perseverative responses is in particular increased with patients with frontal lobe damage (Lezak, 1995). The cards may be sorted according to the colour, form, or number of the symbols on each card. After every sort, the testee receives accuracy feedback that he / she must use to figure out what the correct sorting rule is. The sorting rule changes after every 10 successive correct responses, cycling through colour, form and number. The Card Sorting Test is scored according to the criteria originally recommended by Robert K. Heaton. The norms used are those used by Heaton and represents test performance by 150 healthy subjects [123 males, 27 females; mean age 35.9 years (SD=15.3); mean IQ 114 (11.7)]. The following measures are defined by Ormond Software Enterprises, (1998):

Accuracy & Set Shifting

Correct Sorts: A correct response is one that matches the sorting rule.

Incorrect Sorts: An incorrect response is one that does not match the sorting rule. More than 24 errors are predictive of frontal involvement in focal cases.

Categories Shifted: The sorting rule shifts after every 10 correct consecutive responses. The rule cycles through colour, form and then number. The number of changes is denoted as Categories Achieved and most healthy subjects should achieve at least 4 shifts of the sorting rule.

Perseveration

Perseverative Responses: A perseverative response is defined as one that matches the perseverated-to principle, i.e. a response that would have been correct in the previous stage,

e.g. sorting according to colour when the current rule is form. Not all perseverative responses are errors (see non-perseverative Errors). Twenty or more perseverative responses indicate impairment.

Perseverative Errors: A perseverative error is an incorrect perseverative response. Scores above 13 are predictive of brain damage and above 16 of frontal lobe involvement.

Non-perseverative Errors: A non-perseverative error is one that is incorrect but not perseverative.

Conceptual Ability

Moves To First Category Shift: The number of responses before completion of the first category shift is a measure of conceptual ability.

Conceptual Level Responses: Conceptual level responses are all those correct responses that occurred in runs of three or more. These responses probably reflect an understanding of the correct sorting principle and are unlikely to be due to chance.

Learning & Set Maintenance

Failures to Maintain Set: A failure to maintain set occurs every time the patient makes five correct successive responses, but fails to go on to achieve 10 correct sorts in a row.

Learning-to-Learn: Learning-to-learn refers to the patient's average change in efficiency across successive stages of the Card Sorting Test. This measure is only calculated for subjects who have achieved 3 or more shifts of the sorting rule. Learning-to-learn is calculated as the mean of the differences in the percentage error score for each stage that has been attempted that has at least 10 responses in it. A positive score implies increasing efficiency across categories due to learning, and a negative score implies deterioration in performance over time.

Blood analysis

Analytical blood tests used were the Viral Load Count and the CD4 Lymphocyte count. The Viral Load Count gives a quantitative estimate of the circulating viral particles found in the plasma of infected individuals. Results are reported in copies/ml. A viral load of less than 500 copies/ml has a survival median of more than 10 years, a load of 10000-30000 copies/ml has a survival median of 7.5 years and a viral load of more than 30000 copies/ml has a survival median of 4.4 years (Bartlett, 1999). Methods used for analysis were described in an earlier chapter. The CD4 T Lymphocyte count gives a quantitative indication to what extent the patient has been immune compromised. CD4 cells are reported in cells/mm^3. Only 10% of patients with an AIDS-defining diagnosis have a CD4 count of >200 cells/mm^3 (Bartlett, 1999).

PROCEDURE

Data was collected from October 1999 till June 2000. The blood samples were collected in an EDTA and a clot-wax tube by a registered phlebotomist for CD4 and VLC as requested by the patient's physician. Specimens were identified, barcode labelled and sent by courier in cooled containers to TOGA Laboratories in Johannesburg who specialise in HIV (CD4 and VLC) testing. Venesection and a neuropsychological test battery were performed during the same appointment. Neuropsychological tests were conducted in a quiet private office equipped with a computer and an ordinary writing desk. The researcher scored the neuropsychological test data and combined the data with the blood results in an Excel spreadsheet. Scores were used in their raw form as suggested by Lezak (1995).

Instructions for the Digit Symbol Substitution test were as follows (Appendix A):

Look at these squares. You will notice that each has a digit in the top half and a symbol in the bottom half. Each digit has a separate symbol. Now, at the bottom there are more blocks that contain digits in the top half and an empty bottom half. You must fill in the symbol that belongs to the digit. The first digit is a 2; therefore we use this symbol. (The tester points to the example and completes the first eight squares). Now, I want you to carry on from here by filling in the symbol that belongs to the digit above it. You must complete it in the order that it occurs and you must not skip any one of them. I am going to time you. You must complete as many as you can in ninety seconds.

The Digit Symbol Substitution test was stopped at ninety seconds and scored as follows: a correct substitution scores one point and by adding all the points a total score was obtained for correct substitutions done in ninety seconds. Scores were carried over to the Excel spreadsheet.

Instructions for the Digits Forward test were as follows (Appendix A):

I am going to say a few digits. Listen carefully and when I have finished, you must repeat them in the same order. Here is an example; I say 7 – 9 – 1, then you say...? Here is the next one ... Ready?

A stopwatch was used to time the digits at a rate of 1 per second. The test was stopped when the testee made a mistake in the second pair of digits. The Digits Forward Test was scored by using the highest string with no errors. Each digit in the string scores one point. The scores were carried over to the Excel spreadsheet.

Instructions for the Digits Backward test were as follows (Appendix A):

I am going to say a few digits. Listen carefully and when I have finished, you must repeat them in the reverse order. Here is an example; I say 2 – 4, then you say...? Here is the next one ... Ready?

A stopwatch was used to time the digits at a rate of 1 per second. The test was stopped when the testee made a mistake in the second pair of digits. The Digits Backward Test was scored by using the highest string with no errors. Each digit in the string scores one point. The scores were carried over to the Excel spreadsheet.

The scores of the two digit tests were not combined as in the WAIS-R, because it measured different processes (Lezak, 1995).

Instructions for the Wisconsin Card Sorting test were as follows (Appendix B):

When the test starts, you will see the screen as shown below. Please sort out the deck of cards, using the mouse, by moving each card to the placeholder below the key card you think it matches. Once you have moved a card, you cannot move it again. After each move, you will receive feedback, informing you if the sort was correct or not. Please try to sort the cards correctly. Good luck! Before the test, the testee was given the opportunity to have a practice run to move the cards with the mouse.

The Wisconsin Card Sorting test was scored by the computer and raw scores were carried over to the Excel spreadsheet from a hard copy printout.

Toga Laboratories via the pathologists supplied analysis results for the VLC and CD4. Confidential hardcopy reports were forwarded, with the patient's consent to the referring physician and the researcher. The scores were carried over to the Excel worksheet.

STATISTICAL ANALYSIS

The data analysis was done using the STATISTICA statistical programme. Descriptive statistics were used to describe the results of the blood analysis and performance of the neuropsychological tests.

Pearson's product-moment correlations were computed to investigate any relationships between the performance on the neuropsychological tests and the two markers for HIV progression (CD4 and VLC).

Differences in performance on the various neuropsychological tests were compared for the HIV-positive and HIV-negative subjects using a t -test. The two-sample t -test was selected to test the hypothesis Ho and H_1. The two-sample t -test was also used to compare age between the two groups. This test is a statistical test used to determine whether there is a significant difference between the mean values of two samples.

CHAPTER 8

RESEARCH RESULTS

INTRODUCTION

The purpose of the study was twofold: In the first place to establish whether HIV-positive subjects suffer from neuropsychological impairments and whether there is a difference in the scores on the neuropsychological test between HIV-positive and HIV-negative subjects. Secondly, to establish whether Viral Load Count (VCL) can be used as a marker for early neuropsychological impairment. In this chapter the results of the study will be presented.

RESULTS OF THE STUDY

Descriptive Statistics

Table 8.1 represents the descriptive data obtained for each of the test scores administered as well as for the blood analysis.

Table 8.1 Descriptive Statistics for results of blood analysis and test performance.

	HIV positive			HIV negative		
Variable	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
CD4	383.44	289.12	20	NA	NA	30
Viral Load Count	18042	26086	20	NA	NA	30
Digit symbol substitution	38.100	12.498	20	48.933	7.109	30
Digit Forward	5.850	1.460	20	6.367	1.272	30
Digit Backward	4.000	1.256	20	4.300	0.952	30
Correct Sorts	66.750	19.952	20	73.100	20.996	30
Incorrect Sorts	61.200	19.986	20	54.900	20.996	30
Categories Shifted	3.050	2.564	20	3.500	2.909	30
Perseverative Responses	37.300	30.782	20	24.500	16.639	30
Perseverative Errors	24.150	23.186	20	14.400	11.857	30
Non-perseverative Errors	37.100	17.136	20	40.500	18.288	30
Moves To First Category Shift	23.650	24.014	20	16.567	15.221	30
Conceptual Level Responses	49.350	26.788	20	57.067	28.036	30
Learning-to-Learn	5.850	3.329	20	7.033	3.557	30

STATISTICAL ANALYSIS

T-test

Table 8.2 represents the results of the t-tests performed on each of the neuropsychological tests to see whether or not the performance of the HIV+ and HIV- subjects differ.

Table 8.2 Significance Values for Comparisons between HIV positive (HIV +) and Controls (HIV -)

Variable	**t-value**	**df**	**p-level**
Digit symbol substitution	-3.905*	48	.0002*
Digit Forward	-1.325	48	.1912
Digit Backward	-.960	48	.3421
Correct Sorts	-1.068	48	.2907
Incorrect Sorts	1.050	48	.2947
Categories Shifted	-.561	48	.5773
Perseverative Responses	1.904	48	.0629
Perseverative Errors	1.957	48	.0561
Non-perseverative Errors	-.660	48	.5123
Moves To First Category Shift	1.279	48	.2071
Conceptual Level Responses	-.970	48	.3367
Learning-to-Learn	-1.18	48	.2431

** Marked values are significant at $p < .05$.*

The t-test results showed a significant difference between the HIV positive and the HIV negative group in the performance in the Digit Symbol Substitution test at the $p< 0,5$ level. Although the difference on the Perseverative Errors of the WCST was not

statistically significant, there seems to be an indication that this test is also able to distinguish between the two groups.

The t-test results further showed no significant difference between the HIV positive and the HIV negative group with regard to age.

Pearson's product-momentum correlations

Table 8.3 depicts the correlations between VLC, CD4 and Neuropsychological performance.

Table 8.3 Correlations between VLC, CD4 and performance on Neuropsychological tests

Variable	Correlations (N=20)	
	VLC	CD4
Digit Symbol Substitution	-.46*	-.20
Digit Forward	-.03	-.30*
Digit Backward	-.10	-.08
Correct Sorts	-.06	-.04
Incorrect Sorts	.06	-.04
Categories Shifted	.03	.01
Perseverative Responses	.30*	-.04
Perseverative Errors	.28*	.01
Non-perseverative Errors	-.22	.04
Moves To First Category Shift	.01	.22
Conceptual Level Responses	.03	-.03
Learning-to-Learn	-.01	-.05

** Marked correlations are significant at $p < .05$.*

The results of the Pearson's momentum correlations showed that more neuropsychological tests correlate with the VLC than with the CD4 count. The Digit Symbol Substitution test has a negative correlation with the VLC. The Perseverative Responses test as well as the Perseverative Errors test has a positive correlation with the VLC. Compared to the CD4 count, only the Digit Forward test has a negative correlation.

Hypothesis testing

With regard to the Specific hypotheses derived from the Research hypothesis I, it can thus be said that the:

1. Null hypothesis 1 must be rejected, and alternative hypothesis 1 accepted:
There is a significant difference in the performance on the WAIS-R Digit Symbol Substitution test between HIV+ and HIV- subjects.

2. Null hypothesis 2 must be accepted:
There is no significant difference in the performance on the WAIS-R Digits Forward test between HIV+ and HIV- subjects.

3. Null hypothesis 3 must be accepted:
There is no significant difference in the performance on the WAIS-R Digits Backward test between HIV+ and HIV- subjects.

4. Null hypothesis 4 must be accepted:
There is no significant difference in the performance on the Correct sorts test between HIV+ and HIV- subjects.

5. Null hypothesis 5 must be accepted:
There is no significant difference in the performance on the Incorrect sorts test between HIV+ and HIV- subjects.

6. Null hypothesis 6 must be accepted:

There is no significant difference in the performance on the Categories achieved test between HIV+ and HIV- subjects.

7. Null hypothesis 7 must be accepted:
There is no significant difference in the performance on the Perseverative response test between HIV+ and HIV- subjects.

8. Null hypothesis 8 must be accepted:
There is no significant difference in the performance on the Perseverative errors test between HIV+ and HIV- subjects.

9. Null hypothesis 9 must be accepted:
There is no significant difference in the performance on the Non-perseverative errors test between HIV+ and HIV- subjects.

10. Null hypothesis 10 must be accepted:
There is no significant difference in the performance on the Move to first category shift test between HIV+ and HIV- subjects.

11. Null hypothesis 11 must be accepted:
There is no significant difference in the performance on the Conceptual level response test between HIV+ and HIV- subjects.

12. Null hypothesis 12 must be accepted:
There is no significant difference in the performance on the Failure to maintain set test between HIV+ and HIV- subjects.

With regard to the Specific hypotheses derived from the Research hypothesis II it can thus be said that the:

1. Null hypothesis 1must be rejected and alternative hypothesis 1 accepted:
There is a significant correlation between VLC and performance on the WAIS-R Digit Symbol Substitution test.

2. Null hypothesis 2 must be accepted:
There is no significant correlation between VLC and performance on the WAIS-R Digits Forward test.

3. Null hypothesis 3 must be accepted:
There is no significant correlation between VLC and performance on the WAIS-R Digits Backward test.

4. Null hypothesis 4 must be accepted:
There is no significant correlation between VLC and performance on the Correct sorts test.

5. Null hypothesis 5 must be accepted:
There is no significant correlation between VLC and performance on the Incorrect sorts test.

6. Null hypothesis 6 must be accepted:
There is no significant correlation between VLC and performance on the Categories achieved test.

7. Null hypothesis 7 must be rejected and alternative hypothesis 1 accepted:
There is a significant correlation between VLC and performance on the Perseverative response test.

8. Null hypothesis 8 must be rejected and alternative hypothesis 1 accepted:
There is a significant correlation between VLC and performance on the Perseverative errors test

9. Null hypothesis 9 must be accepted:

There is no significant correlation between VLC and performance on the Non-perseverative errors test.

10. Null hypothesis 10 must be accepted:

There is no significant correlation between VLC and performance on the Move to first category shift test.

11. Null hypothesis 11 must be accepted:

There is no significant correlation between VLC and performance on the Conceptual level responses test.

12. Null hypothesis 12 must be accepted:

There is no significant correlation between VLC and performance on the Failure to maintain set test.

SUMMARY

In this study a meaningful difference was found between the mean values of the HIV positive group and HIV negative control group for the Digit Symbol Substitution test. The study also showed a negative correlation between the VLC and the Digit Symbol Substitution test. There is also a positive correlation between the VLC and both the Perseverative Responses test and the Perseverative Errors test. However, no correlation could be found between the CD4 count and any of these tests. From all the neuropsychological tests used in this study, the CD4 count only correlates negatively with the Digit Forward test (Figure 8.3). This implies the tendency that with a high VLC, the Digit Symbol Substitution test score will be low and the perseverative responses and

perseverative errors will be more. A discussion of these findings will follow in the next chapter.

CHAPTER 9

DISCUSSION OF RESULTS

INTRODUCTION

The purpose of the study was twofold: In the first place to establish whether HIV-positive subjects suffer from neuropsychological impairment and whether there is a difference in the scores on the neuropsychological tests between HIV-positive and HIV-negative subjects. Secondly, to establish whether Viral Load Count (VLC) can be used as a marker for early neuropsychological impairment. A number of variables was investigated, because, based on studies discussed in earlier chapters, it is shown that a high portion of HIV-positive individuals suffer from neuropsychological dysfunction in the early stages of the infection. The neuropsychological impairment already experienced in the earlier phase, may be a runner-up for Aids Dementia that is seen in the later stages of the infection.

For measuring early neuropsychological damage, it was decided to include the following tests in the neuropsychological battery:

1. Digit Symbol Substitution test
2. Digits Forward test
3. Digits Backward test
4. Wisconsin Card Sorting test

The VLC was used for measuring the viral burden the individual is exposed to. It is believed that the higher the VLC, the greater effect it will have on the various target sites.

Once infected by the HIV, there is a continuos challenge to the immune system. Since the HIV primarily depletes the lymphocytes of the immune system, the CD4

lymphocyte count was used as a marker for the effect the HIV has on the immune system. According to the findings of this research, there are significant neuropsychological differences between the HIV-positive and HIV-negative groups. Neuropsychological tests that showed a difference, were the Digit Symbol Substitution test and to a lesser extent two of the Wisconsin Card Sorting sub tests, namely the Perseverative Responses test and the Perseverative Errors test. The Digits Forward and the Digits Backward tests, as well as the remainder of the Wisconsin Card Sorting sub tests could not distinguish between the HIV-positive and the HIV-negative groups.

In this research it was also found that there is a correlation between the VLC and the Digit Symbol Substitution test, and two of the Wisconsin Card Sorting sub tests, namely the Perseverative Responses test and the Perseverative Errors test. There was no correlation found between the VLC and Digits Forward, Digits Backward and the remainder of the Wisconsin Card Sorting sub tests. However, there was a correlation between the CD4 count and the Digits Forward test. In this research no correlation could be found between the CD4 count and the Digit Symbol Substitution test, the Digits Backward or any of the Wisconsin Card Sorting sub tests.

CONCLUSION

This study confirmed that the HIV does cause neuropsychological dysfunction in the early stage of the disease and that the Viral Load Count (VLC) can be used as a marker for early neuropsychological impairment. Since variables like education, handedness and age were controlled, it can be concluded that the HIV status is responsible for most of the variance between the HIV-positive and HIV-negative groups.

Table 9.1 gives a summary of the various variables and the degree to which each neuropsychological- and analytical test discriminate between HIV-positive and HIV-negative groups.

Table 9.1 Discriminating capability and correlation features of the neuropsychological test battery

Variable	Discrimination value	Correlation value with VLC	Correlation value with CD4
Digit symbol substitution	High	High (-)	Medium (-)
Digit Forward	None	None	High (-)
Digit Backward	None	Low (-)	None
Correct Sorts	None	None	None
Incorrect Sorts	None	None	None
Categories Shifted	None	None	None
Perseverative Responses	Medium	High (+)	None
Perseverative Errors	Medium	High (+)	None
Non-perseverative Errors	None	Medium (-)	None
Moves To First Category Shift	None	None	Medium (+)
Conceptual Level Responses	None	None	None
Learning-to-Learn	None	None	None

High are significant at $p < .05$

The various neuropsychological findings can be explained as follows:

As seen from figure 7.2, the Digit Symbol Substitution test and the Wisconsin Card Sorting test measure neuropsychological function over most of the cognitive domains. The Digit Symbol Substitution test and especially the two sub tests of the Wisconsin Card Sorting test, namely the Perseverative Responses test and the Perseverative Errors test, are highly sensitive to neuronal damage but lack specificity (Lezak, 1995). It can therefore be expected that these tests will show first with neuronal damage.

In this study it is shown that the Digit Symbol Substitution test can significantly discriminate between the HIV-positive group and the HIV-negative group. The two sub tests of the Wisconsin Card Sorting test, namely the Perseverative Responses test and the Perseverative Errors test, show a tendency to discriminate between the HIV-positive group and the HIV-negative group. The reason for this apparent insensitivity might be because of the small sample size and the inclusion of individuals that are infected only for a very short period of time. These individuals will have a high VLC with an only recently activated immune system. Neuropsychological test results can therefore also be interpreted that individuals with high VLC and normal neurocognitive screens are most likely to be in the early phase of their infection, or not going to be neuropsychologically affected at all. It is expected that the Perseverative Responses test and the Perseverative Errors test with a bigger sample size and the exclusion of very recently infected persons (less than one year), will also show discriminative properties.

None of the other tests in the battery could discriminate between the HIV-positive group and the HIV-negative group. This can be explained by the fact that tests like the Digits Forward test measure more specific cognitive areas, for example memory, that are still protected by the hierarchical compensatory regions.

The Digit Symbol Substitution test has a significant negative correlation with the VLC. The two sub tests of the Wisconsin Card Sorting test, namely the Perseverative Responses test and the Perseverative Errors test, have a significant positive correlation with the VLC. It can be explained that, since an infective viral infiltration with an activated microglia immune response will be diffuse in nature, therefore neuropsychological tests that measure diffuse damage will be first to register such damage. This means that the higher the VLC, the stronger the challenge on the immune system will be and with a prolonged elevated immune response (microglia), the higher the chance for indirect neural damage. It is acknowledged that not all cases develop like this, but this explanation will surely contribute to explaining the cases with neuropsychological impairment and later dementia. Although the Digits Forward test correlates significantly with the CD4 count, it could not be logically explained.

LIMITATIONS OF THE STUDY

Limitation of this study can be grouped under theoretical, practical and economical aspects

Regarding the theoretical aspects, it is the small sample size (N = 20) that hampered proper statistical differentiation into various sub-populations in order to follow the pattern the VLC follows during progression of the condition. As seen from the progression of the HIV infection, the VLC has a high peak in the initial phase of the infection. During this time, neurocognitive damage is not yet expected, because of the short exposure duration and the plasticity capabilities of the brain (Spreen, 1995). It is only during the second viral load peak that cognitive damage is expected to occur. What further hampered differentiation into various sub-populations, was that 30% of the participants did not know whether they were infected for more than one year or not. It can thus be assumed that the HIV positive group was contaminated by individuals who are not yet cognitive affected but who have a high VLC. These individuals most probably affected the effect of the correlation study.

Furthermore, not all HIV positive individuals are interested in taking medication and therefore referrals for these tests are relatively low. It is also noted that markedly fewer men than women were referred for follow-up investigations. It appeared to the researcher as if men are less willing to pursue the options of therapy. It was also observed that men were more reluctant to participate in a research project or undergo further testing. Although not specifically tested, it appeared as if men were more covert about their HIV serostatus than woman.

On the economical side, a limitation was that, because of the cost involved, when doing the CD4 and VLC, only a relative small sample (N = 20) could be obtained for the purpose of this study. The researcher had to wait until testing was justified for a particular patient. The interval for requiring CD4 and VLC tests is in the region of six to twelve months, depending on the patient's situation.

On the practical side, a limitation was that the patient interview had to be as short as possible for the sake of the patients (thirty minutes at the most). From a trial investigation among HIV positive patients, the researcher learned that patients felt that if

they were kept busy for long periods of time, they would draw attention towards themselves and were afraid that their right to secrecy might be jeopardised.

WHAT WAS LEARNT FROM THE STUDY

At face value, this design does not appear to be complex. It quickly became apparent to the researcher that this was a more complex matter than was thought in the beginning. The HIV and its progression towards disease are complex and not yet fully sorted out as seen from earlier chapters. What was valuable to learn from this study, is the need that HIV positive individuals have for any information that can help them to demystify their condition.

It was also learnt that the more HIV becomes an issue in the media, the more sensitive HIV positive individuals become. HIV positive patients placed a high value on secrecy, and the researcher did his utmost best at all times to respect their concerns.

Concerning Neuropsychological testing, this study indicated that a test like the Digit Symbol Substitution test, may be of great value in predicting the HIV-positive patient's neuropsychological functioning. Because of the sensitivity to brain impairment, its insensitivity to learning on retesting and quick administration time of this test, it can be used as a screening test at clinics and with consultations at the physicians, for the possible prediction of the development of Aids dementia. Preventive anti-viral medication can then be prescribed well in advance when necessary.

IMPLICATIONS FOR FUTURE STUDIES

Since the cost of viral markers is so high, patients will most surely benefit from early and regular neuropsychological testing. Further investigation may even emphasise the need to start antiviral therapy earlier; not only to reduce the viral load, but also to protect the brain from further immune response surges that can have devastating effects on the brain as seen in earlier chapters. Neurological studies could also help to study the efficiency of HIV antiviral drugs and the protection it offers against brain damage.

It can thus be suggested that in future studies a sample of at least 50 HIV-positive candidates will be necessary to be in a position to form subgroups on duration of infection. It can further be suggested that the same neuropsychological battery can be used in future studies.

CONCLUDING REMARKS

Although the researcher found investigations involving HIV-positive individuals morbid and emotionally taxing, it has also served as a stimulus to peruse any idea in an effort to contribute to the fight against this devastating disease. It gave great satisfaction to participate in a study where neuropsychological testing could contribute to the diagnosis and possible preventive treatment to patients.

APPENDIXES

APPENDIX A

N.I.P.N.
N.I.P.R. 82

X. SYFERS VERVANG DEUR SIMBOLE.
X. DIGIT SYMBOL SUBSTITUTION.

NAAM
NAME _____ DATUM
DATE _____

SLEUTEL
KEY

1	2	3	4	5	6	7	8	9
–	⋀	⊐	L	∪	○	∧	×	=

VOORBEELD SAMPLE								TOETS BEGIN TEST BEGINS																		
2	1	3	1	2	4	3	5	3	1	2	1	3	2	1	4	2	3	5	2	3	1	4	6	3		
1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3		
6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7		

AANTAL KORREK 120" NUMBER CORRECT 90"	AANTAL HALF KORREK 120" NUMBER HALF CORRECT 90"	TOTAAL 120" TOTAL 90"

SYFERS IN DIE SELFDE VOLGORDE

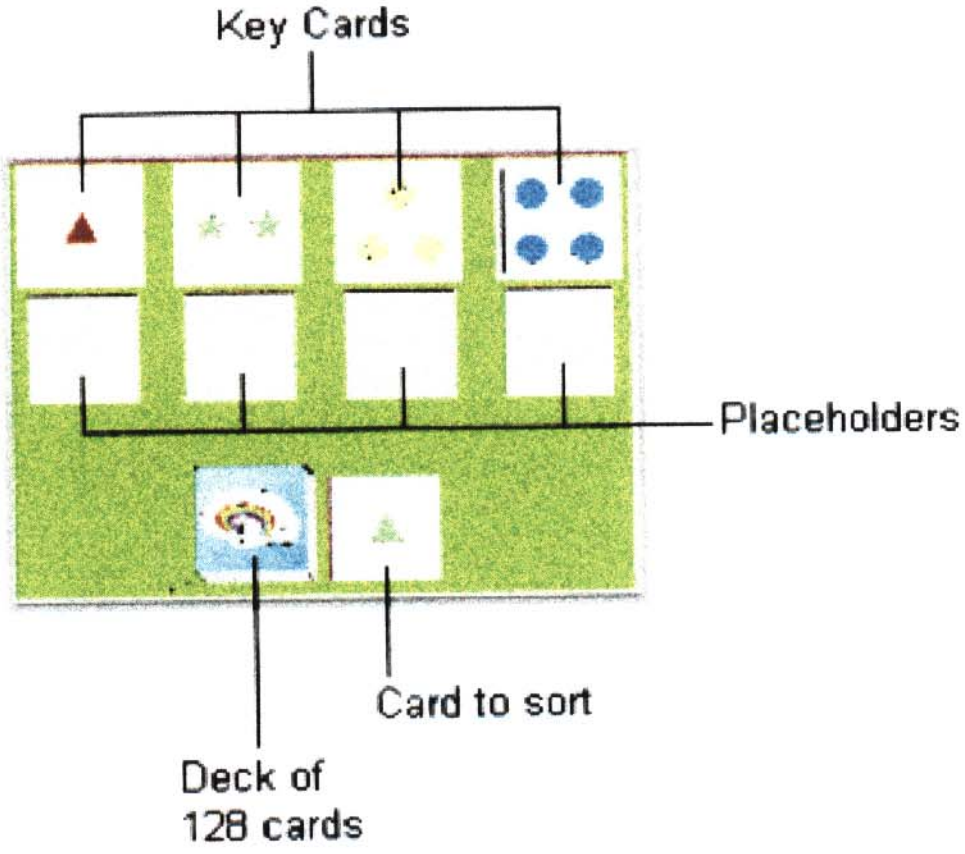
3. 5,8,2 6,9,4,
4. 6,4,3,9 7,2,8,6
5. 4,2,7,3,1 7,5,8,3,6
6. 6,1,9,4,7,3 3,9,2,4,8,7
7. 5,9,1,7,4,2,8 4,1,7,9,3,8,6
8. 5,8,1,9,2,6,4,7 3,8,2,9,5,1,7,4
9. 2,7,5,8,6,2,5,8,4 7,1,3,9,4,2,5,6,8 TELLING_____

SYFERS IN OMGEKEERDE VOLGORDE

2. (2,4) (5,8)
3. 6,2,9 4,1,5
4. 3,2,7,9 4,9,6,8
5. 1,5,2,8,6 6,1,8,4,3
6. 5,3,9,4,1,8 7,2,4,8,5,6
7. 8,1,2,9,3,6,5 4,7,3,9,1,2,8
8. 9,4,3,7,6,2,5,8 7,2,8,1,9,6,5,3 TELLING_____

NAAM:_____ DATUM:_____

APPENDIX B



APPENDIX C

Questionnaire for Participants

ID no _____

Date of testing:_____

Gender: ☐ Male
☐ Female

Age: _____

Highest educational qualification

_____

Occupation: _____

I am willing to participate in a research project concerning the progression of HIV and the possible effects it has on the brain.

_____ _____
Signature of participant Date

1. I have been diagnosed as : ☐ HIV-
☐ HIV +
2. If HIV+, how long ago do you think you have contracted the HI Virus?
☐ < 1 year
☐ > 1year
☐ don't know
3. Are you on medication, HIV therapy included?
☐ no
☐ yes
If yes, name the medication:

_____
4. Do you have chronic conditions or ailments?
☐ no
☐ yes
If yes, please name them

_____

5. Were you ever diagnosed with or treated for psychological problems?

☐ no
☐ yes

If yes, please name them

_____

6. Which is your dominant hand?

☐ right
☐ left
☐ both

7. Do you have computer experience?

☐ no
☐ yes

8. Are you currently employed?

☐ no
☐ yes

9. Have you ever abused drugs or alcohol?

☐ no
☐ yes

If yes, what was the substance you abused?

_____

Thank you for your co-operation. The information you supplied is strictly confidential and will be used for statistical purposes only.

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