

**The role of systemic inflammation and the apolipoprotein E
gene in human immunodeficiency virus-associated cognitive
impairment**

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

Dr Elana van Brakel

VBRELA001

March 2014

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

Abstract

Background: The human immunodeficiency virus-1 (HIV-1) causes cognitive impairment in up to 50% of HIV-infected individuals. High prevalence rates of HIV-associated neurocognitive disorders (HAND) have been reported in South Africa. Systemic inflammation associated with HIV-infection may enhance neuroinflammation, and ultimately neurodegeneration, through signalling from the periphery to the brain. In the South African setting, opportunistic infections remain common. South Africans are predominantly infected with clade C of HIV-1 for which we have limited information about its neurological complications. Indigenous South Africans have a high frequency of the $\epsilon 4$ allele of the apolipoprotein E gene (APOE) and APOE $\epsilon 4$ has been investigated as a risk factor for HAND. A study of the relationships between systemic infection, APOE genotype and cognitive impairment is therefore relevant in our setting.

Aims: This study aimed to define the role of systemic inflammation in the pathogenesis of HAND. We hypothesised that high levels of pro-inflammatory cytokines in the blood would be associated with more severe cognitive impairment. Conversely, better cognition would be associated with high levels of anti-inflammatory cytokines. We also hypothesised that participants with an initial pro-inflammatory cytokine profile would respond better to combination antiretroviral therapy (cART) in terms of cognitive function. Furthermore, the presence of the $\epsilon 4$ allele would be associated with more inflammation as well as greater cognitive impairment prior to cART.

Methods: HIV-positive, cART-naïve participants were recruited from primary care clinics in the Western Cape. Neuropsychological test batteries were used to derive global deficit scores (GDS) at baseline and after at least 9 months of cART. APOE genotyping and measurements of systemic pro- and anti-inflammatory cytokines (IL-1 β , TNF- α , IL-10, and TGF- β) were performed on samples collected at baseline. The GDS, cytokine levels and APOE data were used in correlation analyses.

Results: 114 participants were assessed at baseline and 40 at follow-up. No correlation between systemic cytokine levels and cognitive impairment was found. The $\epsilon 4$ allelic frequency was high in this sample, but was not associated with worse cognitive impairment. IL-10 levels tended to be higher and TGF- β levels tended to be lower in $\epsilon 4$ carriers. TGF- β concentrations were significantly higher in $\epsilon 3$ carriers compared with non- $\epsilon 3$ carriers. Baseline cytokine levels did not predict cognitive response to cART.

Conclusions: This study of young HIV-infected people confirmed the high prevalence of the $\epsilon 4$ allele in our region. While $\epsilon 4$ was not associated with worse cognitive impairment, this association may only be seen in older HIV-infected individuals. The $\epsilon 3$ allele was associated with higher anti-inflammatory cytokine concentrations and may, therefore, confer neuroprotection in HIV-infected patients. Measurements of systemic cytokines appear to have limited value as surrogate markers of neuroinflammation – cerebrospinal fluid measurements may be better. More sophisticated statistical models may be required to relate multiple cytokine measurements to cognitive function.

The role of systemic inflammation and the apolipoprotein E gene in human immunodeficiency virus-associated cognitive impairment

Student: **Dr Elana van Brakel**

Student number: **VBRELA001**

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements for the degree:

Master of Science in Medicine – Neuroscience (MM095)

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of submission:

March 2014

Supervisor:

Assoc Prof M I Combrinck

Divisions of Neurology and Geriatric Medicine

Department of Medicine

UNIVERSITY OF CAPE TOWN

Declaration

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

I empower the university to reproduce for the purpose of research either the whole, or any portion, of the contents in any manner whatsoever.

A handwritten signature in black ink that reads "E. Brakel". The letters are cursive and connected.

Signature:

Date: 16 March 2014

Acknowledgements

I thank the following people whose valuable input made this dissertation possible:

My supervisor, Assoc Prof Marc Combrinck from the Divisions of Neurology and Geriatric Medicine at Groote Schuur Hospital, for his patient, detailed and expert guidance and assistance throughout this project.

Dr John Joska from the Department of Psychiatry at Groote Schuur Hospital who initiated the larger HAND study, for the opportunity to be part of his project and for his guidance and advice.

Dr Helen Cross, Dr Laurian Grace, and Dr Katherine James, my fellow researchers in the Neuroscience group: Helen for help with data collection and sample preparation. Laurian and Katherine for guidance and advice.

Mr Tariq Ganief and Mr Brandon Murugan for assistance with aliquoting the serum samples that I needed from their store of samples.

Drs Judith Hornby and Lindsay Petersen at the Centre for Proteomic and Genetic Research for performing the cytokine assays.

Ms Katya Mauff from the Statistical Consultation Services at the University of Cape Town for consultation and assistance with statistical analyses.

The Department of Neurology at Groote Schuur Hospital, for creating this research opportunity and for providing the research fellowship.

The National Research Foundation for funding this study.

My family and friends for their on-going motivation.

My husband, Riaan: for always believing in me and for your patient love and constant support.

And Ian, our perfect boy, born in the midst of this dissertation: I love you very much!

Contents

Chapter 1: INTRODUCTION	1
1.1 General background and rationale of the study	1
1.1 Problem statement.....	4
1.2 Overview of methodology	6
Chapter 2: LITERATURE REVIEW	7
2.1 Introduction	7
2.2 HIV-1 infection in the CNS	7
2.3 HIV-associated neurocognitive disorders	8
2.3.1 Neuropathogenesis of HAND	8
A. Neuroinvasion (Figure 2.2).....	8
B. Mechanisms of neurodegeneration	10
C. Markers of inflammation	12
D. The link between the peripheral/systemic circulation and inflammation in the CNS.....	20
E. Summary of the neuropathogenesis of HAND (Figure 2.7).....	25
2.3.2 Pathological correlates of HAND	26
2.3.3 Clinical features of HAND.....	27
A. Classification	27
B. Clinical presentation	30
C. Clinical course.....	31
D. Functional impact	31
2.3.4 Management of HAND	32
A. Diagnosis.....	32
B. Treatment.....	35
C. Prognosis	38
2.3.5 Risk or associative factors for HAND	39
2.4 Viral clade	40
2.5 Apolipoprotein E.....	42
2.6 Summary.....	46
Chapter 3: AIMS AND HYPOTHESES.....	48
Chapter 4: METHODOLOGY	50
4.1.1 Participants.....	50

4.1.2	Inclusion criteria	51
4.1.3	Exclusion criteria	51
4.1.4	Study design.....	52
4.1.5	Instruments and measures.....	53
A.	Screening assessments	53
B.	Baseline assessments.....	53
4.1.6	Study procedure	66
4.1.7	Data management and analysis.....	67
4.1.8	Ethical considerations	67
4.1.9	Summary of methods	67
Chapter 5:	RESULTS OF THE STUDY	70
5.1	Participant characteristics	72
5.1.1	Cross-sectional component (n = 114)	72
5.1.2	Longitudinal component (n = 40).....	75
5.2	Presentation of results by hypothesis	78
5.2.1	Hypothesis 1.....	78
5.2.2	Hypothesis 2.....	90
5.2.3	Hypothesis 3.....	94
5.2.4	Hypothesis 4.....	97
5.3	Summary of results	107
5.3.1	Baseline participant characteristics	107
5.3.2	Follow-up participant characteristics	107
5.3.3	Results by hypothesis	107
Chapter 6:	DISCUSSION	109
6.1	Summary of rationale for the study	109
6.2	Summary of what the study entailed.....	110
6.3	Participant characteristics	110
6.4	Results per hypothesis.....	115
6.5	Limitations of the study and recommendations for future research	120
Chapter 7:	CONCLUSIONS	123
Chapter 8:	REFERENCES	124

List of figures

Figure 2.1	HIV-associated neurocognitive disorders (HAND)	8
Figure 2.2	HIV neuroinvasion and multinucleated-giant cell formation	9
Figure 2.3	Mechanisms of neuroprotection and neurodegeneration in AIDS.....	12
Figure 2.4	Common mechanisms by which microglial activation and subsequent pro-inflammatory cytokine release may contribute to neurodegenerative pathology.....	14
Figure 2.5	The relationship between systemic infection, CNS inflammation, neurodegeneration and cognitive impairment in HIV infection	20
Figure 2.6	Schematic representation of the “cross-talk” between components of the BBB and cells of the CNS in microglial activation in AIDS.	23
Figure 2.7	Summary of the neuropathogenesis of HAND	26
Figure 2.8	Proposed decision-tree for diagnosing HIV-associated Neurocognitive Disorders	33
Figure 2.9	Model of CSF HIV-1 infection and its relation to immune activation and treatment responses.....	36
Figure 2.10	Change in Global Deficit Score at baseline and one year of participants initiating cART across groups of severity at baseline.....	37
Figure 2.11	Risk factors for HIV-associated neurocognitive disorders.....	39
Figure 2.12	Current global distribution of HIV-1 subtypes and recombinant forms.....	41
Figure 2.13	APOE allele frequencies in various African populations. In the pie charts, solid sections refer to the APOE ϵ 2 allele, vertically ruled sections to the APOE ϵ 3 allele, and stippled sections to the APOE ϵ 4 allele.	43
Figure 2.14	The relationship between APOE ϵ 4, systemic infection, CNS inflammation, neurodegeneration and cognitive impairment in HIV-1 infection.....	44
Figure 4.1	Bio-Plex sandwich immunoassay.....	63
Figure 4.2	Multiplex immunoassay technology.....	63
Figure 4.3	Flow cytometry-based analysis	64
Figure 4.4	Schematic representation of an immunoassay sandwich-based assay workflow	64
Figure 4.5	Summary of methods relating to original study	68
Figure 4.6	Summary of methods relating to this study	69
Figure 5.1	Flow diagram of participant enrolment and attrition	71
Figure 5.2	GDS groups at baseline	77
Figure 5.3	GDS groups at follow-up.....	

Figure 5.4	GDS groups at baseline for the attrition group	77
Figure 5.5	Comparison of TNF- α across baseline cognitive function groups (outlier removed)	80
Figure 5.6	Comparison of IL-1 β across baseline cognitive function groups (outlier removed)	80
Figure 5.7	Comparison of IL-10 across baseline cognitive function groups (outliers removed)	81
Figure 5.8	Comparison of TGF- β across baseline cognitive function groups.....	81
Figure 5.9	Correlation of IL-10 with baseline GDS (outliers removed).....	83
Figure 5.10	Correlation of TGF- β with baseline GDS.....	83
Figure 5.11	Comparison of baseline CD4 count across groups of cognitive impairment (outlier removed)	84
Figure 5.12	Correlation of TGF- β with baseline CD4 count	86
Figure 5.13	Correlation of IL-10 with baseline CD4 count (outliers removed).....	86
Figure 5.14	Component plot in rotated space	89
Figure 5.15	Box plot of IL-10 by ϵ 4 carrier status (outliers 31 and 55 removed).....	91
Figure 5.16	Box plot of TGF- β by categories of ϵ 4 allele status.....	92
Figure 5.17	Comparison of TGF- β across categories of ϵ 3 allele present or absent	94
Figure 5.18	Presence of the ϵ 4 allele across groups of cognitive functioning	95
Figure 5.19	ϵ 4 allele status across groups of cognitive functioning.....	97
Figure 5.20	Comparison of baseline and follow-up GDS groups	101
Figure 5.21	Comparison of baseline inflammatory profile to GDS change.....	102
Figure 5.22	Comparison of baseline inflammatory profile to GDS change.....	103
Figure 5.23	Comparison of baseline inflammatory profile by groups of GDS change...	104
Figure 5.24	Comparison of baseline inflammatory profile by groups of GDS change...	104
Figure 5.25	Comparison of baseline inflammatory profile across treatment effect groups	105
Figure 5.26	Comparison of baseline inflammatory profile across treatment effect groups	106
Figure 5.27	Correlation of TGF- β with change in GDS	106

List of tables

Table 2-1	Revised research criteria for HAND	29
Table 4-1	Neuropsychological test battery	59
Table 4-2	Summary of the research criteria for HAND	60
Table 4-3	A conversion table for transforming T scores into deficit scores	61
Table 4-4	Assay kits used to determine cytokine profiles in serum samples	62
Table 5-1	Demographic characteristics of the participants.....	72
Table 5-2	Classification into GDS groups based on cognitive scores.....	73
Table 5-3	APOE allelic frequencies (n=113).....	73
Table 5-4	APOE genotypic frequencies (n = 113)	73
Table 5-5	Comparison of baseline characteristics across cognitive function groups	75
Table 5-6	Demographic characteristics of the follow-up group (n = 40).....	76
Table 5-7	Classification into GDS groups based on follow-up cognitive score	77
Table 5-8	Comparison of follow-up and baseline CD4 count	78
Table 5-9	Comparison of median follow-up and baseline CD4 counts.....	78
Table 5-10	Comparison of inflammatory markers across GDS groups	79
Table 5-11	Correlation between baseline GDS and inflammatory markers	82
Table 5-12	Comparison of baseline CD4 across groups of cognitive impairment.....	84
Table 5-13	Comparison of baseline CD4 count with inflammatory markers.....	85
Table 5-14	Component extraction by principal component analysis	87
Table 5-15	Initial exploratory factor analysis	87
Table 5-16	Rotated component matrix	88
Table 5-17	Component matrix for analysis 1.....	89
Table 5-18	Component matrix for analysis 2.....	90
Table 5-19	Comparison of inflammatory markers with the presence of the ϵ 4 allele	91
Table 5-20	Comparison of inflammatory markers across categories of ϵ 4 allele status..	92
Table 5-21	Comparison of inflammatory markers across categories of ϵ 2 allele carrier status	93
Table 5-22	Comparison of inflammatory markers across categories of ϵ 3 carrier status	93
Table 5-23	Presence of the ϵ 4 allele across categories of baseline cognitive functioning	95
Table 5-24	Comparison of ϵ 4 allele status across categories of cognitive functioning....	96
Table 5-25	Change in GDS over time	98

Table 5-26	ART effect on cognitive function over time.....	98
Table 5-27	Comparison of baseline and follow-up GDS groups	100
Table 5-28	Comparison of baseline inflammatory profile to GDS change.....	102
Table 5-29	Comparison of baseline inflammatory profile by groups of GDS change....	103
Table 5-30	Comparison of baseline inflammatory profile across treatment effect	
	groups	105

List of abbreviations

AAN	American Academy of Neurology
AD	Alzheimer's disease
ADLs	Activities of daily living
AIDS	Acquired immunodeficiency syndrome
ANI	Asymptomatic neurocognitive impairment
apoE	Apolipoprotein E
APOE	Apolipoprotein E gene
ART	Antiretroviral therapy
ARV	Antiretroviral
BBB	Blood brain barrier
cART	Combination antiretroviral therapy
CD	Cluster of differentiation
CD4 ⁺	Cluster of differentiation Type 4
CNS	Central nervous system
CPGR	Centre for Proteomics and Genetic Research
CSF	Cerebrospinal fluid
ELISA	Enzyme-linked immunoassay
GDS	Global deficit score
gp120	glycoprotein 120
GSH	Groote Schuur Hospital
HAD	HIV-associated dementia
HAND	HIV-associated neurocognitive disorders
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus Type 1
HIVE	HIV encephalitis
HNRC	HIV Neurobehavioural Research Center
HREC	Human Research Ethics Committee

ICAM	Intercellular adhesion molecule
IHDS	International HIV dementia scale
IL-10	Interleukin 10
IL-1 β	Interleukin 1 beta
iNOS	Inducible nitric oxide synthase
IRIS	Immune reconstitution inflammatory syndrome
LPS	Lypopolysaccharide
MCMD	Minor cognitive motor disorder
MCP-1	Monocyte chemotactic protein-1
MND	Mild neurocognitive disorder
MRI	Magnetic resonance imaging
NF- $\kappa\beta$	Nuclear factor kappa beta
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
PLWHA	People living with HIV/AIDS
RANTES	Regulated on Activation, Normal T cell Expressed
ROS	Reactive oxygen species
SDF-1	Stromal cell derived factor-1
SPSS	Statistical Package for the Social Sciences
Tat	Transcriptional transactivator
TGF- β	Transforming growth factor beta
TNF- α	Tumor necrosis factor alpha
UCT	University of Cape Town
VCAM	Vascular cell adhesion molecule
VCT	Voluntary counselling and testing
vpR	Viral protein R

CHAPTER 1: INTRODUCTION

1.1 General background and rationale of the study

The human immunodeficiency virus type 1 (HIV-1) is a lentivirus that belongs to the family of retroviruses (Cullen 1991; Weiss *et al.* 1985). HIV-1 was first identified in 1983, and it was soon discovered to be morphologically and genetically related to other lentiviruses, known to cause multi-organ disease and death (Gonda *et al.* 1985; Barre-Sinoussi *et al.* 1983).

HIV-1 predominantly infects cluster of differentiation type four (CD4⁺) T lymphocytes in the peripheral blood but also infects other cells of the immune system such as monocytes or macrophages (Ellis *et al.* 2009; Rosenberg and Fauci 1989; McElrath *et al.* 1989).

The central nervous system (CNS) is susceptible to infection by retroviruses and the brain is the most frequently infected organ following HIV-associated lung pathology (Masliah *et al.* 2000; Clements and Zink 1996). HIV-1 is very often associated with debilitating CNS disorders (Grant *et al.* 2005; McArthur *et al.* 2005).

HIV-1 enters the CNS as a passenger within infected monocytes as they cross the blood-brain barrier (BBB). Once inside the brain these monocytes differentiate into macrophages where an HIV reservoir is created (González-Scarano and Martin-Garcia 2005; Rosenberg and Fauci 1989; Koenig *et al.* 1986; Wiley *et al.* 1986). It is likely that HIV-1 infection of the CNS becomes established early after systemic viral infection, and possibly even before seroconversion occurs (Davis *et al.* 1992; Resnick *et al.* 1988).

Upon entering the brain, HIV-1 initiates an inflammatory response (neuroinflammation) by directly activating brain macrophages, microglia, and astrocytes, with the resultant production of excessive neurotoxic pro-inflammatory factors. This immune activation ultimately leads to neuronal damage and loss (neurodegeneration). The latter results in cognitive impairment (Hazleton *et al.* 2010; Wang *et al.* 2006). HIV-associated inflammation, characterized by peripheral monocyte infiltration with activation of brain macrophages and microglia, is therefore believed to play a central role in CNS disease (Deeks 2011; Wang *et al.* 2006).

The brain is however not an “immune-privileged” organ as was previously thought and it does also respond to systemic inflammatory stimuli and immune responses (Wärnberg *et al.* 2009). CNS HIV-1 infection forms an important component of systemic infection and the level of systemic immune activation modulates HIV infection in the CNS (Sinclair *et al.* 2008). A possible trigger of chronic systemic immune activation in HIV-1 infection is elevation of a circulating endotoxin, bacterial lipopolysaccharide (LPS). These elevated levels of LPS are a consequence of translocation of bacterial products from a leaky gut associated with HIV infection (Brenchley *et al.* 2006b). LPS triggers monocyte activation, and activated monocytes play a key role in the pathogenesis of HIV-associated cognitive impairment (Ancuta *et al.* 2008; González-Scarano and Martin-Garcia 2005; McArthur *et al.* 2003; Kaul *et al.* 2001). The numbers of circulating activated monocytes have been clearly associated with the development of HIV dementia (Gartner 2000; Pulliam *et al.* 1997; Tyor *et al.* 1992).

HIV infection leads to increased numbers of circulating monocytes from the bone marrow, and also increased levels of monocytes that circulate in an activated state (Fischer-Smith and Rappaport 2005; Thieblemont *et al.* 1995). The elevated levels of LPS that lead to the immune activated state associated with HIV-infection also cause increased levels of circulating cytokines (Douek 2007; Brenchley *et al.* 2006b). The BBB becomes more leaky and therefore more HIV-infected monocytes can enter the brain (Wang *et al.* 2008; Zhou *et al.* 2006). It thus follows that there is an association between the systemic inflammatory response and the numbers of activated macrophages and microglia in the CNS, neuronal damage and cognitive dysfunction (Glass *et al.* 1995). Numerous studies have recognized systemic inflammation as a key driver of HIV-1 pathogenesis, both in the periphery and in the CNS (Deeks 2009; Brenchley *et al.* 2006b). We can therefore postulate that repeated or chronic systemic infections and inflammation – as is common in HIV disease – may drive and enhance the process of neuroinflammation, and ultimately neurodegeneration, through signalling from the peripheral blood to the CNS.

Neurodegeneration in HIV-infected individuals results in the clinical syndromes of the HIV-associated neurocognitive disorders (HAND). These neurocognitive disorders can range in severity from slight deficits to debilitating dementia (Ellis *et al.* 2009). The classification of HAND comprises three categories based on standardized diagnostic procedures: asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). ANI and MND are characterized by

mild cognitive impairment but with MND there is also mild interference with everyday functioning. HAD (formerly known as AIDS dementia complex) is characterized by severe cognitive impairment causing marked interference with activities of daily living (ADLs). The research criteria further require that the impairment is not due to delirium and cannot be fully explained by comorbid conditions (Antinori *et al.* 2007).

During the early stages of HAND, clinical symptoms may be absent (ANI) or subtle (MND) but they can progress in severity to a disorder of cognitive, behavioural and motor dysfunction (Jannsen *et al.* 1991; Navia *et al.* 1986). HAD is characterized by deficits in learning, motor coordination, verbal fluency, memory, attention and processing speed (Cysique *et al.* 2006).

The $\epsilon 4$ allele of the apolipoprotein E gene (APOE) has been investigated as a risk factor for the development of HAND. The results are, however, controversial with some studies identifying $\epsilon 4$ as a risk factor for developing HAD (Spector *et al.* 2010; Corder *et al.* 1998) while others found no association between the presence of $\epsilon 4$ and an altered rate of progression to HAD (Burt *et al.* 2008; Dunlop *et al.* 1997). It is possible that a correlation between APOE $\epsilon 4$ and HAD is age-dependent (Valcour *et al.* 2004a). The $\epsilon 4$ allele is less effective than $\epsilon 3$ at down-regulating the brain inflammatory response. It has been associated with an increased expression of pro-inflammatory cytokines *in vitro*, and also with the presence of an increased pro-inflammatory immune response *in vivo* - both peripherally and centrally (Vitek *et al.* 2009; Harry *et al.* 2000). APOE $\epsilon 4$ has been shown to accelerate HIV disease progression and increase susceptibility to infection *in vitro* (Burt *et al.* 2008).

As per reports from the developed world (e.g. America and Europe), the widespread use of combination antiretroviral therapy (cART) has significantly altered the nature of HAND, specifically decreasing the incidence of HAD (McArthur 2004; Sacktor *et al.* 2002). The less severe forms of HAND, however, remain common. In general, the initiation of cART improves neuropsychological function, but this improvement is neither full nor universal (Joska *et al.* 2010c). The early cognitive response following cART initiation is likely to be associated with rapid suppression of acute neuroinflammation as well as immune recovery and lower viral loads in both the systemic circulation and in the CNS (Heaton *et al.* 2010; Liner II *et al.* 2008; Gendelman *et al.* 1998).

1.1 Problem statement

HIV-1 is an important global health concern and South Africa has the highest number of people living with HIV/AIDS (PLWHA) according to the WHO/UNAIDS report of 2010 (Joint United Nations Programme on HIV/AIDS 2010).

HIV-1 infection worldwide is classified into subtypes (clades) based on the genetic similarity of the viral strain and the geographic regions where they predominate (Ellis *et al.* 2009). There are at least 10 different genetic HIV-1 subtypes, labelled A to J, responsible for the AIDS pandemic. Sub-Saharan Africa is the region most affected by the HIV epidemic and here HIV-1 clade C infection is most common, accounting for around 50% of infections worldwide. The majority of HIV research has, however, been conducted in Caucasian populations in the developed world – Europe, Australia and the Americas – where HIV infection is almost exclusively due to subtype B. These research findings are responsible for much of our current understanding of the disease progression of HIV-1 and may not be generalizable to HIV clades and human populations in Sub-Saharan Africa or other developing countries (Ellis *et al.* 2009; McCutchan 2006; Kanki *et al.* 1999).

HAND affects around 50% of PLWHA (Heaton *et al.* 2010; Grant 2008; McArthur *et al.* 1993). The first detailed HAND study in South Africa was performed at primary care clinics in Cape Town and high prevalence rates for HAD (25.3%) and MND (42.4%) were reported. These rates were in keeping with other studies from the developing world such as Uganda and India (Joska *et al.* 2010d). It is clear that HAND and, especially, HAD remains prevalent in South Africa despite the use of cART.

African populations have a higher frequency of the $\epsilon 4$ allele of the APOE gene (Burt *et al.* 2008; Eichner *et al.* 2002; Kamboh *et al.* 1989) and up to 37% of native Africans, such as the Khoi San, who live in Southern Africa, are carriers of the $\epsilon 4$ allele (Sandholzer *et al.* 1995). A high frequency of the $\epsilon 4$ variant was also found among IsiXhosa speakers in South Africa, and this was similar to reports of other African populations (Joska *et al.* 2010a).

The use of cART leads to immune recovery and a reduction in the occurrence of opportunistic infections in HIV-infected people. However, in sub-Saharan Africa, opportunistic infections such as tuberculosis, cryptococcal disease, and syphilis remain

common (Sacktor *et al.* 2006). This is also the case in our South African setting where access to antiretroviral therapy (ART) is often delayed and occurs late in the course of the disease (Joska *et al.* 2010d).

The pathogenesis of HAND is not clearly understood but it is believed that HIV enters the CNS very early after infection, resulting in the activation of the macrophages and microglia within the brain. This, in turn, initiates a neuroinflammatory response. There is also mounting evidence that links systemic inflammation with neuroinflammation. We can postulate that repeated systemic infections and inflammation, as commonly occur in HIV disease, may drive and enhance the process of neuroinflammation. The latter ultimately leads to neurodegeneration.

In South Africa HIV-1 clade C is responsible for most of the burden of HIV infection. There are still limited data available about the neurological complications of clade C, as most studies have been done in clade B regions. The frequency of the APOE ϵ 4 allele is high in indigenous southern African populations compared with studies in North America and Europe (Sandholzer *et al.* 1995). A study of the relationship between systemic infection, APOE genotype and cognitive impairment is therefore both biologically important and clinically relevant in our setting.

We hope to provide information about risk or associative factors that might help us to understand why some people with HIV develop cognitive impairment while others do not. This might also help with the early identification of patients at risk for developing HIV-associated dementia and assist us in identifying patients expected to have a poorer neurocognitive response to cART. We hypothesize that a predominantly pro-inflammatory cytokine profile in the peripheral blood of HIV-positive participants, who are not yet on cART (cART-naïve), will correlate with more severe cognitive impairment. Conversely, better cognition will be associated with a predominantly anti-inflammatory cytokine profile. Participants with an initial pro-inflammatory cytokine profile will respond better to cART in terms of cognitive function. The presence of the ϵ 4 allele of APOE will be associated with more inflammation as well as with greater cognitive impairment.

1.2 Overview of methodology

This retrospective, observational study had two components: a cross-sectional baseline analysis correlating various factors with cognitive function, and a longitudinal part. Ethical approval was obtained from the University of Cape Town/Groote Schuur Hospital Human Research Ethics Committee (HREC)(No. REC 263/2007). This study formed part of an existing research project that recruited 167 young adult, HIV-positive, cART-naïve participants from primary care ARV clinics in Cape Town, South Africa.

The cross-sectional component of my project involved a sample of 114 of the original 167 participants who completed baseline assessments. Pro-and anti-inflammatory cytokines were measured in the stored serum samples. Global deficit scores (GDS) (Carey *et al.* 2004a; Heaton *et al.* 1995) were calculated from the neurocognitive tests performed at initial and follow-up visits. These scores were correlated with inflammatory cytokines as well as with the data from APOE genotyping performed in the existing research project.

The longitudinal data of 40 participants who returned for follow-up assessments, after at least 9 months on cART, were analysed. The GDS at follow-up was compared with the GDS at baseline to determine if cognitive function improved, worsened or was maintained after starting ART.

All data, including the results of the cytokine assays, were recorded on a Microsoft Excel spread sheet. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21. The significance level was set at $\alpha = 0.05$.

In the next chapter I shall review the literature on HIV neuropathogenesis, inflammation and apolipoprotein E (apoE).

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter will explore the pathogenesis of HAND with a specific focus on the neuropathogenic role of systemic inflammation and infection. The chapter will start with how HIV-1 infects the CNS and then follow with a detailed discussion of HAND with emphasis on inflammation and inflammatory cytokines. Risk or associative factors of HAND will also be reviewed, with viral clade and apolipoprotein E discussed in detail under separate headings. The chapter will conclude with a summary of the previous research and how it relates to this study.

2.2 HIV-1 infection in the CNS

HIV-1 primarily infects the immune system by binding to CD4⁺ T lymphocytes in the peripheral blood. It is the gradual depletion of the CD4⁺ T cell population that is responsible for the progressively severe immunosuppression that characterizes HIV infection (Schnittman *et al.* 1989; McDougal *et al.* 1985). HIV-1 has also been shown to infect another class of CD4⁺ cells, both *in vitro* and *in vivo*, namely the monocyte/macrophage cells (Rosenberg and Fauci 1989). The CNS is susceptible to infection by retroviruses and particularly by members of the lentivirus family (Clements and Zink 1996). A large number of individuals infected by HIV-1 develop debilitating neurological complications (Joska *et al.* 2011). It is likely that infection of the CNS becomes established early, possibly even before seroconversion occurs. However, it is uncertain if neurological damage begins at this stage or only occurs later after systemic immunosuppression has developed (McArthur *et al.* 2010). In the brain HIV is predominantly found in the macrophages or microglia. Infected monocytes from the periphery cross the blood-brain barrier and differentiate into macrophages. In this way, HIV establishes a reservoir within these perivascular macrophages (González-Scarano and Martin-Garcia 2005; Rosenberg and Fauci 1989). Chronic HIV-1 infection results in neurodegenerative disease (Shapsak *et al.* 2011). Neurodegeneration is, however, not caused by direct infection of neurones, but rather through the infection of macrophages and the resultant inflammatory activation that indirectly cause neuronal damage. Macrophages, therefore, are thought to play a pivotal role in the neuropathogenesis of

HIV-infection and the resulting clinical syndromes of the HIV-associated neurocognitive disorders (Ellis *et al.* 2009; Watkins *et al.* 1990; Price *et al.* 1988).

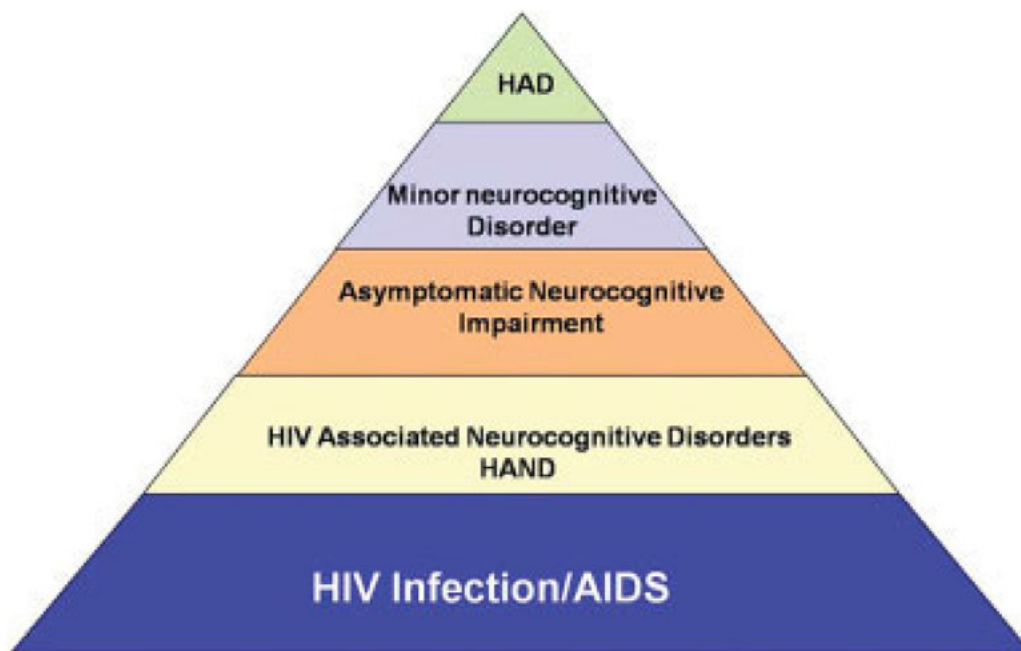


Figure 2.1 HIV-associated neurocognitive disorders (HAND)

(McArthur *et al.* 2010)

2.3 HIV-associated neurocognitive disorders

2.3.1 Neuropathogenesis of HAND

A. Neuroinvasion (Figure 2.2)

HIV-1 is known to enter the CNS within days of primary infection (An *et al.* 1999; Davis *et al.* 1992). Haase and colleagues proposed that HIV and other lentiviruses enter the CNS as passengers in cells that traffic to the brain. The monocyte is a type of CD4⁺ cell in the peripheral circulation that is infected by HIV. The monocyte conceals the virus genome and carries it without detection by the immune system to different sites in the body (Haase 1986; Peluso *et al.* 1985). HIV is able to cross the BBB by this “Trojan Horse” method (a), which remains the most widely accepted model of HIV neuroinvasion (Liu *et al.* 2002). Monocytes traffic to the brain to replenish the perivascular macrophage population

(González-Scarano and Martin-Garcia 2005; Williams *et al.* 2001b; Kaul *et al.* 2001). Once inside the brain they differentiate into macrophages (b) and HIV is believed to establish a reservoir within this perivascular macrophage population (Rosenberg and Fauci 1989; Koenig *et al.* 1986). Perivascular macrophages, situated around CNS blood vessels, are derived from the bone marrow and are continually replaced by monocytes from the circulation. This normal turnover of perivascular cells provides an “open door” for HIV to access the CNS (González-Scarano and Martin-Garcia 2005; Williams *et al.* 2001b) Chemokines are important mediators of the transmigration of monocytes across the BBB and also direct the movement of resident microglia and macrophages. Chemokine (C-C motif) ligand 2 (CCL2) is the most potent chemo-attractant chemokine and plays a key role in the infiltration of HIV-infected monocytes into the CNS and the neuropathogenesis of HAND (Eugenin *et al.* 2006).

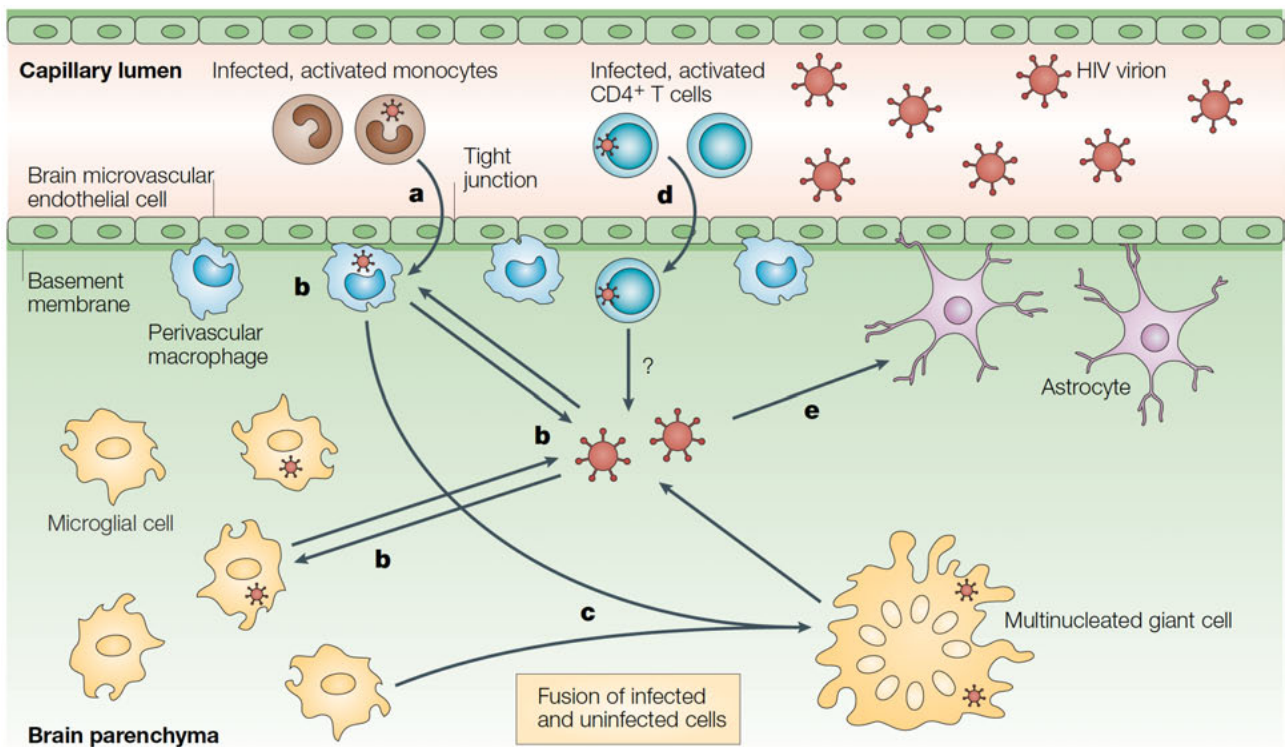


Figure 2.2 HIV neuroinvasion and multinucleated-giant cell formation

(González-Scarano and Martin-Garcia 2005)

Several cell types in the perivascular region of the brain – mainly astrocytes, perivascular macrophages, and microglia – come into direct contact with HIV-infected monocytes from the periphery. Of these, perivascular macrophages and microglia (macrophages of brain

parenchyma) are the most important as they become productively infected with HIV, and are likely to mediate the neurodegeneration seen in patients with HAD (González-Scarano and Martin-Garcia 2005; Williams *et al.* 2001a). Infected CNS cells express HIV-envelope glycoproteins that mediate cell-to-cell fusion with cells that express both CD4 and HIV co-receptor. In the brain this fusion involves infected macrophages and microglia to form multinucleated giant cells (c) that can also produce virus before they die. Multinucleated giant cells are reported to be the hallmark of HIV neuropathology (González-Scarano and Martin-Garcia 2005). HIV-1 might also enter the brain in infected CD4 T⁺ cells, but it is still unclear whether these cells contribute to the pool of replicating virus in the brain (González-Scarano and Martin-Garcia 2005). HIV infection of astrocytes is restricted and not productive (e), as they do not contribute to viral replication (Wang *et al.* 2006). There is no evidence of productive infection of neurones *in vivo* (Sharpless *et al.* 1992). However, Torres-Muñoz and colleagues detected HIV-1 deoxyribonucleic acid (DNA) sequences in brain neurones of a small study sample *in vivo* (Torres-Muñoz *et al.* 2001). Whether this neuronal infection could contribute to neuronal injury and death remains uncertain. Numerous studies have, however, been published that support other mechanisms that mediate the neuropathology of HIV.

In the following section I will elaborate on these pathogenetic mechanisms by discussing the direct and indirect models of neurodegeneration.

B. Mechanisms of neurodegeneration

The exact pathogenesis underlying HIV-1 associated CNS dysfunction remains unclear. The number of HIV-infected cells does not always correlate with the severity and course of clinical disease. This fact supports mechanisms other than direct viral toxicity as the cause of CNS damage (Schouten *et al.* 2011). Shortly after HIV-1 enters the brain and settles in the perivascular macrophages and microglia, these cells become activated and a neuroinflammatory response results. The latter eventually leads to the cognitive impairment observed in some patients (Schouten *et al.* 2011; Hazleton *et al.* 2010). There are at present two main theories of HIV-associated neurodegeneration. Disruption of normal neurological functions can either be caused by a direct mechanism or by an indirect or “bystander” effect. Both these models require initial productive HIV-infection of perivascular macrophages and microglia, and are not mutually exclusive. They might well coexist. Most of the available evidence does, however, support the bystander model as

the predominant mechanism of neurodegeneration in HAND (González-Scarano and Martin-Garcia 2005). Refer to Figure 2.3:

In the direct injury hypothesis, infected cells release HIV proteins, glycoprotein 120 (gp120), transcriptional transactivator (Tat) or viral protein R (vpR) (a). The interaction of these viral proteins and neurones causes damage or injury to the latter (Kaul *et al.* 2005; González-Scarano and Martin-Garcia 2005). It remains uncertain, however, whether the concentrations of viral proteins that are present in the CNS are high enough to mimic the effects observed in *in vitro* experiments (Klasse and Moore 2004; Albright *et al.* 2003). Toxic viral proteins may work in conjunction with factors released from microglia and astrocytes to promote neurodegeneration.

In the indirect, or “bystander” model, neuronal death is mainly a consequence of the neuroinflammatory process. It has even been proposed that after the inflammatory process becomes established, it can be self-propelled and maintained even if the virus were to be cleared from the CNS. Nath and colleagues termed this phenomenon, where HIV-1 proteins act as a trigger to initiate a cascade of inflammation, the “hit and run phenomenon” (Nath *et al.* 1999). Once activated by HIV, macrophages and microglia release numerous soluble products, including cytokines, chemokines, quinolinic and arachidonic acids, platelet activating factor, nitric oxide and growth factors. These factors can exert neurotoxic effects through various mechanisms such as excitotoxicity, oxidative stress and neuronal apoptosis (a). These released factors also further activate macrophages and/or microglia and promotes the proliferation and activation of astrocytes (b). Activated astrocytes modify the permeability of the BBB and promote migration of monocytes to the brain (c). Activation of astrocytes also leads to increased levels of intracellular calcium, glutamate and other neurotoxins resulting in the excitotoxic death of neurons (d).

Although a number of the non-viral products secreted by activated macrophages, microglia and astrocytes, have proven to be neurotoxic, some may promote neuronal survival. Growth factors and some of the β -chemokines might play a neuroprotective role (e) and ultimately neurodegeneration follows a breakdown in the usual balance between neuroprotection and neurotoxicity (Kaul *et al.* 2005; González-Scarano and Martin-Garcia 2005; Albright *et al.* 2003).

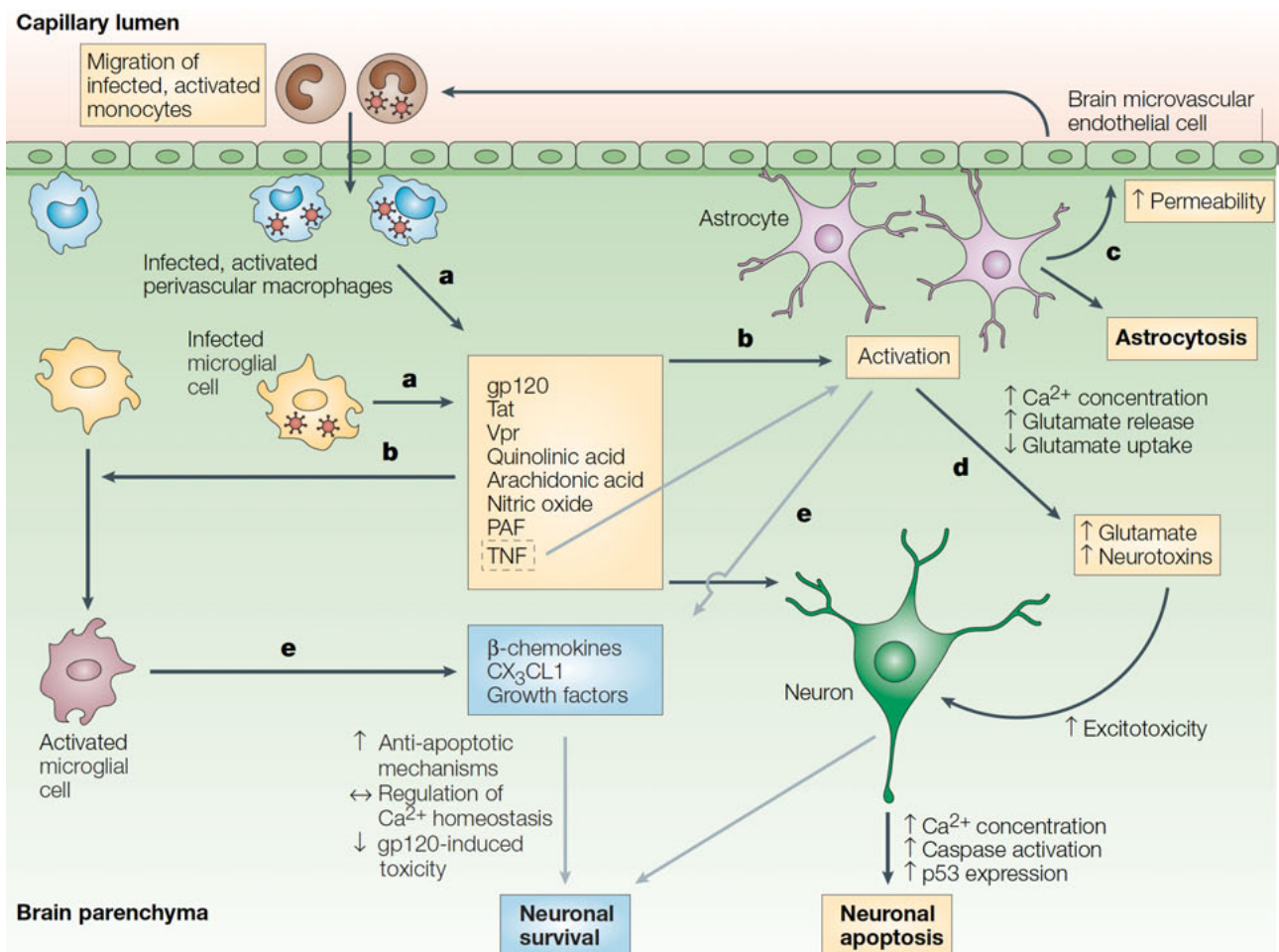


Figure 2.3 Mechanisms of neuroprotection and neurodegeneration in AIDS

(González-Scarano and Martin-Garcia 2005)

In the next section I will elaborate on the process of neuroinflammation by discussing the role of specific cytokines in the pathogenesis of HAND.

C. Markers of inflammation

In the previous section we've noted that HIV-1 does not infect neurones directly but induces damage indirectly through the release of neurotoxic mediators from activated uninfected, and/or HIV-1-infected macrophages and/or microglia. These mediators include both cellular activation products and viral proteins. Activated macrophages accumulate in the brains of infected individuals through monocytes that cross the BBB, carrying virus into the CNS and establishing local infection. Macrophages also accumulate through the recruitment of additional monocytes from the periphery by chemotactic factors released from infected and activated macrophages or microglia in the

CNS. These activated macrophages and microglia release a number of cytokines and other small molecules as well as viral proteins that influence uninfected adjacent cells and activate them, thus amplifying the inflammatory cascade. These viral proteins and cellular products also have neurotoxic properties and can ultimately lead to neuronal injury and death directly, or through inducing activation of astrocytes (Yadav and Collman 2009).

Cytokines are humoral proteins with multiple functions that regulate individual cells and tissues under physiological or pathological conditions. They are important mediators of communication between the brain and endocrine or immune systems, and they play a key role in the induction and regulation of inflammation in the CNS. The latter can cause progression or inhibition of neurodegeneration (Wang *et al.* 2006; Merrill and Benveniste 1996). Multiple pro- and anti-inflammatory cytokines are elevated in the CNS and/ or the cerebrospinal fluid (CSF) of patients with HIV-dementia. Pro-inflammatory cytokines are mostly neurotoxic; anti-inflammatory cytokines are generally viewed as neuroprotective. It is, however, not as simple as this and the functions of different cytokines overlap significantly. The role of a specific cytokine can also change over time (Allan and Rothwell 2003). Cytokines in the CNS can be released as a result of direct viral infection or through the stimulation of uninfected mononuclear phagocytes by shed viral proteins to express elevated levels of cytokines (Rappaport *et al.* 1999; Sundar *et al.* 1991). Many of these cytokines can augment further expression of cytokines leading to a pro-inflammatory environment in the CNS. Additionally, several cytokines are directly or indirectly neurotoxic and can contribute to neuronal injury (Yadav and Collman 2009). The most extensively studied cytokines in HIV-dementia are tumour necrosis factor alpha (TNF- α) and interleukin-1 beta (IL-1 β), which are thought to play a major role in inducing neuronal death (Brabers and Nottet 2006; Wang *et al.* 2006; Zhao *et al.* 2001a; Achim *et al.* 1993). The anti-inflammatory cytokines, interleukin-10 (IL-10) and transforming growth factor beta (TGF- β), on the other hand, have been shown to have neuroprotective effects (Allan and Rothwell 2003). Overall, it is probably the balance between pro- and anti-inflammatory cytokines in the brain that will determine whether injured neurons recover or die (Boche *et al.* 2003; Nguyen *et al.* 2002).

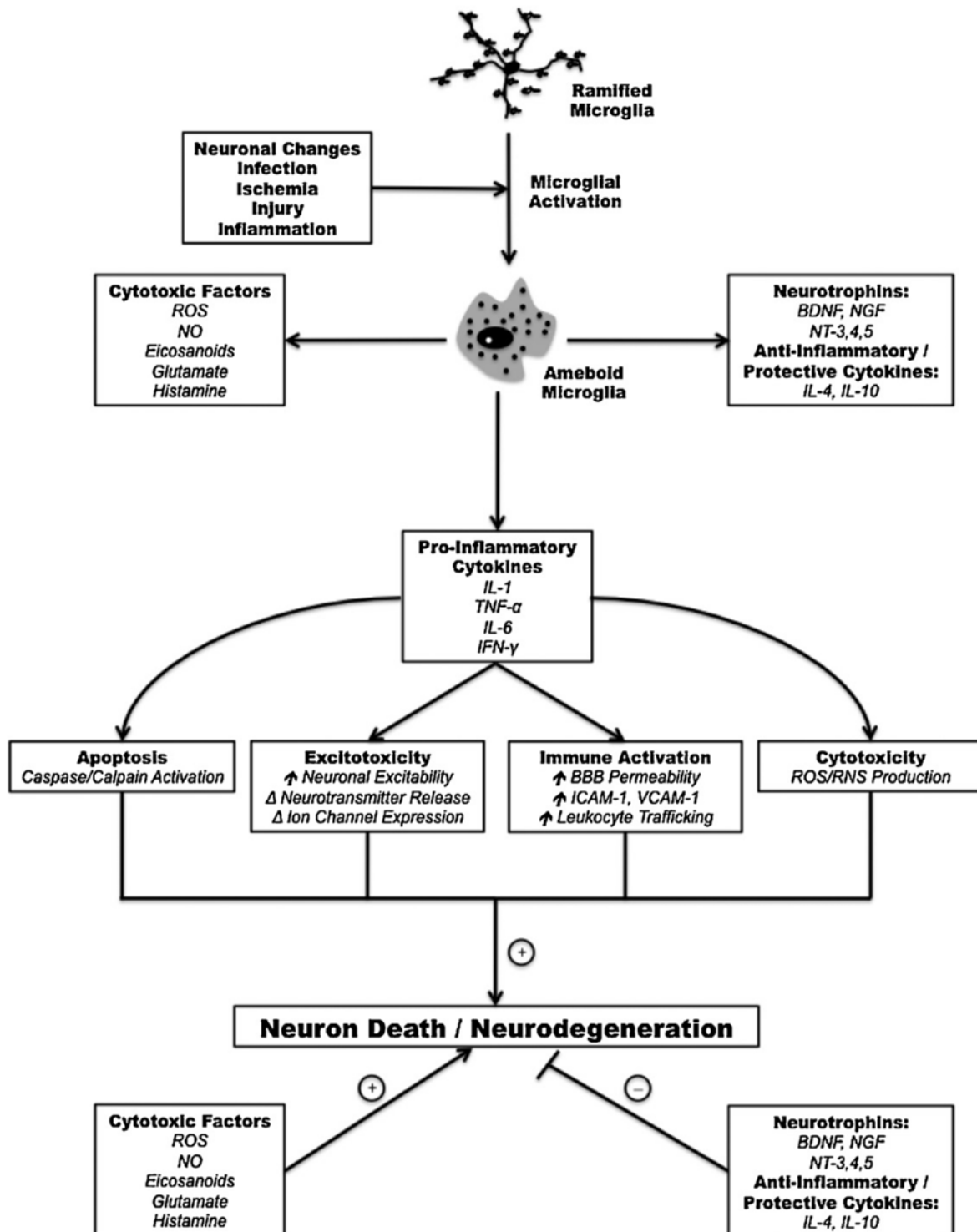


Figure 2.4 Common mechanisms by which microglial activation and subsequent pro-inflammatory cytokine release may contribute to neurodegenerative pathology.

(Smith *et al.* 2012)

Tumour necrosis factor alpha (TNF- α)

TNF- α is elevated in both the brain and CSF of patients with HAD (Tyor *et al.* 1992). When exposed to either gp120 or tat, *in vitro*, macrophages/microglia express elevated levels of TNF- α (Nuovo and Alfieri 1996; Yeung *et al.* 1995).

TNF- α plays an important role in facilitating the entry of HIV-infected cells into the brain: It increases the permeability of the BBB and induces the expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin on astrocytes and endothelial cells. These proteins allow HIV-1 infected monocytes to transmigrate into the CNS and they up-regulate the expression and release of various chemokines in the CNS that attract monocytes and macrophages (Winkler and Beveniste 1998; Fiala *et al.* 1997; Hurwitz *et al.* 1995; Collins *et al.* 1995). Gelbard and colleagues found that TNF- α might exert toxic effects by over stimulating the N-methyl-D-aspartate (NMDA) glutamate receptor on neurones (Gelbard *et al.* 1993). In conjunction with stromal cell-derived factor 1 (SDF-1), a chemokine, TNF- α also increases the release of the neurotransmitter glutamate from astrocytes and microglia (Bezzi *et al.* 2001). In addition, TNF- α inhibits the re-uptake of glutamate by astrocytes. Along with gp120, it also stimulates macrophages and microglia to release L-cysteine, which is a precursor to glutamate (Yeh *et al.* 2000). These resultant excessive levels of extracellular glutamate may contribute to neuronal cell death in HAD (Yadav and Collman 2009).

In vitro exposure of neurons to gp120 and tat results in elevated levels of sphingomyelin and ceramide, a process probably mediated by TNF- α and IL-1 β expressed by macrophages and microglia (Yeung *et al.* 1995). Sphingomyelin is a major class of membrane phospholipids that, when hydrolysed by sphingomyelinase, produces ceramide. Ceramide is a second messenger thought to be involved in apoptosis. Both TNF- α and IL-1 β are thought to activate membrane-associated sphingomyelinase, and sphingomyelin and ceramide levels have been shown to be elevated in the brain tissue and CSF of HIV-1 infected patients with cognitive impairment. TNF- α -induced sphingomyelin and ceramide can, furthermore, activate HIV-1 transcription. There is also evidence that ceramide can lead to the production of neurotoxic radical oxygen species (ROS) (Haughey *et al.* 2004). TNF- α may play a further neurotoxic role by inducing the expression of fractalkine (FKN) in neurones and astrocytes. When FKN binds to its receptor CX3CR1, it induces adhesion, chemo-attraction and activation of other inflammatory cells including macrophages and microglia (Ericksen *et al.* 2003). TNF- α

also induces the production of platelet activating factor (PAF) that is another significant mediator of neurotoxicity in HAD (Gelbard *et al.* 1994).

TNF- α alters the function of astrocytes, macrophages and microglia, which ultimately leads to the release of more pro-inflammatory cytokines. Astrocytes stimulated by TNF- α also appear themselves to be susceptible to apoptosis (Saha and Pahan 2003).

Besides the effects that TNF- α can exert individually, it has also been demonstrated to enhance the toxic effects of other inflammatory mediators present in the brains of patients with HAD (Brabers and Nottet 2006).

TNF- α is pro-inflammatory, can lead to further activation and recruitment of macrophages and microglia, and is also a direct neurotoxin. Its multiple effects ultimately result in an accumulation of excessive amounts of excitatory molecules in the extracellular space. This leads to overstimulation of the NMDA receptor, resulting in excessive calcium (Ca^{2+}) influx and the formation of nitric oxide (NO) and superoxide anion, which results in neuronal apoptosis or necrosis. TNF- α can also activate astrocytes and decrease the uptake of the excitotoxic neurotransmitter glutamate, thus potentiating glutamate neurotoxicity (Yadav and Collman 2009; Brabers and Nottet 2006; Bonfoco *et al.* 1995; Lipton 1994).

There are, however, data suggesting that TNF- α can have neuroprotective properties. TNF α activation of nuclear factor kappa beta (NF- κ B) can protect neurones against apoptosis induced by oxidative and metabolic insults and also by enhancing cellular calcium homeostasis (Glazner and Mattson 2000; Barger *et al.* 1995). Data in support of the neuroprotective properties of TNF- α are, however, incomplete and these have not always been acquired in relation to HAD studies (Brabers and Nottet 2006).

Interleukin-1 beta (IL-1 β)

IL-1 β is another cytokine found to play a central role in the inflammation and neurotoxicity associated with HAD (Brabers and Nottet 2006; Tyor *et al.* 1992). It shares many neurotoxic properties with TNF- α (Yadav and Collman 2009). IL-1 is a macrophage cytokine whose expression is tightly regulated. It is produced as pro-IL-1 β and is then cleaved by the IL-1 β converting enzyme, caspase-1, to produce active IL-1 β (Thornberry *et al.* 1992). Neurones, endothelial cells and astrocytes express caspase-1, but

expression is highest in activated macrophages and microglia (Zhao *et al.* 2001a). IL-1 β activates astrocytes with the subsequent production of TNF- α and inducible nitric oxide synthase (iNOS) via activation of NF- κ B (Jana *et al.* 2005; Zhao *et al.* 2001a). The formation of iNOS results in the expression of NO, which is neurotoxic and also increases BBB permeability (Chao *et al.* 1996). When microglia are exposed to gp120, they produce ROS and also overexpress IL-1 β (Viviani *et al.* 2001). Like TNF- α , IL-1 β is responsible for NMDA receptor-mediated increased levels of toxic Ca²⁺ levels in neurones (Yeh *et al.* 2000). It can also induce expression of ICAM, VCAM and E-selectin on endothelial cells and astrocytes, leading to increased monocyte infiltration into the brain (Winkler and Beveniste 1998; Collins *et al.* 1995). IL-1 β furthermore induces the expression of monocyte chemoattractant protein-1 (MCP-1) by astrocytes, a potent chemo-attractant for mononuclear phagocytes (Oh *et al.* 1999). IL-1B can also induce the expression of ceramide, thought to play a role in ROS formation and neuronal apoptosis (Haughey *et al.* 2004).

Like TNF- α , IL-1 β has been shown to play a neuroprotective role in HAD through the induction of NO production. Depending on its redox status, NO has been shown to limit viral replication and reactivation in acutely infected cells (Mannick *et al.* 1999). IL-1 β can induce the expression of chemokine (C-C motif) ligand 5 (CCL5), also known as Regulated on Activation, Normal T Cell Expressed (RANTES), from astrocytes, which can protect neurones against apoptosis (Kim *et al.* 2004; Bruno *et al.* 2000). RANTES can also prevent HIV-1 infection of macrophages and microglia by competitively binding to its receptor, C-C chemokine receptor type 5 (CCR5) (Brabers and Nottet 2006).

Interleukin-10 (IL-10)

IL-10 is an important anti-inflammatory cytokine that can limit and terminate the inflammatory response. It also regulates the differentiation and proliferation of immune cells. Numerous studies, both *in vivo* and *in vitro*, suggest that IL-10 has a major role in inflammatory, malignant and autoimmune diseases (Asadullah *et al.* 2003). IL-10 controls inflammatory processes by potently down-regulating the expression and actions of pro-inflammatory cytokines such as IL-1 and TNF- α , chemokines, adhesion molecules, as well as antigen-presenting and co-stimulatory molecules in brain monocytes or macrophages (Konsman *et al.* 2002; Kelly *et al.* 2001; Moore *et al.* 2001). IL-10 most likely exerts this anti-inflammatory property by inhibiting the transcription factor NF- κ B. NF- κ B is responsible for the production of numerous pro-inflammatory proteins (Wang *et al.* 1995).

Macrophages are the major source of IL-10. They can be stimulated to produce IL-10 by several endogenous and exogenous factors amongst which are endotoxin and TNF- α (Meisel *et al.* 1996; Platzer *et al.* 1995). IL-10 supports the maturation of monocytes to macrophages (Allavena *et al.* 1998). In general, it inhibits all activities that promote inflammation while enhancing the immuno-suppressive activities of these monocytes and macrophages (Buchwald *et al.* 1999).

Transforming growth factor beta (TGF- β)

TGF- β belongs to a family of multifunctional peptides that control the growth, differentiation and functioning of a variety of target cells (Roberts *et al.* 1990). It is a potent cytokine with an essential role in active immune suppression (Mantel and Schmidt-Weber 2011). Wahl and colleagues demonstrated that TGF- β 1 is present in the brains of AIDS patients, but not in normal brain tissue, and its presence is likely to be neuroprotective (Wahl *et al.* 1991). TGF- β is normally found in high concentrations in HAD (Dhar *et al.* 2006). Monocytes are responsible for TGF- β production, and TGF- β 1 is the isoform most abundantly expressed (Lotz and Seth 1993). TGF- β plays a neuroprotective role in neurodegenerative diseases by limiting inflammation (Flanders *et al.* 1998), and by protecting glial cells against injury by nitric oxide synthase (Bottner *et al.* 2000). TGF- β 1 has been shown to directly down-regulate the production of the pro-inflammatory cytokines IL-1 β and TNF- α . The presence of TGF- β before the excitotoxic insult may make the acute inflammatory response less aggressive (Bogdan *et al.* 1992). TGF- β was one of the first cytokines shown to induce monocyte deactivation (Chantry *et al.* 1989; Tsunawaki *et al.* 1988). It can also stimulate or inhibit expression of other cytokines in macrophages and microglia (Musso *et al.* 1990; Chantry *et al.* 1989).

Although TGF- β has been considered an anti-inflammatory cytokine, Matsumura *et al.* provided evidence that TGF- β acts as a pro-inflammatory cytokine in the brain (Matsumura *et al.* 2008). Their research also showed that the increase in TGF- β concentrations in the CSF preceded that of other pro-inflammatory cytokines in the blood. This implies that the increase in TGF- β in the CSF was not likely to have been induced by cytokines in the serum. A possible alternative system for transmission of the peripheral inflammation/infection signal to the brain has therefore been proposed: peripheral information may be transmitted via the vagus nerve (Romanovsky 2004), leading to this early increase of active TGF- β in the brain (Matsumura *et al.* 2007). Another possible mechanism by which TGF- β contributes to the pro-inflammatory environment is through

chemotaxis of monocytes, thus causing their migration to the brain. Even at very small concentrations, TGF- β is an extremely potent chemotactic agent for human peripheral blood monocytes. At higher concentrations, TGF- β can also activate these recruited monocytes to secrete other pro-inflammatory cytokines such as IL-1 and TNF- α (Wahl *et al.* 1987). The ability of TGF- β to further activate the synthesis of TNF- α is probably a major pathway leading to the destruction of neurones (Rappaport *et al.* 1999; Sawaya *et al.* 1998).

Wahl and colleagues suggested an association between HIV-1 infection and the production of TGF- β 1 (Wahl *et al.* 1991). TGF- β stimulates replication of HIV in infected monocytes and also promotes virus spreading under certain *in vitro* conditions (Lotz and Seth 1993). Tat protein has been shown to increase the expression of TGF- β 1 in the brain (Sawaya *et al.* 1998; Cupp *et al.* 1993). TGF- β 1 levels were significantly elevated in the CSF of patients with HIV-1 infection and CD4<500cells/mm³, compared to HIV-positive patients with CD4>500cells/mm³ and HIV-negative controls (Johnson *et al.* 2004).

From the literature it is clear that TGF- β plays a highly divergent role in the CNS and that its effects are bidirectional, pro-inflammatory or anti-inflammatory, depending on the type and status of the cell that receives its action (Matsumura *et al.* 2008).

Summary

Cytokines comprise a group of small polypeptides with tremendous diversity in their potential actions (Smith *et al.* 2012). It is clear that when cytokines are expressed inappropriately, their deleterious effects can exceed the beneficial role that they play in resistance of the host to infection. Plenty of evidence supports the active participation of pro-inflammatory cytokines in neuronal death, astrogliosis and demyelination found with immune and non-immune brain injury. Overexpression of TGF- β can convert its protective functions to a pathogenic state (Lotz and Seth 1993).

In the above paragraphs, I discussed how HIV-1 invades the brain early when infected monocytes migrate across the BBB. This leads to HIV-1 infection of the resident macrophages and microglia in the brain, which initiates a neuroinflammatory process that ultimately causes the neurocognitive disorders associated with HIV-1. Besides this initial neuroinvasion and the resulting neuroinflammatory process, other factors associated with progressive HIV infection in the periphery may be necessary to eventually trigger the

development of HAND (Gartner 2000). In the following paragraphs I will discuss the link between systemic inflammation and inflammation in the brain.

D. The link between the peripheral/systemic circulation and inflammation in the CNS

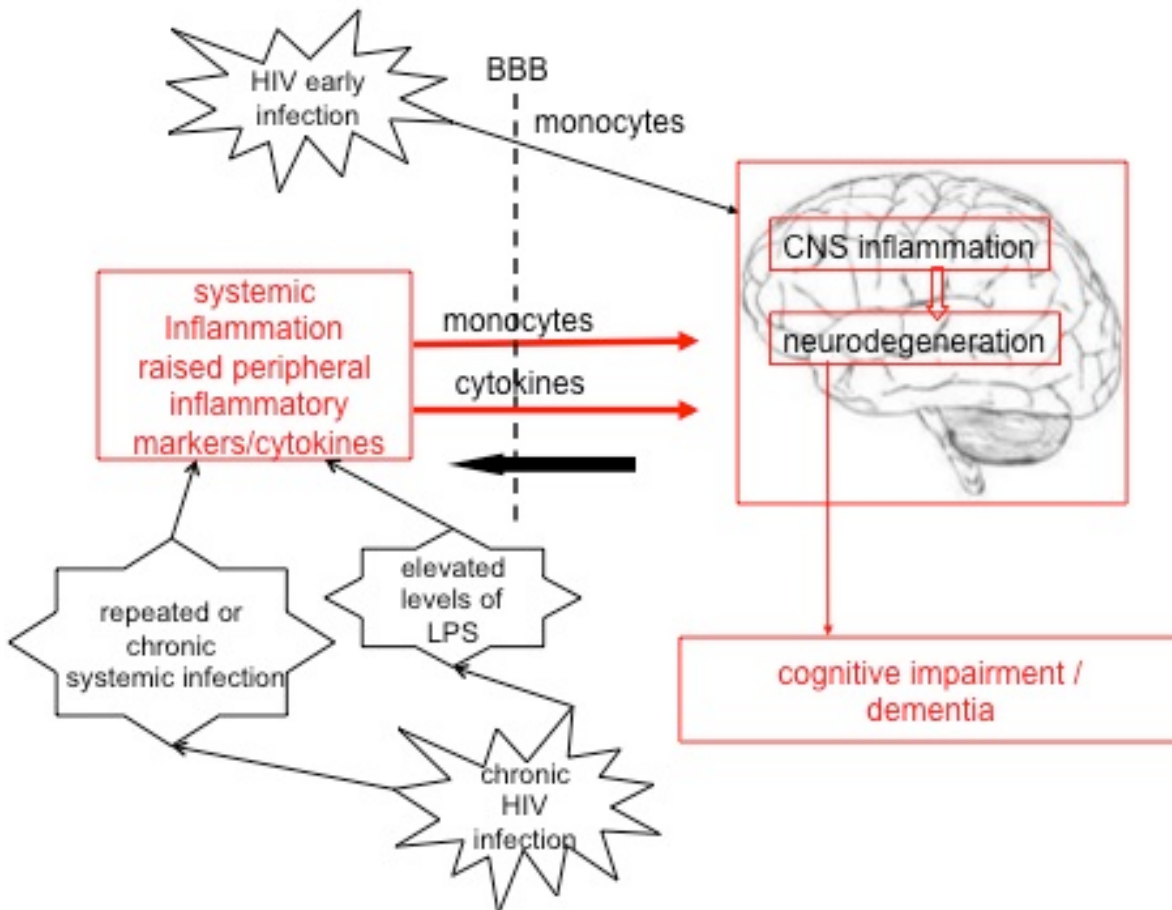


Figure 2.5 The relationship between systemic infection, CNS inflammation, neurodegeneration and cognitive impairment in HIV infection

The role of the BBB in HIV-1 CNS infection

The BBB functions as a protective mechanism for the brain. It restricts the entry of cellular components of the immune system, relatively excluding them from immune surveillance (Berger and Avison 2004). The brain is however not an “immune-privileged” organ as was previously thought and it does also respond to peripheral inflammatory stimuli and immune responses (Wärnberg *et al.* 2009). The CNS can therefore be affected through the actions of peripheral cytokines that cross the BBB.

HIV infection is a well-known cause of BBB disruption, and targets many of the cellular and structural components of the BBB. The initial early entry of HIV, however, occurs against a setting of normal BBB structure and function (“Trojan Horse” mechanism). Later HIV-associated compromise of the BBB, through pro-inflammatory mechanisms or direct impact of HIV proteins, causes the BBB to become more leaky. This may facilitate an accelerated entry of HIV into the CNS, which may have a significant impact on the clinical progression of HIV-associated CNS disease (Wang *et al.* 2008; Zhou *et al.* 2006; Berger and Avison 2004).

Increased monocyte trafficking into the CNS

From the literature it is clear that activated monocytes play a key role in the pathogenesis of HIV-associated cognitive impairment (González-Scarano and Martin-Garcia 2005; McArthur *et al.* 2003; Kaul *et al.* 2001). Monocytes are produced, and also mature, in the bone marrow. Increased numbers of monocytes and resident macrophages have been found in the bone marrow of patients with AIDS (Titius *et al.* 2009; Kaloutsis *et al.* 1994). Uncontrolled HIV replication and the resulting immunodeficiency alter the myeloid differentiation pathway. HIV infection therefore results in increased numbers of circulating and activated monocytes from the bone marrow (Fischer-Smith and Rappaport 2005; Thieblemont *et al.* 1995). These cells invade the CNS and have been clearly associated with the development of HIV dementia (Gartner 2000; Pulliam *et al.* 1997; Tyor *et al.* 1992). Pulliam *et al.* also found a unique subset of activated CD14/CD16 and CD14/CD69 expressing monocytes in patients with AIDS dementia. They demonstrated that the CD69 monocytes are neurotoxic *in vitro* and suggested that this subgroup of monocytes might be the link between the periphery and parenchymal damage in the brain (Pulliam *et al.* 1997). The dramatic increase in the total number of brain macrophages associated with HIV-encephalitis (HIVE), the pathological hallmark of HIV-dementia, thus appears to be due to trafficking of monocytes/macrophages from the periphery into the CNS, and not from local microglial proliferation (Fischer-Smith *et al.* 2004). It therefore follows that certain events in the periphery lead to altered monocyte/macrophage homeostasis with a resultant increased CNS invasion of activated HIV-1 infected monocytes. This is the so-called late invasion model (Fischer-Smith and Rappaport 2005).

Chronic systemic infection and inflammation

Numerous studies have recognized systemic inflammation as a key driver of HIV-1 pathogenesis, both in the periphery and in the CNS (Deeks 2009; Brenchley *et al.* 2006b).

Recent research has also begun to show possible dynamic interrelationships between the CNS and systemic disease (Rausch and Davis 2001). As discussed previously, systemic HIV infection of monocytes and T cells ultimately leads to CNS infection and neurodegeneration through the activation of macrophages and microglial cells in the brain (Nottet and Gendelman 1995). Microglial cells can be activated by HIV-infected and/or uninfected immune-reactive monocytes and lymphocytes (Yoshioka *et al.* 1995). It is possible that microglial cells can be activated in response to systemic infection before or after HIV-infected monocytes enter the brain (Langford and Masliah 2001).

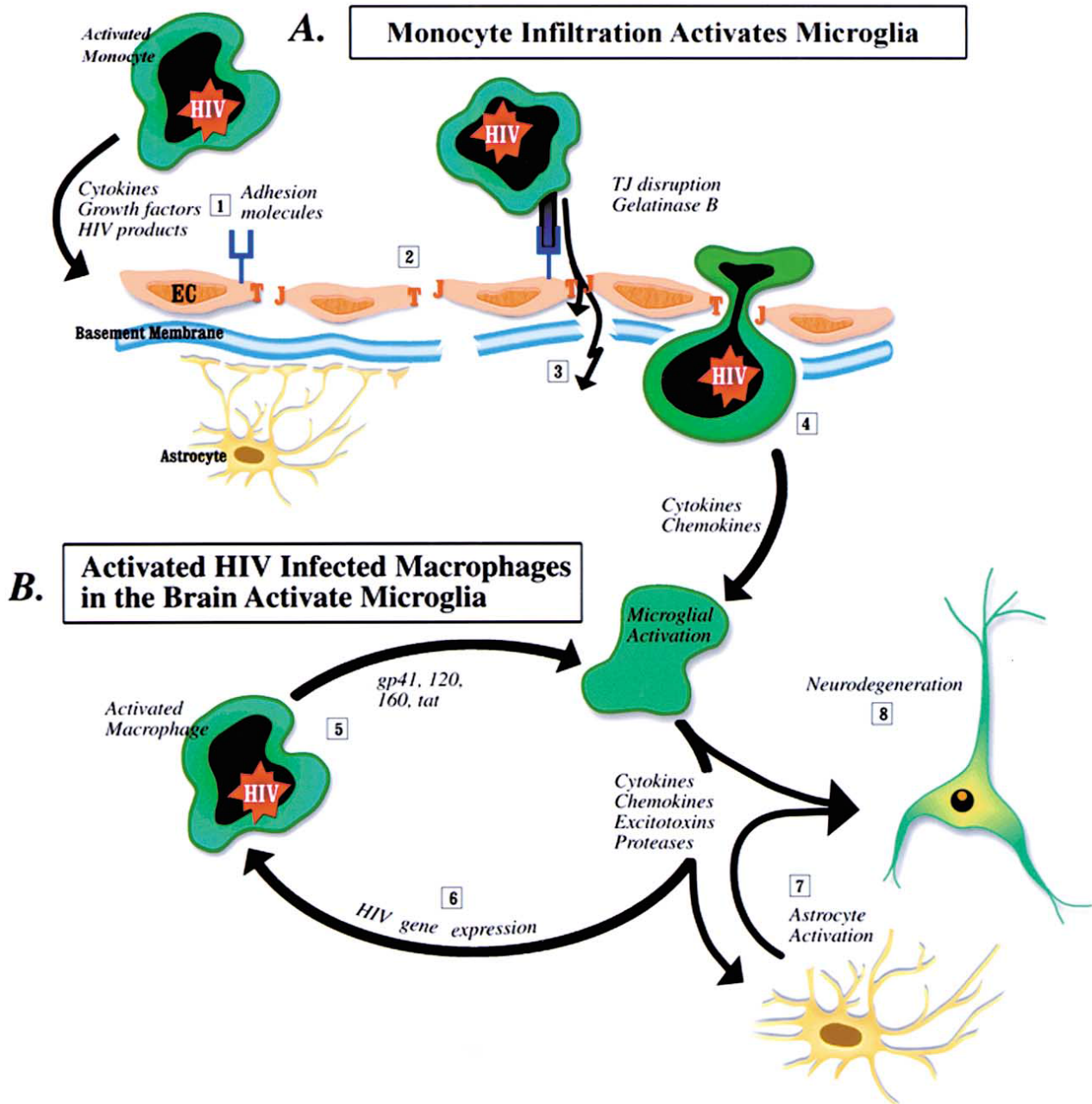


Figure 2.6 Schematic representation of the “cross-talk” between components of the BBB and cells of the CNS in microglial activation in AIDS.

(Langford and Masliah 2001)

During HIV infection, signals originating from activated monocytes in the bloodstream may activate microglial cells through a cascade of stimuli that cross the BBB (Figure 2.6A). Resting microglia may also be activated by activated macrophages/microglia in the brain (Figure 2.6B) (Gebicke-Haerter *et al.* 1996). Systemic and CNS HIV infection therefore act together in the activation of microglia leading to HIV-1 and increased neurodegeneration (Langford and Masliah 2001). Pro-inflammatory mediators produced

systemically following infection can signal to the brain, thus leading to activation of microglial cells that ultimately induce adaptive metabolic and behavioural changes (“sickness behaviour”). Induction of this sickness response is associated with the expression of pro-inflammatory cytokines such as IL-1 β and TNF- α , both in the periphery and in the brain (Hosoi *et al.* 2002). In normal healthy persons this is part of the immune system’s defence against disease (and part of the generation of fever), but in patients with chronic neurodegenerative disease, systemic inflammation leads to accelerated neuroinflammation and increased neuronal death (Minghetti 2005; Perry *et al.* 2003). Ryan *et al.* measured increased levels of soluble TNF- α type II receptor and soluble CD14 in the plasma of HIV-1 infected subjects with cognitive impairment and brain atrophy (Ryan *et al.* 2001). CD14 is found principally on human monocytes and increased release of soluble CD14 has been observed from activated monocytes (Landmann *et al.* 1996). Increased levels of soluble CD14 also correlate with HIV disease progression (Lien *et al.* 1998). These findings support an interplay between peripheral immune responses and cognitive dysfunction in advanced HIV-1 disease (Ryan *et al.* 2001).

Untreated HIV infection is associated with recurrent systemic and opportunistic infections, and therefore also with increased circulating levels of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6. These cytokines can cross the BBB or circulating cytokines can affect the CNS indirectly to produce more cytokines in the CNS (Wong *et al.* 1995; Watkins *et al.* 1995; Hashimoto *et al.* 1991). The peripheral nervous system can influence the brain by sending signals via the peripheral or cranial nerves, especially the vagus nerve. We can, therefore, see that the peripheral and central cytokine compartments appear to be integrated and that they might synergize or inhibit each other (Szelenyi 2001).

Immune activation is a strong predictor of HIV disease progression (Brenchley *et al.* 2006a). A possible trigger of chronic systemic immune activation in HIV-1 infection is elevation of a plasma endotoxin, bacterial lipopolysaccharide (LPS). Elevated levels of LPS are a consequence of translocation of bacterial products across a gut mucosa barrier, associated with HIV-mediated depletion of mucosal CD4 T lymphocytes (Douek 2007; Brenchley *et al.* 2006b). LPS triggers monocyte activation and can also compromise the integrity of the BBB, which permits entry of increasing numbers of HIV-infected monocytes into the brain (Ancuta *et al.* 2008; Zhou *et al.* 2006). Using an *in vivo* novel mouse model, Wang and colleagues demonstrated that HIV-1 infection increased the ability of

monocytes to enter the brain and also increased sensitivity of the BBB to disruption by LPS (Wang *et al.* 2008). Through these mechanisms circulating LPS may therefore be linked to the development of HAD. The elevated levels of LPS that lead to an immune activated state associated with HIV-infection also causes increased levels of circulating cytokines (Douek 2007; Brenchley *et al.* 2006b).

From the above evidence, it follows that there is an association between the systemic inflammatory response and the numbers of activated macrophages and microglia in the CNS, neuronal damage, and cognitive dysfunction (Glass *et al.* 1995). We can therefore postulate that repeated or chronic systemic infections and inflammation – as is common in HIV – may drive and enhance the process of neuroinflammation, and ultimately that of neurodegeneration and the development of HAND, through signalling from the peripheral blood to the CNS (Gannon *et al.* 2011; Ancuta *et al.* 2008).

E. Summary of the neuropathogenesis of HAND (Figure 2.7)

During HIV-1 infection, chronic immune activation in the periphery develops as a result of anti-HIV-1 immune responses, elevated levels of LPS due to microbial translocation from the gut, and viral proteins such as gp120 and Tat. Immune activation is believed to drive systemic immunopathogenesis but also leads to an expanded subset of activated monocytes, some of which are also infected with HIV-1. These monocytes have enhanced migratory capacity and traffic through the BBB, which is compromised by the action of viral proteins, pro-inflammatory mediators, and LPS. Once inside the CNS, these cells differentiate into macrophages and release viral particles that infect other cells with HIV-1. Activated macrophages also release viral proteins, cytokines (e.g. TNF- α , IL-1 β), and chemokines that activate neighbouring macrophages and microglia and therefore sustain the neuroinflammatory response. These factors also activate astrocytes. Released chemokines such as MCP-1 and SDF-1 α further recruit monocytes into the CNS. Some of these chemokines also up-regulate adhesion molecules on various cell types that further enhance recruitment of inflammatory cells. These processes lead to accumulation of activated monocytes/macrophages in the brain that correlates with neurological injury. Neuronal injury results from the combined effects of neurotoxic viral proteins released from infected monocytes/macrophages, neurotoxic cytokines released from infected and non-infected activated cells, and soluble macrophage activation products like quinolinic acid and platelet activating factor that lead to neuronal cell death.

Dysfunctional astrocytes also contribute through the dysregulated homeostasis of the excitotoxic neurotransmitter, glutamate (Yadav and Collman 2009).

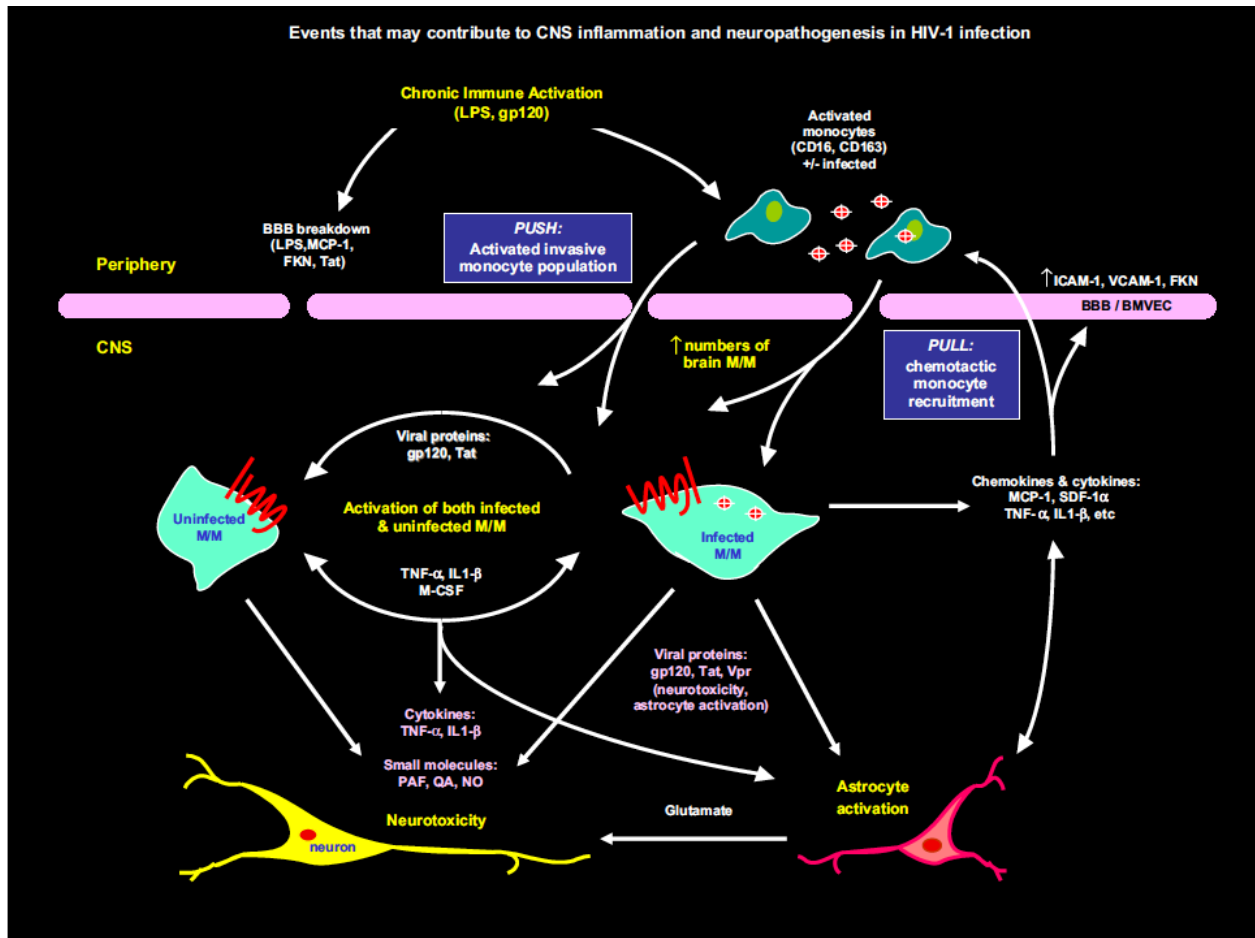


Figure 2.7 Summary of the neuropathogenesis of HAND

(Yadav and Collman 2009)

Next I shall examine briefly the neuropathological and clinical features of HAND.

2.3.2 Pathological correlates of HAND

Chronic neuroinflammation results in the neuropathological abnormalities collectively called HIV-1 encephalitis (HIVE) (Yadav and Collman 2009; Langford *et al.* 2003; Zheng and Gendelman 1997). Simian immunodeficiency virus (SIV) encephalitis models and human autopsies have been crucial in advancing our understanding of the pathology of HAND (McArthur *et al.* 2010). Neuropathologically, HIVE is characterized by: the

infiltration of macrophages; the formation of microglial nodules and multinucleated giant cells (MNGC) through fusion of HIV-infected macrophages in central white matter and deep grey matter; widespread reactive astrogliosis and the loss of neurons; and myelin pallor – the loss of myelin surrounding neuronal axons, indicating damage to oligodendrocytes (Hazleton *et al.* 2010; Lawrence and Major 2002; Gendelman *et al.* 1994). It is interesting that these pathological features are most closely associated with the clinical signs of HAD, and that the presence of macrophages and microglia is a better correlate of HAD than is the presence of HIV-infected cells or the viral antigen load in the brain (Kaul *et al.* 2005; Glass *et al.* 1995).

Since the advent of cART, HIVE is now believed to have reduced considerably, while more subtle neuropathological alterations such as brain infiltration of blood-borne monocytes and more limited gliosis are common (Kraft-Terry *et al.* 2010; Everall *et al.* 2005). Vago and colleagues did a retrospective study of 1597 autopsy cases in Italy and determined the frequency of HIVE as 54% before the introduction of cART with a subsequent 60% decline in the cART era (Vago *et al.* 2002).

The neuropathological changes and most productive HIV infection are seen within the basal ganglia, brainstem and deep white matter (Brew *et al.* 1995; Kure *et al.* 1991). This regional predisposition probably accounts for the mostly subcortical pattern of the clinical deficits in HAND (McArthur *et al.* 2010).

2.3.3 Clinical features of HAND

A. Classification

Since 1991, the guidelines published by the AIDS Task Force of the American Academy of Neurology (AAN) were used to diagnose the cognitive impairment related to HIV-1 infection. These criteria defined two levels of HIV-associated cognitive impairment: HIV-dementia (HIV-D) and minor cognitive and motor disorders (MCMD). In 1995, Grant and Atkinson expanded the AAN system to include the additional diagnosis of “sub-syndromic neurocognitive impairment”. This was used to characterize patients with mild neurocognitive deficits that did not interfere with daily functioning (Grant and Atkinson 1995). In 2007 the HIV Neurobehavioral Research Center (HNRC) proposed a refinement of the AAN criteria which recognized the following three conditions: asymptomatic

neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). ANI and MND are defined as scoring at least one standard deviation below the mean of a control population in at least two domains of cognitive functioning. However, in MND there is mild impairment in ADLs. MND is similar to MCMD as previously defined by the AAN criteria. HAD is defined by a score of at least two standard deviations below the mean of a control population in at least two cognitive areas, and with marked impairment in ADLs. The revised research criteria require that the impairment is not due to delirium and cannot be fully explained by comorbid conditions (Antinori *et al.* 2007).

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

Table 2-1 Revised research criteria for HAND

(Antinori *et al.* 2007)

B. Clinical presentation

HAND is a spectrum of disorders ranging from mild impairment to severe dementia (Ellis *et al.* 2009). Individuals with HIV can develop cognitive impairment in a number of different domains and the clinical features of HAND are both subcortical and cortical (Hazleton *et al.* 2010). The domains most affected by HAND are attention, learning and memory, motor function, psychomotor speed, executive function, and language (Power *et al.* 2009; McArthur *et al.* 2005). During the early stages of HAND, clinical symptoms may be absent (ANI) or subtle (MND) but they can progress in severity to a debilitating dementia (HAD). When clinically evident, symptoms fall into the main categories of cognitive, behavioural and motor dysfunction (Nath and Berger 2004; Jannsen *et al.* 1991; Navia *et al.* 1986).

MND may have functional consequences on work and medication adherence, but can present a diagnostic challenge because individuals typically present with vague cognitive complaints and a relatively normal neurological examination. Neuropsychological tests are useful tools for demonstrating early cognitive dysfunction (Nath and Berger 2004).

In the pre-cART era HAD most commonly presented in patients with advanced immunosuppression (e.g. CD4 cell counts below 200 and elevated viral loads), co-existing systemic disease and clinical hallmarks of advanced AIDS. Since the start of the cART era dementia is sometimes the presenting or only evidence of HIV infection before the patient exhibits any other illness characteristic of impaired immunity. CD4 cell counts can now be normal or near normal when a patient presents with HAD (Dore *et al.* 2003; Price *et al.* 1988; Navia and Price 1987). The early stages of HAD are frequently characterised by the symptoms of memory loss, mental slowing, reading and comprehension difficulties, as well as apathy. The initial symptoms of dementia due to HIV can be subtle and can be overlooked or misdiagnosed as depression. Typical cognitive deficits of HAD are: memory loss selective for impaired retrieval; impaired ability to manipulate acquired knowledge; personality changes that are characterized by apathy, inertia and irritability; and general slowing of all thought processes (McArthur *et al.* 2003). Gait disturbance, with non-specific stumbling and tripping, and impairment of fine manual dexterity are common early motor manifestations. Motor examination findings reveal impaired rapid eye and limb movement, diffuse hyperreflexia, frontal lobe release signs, and sometimes Parkinsonism

(McArthur *et al.* 2003). Clinical presentation can, however, vary considerably between affected individuals (Navia *et al.* 1986).

C. Clinical course

Like the clinical presentation, the course of HAND also varies considerably. There is now evidence that affected individuals may show marked recovery of their cognitive functions with effective combination antiretroviral therapy (cART), or worsening of HAND with advanced AIDS (Woods *et al.* 2009). HIV-infected individuals may develop ANI, then progress to MND, and then HAD but clinical progression can also fluctuate (Hazleton *et al.* 2010; Bouwman *et al.* 1998). Navia *et al.* described the typical presentation of HIV-dementia and found the course of the disease, in most patients, to be steadily progressive, interrupted by episodes of abrupt acceleration. In 20% of patients a slowly progressive course was observed (Navia *et al.* 1986). The presence of MND may be a predictor of HAD, or is at least associated with the neuropathological changes of HIV encephalitis (Joska *et al.* 2010d; Cherner *et al.* 2002). MND may also indicate a worse prognosis in AIDS (Sacktor *et al.* 1996). Several studies, however, do suggest that normalization of symptoms is possible (Valcour *et al.* 2004a; Sacktor *et al.* 2002; Heaton *et al.* 1995). Antinori *et al.* found that a substantial proportion of HIV-infected persons' symptoms fluctuate between normal to abnormal (Antinori *et al.* 2007).

D. Functional impact

HIV-positive patients with neuropsychological impairment are at higher risk of dying than those without impairment (Ellis *et al.* 1997). Neurocognitive disorders associated with HIV are present in up to 50% of HIV-positive patients with the milder forms of HAND being present in at least 30% to 40% of symptomatic HIV-positive adults (Joska *et al.* 2010d; Sacktor *et al.* 2002). The milder forms of HAND are more prevalent in the cART era and survival is considerably longer (Antinori *et al.* 2007). Despite the remarkable improvement in survival rates since the introduction of cART, HAND remain a significant public health concern (Woods *et al.* 2009) and may occur even in those patients who do not have any other evidence of active HIV disease (Antinori *et al.* 2007). These disorders, even the milder forms of HAND, impact negatively on social and occupational functioning. Individuals may be non-adherent to cART regimens, and they may miss clinical appointments. They may also be more vulnerable to sexual abuse and more likely to

engage in high-risk behaviours (Alfahad and Nath 2013; Gras and Kaul 2010; Gorman *et al.* 2009).

2.3.4 Management of HAND

A. Diagnosis

The diagnosis of HAND is primarily clinical. It relies on a neuropsychological, psychiatric and medical evaluation to identify the clinical syndrome and to exclude any alternative diagnoses or confounding conditions (Barber *et al.* 2013; Joseph *et al.* 2009).

Based on current diagnostic nomenclature, the diagnosis of HAND requires assessment of at least five domains of neurocognitive functioning known to be affected by HIV infection: executive function, episodic memory, speed of information processing, motor skills, attention/working memory, language, and sensoriperception (Antinori *et al.* 2007). In an ideal environment, these domains would be assessed using a performance-based neurocognitive test battery and interpreted using demographically appropriate normative data (Woods *et al.* 2009). In busy primary care settings and countries with limited resources, clinicians usually do not have access to a detailed neuropsychological test battery and therefore use clinical assessments or brief screening tools such as the International HIV Dementia Scale (IHDS) (Woods *et al.* 2009; Sacktor *et al.* 2005).

The diagnosis of HAND often depends on demonstrating a decline in everyday functioning, but the assessment of functional impairment is an intricate and complicated process (Woods *et al.* 2009). There are also currently no widely agreed-upon clinical measures of everyday functioning. The assessments used in research settings are lengthy to administer and therefore not widely used in a clinic setting (Moore *et al.* 2007). Assessments of everyday functioning rely on self-report but are usually complemented by reports from partners or caregivers where patients have cognitive impairment or poor insight and judgement. Self-reported functional abilities are often not accurate (Woods *et al.* 2009).

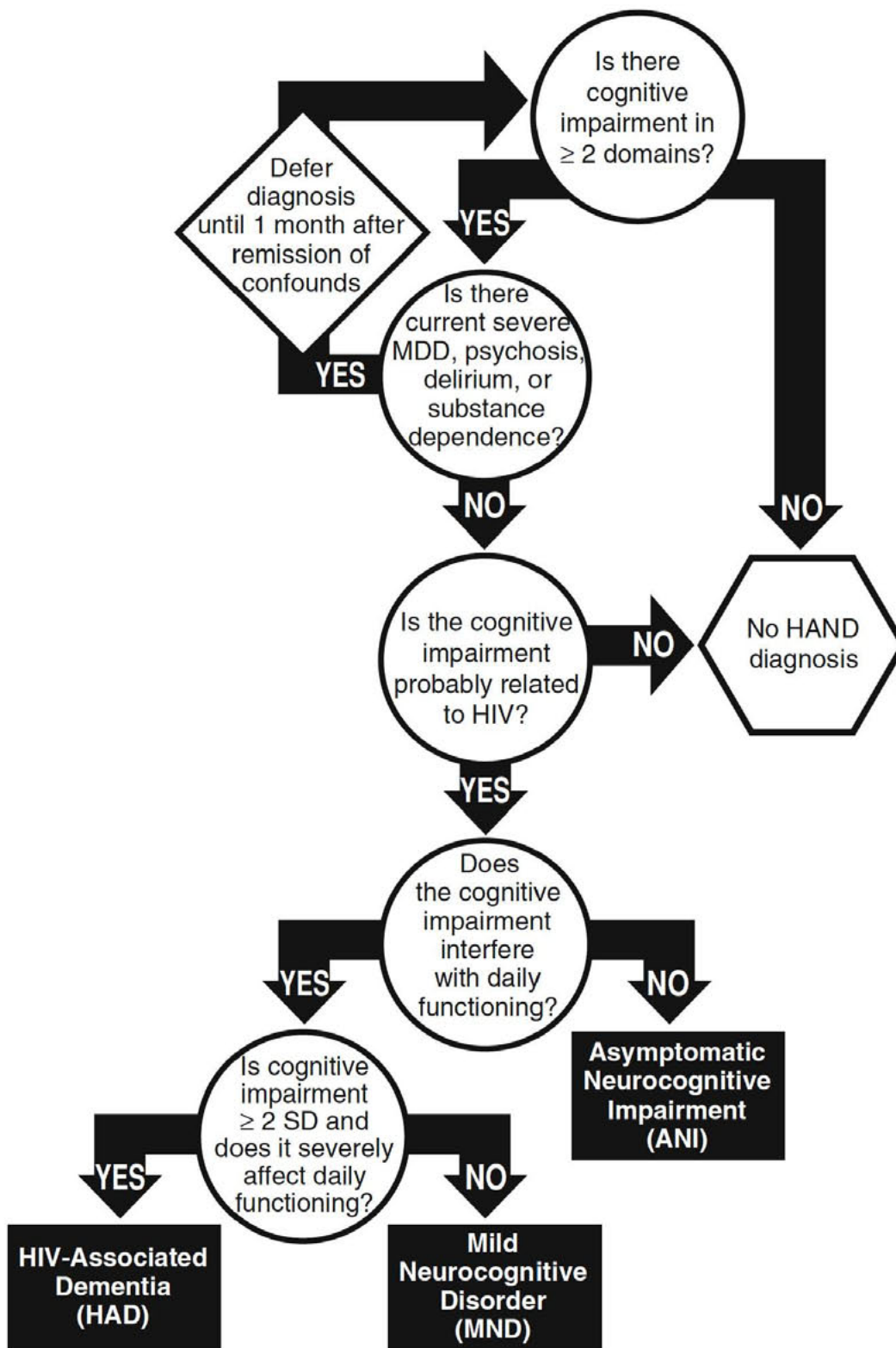


Figure 2.8 Proposed decision-tree for diagnosing HIV-associated Neurocognitive Disorders

(Woods *et al.* 2009)

Another approach used to detect HIV-associated cognitive impairment is the Global Deficit Score (GDS), which summarizes the results of a reduced subset of neuropsychological tests. Demographically corrected test data on individual neuropsychological measures are converted to deficit scores ranging from no impairment to severe impairment. The deficit scores are averaged to create a global deficit score. The GDS weights neuropsychological data by considering both the number and severity of deficits in an individual's performance throughout the test battery, giving relatively less weight to superior performances and/or those within normal limits. The GDS has been shown to effectively predict mild cognitive impairment in HIV infected subjects (Carey *et al.* 2004a; Heaton *et al.* 1995). It is a useful tool for classifying cognitive impairment in HIV, as it does not require the use of functional assessments like the revised criteria for HAND. The GDS is a more objective measure, because HIV-positive patients often visit healthcare centres alone and without someone to provide collateral information (Valcour *et al.* 2011).

Medical evaluation involves a general physical and a neurological examination. The neurological examination should assess the presence of peripheral neuropathy, motor tone and power, involuntary movements, primitive reflexes and gait. Imaging studies, such as magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), are commonly used in developed countries to exclude CNS opportunistic infections, and also to identify the characteristic radiological changes present in HAD. These imaging studies are, however, not always available in resource-poor countries such as South Africa. CSF analysis is indicated in febrile patients or those with acute encephalopathy to exclude cryptococcal or tuberculosis (TB) meningitis. In the more typical non-febrile patients it is generally not needed, provided that imaging can be used to exclude CNS opportunistic infections (McArthur *et al.* 2005).

There is still an urgent need for an objective, scalable and quantitative biomarker profile for HAND. The ideal biomarker for HIV-associated cognitive impairment would: 1) diagnose HAD and the milder forms of HAND, 2) identify patients at risk, 3) detect disease progression, 4) confirm arrested or static encephalopathy, and 5) measure treatment response. It is not plausible that a single biomarker would be sufficient for all these aims and the search should rather be for complementary markers (McGuire 2009). Given that systemic and localized inflammation is considered an underlying pathogenic mechanism of HAND, the change in CSF cytokine levels during HIV might help to identify HAND. In a preliminary study by Yuan *et al.*, there was an association between the increased levels of

cytokines in the CSF and HIV-associated neurocognitive impairment. They concluded that cytokines might provide a biomarker profile for HAND (Yuan *et al.* 2013). The CSF compartment may, however, not be the most promising compartment for biomarker research in HIV-associated dementia. Given the trafficking of the monocytes and macrophages and their important role in the pathogenesis of HAND, the blood compartment may be more informative and also more accessible (McGuire 2009).

Dementia remains one of the most feared complications of HIV and it continues to pose a challenge to the clinician both in terms of diagnosis and also treatment (Nath *et al.* 2008).

B. Treatment

The development of cART has significantly altered the nature of HIV-associated cognitive disorders although they continue to be a major clinical problem among HIV-infected individuals (Grant 2008; Sacktor *et al.* 2002). The incidence of HAD has decreased but the milder forms of neurocognitive impairment remain prevalent (Nath *et al.* 2008). However, because of the increased numbers of PLWHA and on cART, the prevalence of HAD is actually rising (McArthur 2004). The clinical presentation of HAD has also changed in the cART era (Brew 2004). Cysique and colleagues compared the prevalence and patterns of neuropsychological impairment in pre- and post-cART cohorts in Australia and found no significant differences between the prevalence of impairment between the two groups. They did, however, find a difference in the pattern of neuropsychological impairment in the post-cART group. There was an improvement in attention, verbal fluency and visuoconstruction deficits, but learning efficiency and complex attention ability seemed to have deteriorated. It appears that the subcortical features that were previously thought to be characteristic are less prominent now (Cysique *et al.* 2004). In general, cART does improve neuropsychological function and this improvement is noted in a number of different cognitive domains. Improvement is, however, neither full nor universal. It is possible that remaining deficits reflect a loss of cortical neurones and that the reversible deficits reflect damage to white matter. It is also possible that cART has a greater treatment impact on the more severe forms of HAND than on the milder forms (Joska *et al.* 2010c).

Several mechanisms to explain neuropsychological improvement have been proposed in the literature (Cysique *et al.* 2009) (Figure 2.9). It is possible that systemic treatment

alone is enough to have a beneficial effect on the CNS (Sinclair *et al.* 2008). ART markedly improves morbidity and mortality in HIV by reducing plasma viral load to undetectable levels and restoring immune function (Letendre *et al.* 2004). These lower viral loads and improvement in CD4 counts and immune function may benefit the CNS by reducing the number of circulating activated monocytes, leading to a reduction in their migration into the brain. This decreases the load of viral proteins that the brain is exposed to as well as reducing levels of immune activation, neuroinflammation and the production of neurotoxic chemicals (Liner II *et al.* 2008; Sinclair *et al.* 2008; McArthur *et al.* 2004; Brew 2004). Alternatively it is also possible that ART improves neurological function by acting locally within the brain. For this to be effective, we need to use antiretroviral drugs with good CNS penetration. The effect of ART within the brain may be the key to maintaining a lower viral load in the CSF and decreasing local immune activation and neuroinflammation (Liner II *et al.* 2008).

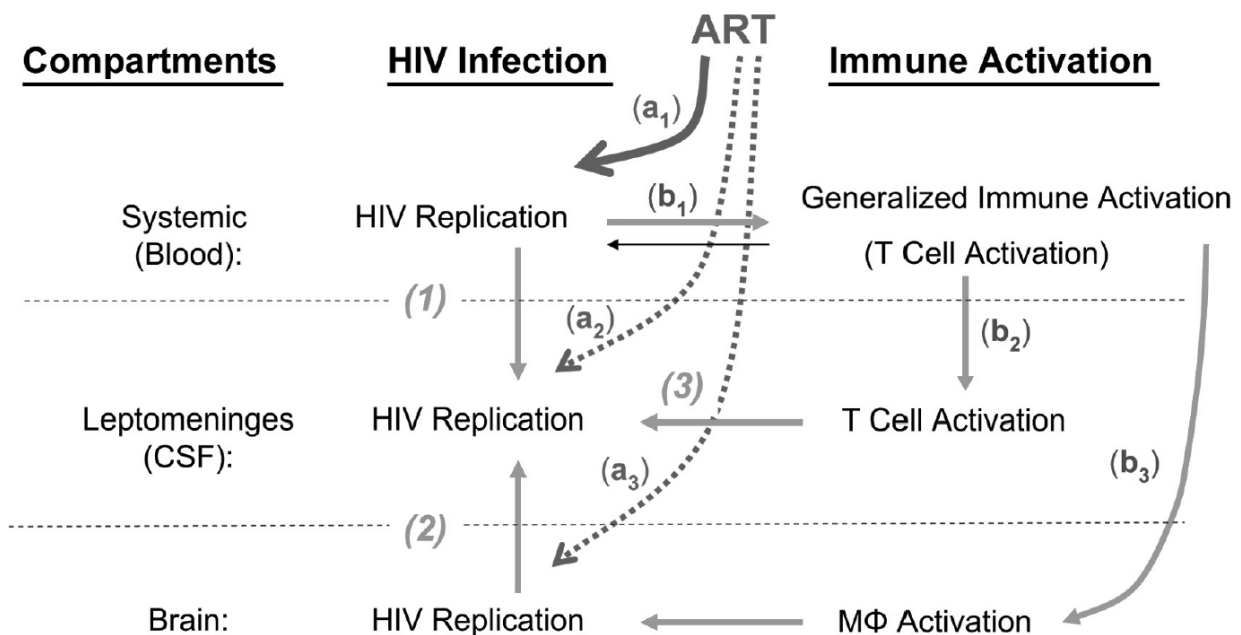


Figure 2.9 Model of CSF HIV-1 infection and its relation to immune activation and treatment responses.

(Sinclair *et al.* 2008)

Neurological improvement due to cART remains variable. In some individuals improvement occurs within the first few weeks after initiation of cART, while in the majority of individuals improvement may occur after up to a year on treatment (Joska *et al.* 2010c).

The early cognitive response due to cART might be associated with a greater degree of baseline impairment and neuroinflammation as well as with good systemic viral suppression on cART (Wojna and Nath 2006). Joska *et al* reported on the impact of cART in a prospective study in South Africa and found that individuals most impaired at baseline (i.e. those with low CD4 counts, high viral loads, and high levels of inflammation) were more likely to improve at follow-up after one year than those who were less impaired (Figure 2.10) (Joska *et al.* 2012).

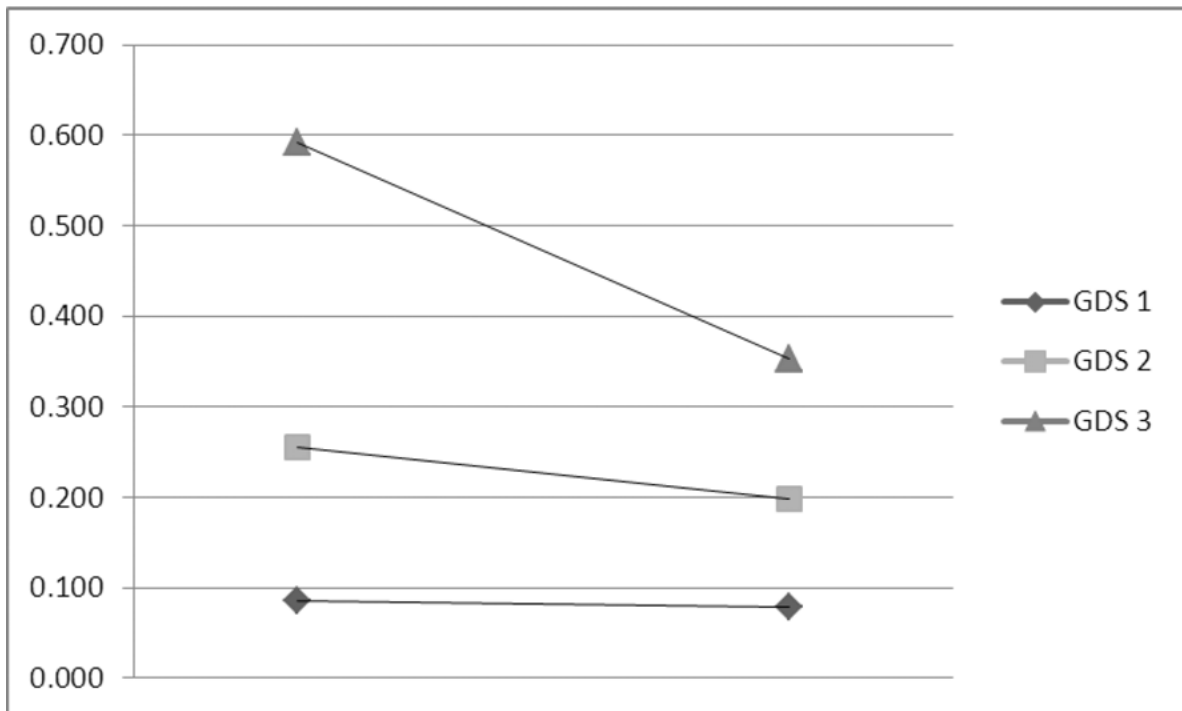


Figure 2.10 Change in Global Deficit Score at baseline and one year of participants initiating cART across groups of severity at baseline.

(Joska *et al.* 2012)

Various other treatment modalities have been investigated, but to date these have not been proven to be successful options. Early initiation of an effective cART regime remains the gold standard in treating HAND, and needs to be combined with a focus on controlling the comorbidities that can have a major impact on HAND (Alfahad and Nath 2013).

C. Prognosis

The effective use of cART is associated with a significant reduction in the disease burden of HAD. People who initiate cART before they have severe immunosuppression and who achieve plasma and CSF viral suppression after starting treatment with CSF penetrating regimens benefit the most from cART (Letendre *et al.* 2004; Sacktor *et al.* 2003). Prior to the introduction of cART, HIV-dementia usually followed a progressive course over 3 to 9 months, leading to severe neurological deficits and eventually death (Navia *et al.* 1986). With cART, survival rates have improved from a mean of 5 months to 38.5 months (Dore *et al.* 2003). In South Africa, with the largest HIV epidemic in the world despite one of the biggest anti-retroviral rollout programmes, people with HIV-infection still enter treatment late or not at all. Given that more than 20% of individuals in primary care in the Western Cape province of South Africa have HIV-associated cognitive impairment, there are significant implications for care (Joska *et al.* 2011; Joska *et al.* 2010b).

It is interesting that not all HIV-positive individuals develop cognitive impairment and a variety of host and viral factors have been identified as associated with an increased risk of developing HAND. These factors will be discussed in the next section with specific focus on viral clade and the apolipoprotein E gene.

2.3.5 Risk or associative factors for HAND

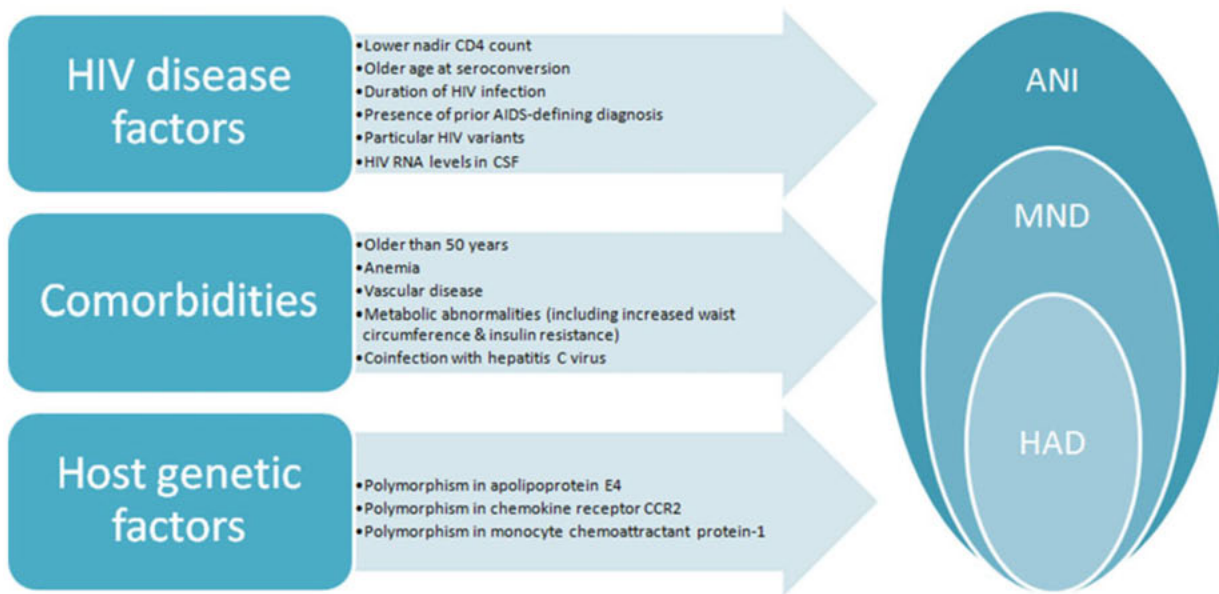


Figure 2.11 Risk factors for HIV-associated neurocognitive disorders.

(Alfahad and Nath 2013)

Of the various clinical factors associated with HAND, some are especially important in the South African setting. HAND has been consistently associated with low CD4 cell count and late stage disease (Heaton *et al.* 2010). This is also the case in South Africa where the HIV epidemic is greatest and access to cART is still limited (Joska *et al.* 2011). Methamphetamine abuse is a major problem in South Africa and not only increases HIV risk behaviour, but has also been associated with increased risk of neuropsychological impairment and both pathological and radiological evidence of neurodegeneration (Hult *et al.* 2008; PlüDdemann *et al.* 2008; Langford *et al.* 2003).

In South Africa, the majority of HIV-infected people attending clinics are women and many of them present with late stage disease. Depression is a common co-morbidity with HAND and women are more prone to suffer from depression (Kessler *et al.* 2005).

Several pathological conditions as well as physiological ageing may be associated with cognitive, neurological or psychiatric dysfunction and may potentially confound the diagnosis of HAND. There is substantial evidence that advancing age (> 50 years) may make PLWHA vulnerable to developing HAND (Valcour *et al.* 2004b). Some studies

attribute this to the ageing brain itself or to the duration of HIV disease (Brew *et al.* 2009). Neuropathological similarities exist between Alzheimer's disease (AD) and HAND (Green *et al.* 2005; Esiri *et al.* 1998).

Individuals may also be genetically more susceptible to developing cognitive problems (Schouten *et al.* 2011). Host genetic factors include the $\epsilon 4$ allele of APOE that has been associated with an increased risk of HAD especially in the elderly (Valcour *et al.* 2004a). Polymorphisms in cytokine and chemokine pathways and innate immune responses have been implicated in HIV-associated neurocognitive dysfunction (Singh *et al.* 2004). Gene variants in inflammatory proteins may modulate neurodegenerative diseases such as AD and HAND in which neuroinflammation plays a significant role in the pathogenesis (Jayadev and Garden 2009).

Viral genetic factors, such as Tat derived from different viral clades, may also affect neurotoxicity, although it is not yet evident whether viral clade does have an important influence on acquiring HAND (Mishra *et al.* 2008; Rao *et al.* 2008).

It is thus clear that there are various underlying pathophysiological mechanisms that can impact on the development and clinical picture of HAND. Thus the entity of HAND has to be considered as a complex syndrome and not just a single entity (Alfahad and Nath 2013).

2.4 Viral clade

HIV-1 strains are grouped according to the sites they prefer to replicate in. T-tropic viruses prefer to replicate in T lymphocytes and M-tropic viruses in macrophages. M-tropic HIV-1 is most commonly identified within the brain (McArthur *et al.* 2010). HIV-1 Group M has further been divided into 10 subtypes labelled A to J. These HIV-1 subtypes, also called clades, are genetically linked viral strains that are, in some cases, also linked geographically or epidemiologically. HIV-1 clades display an uneven global distribution with the most prevalent subtypes being A, B, and C (see Figure 2.12) (Taylor *et al.* 2008; Mishra *et al.* 2008; Hemelaar *et al.* 2006). Sub-Saharan Africa is the region most affected by the HIV epidemic and here HIV-1 clade C infection is most common, accounting for around 50% of infections worldwide (Buonaguro *et al.* 2007).

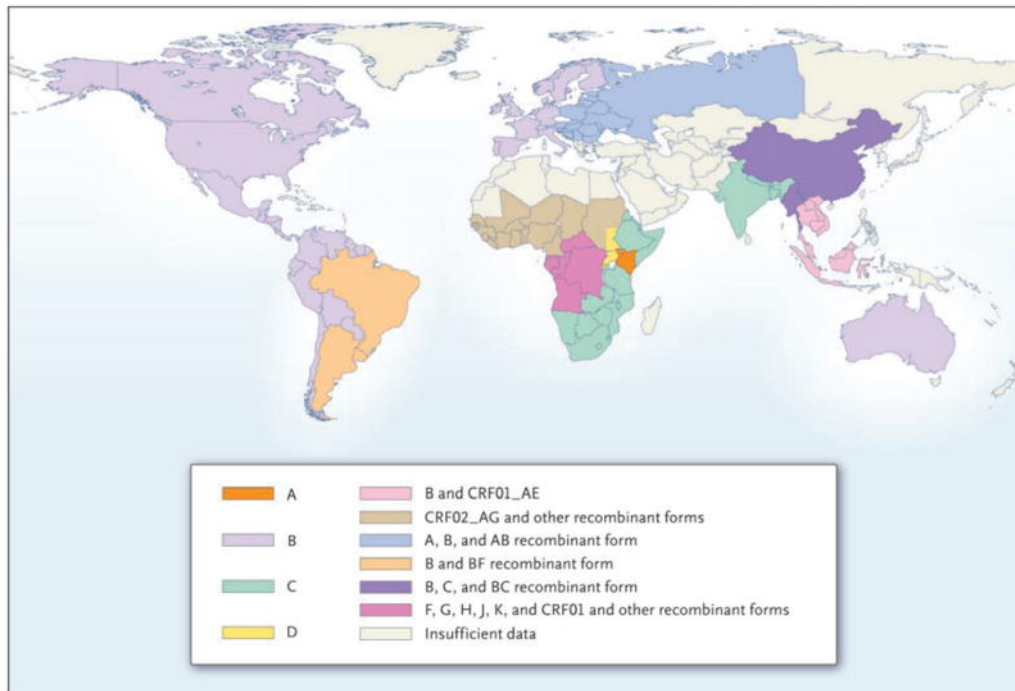


Figure 2.12 Current global distribution of HIV-1 subtypes and recombinant forms.

(Taylor *et al.* 2008)

Viral subtypes may differ in their characteristics and their interaction with the human host, which may influence pathogenesis, transmission, diagnosis, disease progression, and treatment of HIV (Taylor *et al.* 2008; Hemelaar *et al.* 2006; Kanki *et al.* 1999). The majority of HIV research has been conducted in populations of European descent – Europe, Australia and the Americas – where HIV infection is almost exclusively due to subtype B. These research findings are responsible for much of our current understanding of the disease progression of HIV-1 and may not be generalizable to HIV clades and human populations in sub-Saharan Africa or other developing countries where different clades predominate (Ellis *et al.* 2009; McCutchan 2006; Kanki *et al.* 1999).

Previous laboratory studies have showed that clade-specific differences in neuropathogenicity exist among clade B and clade C infected subjects with neurological deficits being more common in clade B than in clade C prevalent areas (Mishra *et al.* 2008; Rao *et al.* 2008). The exact mechanisms underlying these differences in neuropathogenesis by both subtypes remain unclear, but have been ascribed to variations in the neurotoxic region of the regulatory viral protein, Tat (McArthur *et al.* 2010; Gandhi *et al.* 2009). Clade C has been reported as less neurovirulent than clade B resulting in milder cognitive dysfunction (Mishra *et al.* 2008). Gandhi *et al.* demonstrated lower levels of pro-inflammatory and higher levels of anti-inflammatory cytokine expression caused by

Tat C compared with Tat B, which might contribute to the lower neuropathogenicity of clade C (Gandhi *et al.* 2009).

Recent publications have reported conflicting results relating to the clade C risk for acquiring HAND. A study in Ethiopia did not find impairment on the IHDS of HIV-positive cART-naïve patients infected with clade C when compared to HIV-negative controls (Clifford *et al.* 2007). In contrast, the Australian Pacific NeuroAIDS Consortium found substantial deficits in cognitive and motor function in subjects infected with clade C HIV-1 (Wright *et al.* 2008). A study in China found HAND deficits that were comparable with those reported in western countries with mostly clade B infection (Heaton *et al.* 2008). Similarly, a study of HIV-positive, cART-naïve subjects in South Africa where clade C predominates, found the prevalence of MND and HAD to be 42.4 and 25.4% respectively (Joska *et al.* 2010d). These, and several other, studies in distinct clade C cohorts worldwide suggest that individuals infected with clade C may be at equal risk of developing HAND.

2.5 Apolipoprotein E

Apolipoprotein E (apoE) is a plasma protein originally studied for its role in lipid metabolism and transport of lipids among various cells of the body (Weisgraber 1994; Mahley 1988). ApoE is expressed in three common isoforms designated apoE2, apoE3 and apoE4. The gene for apoE is located on human chromosome 19 within the same genomic region that has previously been associated with late-onset familial AD (Mahley and Rall Jr 2000; Poirier *et al.* 1993). The apoE isoforms have been shown to play key roles in other biological processes not related to lipid metabolism such as modulating innate and acquired immune responses *in vitro* and *in vivo*, and being a risk factor for AD (Burt *et al.* 2008; Laskowitz *et al.* 2001; Poirier *et al.* 1993). ApoE is the main apolipoprotein produced by astrocytes and microglia in the CNS and apoE has been shown to play a role in the CNS response to chronic HIV infection (Kuhlmann *et al.* 2010b; Urosevic and Martins 2008; Lynch *et al.* 2003; Saura *et al.* 2003).

The frequency of the three alleles – epsilon-2 (ϵ 2), epsilon-3 (ϵ 3), and epsilon-4 (ϵ 4) – of the apoE gene (APOE) vary substantially between different ethnicities around the world, in particular between individuals of European and African descent (Gerdes 2003). APOE ϵ 3

is said to be the most common in human populations everywhere, but high APOE $\epsilon 4$ frequencies have been reported in African populations (Eichner *et al.* 2002; Zekraoui *et al.* 1997). Sandholzer found an allelic frequency of 37% for the Khoi San (“Bushman”) of southern Africa (Sandholzer *et al.* 1995). The higher frequency of $\epsilon 4$ in individuals of African ethnicity may have implications for the rate of occurrence of neurodegenerative conditions in the southern African setting (Joska *et al.* 2010a).

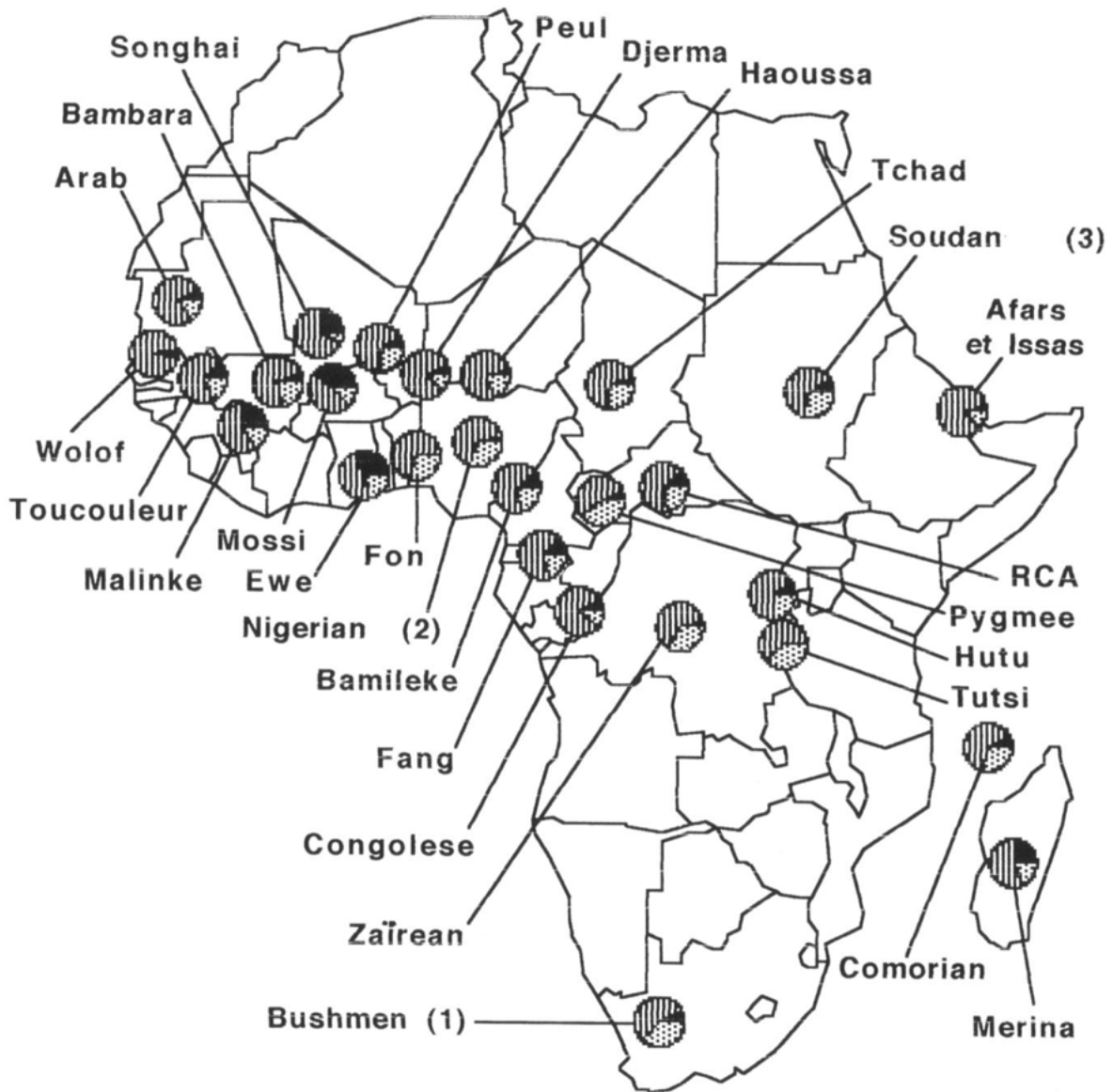


Figure 2.13 APOE allele frequencies in various African populations. In the pie charts, solid sections refer to the APOE $\epsilon 2$ allele, vertically ruled sections to the APOE $\epsilon 3$ allele, and stippled sections to the APOE $\epsilon 4$ allele.

(Zekraoui *et al.* 1997)

APOE $\epsilon 4$ has been identified as a risk factor for AD and has also been investigated as a host risk factor for the development of HAND (Saunders *et al.* 1993a). There are similarities between neurodegeneration in AD and that of HAD with AIDS patients possibly having an increased risk for developing amyloid plaques in the cerebral cortex (Esiri *et al.* 1998).

Multiple mechanisms of apoE modulation of neurodegeneration have been proposed (Figure 2.14):

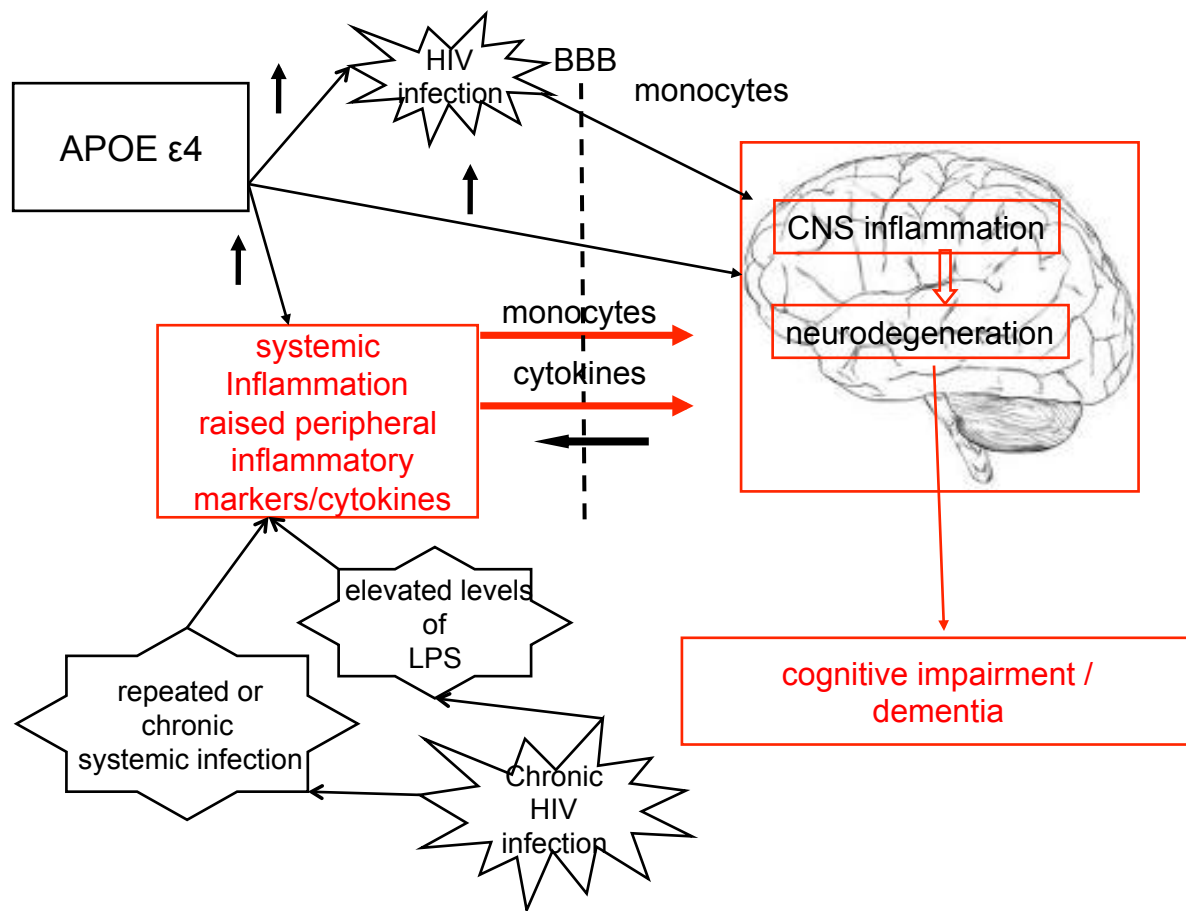


Figure 2.14 The relationship between APOE $\epsilon 4$, systemic infection, CNS inflammation, neurodegeneration and cognitive impairment in HIV-1 infection.

Chronic neuroinflammation is a well-established characteristic of HAND as well as of AD (Smits *et al.* 2000). A study done by Harry *et al.* suggested a regulatory role for apoE in maintaining a critical balance between various pro-inflammatory cytokines. An age-dependent increase in the expression of pro-inflammatory cytokines TNF- α and IL-6 in the blood after LPS injection was observed in APOE $\epsilon 4$ transgenic mice (Lynch *et al.* 2003; Harry *et al.* 2000). Vitek and colleagues demonstrated a more pro-inflammatory

phenotype that included altered cell morphology and increased production of cytoactive factors in microglia derived from APOE ϵ 4 transgenic mice compared to ϵ 3 transgenic mice (Vitek *et al.* 2009). ApoE4 was also found to be less effective than apoE2 and apoE3 at suppressing microglial activation and brain inflammation (Laskowitz *et al.* 2001; Barger *et al.* 1995). Cutler and colleagues found elevated levels of sphingomyelin, ceramide and cholesterol in the brains of HAD patients with the ϵ 4 allele compared to those with the ϵ 3 allele (Cutler *et al.* 2004). This disturbance in sphingolipid metabolism may make neurones more vulnerable to injury through oxidative stress, and may, therefore, play a role in the initiation and progression of dementia in HIV-1 infected patients with the APOE ϵ 4 genotype (Cutler *et al.* 2004; Haughey *et al.* 2004). Additionally, apoE3 has been shown to protect neurones against HIV-1 Tat toxicity *in vitro* while apoE4 does not have any protective properties against Tat-induced oxidative effects (Pocernich *et al.* 2004). In summary, it seems that apoE plays an important role in modifying both systemic and brain inflammatory responses. These effects seem to be specific to the ϵ 4 allele as it is less effective than ϵ 3 at down-regulating the brain's inflammatory response and has also been associated with an increased expression of pro-inflammatory cytokines *in vivo*, both peripherally and centrally. The apoE4 isoform has also been shown to affect lipid and sterol metabolism in the brain, increasing the susceptibility of neurones to oxidative insults.

Another mechanism by which apoE may influence the risk of acquiring HAND is through directly affecting the dynamics of HIV infectivity (Jayadev and Garden 2009). Burt *et al.* found the presence of the ϵ 3 allele to be associated with a slower HIV disease progression. The presence of two ϵ 4 alleles was, however, associated with an accelerated HIV disease course. ApoE4 also predisposes cells to a significantly higher rate of HIV cell infection *in vitro* by facilitating a greater frequency of fusion events than apoE3 (Burt *et al.* 2008). Carrying the ϵ 4 allele has been associated with an increased steady-state viral load (Kuhlmann *et al.* 2010a). In summary, the available evidence indicates that the presence of the ϵ 4 allele has detrimental effects on the course of HIV infection through accelerated virus entry, higher viral loads and faster disease progression.

Published clinical reports relating APOE ϵ 4 to HAD have produced conflicting results. Corder and colleagues reported HAD as being twice as prevalent in carriers of the ϵ 4 allele compared with non-carriers. Subjects with the ϵ 4 allele had excess dementia, demonstrated little improvement over a series of neuropsychological tests and also had slower mental speed than subjects with the ϵ 3 allele (Corder *et al.* 1998). Valcour *et al.*

observed that, after controlling for age and diabetes status, the presence of an APOE ϵ 4 allele was associated with a threefold independent increase in the risk for HAD in older (i.e. ≥ 50 years of age) HIV-positive individuals. No increased risk related to APOE ϵ 4 was, however, demonstrated in younger participants in the group (Valcour *et al.* 2004b). In a HIV-infected Chinese cohort, the ϵ 4 allele was found to be present in a greater proportion of cognitively impaired people than those with other apoE isoforms (Spector *et al.* 2010).

In contrast, several studies have failed to demonstrate a relationship between APOE ϵ 4 and HAD. Although the ϵ 4 allele was associated with an accelerated HIV disease course, Burt *et al.* did not detect an association between APOE genotype and the rate of progression to HAD (Burt *et al.* 2008). Other studies also did not find statistically significant relationships between apoE genotypes and the risk of HAND (Morgan *et al.* 2013; Dunlop *et al.* 1997). The first Southern African study to evaluate the relationship between apoE genotype and HAND was done by Joska *et al.* in 2010. This group also found no differences in the allelic distributions of apoE across the different HAND categories. In fact, they noted a significantly lower frequency of the ϵ 4 allele in adults with HAD compared to those without HAD (Joska *et al.* 2010a).

In summary, the apoE allelic variants occur with different frequencies across different populations around the world. The ϵ 4 allele is thought to be the most common in African populations, and particularly in southern Africa where a high prevalence of ϵ 4 was reported amongst the native Khoi San population. Multiple mechanisms of apoE modulation of neurodegeneration in HIV-positive individuals have been proposed. Most studies of apoE have been conducted in countries where HIV-1 clade B is predominant, and results have been controversial. Large prospective cohort studies of HIV-1 clade C-positive subjects of African descent are needed to further examine the relationship between apoE genotype and HAND.

2.6 Summary

HIV-1 enters the CNS early after infection. A low-grade inflammatory response (neuroinflammation) is initiated as a result, and this can lead to neurodegeneration and cognitive impairment in up to 50% of HIV-positive individuals. The spectrum of HAND

ranges from mild impairment to severe dementia. Repeated or chronic systemic infections may enhance neuroinflammation and consequently drive neurodegeneration through signalling from the peripheral blood to the CNS. The widespread use of cART has significantly altered the nature of HAND, specifically decreasing the incidence of dementia due to HIV. The less severe forms of HAND, however, remain common. Generally the initiation of cART improves neuropsychological function, but this improvement is neither full nor universal. Not all HIV-positive individuals develop HAND, and a variety of host and viral factors have been associated with an increased risk of developing HAND. Of particular importance in the South African setting is viral clade and apoE genotype. HIV-1 clade C predominates in southern Africa and has been associated with a potentially higher risk for moderate-to-severe HAND complications. The $\epsilon 4$ allele of APOE is prevalent in African populations and in Southern Africa, and has been associated with an accelerated HIV disease progression and an increased risk of developing HAND.

CHAPTER 3: AIMS AND HYPOTHESES

AIMS

This study aims to define the role of systemic inflammation in the pathogenesis of HAND as well as to determine why some people with HIV develop neurocognitive impairment while others do not. The study will provide information about risk or associative factors in an attempt to identify patients at risk for developing HIV-associated dementia. Furthermore it will aim to identify those patients expected to have a poorer neurocognitive response to cART. We shall use baseline information to predict cognitive changes following cART.

PRIMARY OBJECTIVE

- We shall measure levels of pro-inflammatory and anti-inflammatory cytokines in the peripheral blood of cART-naïve individuals and relate these to their cognitive status.

SECONDARY OBJECTIVES

- We shall determine whether initial cytokine profiles predict improvement in cognitive scores as a response to cART, by comparing cognitive scores at baseline with follow-up cognitive scores after at least 9 months on cART.
- We shall determine whether the presence of the APOE ε4 allele is associated with increased systemic inflammation and higher levels of pro-inflammatory cytokines compared with APOE ε2 or ε3.
- We shall determine whether the presence of APOE ε4 is associated with worse cognitive impairment at baseline, compared with APOE ε2 or ε3.
- Potential confounding effects (age, education, CD4 count) shall be taken into consideration in the above analyses.

HYPOTHESES

Hypothesis 1

In the initial cross-sectional analysis of cART-naïve participants, a predominantly pro-inflammatory cytokine profile in the peripheral blood will correlate with more severe cognitive impairment. Conversely, better cognition will be associated with a predominantly anti-inflammatory cytokine profile.

That is, a predominance of pro-inflammatory cytokines will be associated with greater cognitive impairment whereas more anti-inflammatory cytokines will be associated with less cognitive impairment.

The potential confounding effects of disease severity, as represented by the CD4 count, will be considered in testing this hypothesis.

Hypothesis 2

In the initial cross-sectional analysis, the presence of the $\epsilon 4$ allele of APOE will be associated with increased levels of systemic inflammation (higher levels of pro-inflammatory cytokines) compared with the presence of the $\epsilon 2$ or $\epsilon 3$ alleles.

In other words, the presence of the $\epsilon 4$ allele will be associated with higher levels of pro-inflammatory cytokines. Conversely, the absence of the $\epsilon 4$ allele will be associated with lower levels of pro-inflammatory cytokines.

Hypothesis 3

In the initial cross-sectional analysis, individuals in possession of the $\epsilon 4$ allele of the APOE gene will have greater cognitive impairment than those with the $\epsilon 2$ or $\epsilon 3$ alleles.

That is, the presence of the $\epsilon 4$ allele will be associated with worse cognitive function. Conversely, the absence of the $\epsilon 4$ allele will be associated with better cognitive function.

Hypothesis 4

Participants with an initial pro-inflammatory cytokine profile at baseline will respond better to cART in terms of cognitive function than those with an initial anti-inflammatory profile.

That is, a predominantly pro-inflammatory profile will be associated with greater improvement in cognitive function, while a predominantly anti-inflammatory profile will be associated with less improvement in cognitive function.

CHAPTER 4: METHODS

In 2007 a collaborative research programme was initiated by the Department of Medicine (Division of Neurology) and the Department of Psychiatry at UCT. The aim of this project was to investigate the neurocognitive disorders found in young adults with HIV/AIDS commencing anti-retroviral treatment in the Western Cape of South Africa.

My research project forms part of this larger study and is a cross-sectional, correlative analysis with a longitudinal component. For my data analysis I utilized the participant information that was collected at baseline and follow-up visits, in the existing study prior to the start of my project. Additionally, for my laboratory analysis, I used frozen serum samples that were collected at baseline and stored at -80°C. APOE genotyping was also completed prior to the start of my project and I was able to use those results in my analyses.

4.1.1 Participants

Research nurses from voluntary counselling and testing (VCT) clinics at Khayelithsa Site C, Woodstock Community Health Care Centre, and Mitchells Plain Community Health Care Centre in the Cape Town metropolitan area were responsible for screening and recruitment of the study participants. Recruitment commenced in February 2008 and continued until August 2009. These participants were all between the ages of 18 and 35 years old, HIV-positive and cART naïve, but in the pre-treatment phase of counselling to start cART.

In the larger original study 167 participants of the 283 participants that were screened completed baseline assessments. Reasons for not attending the full assessment included: financial constraints, the inability to leave work and travel in and out of Cape Town. Of the 167 baseline participants, 109 participants completed follow-up cognitive assessments after one year. Of the 109 follow-up participants, 82 were known to have started cART and 22 were known not to have started cART.

93 HIV-negative age-matched controls were recruited by invitation from the same VCT clinics. These control participants underwent the full neuropsychological battery to assess their cognitive function in order to create a set of “normal” scores for each test. This

allowed for the calculation of a “normal” population-specific mean and standard deviation for each test. These values were used to calculate standard scores (Z and T scores) for each HIV-positive participant being tested by the same neuropsychological battery.

From the group of 167 baseline participants who also met inclusion criteria for my study, I was able to randomly select a sample of 114 participants for the cross-sectional component of my study. Of the 114, a group of 40 subjects met the inclusion criteria and were used for the longitudinal part of my study. I did not include an HIV-negative control group because I was primarily interested in comparing inflammation and cognitive impairment within a group of HIV-positive patients.

4.1.2 Inclusion criteria

Original study:

- Young adults between the ages of 18 and 35 years. This age range was chosen to limit the confounding effect of age and age-related neurodegeneration or cerebrovascular disease
- A recent positive diagnosis of HIV infection made within the last 6 months, including initial and confirmatory tests
- No previous use of antiretroviral medications (cART-naïve)
- Qualification for, and enrolment into, an antiretroviral treatment programme with routine follow-up via this local rollout clinic
- At least 7 years of formal education

This study:

- Inclusion criteria for original study
- Complete data (cognitive scores and CD4 count)
- Available serum samples for cytokine analysis
- At follow-up, on cART for at least 9 months to limit immune reconstitution inflammatory syndrome (IRIS) as a possible cause for symptoms

4.1.3 Exclusion criteria

Original study:

To diagnose HAND it is necessary to exclude other potential causes of cognitive impairment, therefore subjects with the following conditions were excluded:

- Presence of uncontrolled general medical conditions such as diabetes mellitus, epilepsy, and active tuberculosis requiring admission or second-line treatment
- Presence of an identified central nervous system neurological condition such as lymphoma, untreated neurosyphilis, and cryptococcal infection. Those who previously had such conditions and were deemed to have been fully treated were eligible for inclusion.
- History of severe mental illness (psychotic disorder or active major depression)
- Abuse of alcohol or other psycho-active substances within the preceding three months (current substance abuse / dependence)
- History of head injury with loss of consciousness of >30 minutes duration, and/or requiring overnight admission to hospital
- Participants who for any reason, could not undergo the full assessment including the blood tests and neuro-imaging studies
- Participants who refused to sign informed consent

This study:

- Exclusion criteria for original study
- Incomplete data (cognitive scores)
- No serum available
- At follow-up on cART for less than 9 months

4.1.4 Study design

The larger, original study was designed as a prospective controlled analytical study.

My component of this larger study is a cross-sectional correlative analysis with a longitudinal component.

In the cross-sectional part of the study, the levels of four selected pro- and anti-inflammatory cytokines in the peripheral blood of HIV-positive participants were measured and correlated with their cognitive function as assessed at baseline before starting cART.

I also used the apolipoprotein E genotype data available for all participants to investigate the relationships between the $\epsilon 4$ allele, systemic inflammation (concentrations of inflammatory cytokines) and cognitive impairment.

In the longitudinal part of the study, I compared the cognitive scores as assessed at follow-up after at least 9 months of cART therapy, with the baseline (pre-cART) cognitive assessment and cytokine profile to determine whether the baseline cytokine profile could predict subsequent changes in cognition on cART.

4.1.5 Instruments and measures

A. Screening assessments

At the screening clinics, patients completed the following questionnaires that were used to identify any conditions that would exclude them from the study:

- The Mini International Neuropsychiatric Interview (MINI): To exclude those with a history of severe mental illness (Sheehan *et al.* 1998);
- The Centers for Epidemiological Study-Depression Scale: To exclude participants with active major depression (Myers and Weissman 1980);
- The Alcohol Use Disorders Identification Test (AUDIT): To identify participants with a history of recent substance abuse (Saunders *et al.* 1993b)

B. Baseline assessments

Baseline assessments involved a clinical examination, cognitive/neuropsychological test battery and the collection of functional, quality of life and socio-demographic data (age, weight, height, gender and home language). A total of 30 millilitres of blood was also collected for routine laboratory tests, APOE genotyping and cytokine analysis.

Clinical evaluation

The clinical evaluation involved a general physical and a neurological examination. The neurological investigation focussed on detecting signs of peripheral neuropathy (visual analogue scale and assessment of vibration sense), an assessment of motor tone and power, involuntary movements, primitive reflexes and a timed gait test for motor slowing. An assessment of cognition was performed using the International HIV dementia scale

IHDS, a screening tool for HIV-associated dementia (Power *et al.* 1995). The latter has also been validated for use in developing countries (Sacktor *et al.* 2005). In addition, the Mini Mental State Examination (MMSE) was used as a screening tool for cognitive impairment (Folstein *et al.* 1975). Neurological findings were used to calculate a neurological raw score that was used to determine HAND category (Joska *et al.* 2010d).

Special investigations

Blood specimens for determining full blood count, kidney and liver function tests, C-reactive protein, calcium, magnesium, phosphate, total protein, glucose, folate, vitamin B12 and iron studies were collected at the first clinic visit. Lowest pre-treatment CD4 cell count (nadir CD4) and viral load data were obtained from the clinic records at the recruitment sites. Blood specimens were also used for APOE genotyping and freshly spun serum stored for measurement of inflammatory cytokines.

DNA was extracted from whole blood samples and APOE genotyping was performed at the Department of Chemical Pathology (UCT Medical School) using the restriction enzyme digestion method described by Hixon (Hixson and Vernier 1990).

Whole blood samples for inflammatory markers were centrifuged at 4000rpm for 10 minutes immediately after collection and the serum supernatant was aspirated, aliquotted, and stored at -80°C for subsequent analyses.

Neuro-imaging was performed using MRI at the Cape Universities Brain Imaging Centre (CUBIC) at Stellenbosch University Medical School in collaboration with the medical imaging unit at UCT. The purpose of neuro-imaging for my study was primarily to exclude intracranial or mass lesions.

Cognitive tests and neuropsychological assessment

The domains most affected by HAND are attention, learning and memory, motor function, psychomotor speed, executive function, psychomotor speed, executive function and language (Power *et al.* 2009; McArthur *et al.* 2005). All participants underwent a battery of cognitive tests. The tests were translated into Xhosa and Afrikaans and validated by back-translation into English. Changes to word lists were made to reflect local language and idioms. The test battery was selected so that the tests represented the domains typically affected by HIV (Butters *et al.* 1990). These specific tests were also commonly used in

international settings, and thus made our findings comparable. The selected battery was based on that used by the San Diego HIV Neurobehavioral Research Center (HNRC) (located at the University of California in San Diego) (Carey *et al.* 2004b). The battery comprised the following tests:

- Attention (Mental Alteration Test and Mental Control Test)
- Learning and memory (Hopkins Verbal Learning Test and Brief Visuospatial Memory Test)
- Motor (Finger Tapping and Grooved Peg Board – dominant and non-dominant hands)
- Psychomotor speed (Trail Making part A, Color Trails 1 and Digit Symbol Coding)
- Executive function (Color Trails 2, Stroop Color Word test, Wisconsin Card-sorting Test and Rey Complex Figure)
- Language (Category fluency animals and Category fluency fruit and vegetables)

Table 4-1 further explains how the tests are done and the aspect of cognitive function being tested.

Cognitive tests according to domain	
a) Attention	
Mental Alternation Test (MAT) (Jones <i>et al.</i> 1993)	<p>Participants are asked to count to 20 and then to recite the alphabet. Thereafter they must recite both at the same time, in ascending sequence and alternating between numbers and letters, in a set time period.</p> <ul style="list-style-type: none"> • Tests sequencing ability and switching between categories

<p>Weschler Memory Scale III (Mental Control Test) (Wechsler 1997a)</p>	<p>Participants must recite known sequences such as the numbers 1-20, days of the week, or months of the year. The task is then repeated with the sequence in reverse. The tests are timed.</p>
<p>b) Learning and Memory</p>	
<p>The Hopkins Verbal Learning Test (HVL) (Brandt 1991)</p>	<p>3 trials of recall (immediate and 15 min delayed) of a 12-item, semantically categorized list. This is followed by yes/no recognition of the words within a longer list of other words that are similar.</p> <ul style="list-style-type: none"> • A brief assessment of verbal learning and memory based on recognition and recall; total learning
<p>The Brief Visuospatial Memory Test (BMVT) (Benedict 1997)</p>	<p>Participants are shown 6 figures for 10 sec each and are then required to replicate exact copies, immediately and at 25 min delayed recall. They are also asked to identify the 6 figures from a list of 12 similar figures.</p> <ul style="list-style-type: none"> • Tests visuospatial and recall ability; total learning
<p>c) Motor function</p>	
<p>Finger Tapping Test (Halstead 1947)</p>	<p>Participants must tap on a key counter using the index finger of the dominant hand for 10 sec. This is repeated until 5 consecutive trials within 5 taps of each other, or a total of 14 trials are obtained. The test is then repeated with the non-dominant hand.</p> <ul style="list-style-type: none"> • Tests motor speed and motor control

<p>Grooved Pegboard Test (Klove 1963)</p>	<p>Participants are required to place 25 small metal pegs into 25 holes on a metal board. All pegs are similar with a ridge along one side, which corresponds to a randomly positioned slot in each hole on the board. Each peg must, therefore, be rotated to match the slot on the hole before it will fit. Pegs should be placed as fast as possible, first with dominant and then non-dominant hand.</p> <ul style="list-style-type: none"> • Tests manual dexterity, co-ordination and motor speed for dominant and non-dominant hands
<p>d) Psychomotor Speed</p>	
<p>Trail Making Test A (Reitan and Davison 1974)</p>	<p>The participant is presented with a series of randomly arranged circles, each of which contains a different number from 1-25. The task is to connect the circles in ascending order as fast as possible.</p> <ul style="list-style-type: none"> • Tests visual attention, task switching and mental flexibility
<p>Color Trails I (D'Elia 1996)</p>	<p>The participant is presented with a series of randomly arranged circles, each with a number from 1-25. Circles alternate in colour and the task is to connect the consecutive numbers in an ascending sequence, which also implies alternating colours.</p> <ul style="list-style-type: none"> • Tests visual attention, ability to switch between tasks and mental flexibility

<p>Wechsler Adult Intelligence Scale, 3rd Edition – WAIS III (Digit Symbol Coding) (Wechsler 1997b)</p>	<p>Participants are presented with paired digits and symbols. A list of digits is then given and the participant must write the appropriate symbol next to each digit as fast as possible.</p> <ul style="list-style-type: none"> • Assesses processing speed
<p>e) Executive Function</p>	
<p>Color Trails 2 (D'Elia 1996)</p>	<p>The test is made up of colour-number pairs and the colours should be alternated when chronologically connecting the numbers.</p> <ul style="list-style-type: none"> • Tests visual attention, ability to switch between tasks and mental flexibility
<p>Stroop Color Word Test (SCWT) (Stroop 1935)</p>	<p>The participant is first asked to read the names of colours printed in black and white. Next, the participant is presented with a page where the names of colours are printed in conflicting colours (e.g. BLUE written in green ink). The test is to name the colour that they see and not read the name that is written.</p> <ul style="list-style-type: none"> • Tests executive function, processing speed, selective attention and cognitive flexibility
<p>Wisconsin Card Sorting Test (WCST) (Heaton <i>et al.</i> 1993)</p>	<p>This test uses cards with different shapes, colours and quantities. Participants must match another set of cards to the first cards without being told how the cards are to be matched (according to shape, colour or quantity). They are only informed when cards are correctly or incorrectly matched. This task tests the ability to form strategies, to shift strategies and to learn.</p> <ul style="list-style-type: none"> • Tests executive function, attention, set-shifting, modulating impulsive behaviour

<p>The Rey Osterrieth Complex Figure (RCF) (Corwin and Bylsma 1993; Rey and Osterrieth 1993)</p>	<p>Participants are presented with a complex figure, using different coloured lines that they must first copy and then draw from memory at 3 and 30 min after the initial copying task.</p> <ul style="list-style-type: none"> • Tests visuospatial ability, attention, planning and working memory
<p>f) Language</p>	
<p>Category fluency animals and fruit & vegetables (Butters <i>et al.</i> 1987)</p>	<p>Participants must generate orally as many different kinds of animals, fruits and veg as possible within a given time limit (1 min for each category).</p> <ul style="list-style-type: none"> • Tests language fluency

Table 4-1 Neuropsychological test battery

Neuropsychological interviews were performed to assess quality of life and functioning in activities of daily living:

- The Patient’s Assessment of Own Functioning (PAOFI) (Chelune *et al.* 1986)
- Quality of Life Enjoyment and Satisfaction Scale (QLESQ) (Endicott *et al.* 1993)

These instruments were also translated into Xhosa and Afrikaans and the translation was validated through back translation into the first language. Ratings on the PAOFI were used to generate a rank score for functional impairment. This rank score was also used to determine HAND category.

Due to the lack of validated normative data for our population, z-scores were calculated from the HIV-negative control data and then used to determine the degree of impairment.

The neuropsychological test battery, together with the scores from the neuromedical assessment and the functional impairment scores were used to determine neurocognitive disorder status. Based on these scores, participants with impairments were classified into one of the three HAND categories as described by Antinori and colleagues in 2007: (1) HIV-1-asymptomatic neurocognitive impairment (ANI), (2) HIV-1-associated mild

neurocognitive disorder (MND), and (3) HIV-1-associated dementia (HAD) (Antinori *et al.* 2007) (Table 4-2 provides a summary of the revised criteria.)

HAND category	Diagnostic entity	Cognitive performance	Functional performance
0	Normal cognition	Normal	Normal
1	ANI	Acquired impairment in at least 2 cognitive domains (<1SD)	Does not impact on daily functioning
2	MND	Acquired impairment in at least 2 cognitive domains (<1SD)	Interferes with daily functioning to at least a mild degree (e.g. work inefficiency, reduced mental acuity)
3	HAD	Acquired impairment in at least 2 domains, typically in multiple domains with at least 2 domains with severe impairment (<2SD)	Marked impact on daily functioning.

Table 4-2 Summary of the research criteria for HAND

(Valcour *et al.* 2011)

A Global Deficit Score (GDS) was also calculated for each participant as a quantitative measure of global cognitive deficits. The raw data from the neuropsychological test battery were converted to demographically corrected, standard scores (t-scores) using the normative data from the control group. The GDS is a useful way to summarize the results on neuropsychological testing and is calculated by converting the demographically corrected standard scores on individual neuropsychological tests to a deficit score ranging from 0 (no impairment) to 5 (severe impairment) – see Table 4.3 (Carey *et al.* 2004a; Heaton *et al.* 1995).

T scores	Deficit Score	Degree of impairment
≥ 40	0	Normal
39-35	1	Mild
34-30	2	Mild-moderate
29-25	3	Moderate
24-20	4	Moderate-severe
≤ 19	5	Severe

Table 4-3 A conversion table for transforming T scores into deficit scores

(Carey *et al.* 2004b)

An average GDS was then calculated for each participant with higher scores indicating greater impairment. Based on these scores, participants were grouped into three GDS groups: non-impaired (GDS ≤ 0.25), mild-moderately impaired (0.25 < GDS ≤ 0.75), and severely impaired (GDS > 0.75). These 3 groups were shown to correlate with the HAND categories of normal function, ANI, MND and HAD (Joska *et al.* 2012). The GDS has shown good sensitivity in detecting milder neuropsychological impairment in patients with HIV (Heaton *et al.* 1995). It is a useful tool for classifying cognitive impairment in HIV, as it does not require the use of functional assessments like the revised criteria for HAND. The GDS is a more objective measure, because HIV-positive patients often visit healthcare centres alone, without someone to give collateral information. The latter is problematic especially if the participant lacks insight (Valcour *et al.* 2011). For these reasons, I decided to use the global deficit scores in my correlation analyses with inflammatory markers and apolipoprotein E.

Cytokine measurement

Serum samples for inflammatory markers were processed at the Centre for Proteomics and Genetic Research (CPGR) at the Institute for Infectious Diseases and Molecular Medicine (IIDMM) at the UCT Medical School. These markers (analytes) included the pro-inflammatory cytokines – IL-1 β and TNF- α , and the anti-inflammatory cytokines – IL-10 and TGF- β 1. These cytokines were selected because experimental and clinical studies have shown that their expression is rapidly inducible by an inflammatory insult to the CNS. It is also thought to be the balance between pro-and anti-inflammatory cytokines in the brain that may have a profound impact on neuronal function (Boche *et al.* 2003). Cytokine analysis was done by simultaneous multi-analyte detection at the CPGR laboratory. After

delivery to the CPGR laboratory the serum samples were thawed once on ice and then stored at 4°C until time of assay. Each sample was assayed, in duplicate, according to the manufacturer’s instructions included in the assay kits.

Assay Name	Analytes
Bioplex Pro™ Assays Cytokine, Chemokine and Growth Factors BIO-RAD (www.bio-rad.com)	TNF-α, IL-1β, and IL-10
Fluorokine® MAP TGF-B Multiplex Kit R&D Systems (www.RnDSystems.com)	TGF-β1

Table 4-4 Assay kits used to determine cytokine profiles in serum samples

The Bio-rad Bio-plex 200 analyzer – a dual laser, flow-based sorting and detection platform – was used for both the Bioplex Pro™ and Fluorokine® MAP kits. (The Fluorokine MAP multiplex kits are designed to use with a Luminex® or BioRad® BioPlex® analyser.)

Multiplex cytokine assays allow researchers to simultaneously measure the levels of multiple cytokines in a single, and minimal volume (as little as 12.5 microlitres) sample of a matrix such as serum. This can be done in just three to four hours.

The Bio-Plex® suspension array system is built on the three core elements of xMAP technology:

- Fluorescently dyed micro particles (also called beads), each with a distinct colour code to allow detection of individual analytes within a multiplex suspension.
- Dedicated flow cytometer with two lasers and associated optics to measure the different molecules that are bound to the surface of the beads.
- A high-speed digital signal processor that efficiently manages the fluorescence data.

The principle of the assay is similar to that of a sandwich enzyme-linked immunoassay for cytokine analysis (ELISA) (See Figure 4.1).

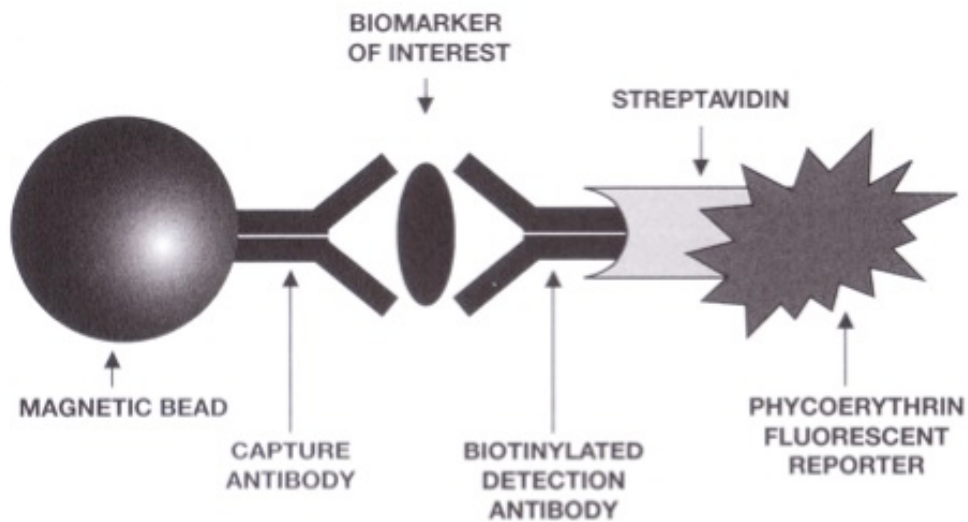


Figure 4.1 Bio-Plex sandwich immunoassay

From: Bio-Plex Pro™ Assays Cytokine, Chemokine, and Growth Factors Instruction Manual. Bio-Rad Laboratories, Inc.

Analyte-specific antibodies are pre-coated onto the fluorescently dyed and colour-coded beads (Figure 4.2 a). The beads, standards and samples are then pipetted into wells and the immobilized antibodies bind the analytes of interest. After a series of washes to remove unbound substances, a biotinylated detection antibody is added to each well to create a sandwich complex (Figure 4.2 b). Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin (streptavidin-PE) conjugate is added to form the final detection complex (Figure 4.2 c). Phycoerythrin serves as a fluorescent indicator, or reporter.

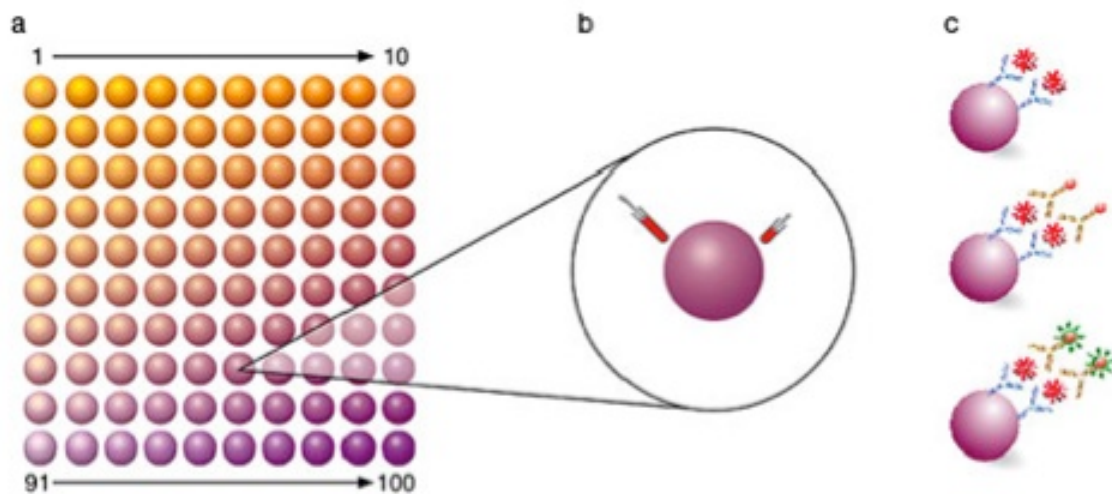


Figure 4.2 Multiplex immunoassay technology

From: <http://www.bio-rad/evportal/en/ZA/LSR/Solutions/LUSM0E8UU/Multiplex-Immunoassays>

A final wash removes unbound streptavidin-PE and the micro particles are again suspended in buffer and read using the Bio-Plex 200 analyser. When the multiplex assay suspension is drawn into the analyser, a red (635 nanometre) laser illuminates the fluorescent dyes within each bead and identifies which analyte is being detected. At the same time a green (532 nanometre) laser stimulates the phycoerythrin to generate a reporter signal, which is detected by a photomultiplier tube.

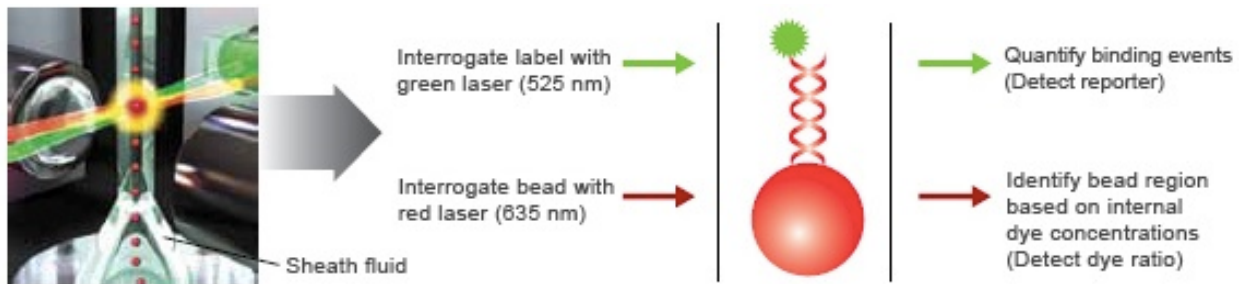


Figure 4.3 Flow cytometry-based analysis

From: <http://www.bio-rad/evportal/en/ZA/LSR/Solutions/LUSM0E8UU/Multiplex-Immunoassays>

A high-speed digital processor manages the data output and Bio-Plex Manager™ software presents the data as Median Fluorescence Intensity (MFI) as well as concentration (picogram/millilitre (pg/mL)). The concentration of analyte bound to each bead is proportional to the phycoerythrin-derived report signal.

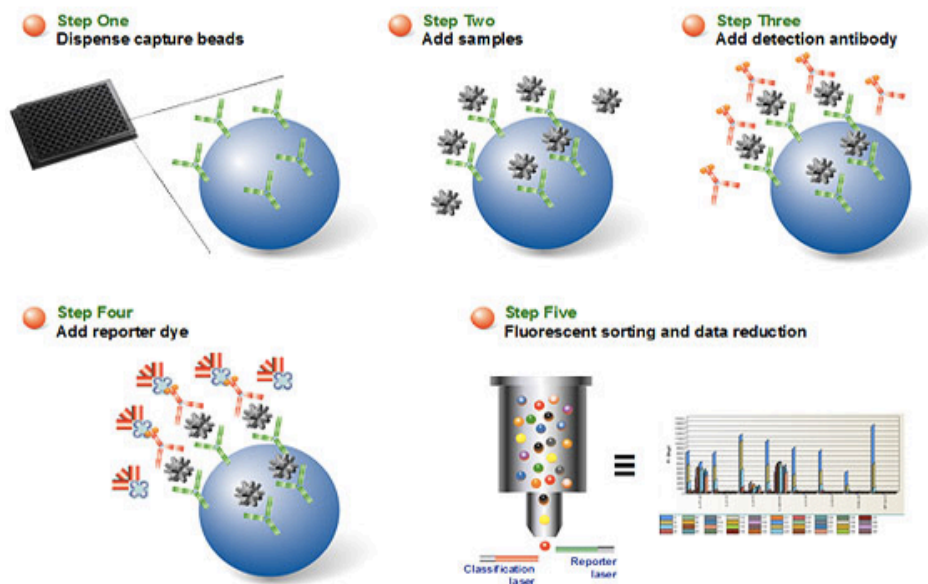


Figure 4.4 Schematic representation of an immunoassay sandwich-based assay workflow

From:

http://www.biorad.com/evportal/en/ZA/evolutionPortal.portal?_nfpb=true&_pageLabel=SolutionsLandingPage&catID=LUSM0ZMNI

The use of antibody microarray assays provides a reliable method for analysing profiles of inflammatory mediators with limited amounts of serum samples (Knight *et al.* 2004). The majority of published studies have generally shown fairly good correlations between these assays and ELISAs for most cytokines tested (Ray *et al.* 2005; Kellar and Douglass 2003; Prabhakar *et al.* 2002; Chen *et al.* 1999). The degree of correlation, however, varied widely and was mostly ascribed to differences in the antibodies that were used (Elshal and McCoy 2006). Multiplex approaches may compromise the ability of such assays to accurately measure the actual levels of cytokines (Siawaya *et al.* 2008). DuPont and colleagues examined the correlation of ELISA and multiplex bead assays for measuring the quantity of a variety of cytokines. Although they demonstrated excellent correlations for seven cytokines, which included IL-1 β , IL-10 and TNF- α , they found significant variation between the absolute cytokine concentrations determined by ELISA and the multiplex kits. These differences were again ascribed to differences in antibody pairs and sample diluents that were used (dupont *et al.* 2005). As an alternative to using a multiarray platform for cytokine quantitation, it can be used to look at relative change rather than absolute cytokine levels, with results being expressed as percentage change from a baseline level (Toedter *et al.* 2008).

We elected to use multianalyte detection for our cytokine analysis, because it allowed us to use much smaller serum concentrations than we would have needed for standard ELISAs. Because we were only interested in analysing four cytokines we trusted that the results of our analyses would be accurate and comparable, with less cross-reaction. We also trust that the manufacturers' kits have been optimized to eliminate or minimize any artefacts due to multiplexing, given rigorous adherence to the manufacturers' protocols. The CPGR reported that manufacturer's instructions for the Bio-Plex Pro™ Multiplex Cytokine Assay (IL-1 β , TNF- α and IL-10) were followed without any deviation. The manufacturer's protocol for the Fluorokine® MAP TGF- β Multiplex Kit was followed, but an additional centrifugation step was included for all samples following activation and prior to performing the assay. This centrifugation step, in a bench-top centrifuge (10000g for 5 min), was necessary to remove precipitates that formed following activation. Each of the plates used in our assay had an internal quality control, which consisted of the generation of a standard curve for each analyte using the reference cytokine concentrations supplied by the manufacturers. All plates passed quality control. Blank and negative control samples were also included on the plate, which did not elicit a response as would be expected.

Upon completion of the assays, CPGR provided us with an analytical report depicting the study procedure that was followed as well as all results generated.

4.1.6 Study procedure

Patients attending the Khayelitsha site C, Woodstock and Mitchells Plain primary care ARV clinics were screened for inclusion and exclusion criteria by clinic staff. Potential subjects were then properly screened by an investigator and enrolled into the study after giving written informed consent. After the informed consent, subjects completed the self-report questionnaires and were then referred to the Department of Psychiatry at Groote Schuur Hospital for a baseline assessment. The baseline assessment included the collection of socio-demographic data, anthropometric measurements, a clinical including neurological assessment, and the neuropsychological test battery. Blood was taken for baseline laboratory tests and was also stored for genetic and proteomic investigations. APOE genotyping was performed at the Department of Chemical Pathology (UCT Medical School). Brain imaging was performed at the Cape Universities Brain Imaging Centre located at the Tygerberg Campus of the University of Stellenbosch on a second visit before the subjects started cART. Participants were initially assessed between January 2008 and December 2009. Re-assessments were done at 6 months to 1 year after commencement of cART to determine whether there were any changes in cognitive function from baseline. At these follow-up visits anthropometry, the clinical assessment and neuropsychological test battery was repeated, but blood for follow-up serum samples was not collected.

For the present study, I utilized the data and serum that were collected and recorded at the baseline and follow-up visits that formed part of the original project. Data collected for the cross-sectional part of my study included socio-demographic information, cognitive data (GDS), apolipoprotein E genotype and baseline CD4 count. HIV infection is commonly accompanied by syphilis co-infection (Lynn and Lightman 2004). A syphilis test was, however, not performed at the baseline visit. The results of serological tests for syphilis that were performed around the time of study recruitment, were obtained from the clinic records and from the National Health Laboratory Service (NHLS) database for 90 of the 114 participants in my study. Three of these tested weakly positive and 1 positive, but there was sufficient time before enrolment into the original study for participants to have

completed treatment for the positive syphilis serology. Data for the longitudinal part of my study included the time between baseline and follow-up assessments, duration on cART, follow-up GDS and CD4 counts at follow-up (not available for all participants). All serum samples stored at -80°C were thawed on ice and then aliquotted into specimen tubes (100µL per tube). The prepared samples were transported on ice to the CPGR laboratory for storage in their freezer at -20°C until the time of analysis.

4.1.7 Data management and analysis

All data, including the results of the cytokine assays, were recorded on a Microsoft Excel spread sheet. Data were prepared for analysis and then imported into the IBM® Statistical Package for the Social Sciences (SPSS) version 21 software package for statistical analysis. The level of statistical significance was set at $\alpha = 0.05$. Distributions of the variables were determined and non-parametric methods were used as appropriate. The specific analyses performed will be discussed in more detail in the results section.

4.1.8 Ethical considerations

This study formed part of a collaborative project between the Departments of Medicine (Division of Neurology) and Psychiatry at UCT. Ethical approval for the study, including an amendment for the cytokine assays (19 May 2008), was obtained from the University of Cape Town/Groote Schuur Hospital Human Research Ethics Committee (HREC) (No. REC 263/2007). The study adhered to the principles of the 2008 revised International Helsinki Declaration (World Medical Association 2009), the South African Guidelines for Good Clinical Practice and the Medical Research Council Ethical Guidelines for Research.

4.1.9 Summary of methods

An overview of the methods described above is presented in figures 4.5 and 4.6.

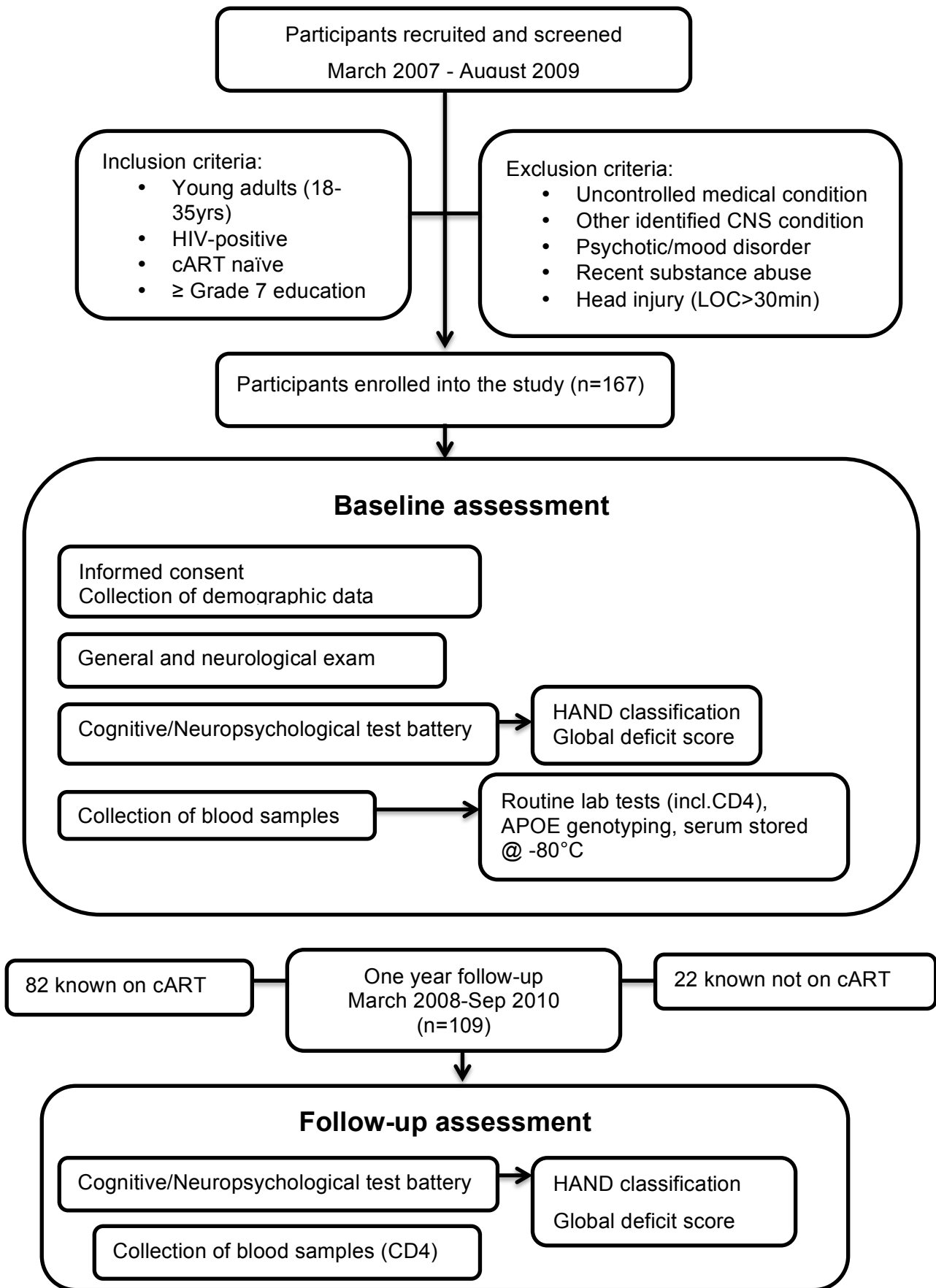


Figure 4.5 Summary of methods relating to original study

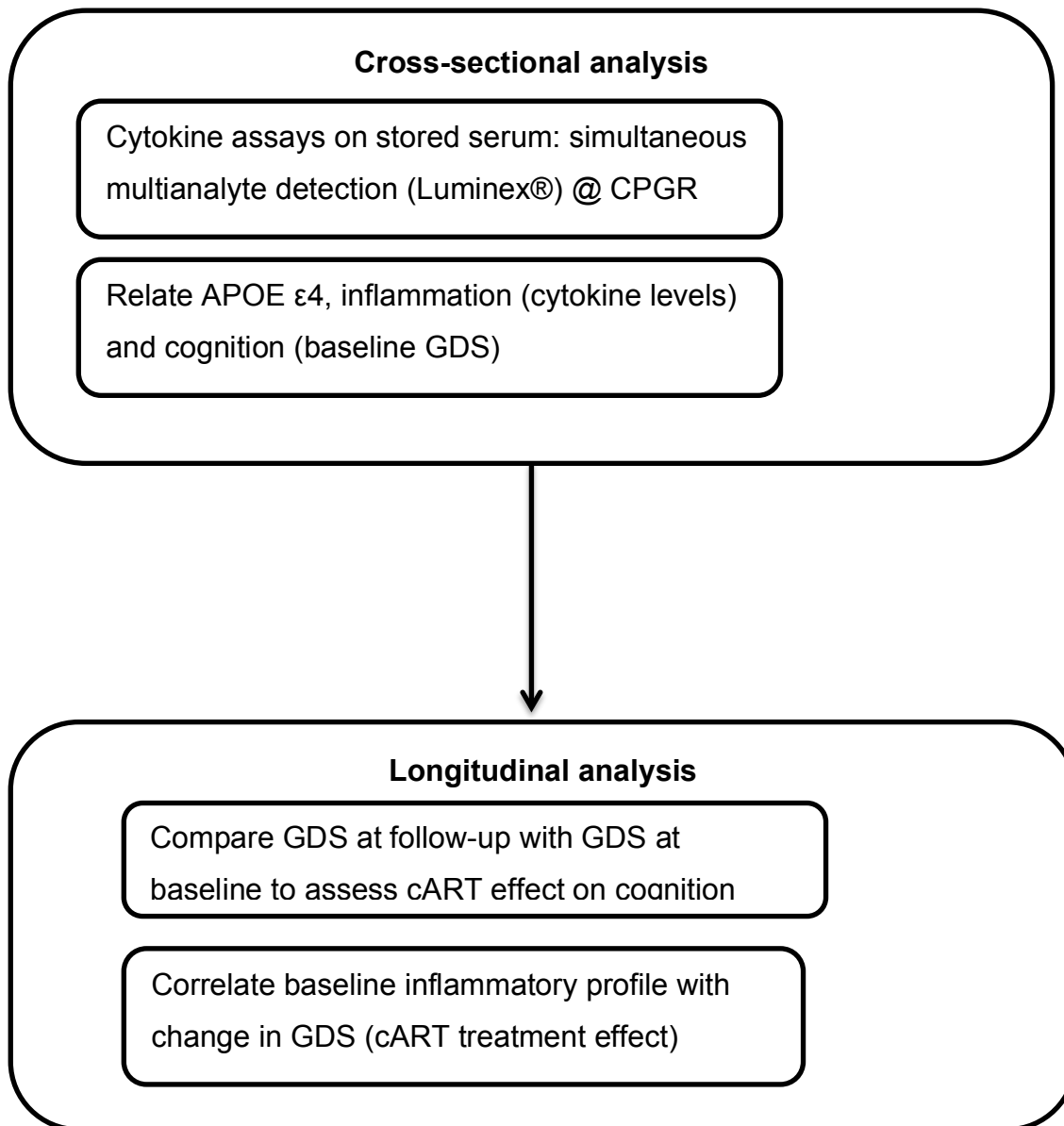


Figure 4.6 Summary of methods relating to this study

CHAPTER 5: RESULTS OF THE STUDY

This chapter will begin with a description of the demographic characteristics of the participants both in the cross-sectional and the longitudinal components of the study. The CD4, APOE and cognitive data will also be described in this section. Statistical analyses were conducted using SPSS® version 21. Frequency tables and bar charts were used to display categorical demographic data (gender, home language, years of education) while summary statistics and histograms were used to display continuous variables (age at first assessment, baseline CD4 count, baseline GDS, inflammatory markers). Normality of the data was assessed by means of the Shapiro-Wilk test of normality. It was determined that all variables were skewed in their distribution (SW, $p < 0.05$). The appropriate non-parametric methods were then used to analyse the data. The results of specific hypothesis testing will be presented in the next section. The chapter will conclude with a summary in general terms of the results obtained.

This study aimed to define the role of systemic inflammation in the pathogenesis of HAND as well as to determine why some people with HIV develop neurocognitive impairment while others do not. The study will provide information about risk or associative factors present at baseline that might predict cognitive response to ART, and identify patients at risk for developing neurocognitive impairment and HIV-dementia.

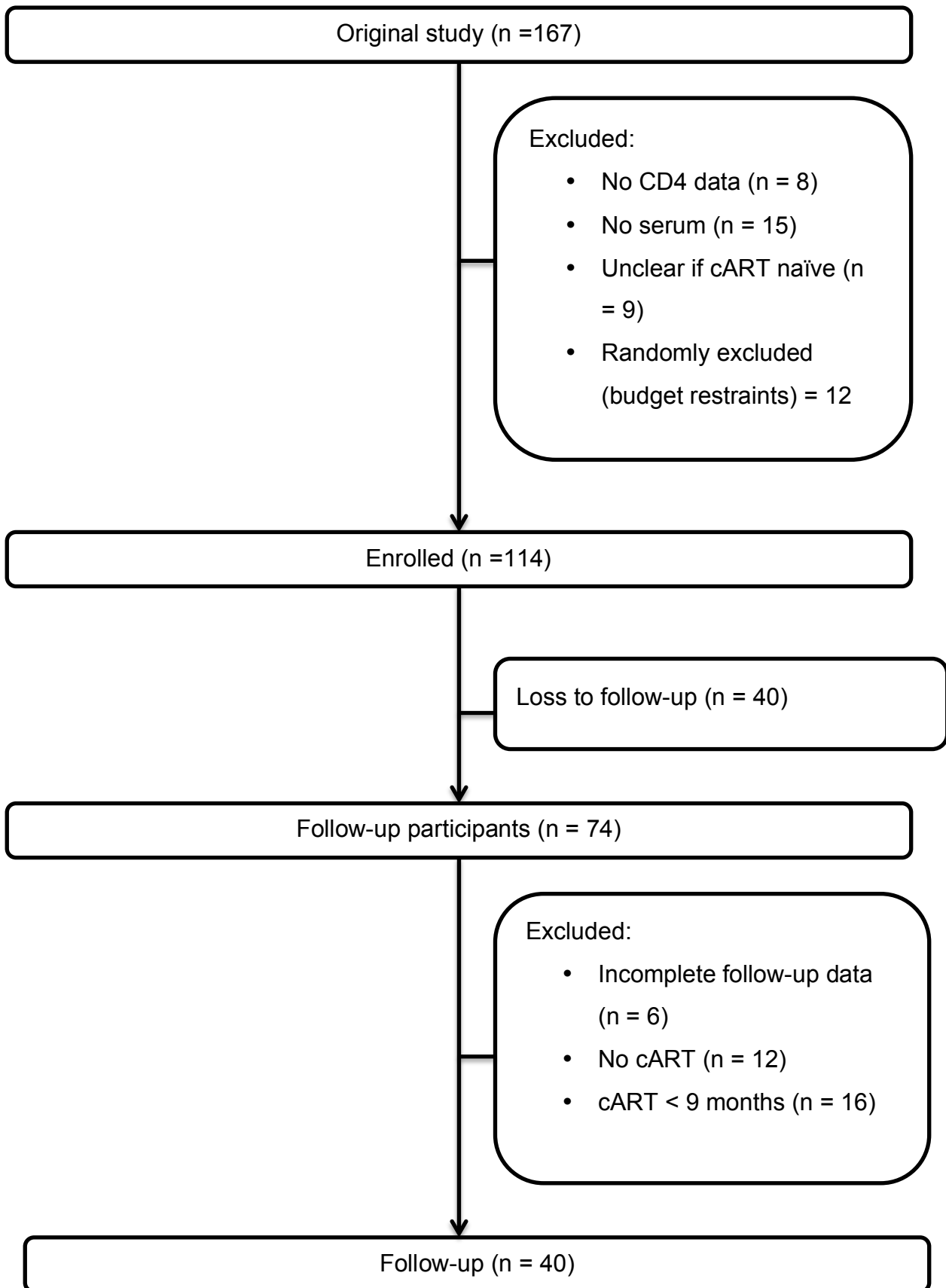


Figure 5.1 Flow diagram of participant enrolment and attrition

5.1 Participant characteristics

5.1.1 Cross-sectional component (n = 114)

A total of 114 HIV-positive, cART-naïve participants were evaluated. The majority of the sample were women (n = 91, 80%) and isiXhosa speaking (n = 101, 88.6%). The median age at first assessment was 30 years (IQR = 27-32.5) and the median number of years of school education was 10 (IQR = 9-11.25). The group had a median baseline CD4 count of 177 cells/μL and a median baseline GDS of 0.5 (mild to moderate cognitive impairment). 55 participants (48.2%) were carriers of the ε4 allele of APOE (See Table 5-1).

Median age at first assessment (IQR) (years)	30 (27-32.5)
Gender, n (%)	Female 91 (80%) Male 23 (20%)
Median years of education (IQR) (years)	10 (9-11.25)
Home Language, n (%)	isiXhosa 101 (88.6) English 7 (6.1) Sotho 3 (2.6) Afrikaans 1 (0.9) Shona 1 (0.9) Shangaan 1 (0.9)
Median baseline CD4 (IQR) (cells/μL)	177 (119-217.5)
Median baseline GDS (IQR)	0.5 (0.1-0.8)
APOE ε4 allele, n (%)	Present 55 (48.2%) Absent 58 (50.8%)

n = count, IQR = interquartile range

Table 5-1 Demographic characteristics of the participants

In order to analyse the participants in terms of cognitive functional ability, three groups were created utilizing the GDS criteria, as described in the methods section (see section 4.1.5, p 60). Based on this classification, 32 of 114 individuals (28.1%) had severe cognitive impairment while 42 of 114 participants (36.8%) were mild to moderately impaired in terms of cognitive function. 40 of 114 individuals (35.1%) had no cognitive impairment (see Table 5-2).

Baseline GDS group	No (Frequency %)
1. Normal (GDS < 0.25)	40 (35.1%)
2. Mild-moderate impairment (0.25 > GDS < 0.75)	42 (36.8%)
3. Severe impairment (GDS > 0.75)	32 (28.1%)

Table 5-2 Classification into GDS groups based on cognitive scores

The allelic and genotypic frequencies of the APOE gene in the study sample are tabulated in Table 5-3 and Table 5-4. The $\epsilon 3$ allele was found to be most prevalent (60.6%) and the frequency of the $\epsilon 4$ allele in the study sample was 28.8%. The frequency of the homozygous $\epsilon 4$ genotype was 8.8%.

Allele	Allelic frequency
$\epsilon 2$	10.6%
$\epsilon 3$	60.6%
$\epsilon 4$	28.8%

Table 5-3 APOE allelic frequencies (n=113)

Genotype	Genotypic frequency
$\epsilon 2\epsilon 2$	0.9%
$\epsilon 2\epsilon 3$	13.2%
$\epsilon 2\epsilon 4$	6.1%
$\epsilon 3\epsilon 3$	36.8%
$\epsilon 3\epsilon 4$	33.3%
$\epsilon 4\epsilon 4$	8.8%

Table 5-4 APOE genotypic frequencies (n = 113)

Baseline characteristics of the study sample were compared across the categories of baseline cognitive function (GDS groups). The groups were similar in terms of gender distribution, home language and median age at first assessment. The only statistically significant difference was in terms of education. The cognitively normal group had higher median years of education compared to the other two groups. The severely impaired group had a lower median CD4 count at baseline, but this difference was not significant. The presence of the $\epsilon 4$ allele of APOE did not differ significantly between the groups. 50% of the severely impaired group were carriers of the $\epsilon 4$ allele, but 55% of the cognitively normal group were also $\epsilon 4$ carriers. The comparison of baseline characteristics and the statistical results are shown in Table 5-5.

	GDS group			Statistical value	
	Normal GDS < 0.25	Mild-Moderate Impairment 0.25≥GDS>0.75	Severe Impairment GDS ≥0.75	X ² (df)	P
	n = 40	n = 42	n = 32		
Women (%)	35 (87.5%)	31 (73.8%)	25 (78.1%)	2.464 (2)	0.292
Xhosa speaking (%)	36 (90%)	37 (88.1%)	28 (87.5%)	12.513 (10)	0.292
Median Age at first assessment (IQR)(years)	29.5 (26-31)	30 (26-33)	30 (27.3-33)	2.426 (2)	0.297
Median years of education (IQR)	11 (10-12)	10 (9-12)	10 (9-11.3)	13.241 (2)	0.001**
Median baseline CD4 count (IQR)	202.6 ± 117.14	202.25 ± 130.55	184.06 ± 165.78	3.443 (2)	0.179
ε4 allele present (%)	22 (55%)	17 (41.5%)	16 (50%)	1.517 (2)	0.620

n = count; IQR = interquartile range; X² = Chi-square statistic; df = degrees of freedom between groups and within groups; p = p value

Table 5-5 Comparison of baseline characteristics across cognitive function groups

5.1.2 Longitudinal component (n = 40)

A total of 40 participants returned for a follow-up visit and were evaluated. The majority of the sample was women (n = 31, 78%) and isiXhosa speaking (n = 38, 95%). The group had a median follow-up CD4 count of 368.5 cells/μL (IQR = 274.5-571) and median follow-

up GDS of 0.1 (cognitively normal). The median time between the baseline and follow-up visits was 12.5 months (IQR = 12-14) and median duration time of ART was 12 months (IQR = 10-13). All participants were therefore on ART for at least 9 months prior to their follow-up assessment. 20 (50%) participants of the follow-up sample were carriers of the ϵ 4 allele of APOE (see Table 5-6).

Gender, n (%)	Female 31 (78%) Male 9 (23%)
Home Language, n (%)	isiXhosa 38 (95%) English 1 (3%) Sotho 1 (3%)
Median follow-up CD4 (IQR) (cells/ μ L)	368.5 (274.5-571)
Median follow-up GDS (IQR)	0.1 (0.00-0.38)
Median months between visits (IQR)	12.5 (12-14)
Median duration of cART (IQR)	12 (10-13)
APOE ϵ 4 allele, n (%)	Present 20 (50%) Absent 19 (47.5%) Not available 1 (2.5%)

n = count; IQR = interquartile range

Table 5-6 Demographic characteristics of the follow-up group (n = 40)

At the one-year follow-up cognitive assessment, and after at least 9 months of ART, 35.1% of the participants were classified into the cognitively normal GDS group and only 7.5% were classified into the group with severe impairment (Table 5-7). Figure 5.2 shows the GDS groups at baseline and Figure 5.3 shows the follow-up GDS groups. The distribution of baseline GDS groups for 38 participants that did not return for a follow-up visit (attrition group) is displayed in Figure 5.4. There were 29% in the cognitively normal group, 34% were mild to moderately impaired and 37% were in the group with severe cognitive impairment.

Follow-up GDS group	Frequency (%)
1. Normal (GDS < 0.25)	27 (35.1%)
2. Mild-moderate impairment (0.25 > GDS < 0.75)	10 (25%)
3. Severe impairment (GDS > 0.75)	3 (7.5%)

Table 5-7 Classification into GDS groups based on follow-up cognitive score

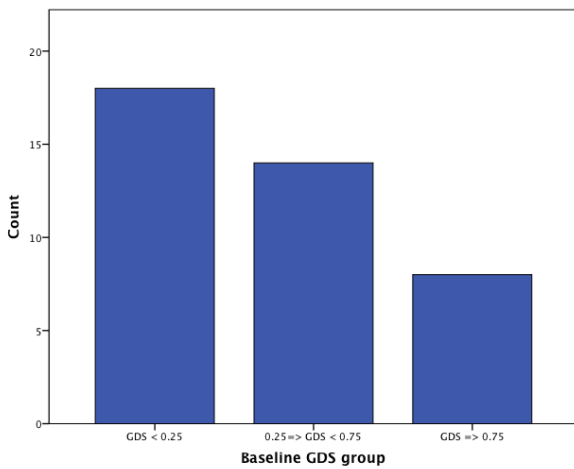


Figure 5.2 GDS groups at baseline

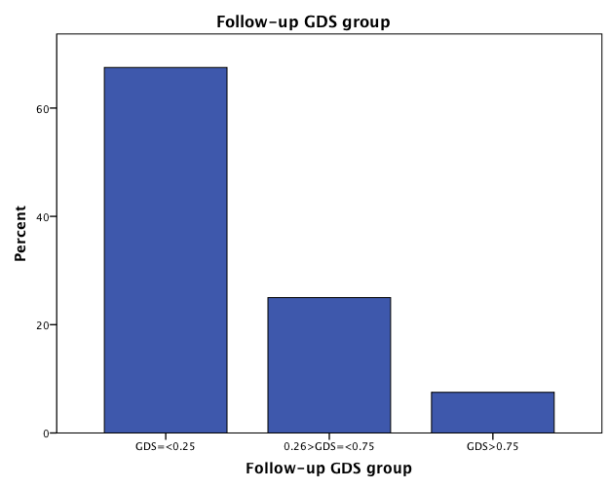


Figure 5.3 GDS groups at follow-up

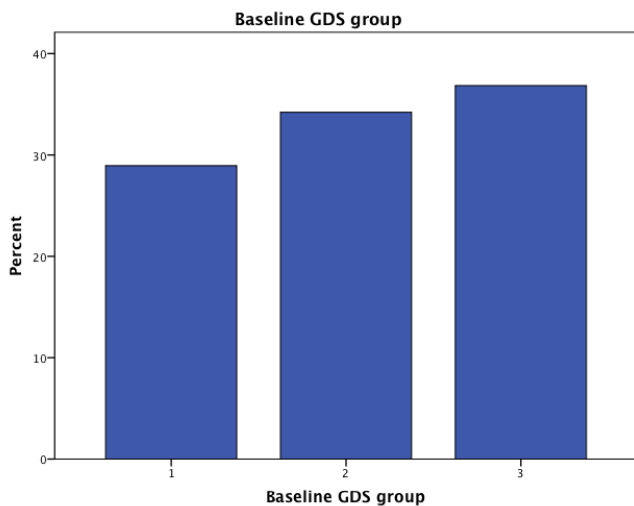


Figure 5.4 GDS groups at baseline for the attrition group

The change in cognitive function from baseline to follow-up will be discussed in more detail in the following section.

The baseline and follow-up CD4 counts are compared in Tables 5-8 and 5-9. Only 1 participant did not have an improvement in CD4 count at the follow-up visit. There was a significant improvement in CD4 count from baseline to follow-up after initiation of cART ($Z = -5.484$, $p < 0.05$).

Follow-up CD4-Baseline CD4	n (Count)
Follow-up CD4 < Baseline CD4	1
Follow-up CD4 > Baseline CD4	39
Total	40

Table 5-8 Comparison of follow-up and baseline CD4 count

Baseline CD4 (cells/ μ L)	Follow-up CD4 (cells/ μ L)	Wilcoxon Signed Ranks
Median (IQR)	Median (IQR)	$Z = -5.484$
174.5 (131.5-208)	368.5 (274.5-571)	$p = 0.000^{**}$

IQR = interquartile range, Z = Wilcoxon Signed Ranks statistic, p = p-value

Table 5-9 Comparison of median follow-up and baseline CD4 counts

5.2 Presentation of results by hypothesis

5.2.1 Hypothesis 1

Hypothesis one stated that in the initial cross-sectional analysis of cART-naïve participants, a predominantly pro-inflammatory cytokine profile in the peripheral blood will correlate with more severe cognitive impairment. The converse should therefore also be true, i.e. better cognition will be associated with a predominantly anti-inflammatory cytokine profile. In other words, higher concentrations of pro-inflammatory markers in the serum will be observed in the group with greater cognitive impairment than in the groups with no or less cognitive impairment. Conversely, greater concentrations of anti-inflammatory markers will be observed in the group with less cognitive impairment. As discussed in Section 2.3.1 C (p 13), the markers TNF- α and IL-1 β were considered “pro-inflammatory” and the markers IL-10 and TGF- β “anti-inflammatory”. This hypothesis was

tested by two different methods. In the first method the variables were used individually in the analyses. The second method involved the creation of an inflammatory profile through factor analysis. The baseline CD4 count was explored across categories of GDS and also correlated with the individual cytokines. CD4 count is a proxy marker for AIDS and could therefore be a potential confounding factor in both the cytokine concentration levels and the degree of cognitive impairment.

Method 1

The inflammatory markers were first compared across baseline GDS groups. The Kruskal Wallis Test was used to determine whether or not the median values of these continuous valued variables differed across the categories of GDS groups. The Kruskal Wallis Test is used in instances where the continuous valued variables are not normally distributed, and there are more than two categories in the grouping variable. The median concentration of TNF- α was the highest in the severely impaired group and the median concentration of TGF- β was the highest in the cognitively normal group. There were, however, no significant differences for any of the tested variables ($p > 0.05$; Table 5-10).

Variable	GDS group			Kruskal Wallis Test	
	Normal	Mild-moderately impaired	Severely impaired	X ² (df)	P-value
	Median (IQR)	Median (IQR)	Median (IQR)		
TNF- α	4.5 (4.0-6.0)	4.5 (4.0-5.5)	5.0 (4.0-6.2)	1.1 (2)	0.577
IL-1 β	3.5 (2.8-4.0)	3.4 (2.5-4.0)	3.4 (2.5-4.5)	0.89 (2)	0.641
IL-10	7.0 (6.5-9.0)	7.9 (5.5-11.0)	7.9 (6.2-11.4)	0.278 (2)	0.87
TGF- β	968.3 (692.5-1235)	867.5 (635.3-1200)	911.7 (108.7-1327.5)	1.226 (2)	0.542

IQR = interquartile range; X² = Chi-square statistic; (df) = degrees of freedom between groups and within groups; p = p value

Table 5-10 Comparison of inflammatory markers across GDS groups

These results are represented graphically by box and whisker plots (Figures 5.5 to 5.8). They show median values, interquartile range (25th to 75th percentile) and minimum and maximum values of the continuous variables not considered to be outliers for each category. As is shown in Figures 5.4 to 5.6, there were outliers. However, removal of these outliers did not alter the results and the differences between the median concentrations of the inflammatory markers across GDS groups remained non-significant.

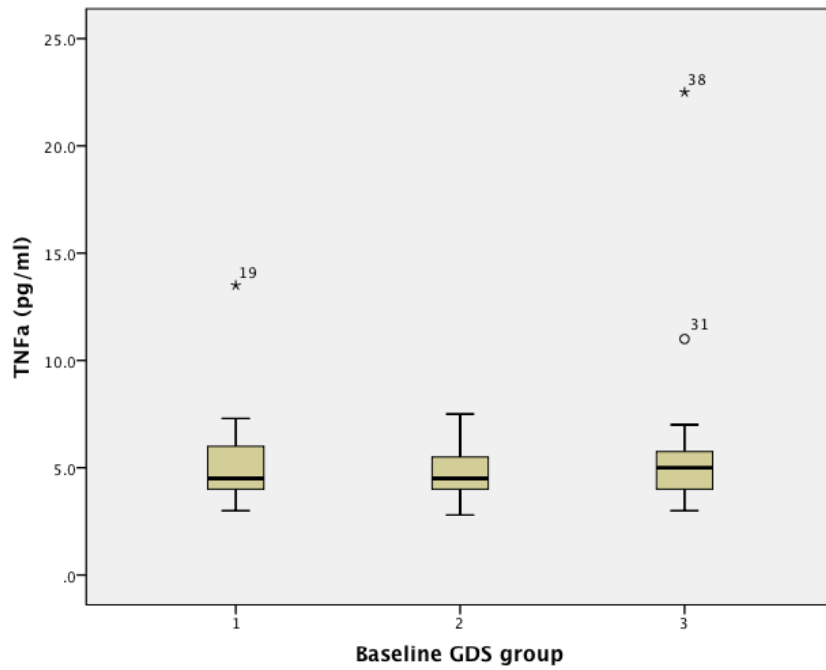


Figure 5.5 Comparison of TNF- α across baseline cognitive function groups (outlier removed)

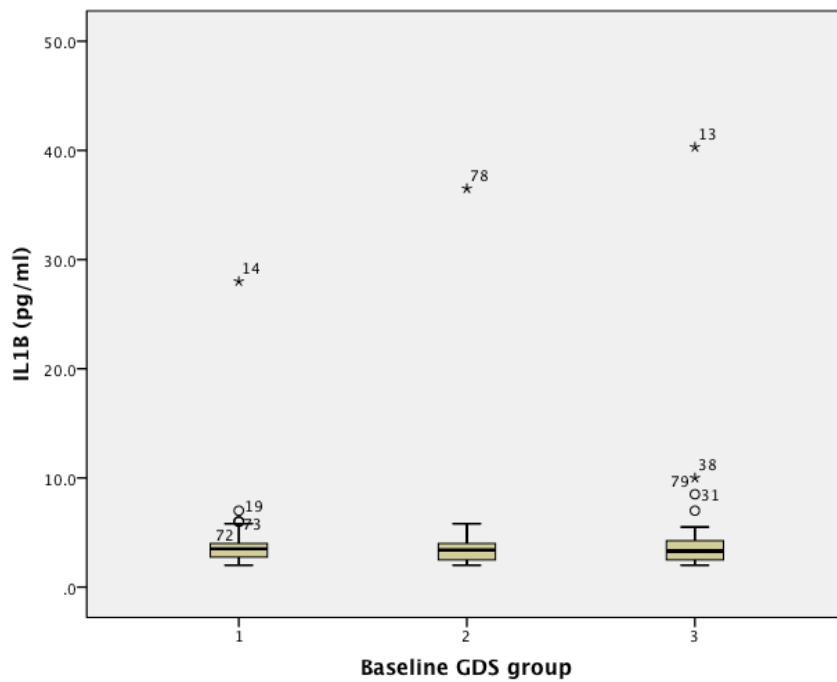


Figure 5.6 Comparison of IL-1 β across baseline cognitive function groups (outlier removed)

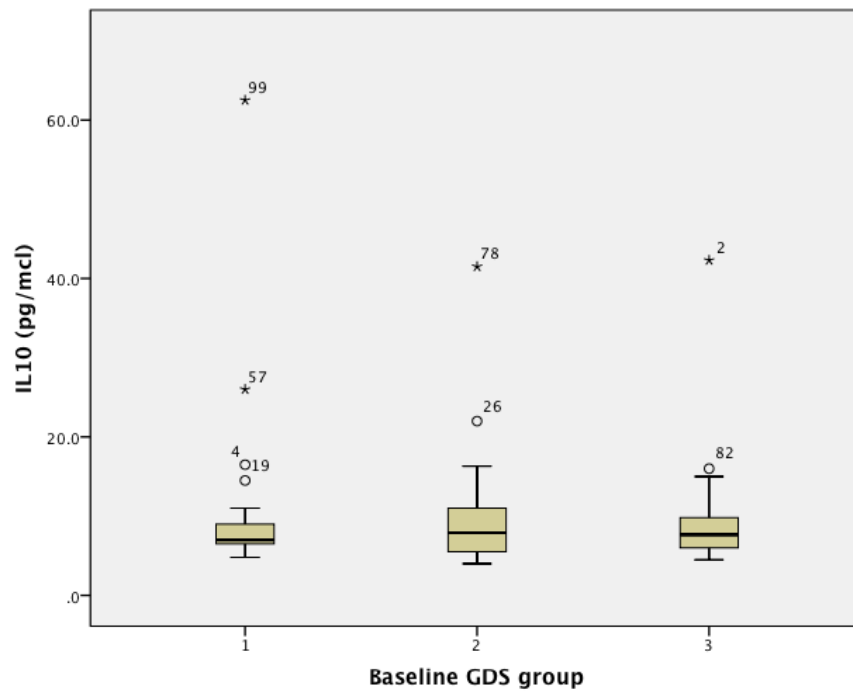


Figure 5.7 Comparison of IL-10 across baseline cognitive function groups (outliers removed)

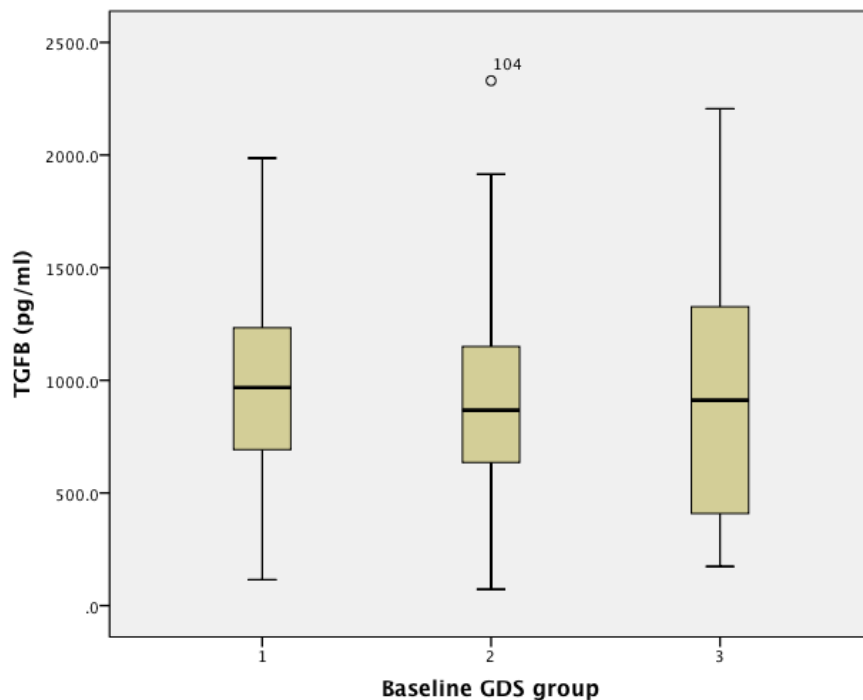


Figure 5.8 Comparison of TGF- β across baseline cognitive function groups

I also treated the GDS as a continuous variable and looked at correlations between baseline GDS and the inflammatory markers (Table 5-11). A higher global deficit score implied worse cognitive impairment. Correlation is the Spearman Rank correlation.

No significant correlations were found between the baseline GDS and any of the inflammatory markers. TNF- α was significantly positively correlated with IL-1 β (correlation coefficient = 0.538, $p < 0.0001$), and IL-10 was significantly positively correlated with both IL-1 β and TNF- α (correlation coefficients of 0.372 and 0.258 respectively, with $p < 0.0001$ and $p = 0.0006$). After removing the outliers 31 and 55 for IL-10 and rerunning the analysis, the correlation remained non-significant (correlation coefficient = 0.069 and p -value = 0.468). The scatterplots for IL-10 and TGF- β are displayed in Figures 5.9 and 5.10.

Variable	Statistic	Baseline GDS	IL-1 β	TNF- α	IL-10	TGF- β
Baseline GDS	Correlation Coefficient Sig. (2-tailed) n	1 114				
IL-1β	Correlation Coefficient Sig. (2-tailed) n	0.002 0.986 114	1 114			
TNF-α	Correlation Coefficient Sig. (2-tailed) n	0.035 0.711 114	0.538** <0.001 114	1 114		
IL-10	Correlation Coefficient Sig. (2-tailed) n	0.11 0.242 114	0.372** <0.001 114	0.258** 0.0006 114	1 114	
TGF-β	Correlation Coefficient Sig. (2-tailed) n	-0.078 0.411 113	-0.159 0.093 113	-0.15 0.114 113	-0.118 0.212 113	1 113

** Correlation is significant at the 0.01 level (2-tailed); Sig. = Significance (p-value); n = count

Table 5-11 Correlation between baseline GDS and inflammatory markers

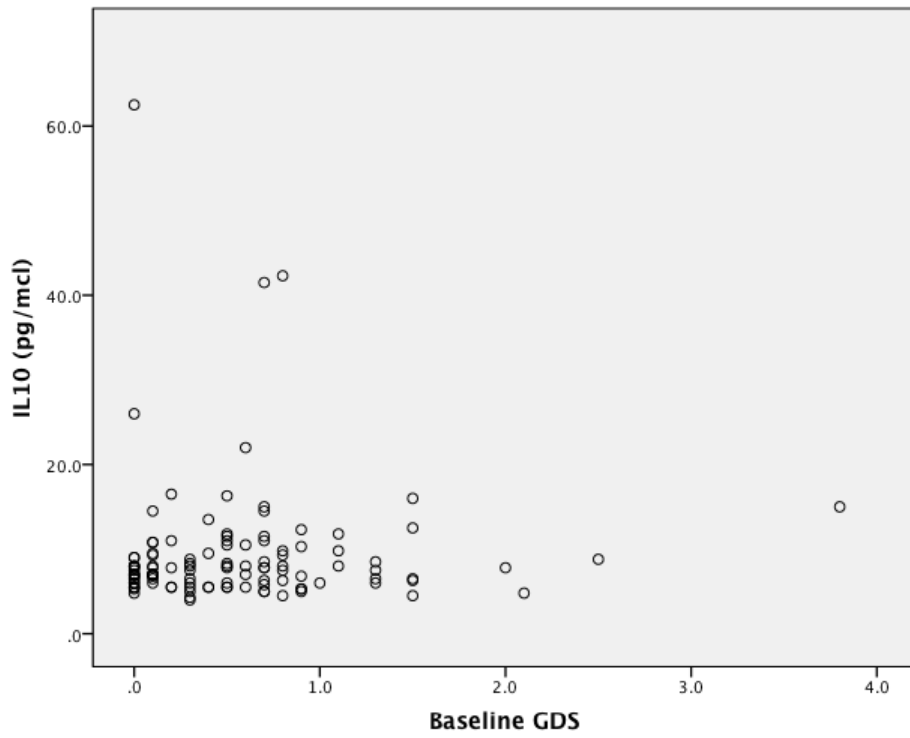


Figure 5.9 Correlation of IL-10 with baseline GDS (outliers removed)

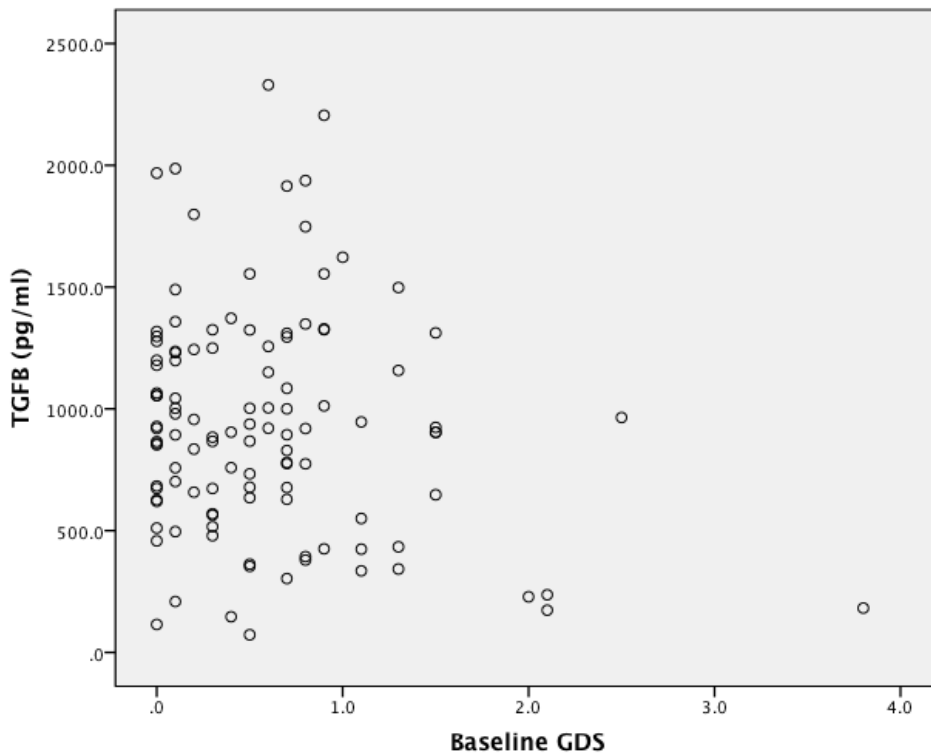


Figure 5.10 Correlation of TGF- β with baseline GDS

I also examined the baseline CD4 count across the categories of GDS (or GDS groups) (Table 5-12).

Variable	GDS group			Kruskal Wallis Test	
	Normal	Mild-moderately impaired	Severely impaired	χ^2 (df)	P-value
	n = 40	n = 42	n = 32		
	Median (IQR)	Median (IQR)	Median (IQR)		
Baseline CD4	184 (147-216)	178 (118-241)	149 (96-197)	3.443 (2)	0.179

N = count; IQR = interquartile range; χ^2 = chi-square statistic; df = degrees of freedom

Table 5-12 Comparison of baseline CD4 across groups of cognitive impairment

The median baseline CD4 count in GDS group 3, the group with severe cognitive impairment, was lower than the CD4 count in the other two groups. There were, however, no significant differences between the three GDS groups in terms of baseline CD4 counts. After removing outlier 69, the test remained non-significant ($p = 0.099$). Fig 5.11 displays the box and whisker plot for the comparison of baseline CD4 count across the groups of cognitive impairment after removal of outlier 69. The groups are therefore similar in terms of the baseline CD4 count.

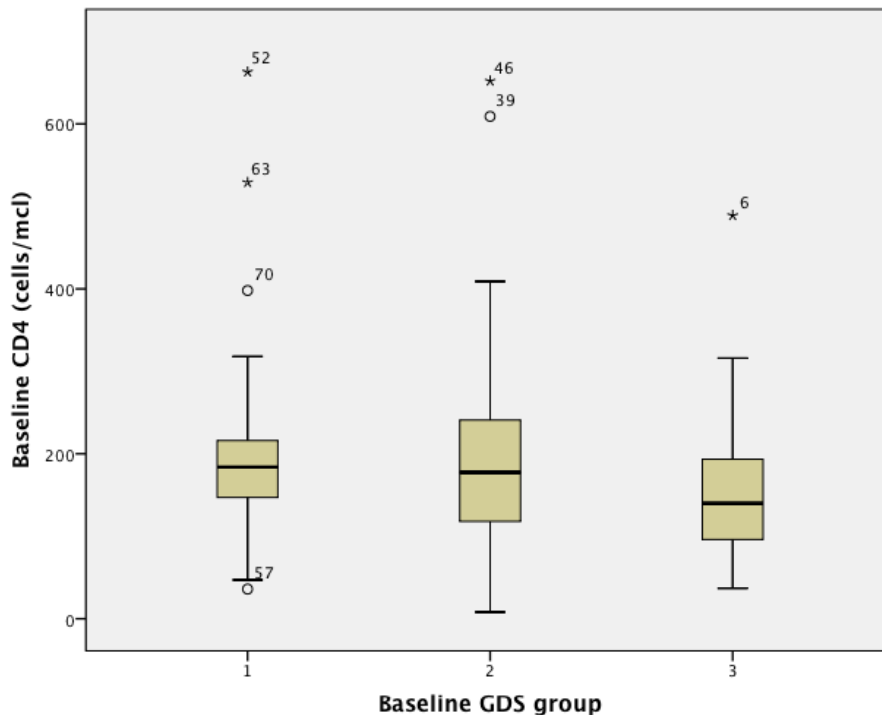


Figure 5.11 Comparison of baseline CD4 count across groups of cognitive impairment (outlier removed)

The baseline CD4 count was also compared with the individual inflammatory markers (Table 5-13). I used a Spearman rank correlation analysis because the variables were non-normally distributed.

There was no correlation between the baseline CD4 counts and the concentrations of the inflammatory markers. The correlations highlighted (**) were already discussed above. After removing the outliers for IL-10, the test was re-run. There was still no significant correlation between IL-10 and baseline CD4 count (Correlation coefficient = 0.102, p = 0.284, n = 112). For TNF- α and IL-1 β outlier 55 was removed. After re-running the test, the correlation coefficients were 0.1226 and -0.137 respectively, with p = 0.148 and 0.147. Scatter plots were used to graphically represent these results. See Figures 5.12 and 5.13 for TGF- β and IL-10 (similar trends were seen for IL-1 β and TNF- α .)

Variable	Statistic	Baseline CD4	IL-1 β	TNF- α	IL-10	TGF- β
Baseline CD4	Correlation Coefficient	1				
	Sig. (2-tailed)					
	n	114				
IL-1β	Correlation Coefficient	-0.116	1			
	Sig. (2-tailed)	0.219				
	n	114	114			
TNF-α	Correlation Coefficient	0.142	0.538**	1		
	Sig. (2-tailed)	0.132	<0.001			
	n	114	114	114		
IL-10	Correlation Coefficient	-0.104	0.372**	0.258**	1	
	Sig. (2-tailed)	0.27	<0.001	0.006		
	n	114	114	114	114	
TGF-β	Correlation Coefficient	0.087	-0.159	-0.15	-0.118	1
	Sig. (2-tailed)	0.358	0.093	0.114	0.212	
	n	113	113	113	113	113

** Correlation is significant at the 0.01 level (2-tailed)

Table 5-13 Comparison of baseline CD4 count with inflammatory markers

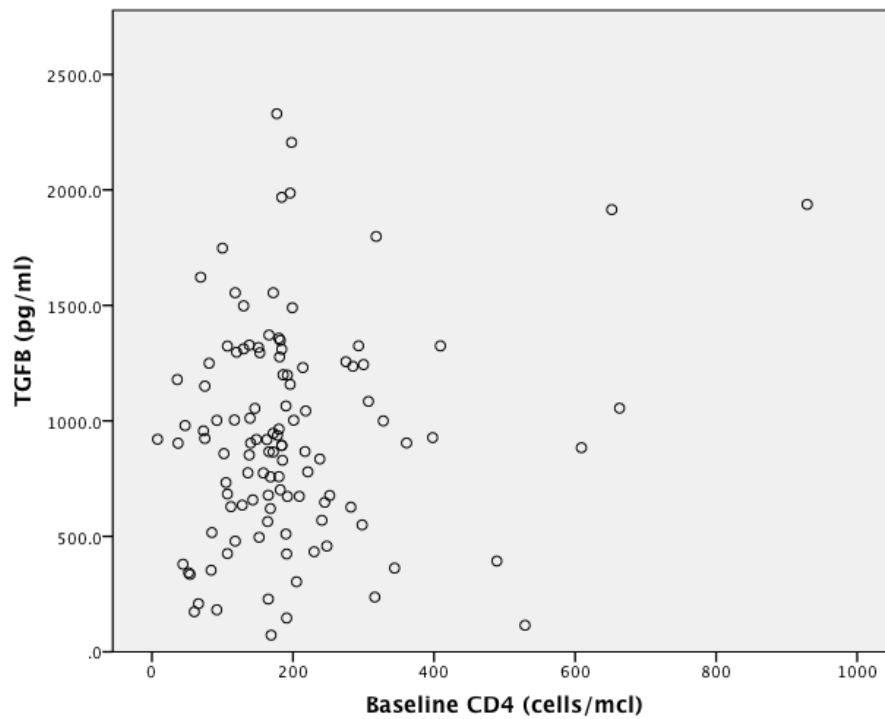


Figure 5.12 Correlation of TGF- β with baseline CD4 count

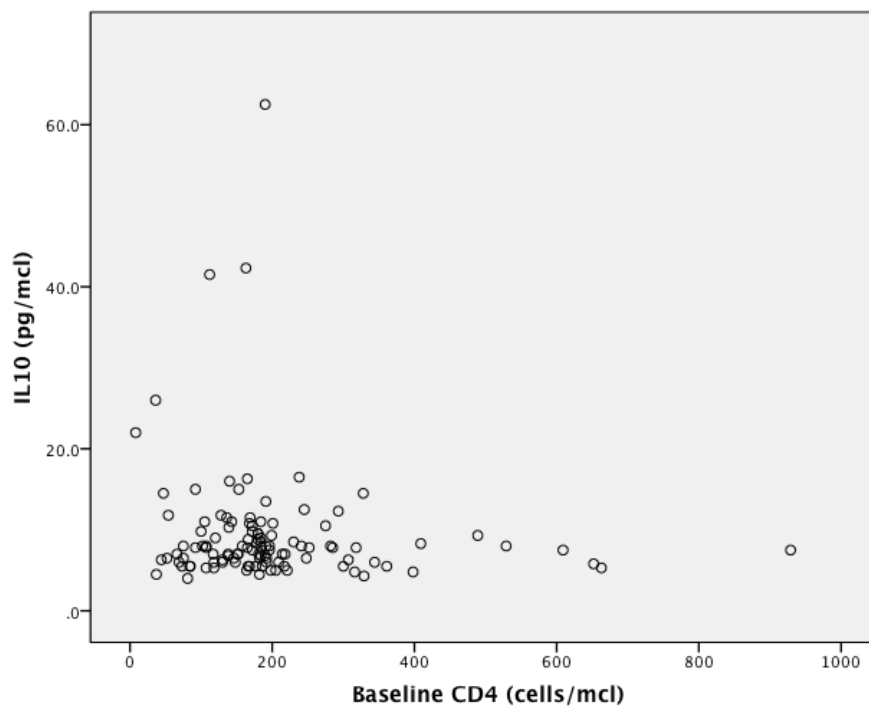


Figure 5.13 Correlation of IL-10 with baseline CD4 count (outliers removed)

Method 2 – Creating an inflammatory profile

An inflammatory profile was created by factor analysis. Factor analysis is a statistical variable reduction procedure to reduce the number of variables and to classify variables.

Factor analysis enabled me to determine which cytokines grouped together as well as how they grouped together based on their weighting. The first step in factor analysis is the identification of the factors, i.e. how many factors are there? Principal component analysis (PCA) was the method used to extract the factors (see Table 5-14). The Kaiser criterion was used to determine the ideal number of factors to extract by using the eigenvalues, which are the amount of variance accounted for by a given factor. The Kaiser criterion only retain factors with eigenvalues > 1, i.e. the factor extracts at least as much variance as the equivalent of one original variable. See Table 5-15 for the results of the initial exploratory factor analysis. Low communalities (< 0.3) indicate that the corresponding variables have high levels of uniqueness and are not contributing to any particular factor. None of my variables had low communalities (Tulsa 2010; Osborne and Costello 2009; Santos and Clegg 1999).

Communalities	Initial	Extraction
IL-1 β	1	0.997
TNF α	1	0.996
IL-10	1	0.596
TGF- β	1	0.568

Table 5-14 Component extraction by principal component analysis

Total Variance Explained			
Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	2.019	50.477	50.477
2	1.137	28.436	78.913
3	0.838	20.946	99.859
4	0.006	0.141	100

Table 5-15 Initial exploratory factor analysis

The first two factors with eigenvalues > 1 were extracted and then a rotational strategy was applied to obtain a clear pattern of the loadings of these factors, that is, factors that are somehow clearly marked by high loadings for some variables and low loadings for others (see Table 5-16).

Rotated component matrix	Component	
	1	2
IL-1 β	0.997	0.048
TNF α	0.997	0.045
IL-10	-0.002	0.772
TGF- β	-0.073	-0.75

Table 5-16 Rotated component matrix

The values in the table are the factor loadings, which is the correlation between the measured cytokine concentrations and the new extracted variables (factors). The factor loadings are used to interpret the factors, in other words, to find a common link between factors that load highly (are highly correlated with the factor). This explains which variables are grouping together and what the inherent meaning of this grouping is. Figure 5.14 displays how the components (factors) plotted in rotated space. We can see that the “pro-inflammatory” cytokines grouped together as component 1. This component was selected for further analyses and for the calculation of the regression (REGR) factor score for each participant.

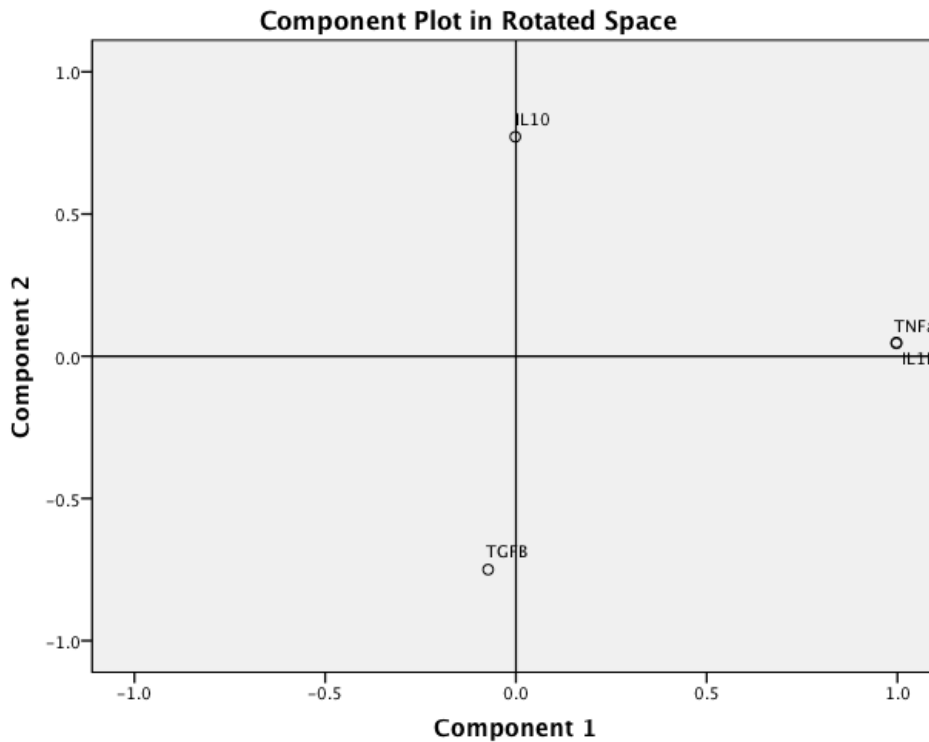


Figure 5.14 Component plot in rotated space

Component 1: IL-1 β and TNF α ; Component 2: IL-10 and TGF- β

After removing the outliers for IL-1 β , TNF- α and IL-10, the PCA was re-run (analysis 1) which resulted in the component matrix in Table 5-17. IL-10 was then removed from the analysis and the PCA (analysis 2) resulted in the component matrix in Table 5-18. IL-1B had the highest loading in analysis 1 and, therefore, carried the most weight while TNF- α carried the most in analysis 2. The REGR factor scores relating to these two analyses can thus be considered “pro-inflammatory”.

Component matrix	Component
	1
IL-1B	0.736
TNF- α	0.531
IL-10	0.576
TGF- β	-0.573

Table 5-17 Component matrix for analysis 1

Component matrix	Component
	1
IL-1B	0.676
TNF- α	0.705
TGF- β	-0.655

Table 5-18 Component matrix for analysis 2

These new continuous variables (REGR factor score for analysis 1 and REGR factor score) were non-normally distributed. There was no significant difference when REGR factor scores were compared across categories of GDS (Kruskal Wallis test: $p = 0.982$ and $p = 0.952$ for factor score analysis 1 and 2 respectively). Likewise, when there was no correlation between the factor scores and GDS (Spearman correlation coefficient 0.014, $p = 0.882$ and Spearman correlation coefficient -0.006, $p = 0.947$ for factor score analysis 1 and 2 respectively). REGR factor score for analysis 1 and analysis 2 were correlated ($p < 0.001$). There was also no correlation between the factor scores and baseline CD4 ($p = 0.235$ and $p = 0.367$ for analysis 1 and analysis 2 respectively).

In summary, there were no correlations between individual cytokine levels and the degree of cognitive impairment as measured by the GDS category and individual scores. Likewise, the systemic cytokine profiles at baseline were not related to the degree of cognitive impairment. These results were not confounded by participant's ages or their CD4 counts which did not differ significantly across the GDS groups.

5.2.2 Hypothesis 2

Hypothesis 2 stated that in the initial cross-sectional analysis, carriers of the APOE $\epsilon 4$ allele would have higher systemic levels of cytokines compared with non- $\epsilon 4$ ($\epsilon 2$, $\epsilon 3$) carriers.

The individual cytokines were compared across two groups: $\epsilon 4$ allele absent and $\epsilon 4$ allele present (see Table 5-19). There was a tendency for IL-10 levels to be higher in the $\epsilon 4$ carrier group compared with the non- $\epsilon 4$ carriers, but this trend did not reach statistical significance ($p = 0.079$). See Figure 5.15 for the box plot comparing IL-10 by $\epsilon 4$ carrier status.

Variable	ε4 allele		Kruskal-Wallis Test	
	No	Yes	X ² (df)	P-value
	n = 58	n = 55		
	Median (IQR)	Median (IQR)		
IL-1β	3.2 (2.8-4.0)	3.5 (2.5-4.5)	0.42 (1)	0.517
TNF-α	4.5 (4.0-5.5)	4.5 (4.0-5.5)	0.234 (1)	0.629
IL-10	7.3 (5.5-9.3)	8.0 (6.5-10.8)	3.077 (1)	0.079**
TGF-β	904.5 (635.3-1277)	919.8 (628.5-1179)	0.004 (1)	0.951

n = count; IQR = interquartile range; χ^2 = Chi-square statistic; df = degrees of freedom

Table 5-19 Comparison of inflammatory markers with the presence of the ε4 allele

Kruskal Wallis Test: $\chi^2 = 3.225$, df = 1, p = 0.079

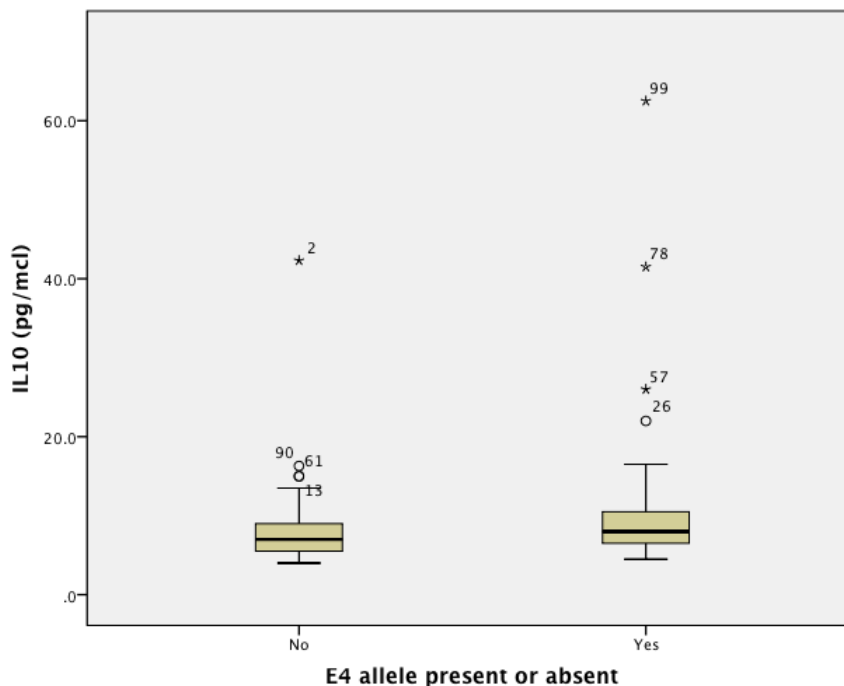


Figure 5.15 Box plot of IL-10 by ε4 carrier status (outliers 31 and 55 removed)

The cytokines were also compared across categories of ε4 allele status (absent, heterozygous or homozygous) (Table 5-20). Again, there were no significant differences across the three categories as determined by the Kruskal Wallis test. Systemic TGF-β concentrations tended to be lower in the ε4 homozygous group compared with the other two groups. This trend, again, did not reach statistical significance. The box plot for TGF-β is displayed below (Figure 5.16).

Variable	ε4 allele status			Kruskal Wallis Test	
	Absent	Heterozygous	Homozygous	X ² (df)	P-value
	n = 58	n = 45	n = 10		
	Median (IQR)	Median (IQR)	Median (IQR)		
IL-1β	3.2 (2.8-4.0)	3.5 (2.5-4.5)	2.8 (2.0-4.0)	2.067 (2)	0.356
TNF-α	4.5 (4.0-5.5)	4.5 (4.0-5.5)	4.8 (4.3-6.0)	0.446 (2)	0.8
IL-10	7.3 (5.5-9.3)	8.0 (6.3-11.0)	8.0 (7.8-8.5)	3.101 (2)	0.212
TGF-β	904.5 (635.3-1277)	956.8 (683.5-1256.3)	765.4 (569.5-858.3)	4.878 (2)	0.087

n = count; IQR = interquartile range; χ^2 = Chi-square statistic; df = degrees of freedom

Table 5-20 Comparison of inflammatory markers across categories of ε4 allele status

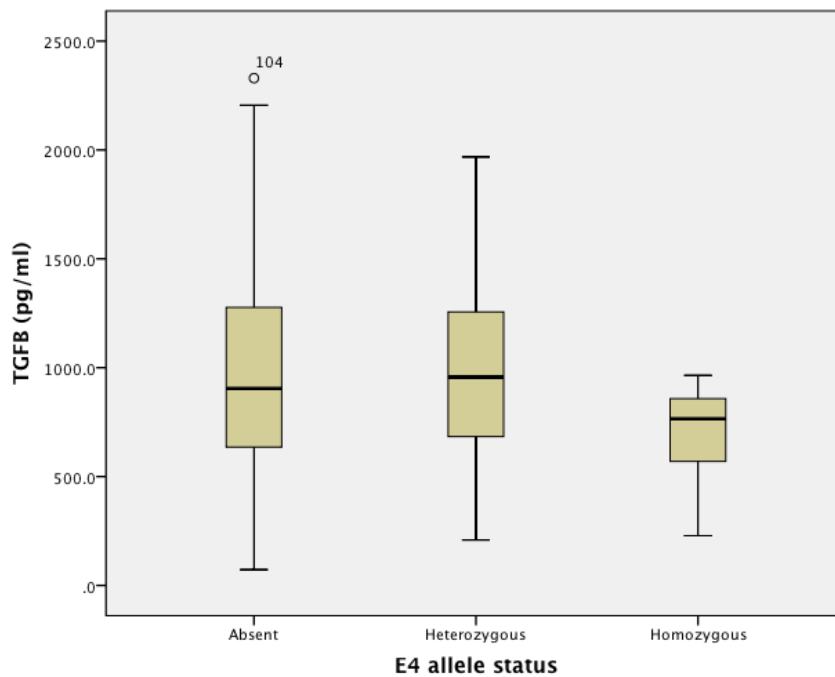


Figure 5.16 Box plot of TGF-β by categories of ε4 allele status

I also compared the cytokines across the categories of ε2 and ε3 allele present or absent (see Tables 5-21 and 5-22). The presence or absence of ε2 did not make any difference to the cytokine concentrations. However, a statistically significant association was found in TGF-β levels and the presence of the ε3 allele ($\chi^2 = 4.508$, df = 1, p = 0.034). TGF-β concentrations were higher in the ε3 group compared with the non-ε3 group. See Figure 5.17 for the box plot displaying this result.

Variable	ε2 allele		Kruskal Wallis Test	
	No	Yes	X ² (df)	P-value
	n = 23	n = 89		
	Median (IQR)	Median (IQR)		
IL-1β	3.5 (3.0-4.0)	3.5 (2.5-4.5)	0.750 (1)	0.387
TNF-α	5.0 (3.8-5.5)	4.5 (4.0-5.5)	0.036 (1)	0.849
IL-10	8.3 (7.0-11.0)	7.5 (6.0-9.65)	2.936 (1)	0.087
TGF-β	893.3 (425.3-1298)	919.8 (631.9-1240.2)	0.209 (1)	0.647

n = count; IQR = interquartile range; χ^2 = Chi-square statistic; df = degrees of freedom

Table 5-21 Comparison of inflammatory markers across categories of ε2 allele carrier status

Variable	E3 allele		Kruskal Wallis Test	
	No	Yes	X ² (df)	P-value
	N = 18	N = 94		
	Median (IQR)	Median (IQR)		
IL-1β	3.5 (2.5-4.2)	3.4 (2.5-4.0)	0.490 (1)	0.825
TNF-α	5.0 (4.0-5.63)	4.5 (4.0-5.5)	0.632 (1)	0.427
IL-10	8.0 (7.0-11.2)	7.5 (6.0-10.35)	2.407 (1)	0.121
TGF-β	832.15 (510.9-930.6)	932.55 (644.3-1295.6)	4.508 (1)	0.034

n = count; IQR = interquartile range; χ^2 = Chi-square statistic; df = degrees of freedom

Table 5-22 Comparison of inflammatory markers across categories of ε3 carrier status

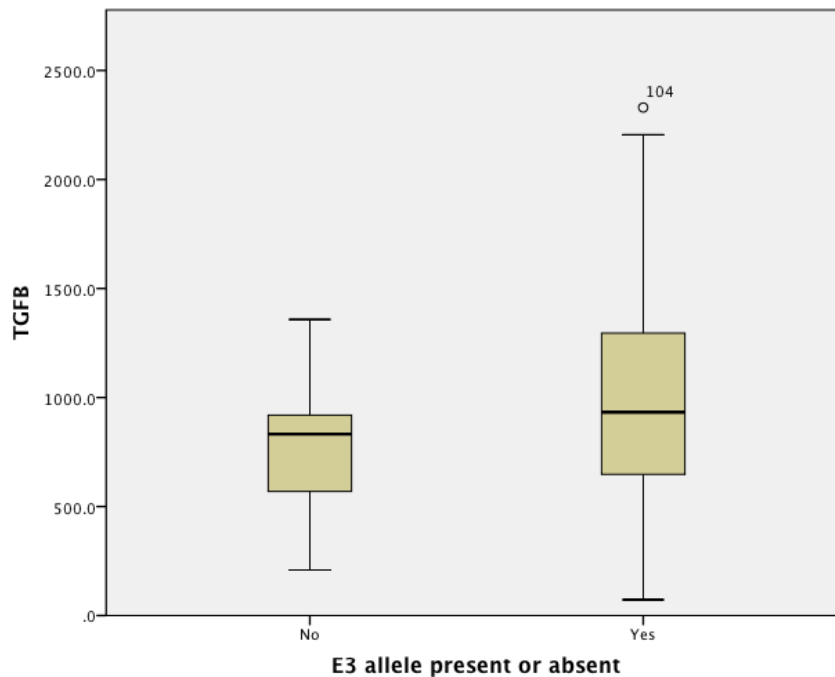


Figure 5.17 Comparison of TGF- β across categories of $\epsilon 3$ allele present or absent

The comparison of the REGR factor scores across categories of $\epsilon 4$ carrier status did not reveal any significant differences (Chi-square test: $p = 0.18$ and $p = 0.575$ for analysis 1 and 2 respectively). Likewise, the comparison of the REGR factor scores across categories of $\epsilon 4$ allele status was not significantly different (Kruskal Wallis test: $p = 0.252$ and $p = 0.307$ for analysis 1 and 2 respectively).

In summary, there was a tendency for IL-10, an anti-inflammatory cytokine, to be higher in $\epsilon 4$ carriers. However, this did not quite reach statistical significance. There was also a tendency for the concentrations of TGF- β , another anti-inflammatory cytokine, to be lower in the $\epsilon 4$ homozygous group compared with $\epsilon 4$ heterozygous and non- $\epsilon 4$ carriers. TGF- β concentrations were significantly higher in $\epsilon 3$ carriers compared with non- $\epsilon 3$ carriers.

5.2.3 Hypothesis 3

Hypothesis 3 stated that in the cross-sectional analysis, $\epsilon 4$ carriers would have greater cognitive impairment (i.e. worse cognitive function) than $\epsilon 4$ non-carriers. To test this hypothesis, the presence of the $\epsilon 4$ allele was compared across the categories of cognitive functioning. Cross-tabulation with contingency tables, clustered bar charts and chi-squared tests of association were used. There was no significant association between the presence of the $\epsilon 4$ allele and the cognitive function group: $\chi^2 = 1.517$, $df = 2$, $p = 0.468$

(see Table 5-23). The results are displayed graphically in a clustered bar chart (see Figure 5-18).

GDS Group	Statistic	ε4 allele present		Total
		No	Yes	
1	Count (n =)	18	22	40
	% Within baseline GDS group 1	45%	55%	100%
	% Within ε4 allele carrier group	31%	40%	35.4%
2	Count (n =)	24	17	41
	% Within baseline GDS group 2	58.5%	41.5%	100%
	% Within ε4 allele carrier group	41.4%	30.9%	36.3%
3	Count (n =)	16	16	32
	% Within baseline GDS group 3	50%	50%	100%
	% Within ε4 allele carrier group	27.6%	29.1%	28.3%
Total	Count (n=)	58	55	113
	% Within baseline GDS group	51.3%	48.7%	100%
	% Within ε4 allele carrier group	100%	100%	100%

Table 5-23 Presence of the ε4 allele across categories of baseline cognitive functioning

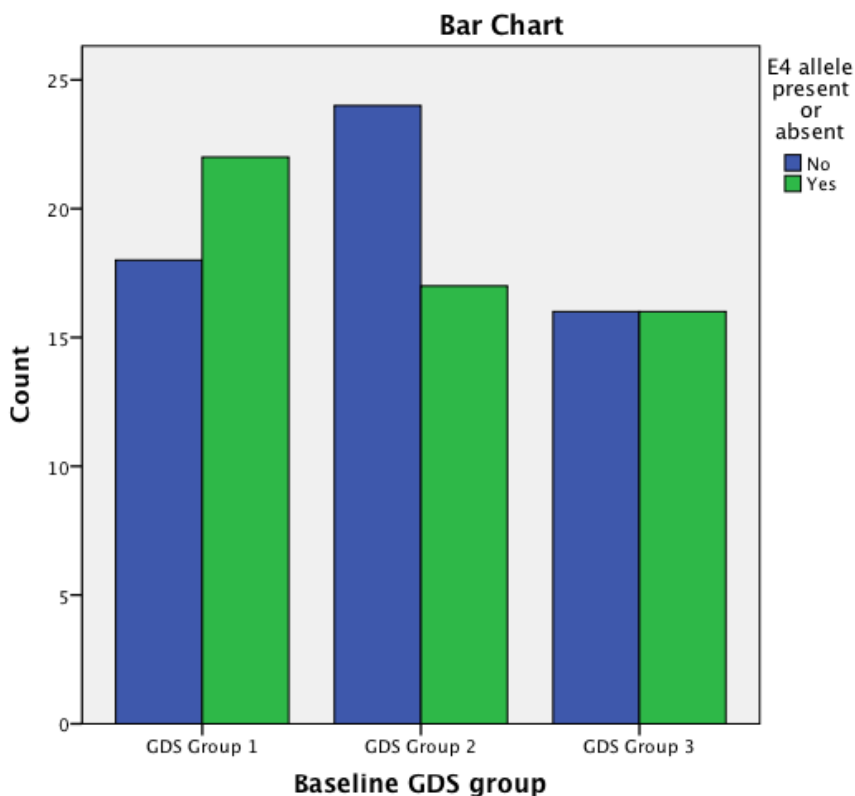


Figure 5.18 Presence of the ε4 allele across groups of cognitive functioning

The status of the $\epsilon 4$ allele, whether absent (0 $\epsilon 4$ alleles), heterozygous (1 $\epsilon 4$ allele) or homozygous (2 $\epsilon 4$ alleles), was also compared across GDS groups of cognitive functioning (see Table 5-24). No significant associations were found: $\chi^2 = 1.523$, $df = 4$, $p = 0.823$. The results are displayed graphically in a clustered bar chart (see Figure 5-19).

GDS Group	Statistic	$\epsilon 4$ allele status			Total
		Absent	Heterozygous	Homozygous	
1	Count (n =)	18	18	4	40
	% Within baseline GDS group 1	45%	45%	10%	100%
	% Within $\epsilon 4$ allele status	31%	40%	40%	35.4%
2	Count (n =)	24	14	3	41
	% Within baseline GDS group 2	58.5%	34.1%	7.3%	100%
	% Within $\epsilon 4$ allele status	41.4%	31.1%	30%	36.3%
3	Count (n =)	16	13	3	32
	% Within baseline GDS group 3	50%	40.6%	9.4%	100%
	% Within $\epsilon 4$ allele status	27.6%	28.9%	30%	28.3%
Total	Count (n =)	58	45	10	113
	% Within baseline GDS group	51.3%	39.8%	8.8%	100%
	% Within $\epsilon 4$ allele status	100%	100%	100%	100%

Table 5-24 Comparison of $\epsilon 4$ allele status across categories of cognitive functioning

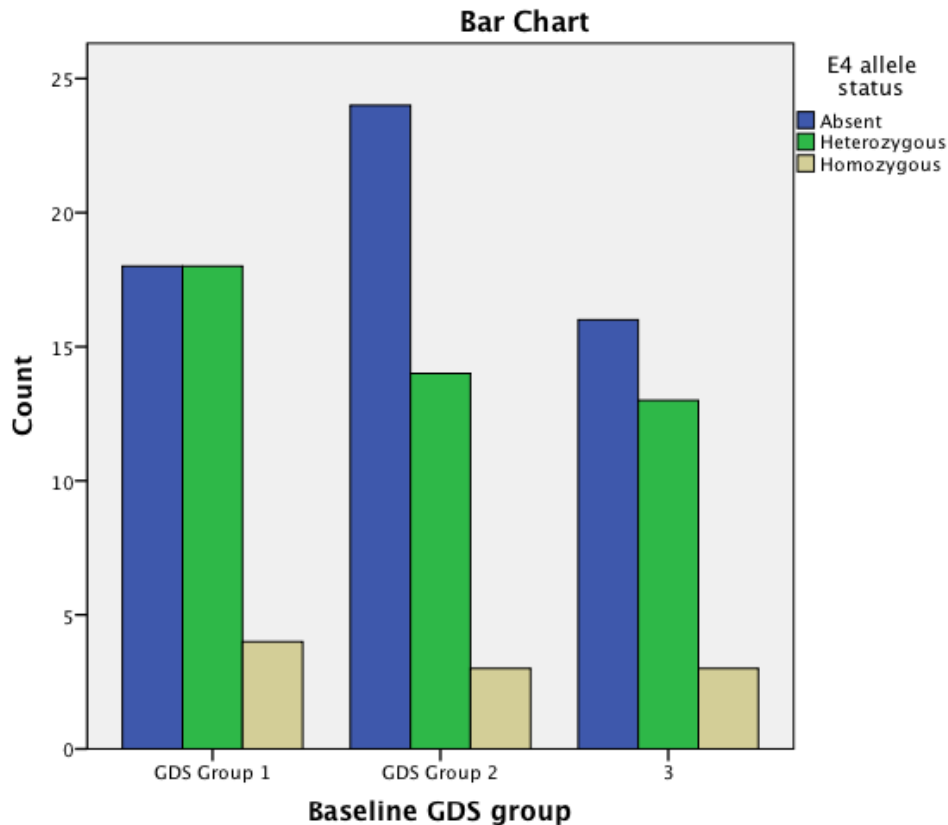


Figure 5.19 ϵ 4 allele status across groups of cognitive functioning

The presence of the ϵ 2 and ϵ 3 alleles was also compared across groups of cognitive function. There were no statistically significant results (Chi-square: $\chi^2 = 4.030$, $df = 2$, $p = 0.133$ for ϵ 2 and Chi-square: $\chi^2 = 4.731$, $df = 2$, $p = 0.94$ for ϵ 3).

In summary, there was no significant association between ϵ 4 carrier status and cognitive impairment. The presence of the ϵ 4 allele was not associated with greater cognitive impairment compared with ϵ 2 and ϵ 3 carriers.

5.2.4 Hypothesis 4

Hypothesis 4 stated that participants with an initial pro-inflammatory cytokine profile at baseline would respond better to cART in terms of cognitive function than those with an initial anti-inflammatory profile. A predominantly pro-inflammatory baseline profile would be associated with more improvement in cognitive function at follow-up. Conversely, a predominantly anti-inflammatory baseline profile would be associated with less improvement in cognitive function at follow-up.

To test this hypothesis, a new variable called “GDS change” was created to reflect the change in the global deficit score over time. This variable had three categories describing the status of cognitive function: maintained, worsened, and improved. In the sample of

patients who returned for follow-up, 60% had improved cognitive function after at least 9 months of cART. 17,5% worsened and 22.5% maintained their level of cognitive functioning when re-tested at their follow-up visit (see Table 5-25).

GDS change	Frequency	Percentage	Valid percentage	Cumulative percentage
Maintained	9	22.5	22.5	22.5
Worsened	7	17.5	17.5	40
Improved	24	60	60	100
Total	40	100	100	

Table 5-25 Change in GDS over time

A second variable called “GDS change 2” was then created to describe the treatment effect over time. This variable has two categories: positive treatment effect and negative treatment effect. The positive treatment effect category included all participants who improved or maintained their cognitive function at follow-up after initiating cART. The negative treatment effect category included all participants whose cognitive function worsened. From Table 5-26 we can see that there was a positive treatment effect in 82.5% of the follow-up sample.

GDS change 2	Frequency	Percentage	Valid percentage	Cumulative percentage
Positive treatment effect	33	82.5	82.5	82.5
Negative treatment effect	7	17.5	17.5	100
Total	40	100	100	

Table 5-26 ART effect on cognitive function over time

A comparison was then made between the baseline and follow-up GDS groups by means of cross-tabulation and the McNemar test: $\chi^2 = 10.07$, $df = 3$, $p = 0.018$. There was a significant correlation between the change in GDS group over time and the baseline GDS group (see Table 5-27). Individuals in baseline group 1 were more likely to be in follow-up group 1 (normal) than those in groups 2 (mild-moderately impaired) and 3 (severely impaired). Similarly, individuals originally in group 2 were more likely to be in follow-up

group 1 than those originally in group 3. Those in groups 2 and 3 were more likely to be in follow-up groups 2 or 3 than group 1. (Also refer to the clustered bar chart in Figure 5.20.)

In other words: Individuals who had normal cognitive function at baseline were more likely to maintain their cognitive function (remain normal) at follow-up than those who were impaired at baseline. It was more likely that an individual with mild to moderate cognitive impairment (group 2) at baseline would improve to normal at follow-up than it was for an individual with severe cognitive impairment (group 3) at baseline. It was also more likely that individuals with mild to moderate (group 2) or severe cognitive impairment (group 3) at baseline would still be in follow-up groups 2 and 3 than in the cognitively normal group.

Baseline GDS group	Statistic	Follow-up GDS group			Total
		Normal (GDS group 1)	Mild-moderately impaired (GDS group 2)	Severely impaired (GDS group 3)	
1	Count (n =)	17	1	0	18
	% Within baseline GDS group 1	94.4%	5.6%	0.0%	100%
	% Within follow-up GDS group 1	63.0%	2.5%	0.0%	45.0%
2	Count (n =)	9	4	1	14
	% Within baseline GDS group 2	64.3%	28.6%	7.1%	100%
	% Within follow-up GDS group 2	33.3%	40.0%	33.3%	35.0%
3	Count (n =)	1	5	2	8
	% Within baseline GDS group 3	12.5%	62.5%	25.0%	100%
	% Within follow-up GDS group 3	3.7%	50.0%	66.7%	20.0%
Total	Count (n =)	27	10	3	40
	% Within baseline GDS group	67.5%	25.0%	7.5%	100%
	% Within follow-up GDS group	100%	100%	100%	100%

Table 5-27 Comparison of baseline and follow-up GDS groups

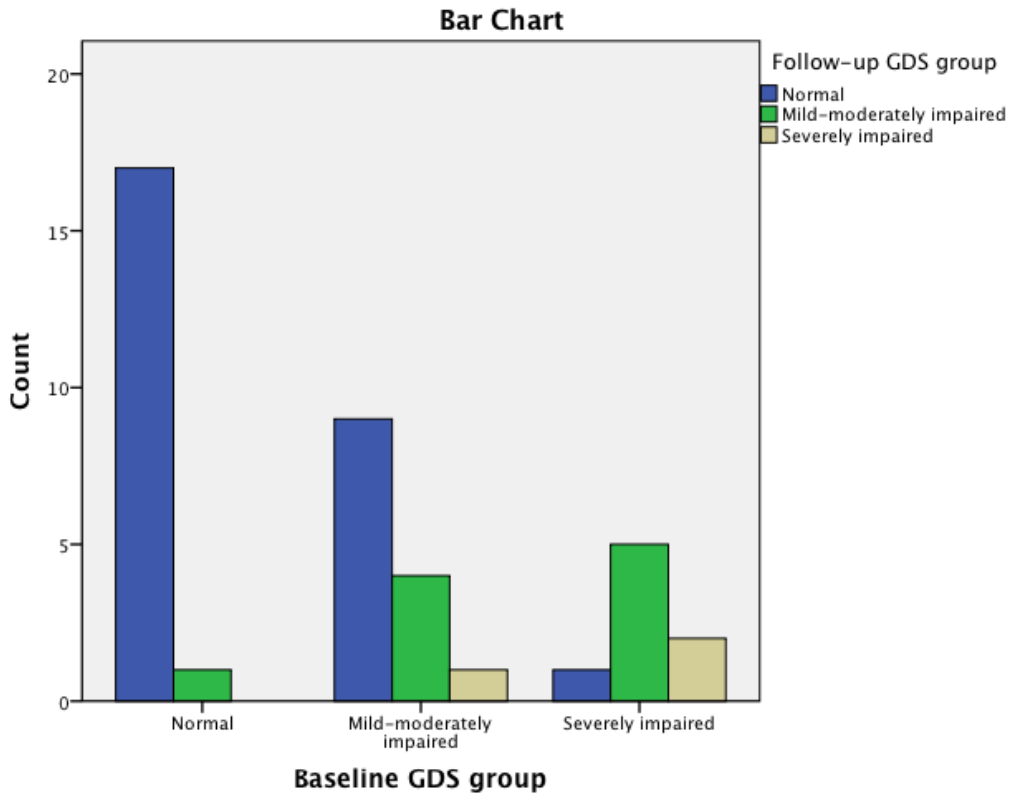


Figure 5.20 Comparison of baseline and follow-up GDS groups

The next step was to compare the inflammatory profile, described in Hypothesis 1, to the change in GDS. For this analysis, GDS change was treated as a continuous variable and the Spearman rank correlation was used. The inflammatory profile at baseline was not significantly correlated with the change in GDS (see Table 5-28)(Figures 5.21 and 5.22).

Variable	Statistic	GDS 2 – GDS 1	REGR factor score 1 for analysis 1	REGR factor score 1 for analysis 2
GDS 2 – GDS 1	Correlation			
	Coefficient	1		
	Sig. (2-tailed)			
	N	40		
REGR factor score 1 for analysis 1	Correlation			
	Coefficient	-0.079	1	
	Sig. (2-tailed)	0.631		
	N	39	39	
REGR factor score 1 for analysis 2	Correlation			
	Coefficient	-0.076	0.910**	1
	Sig. (2-tailed)	0.647	0	
	N	39	39	39

Table 5-28 Comparison of baseline inflammatory profile to GDS change

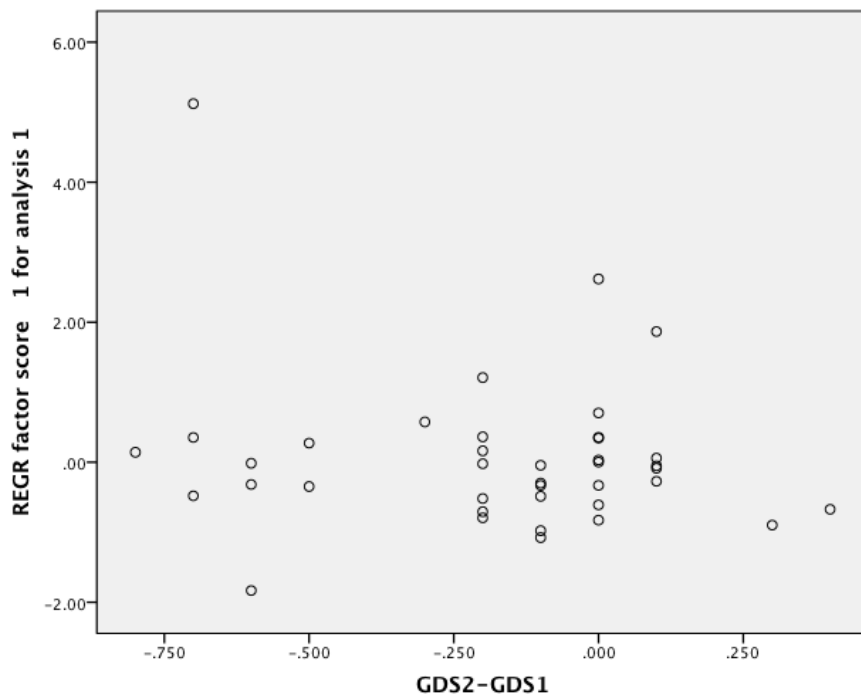


Figure 5.21 Comparison of baseline inflammatory profile to GDS change

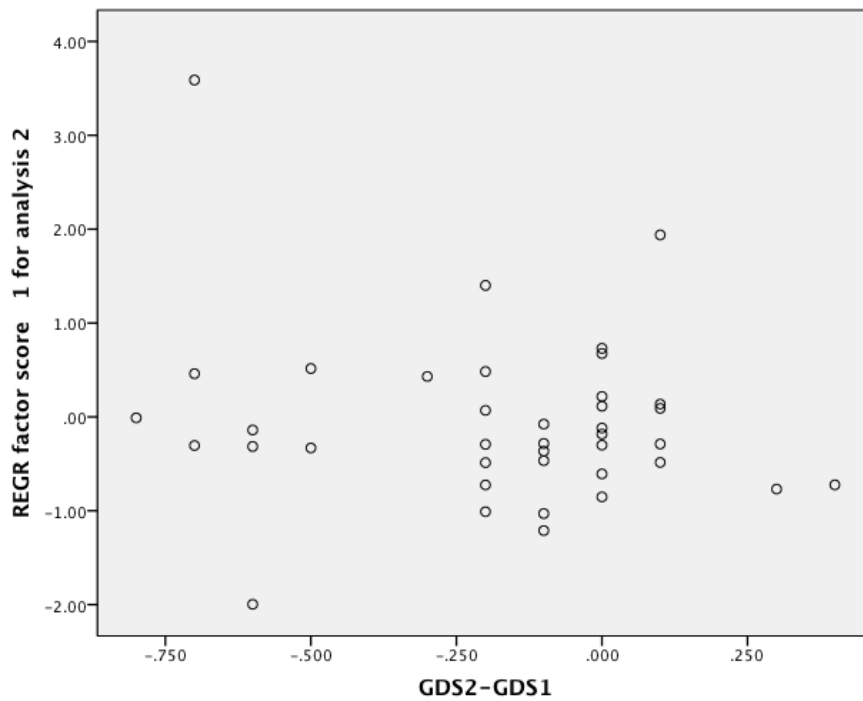


Figure 5.22 Comparison of baseline inflammatory profile to GDS change

The GDS change was also treated as a categorical variable with the three categories: maintained, worsened, and improved. The continuous inflammatory variables (factor scores for analyses 1 and 2) were then examined by categories of GDS change. There were no significant differences between the two continuous inflammatory variables in terms of the change in GDS group (see Table 5-29 and Figures 5.23 and 5.24).

Variable	GDS change group	Median (IQR)	Kruskal Wallis Test
			p-value
REGR factor score 1 for analysis 1	Maintained	0.03 (-0.47-0.53)	0.566
	Worsened	-0.09 (-0.67-0.06)	
	Improved	-0.52 (-0.52-0.27)	
REGR factor score 1 for analysis 2	Maintained	-0.12 (-0.45-0.45)	0.719
	Worsened	-0.29 (-0.72-0.14)	
	Improved	-0.49 (-0.49-0.43)	

Table 5-29 Comparison of baseline inflammatory profile by groups of GDS change

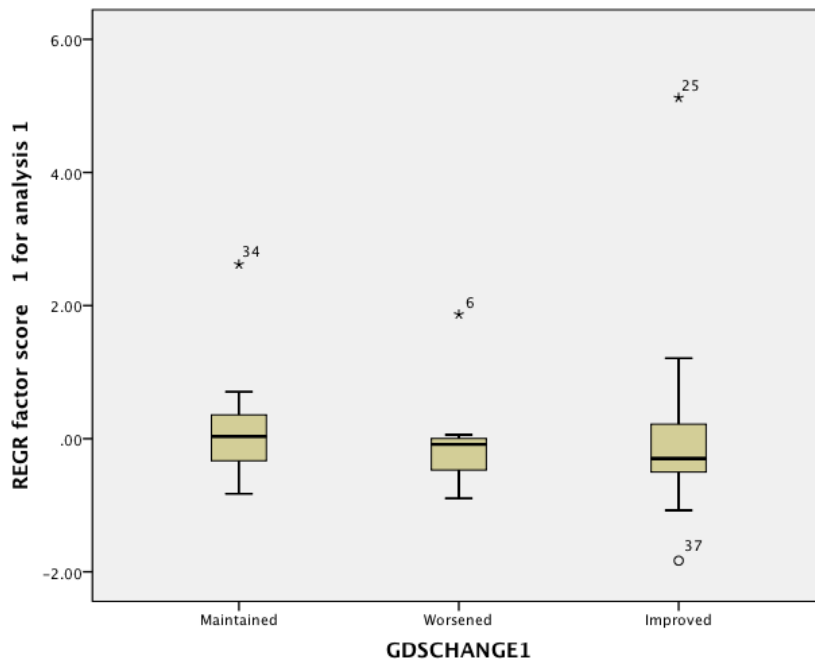


Figure 5.23 Comparison of baseline inflammatory profile by groups of GDS change

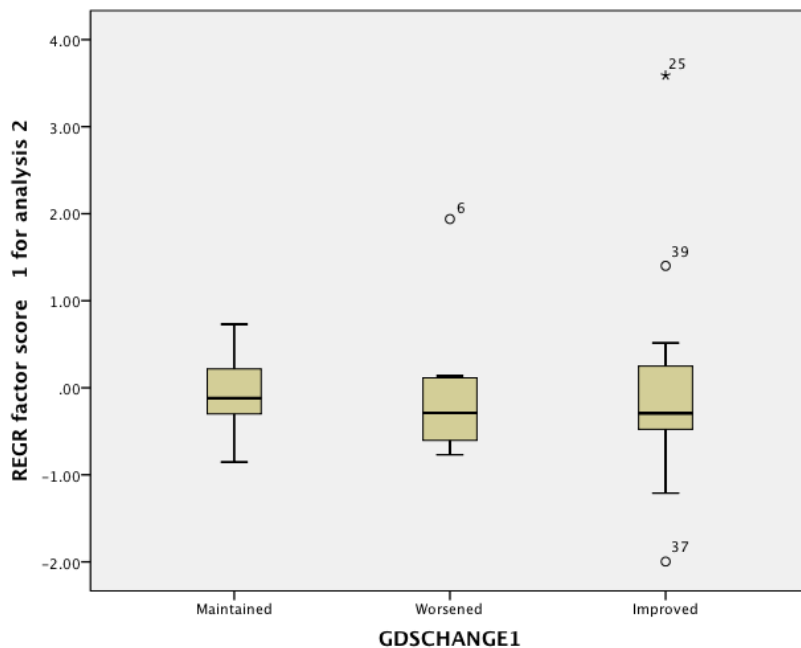


Figure 5.24 Comparison of baseline inflammatory profile by groups of GDS change

The continuous inflammatory variables were also examined across the categories of GDS change (positive or negative treatment effect). The Mann-Whitney test (non-parametric t-test) was used, and again there were no significant differences across the groups. (See Table 5-30 and Figures 5.25 and 5.26.)

Variable	GDS change group	Median (IQR)	Mann-Whitney Test
			p-value
REGR factor score 1 for analysis 1	Positive treatment effect	-0.03 (-0.51-0.35)	0.788
	Negative treatment effect	-0.09 (-0.67-0.06)	
REGR factor score 1 for analysis 2	Positive treatment effect	-0.23 (-0.48-0.38)	0.957
	Negative treatment effect	-0.29 (-0.72-0.14)	

Table 5-30 Comparison of baseline inflammatory profile across treatment effect groups

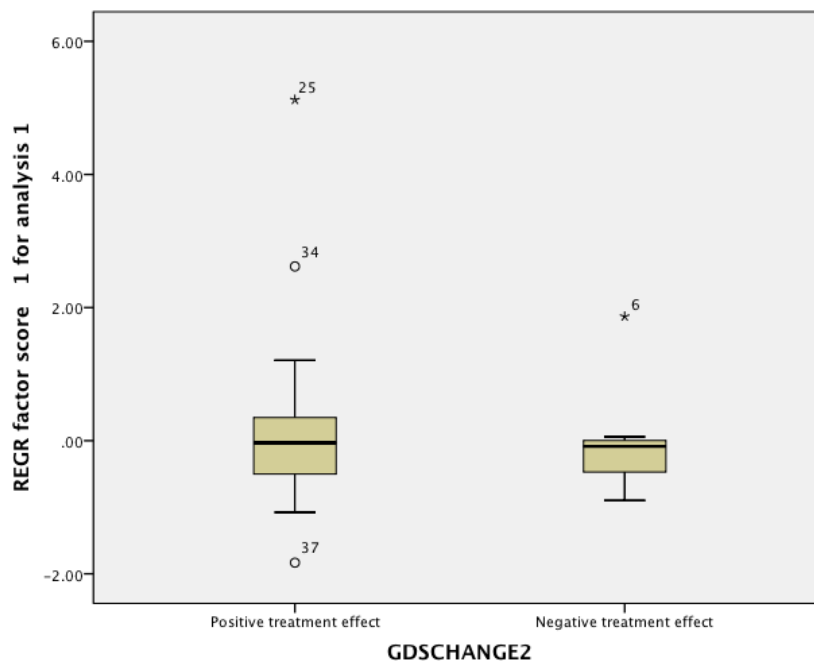


Figure 5.25 Comparison of baseline inflammatory profile across treatment effect groups

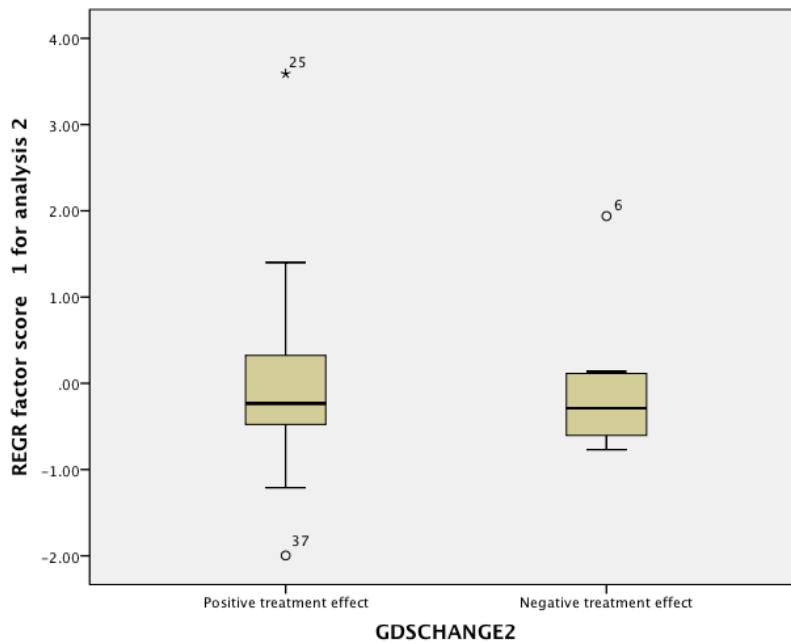


Figure 5.26 Comparison of baseline inflammatory profile across treatment effect groups

The individual baseline cytokine concentrations were also compared to the change in GDS. There were no statistically significant correlations or associations between baseline cytokine concentration and change in GDS at follow-up. Figure 5.27 displays the scatter plot for the correlation of TGF- β with change in GDS (Spearman's correlation coefficient = 0.272, $p = 0.09$). (Similar trends were seen for IL-1 β , TNF- α , and IL-10.)

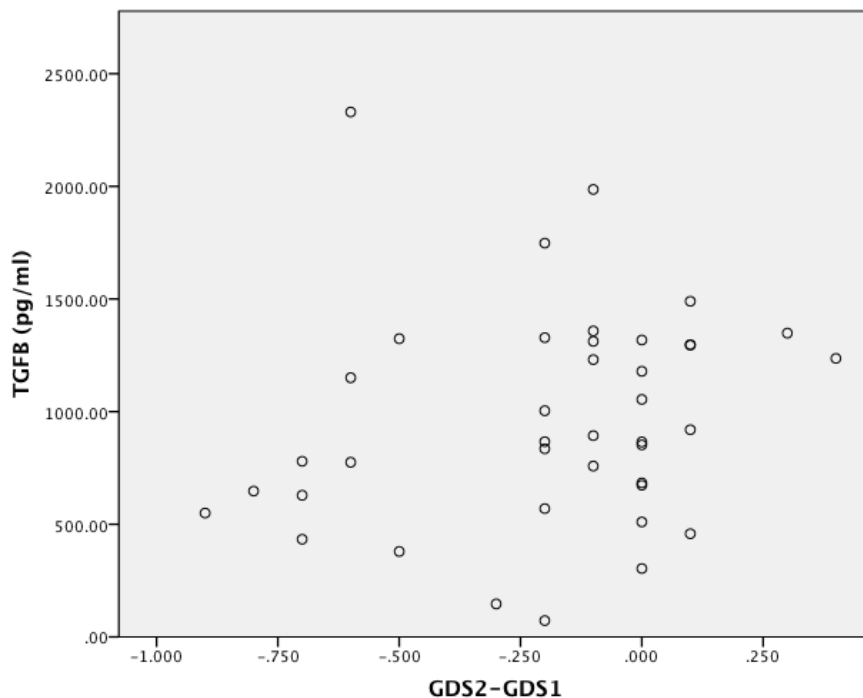


Figure 5.27 Correlation of TGF- β with change in GDS

In summary, there was no significant correlation or association between the individual cytokine levels or systemic cytokine profile and the cognitive response to cART.

5.3 Summary of results

5.3.1 Baseline participant characteristics

- 114 participants were studied at baseline.
- Participants were primarily female and isiXhosa-speaking.
- The median age (IQR) was 30 (27-32.5) years.
- At baseline, 40 participants had no cognitive impairment (GDS group 1), 42 had mild-moderate cognitive impairment (GDS group 2), and 32 were severely impaired (GDS group 3).
- The median CD4 count (IQR) was 177 (119-217.5) cells/ μ L and did not differ significantly amongst the 3 GDS groups.
- The median number of years of education for all participants (IQR) was 10 (9-11.25), but participants in GDS group 1 had significantly higher years of education compared with the other 2 groups.
- The ϵ 4 allelic frequency in the study population was 0.29.
- The proportion of ϵ 4 carriers did not differ significantly amongst the 3 GDS groups.

5.3.2 Follow-up participant characteristics

- Of the original baseline participants, 40 were studied at least 9 months after ART.
- The distribution of participants in the GDS groups at the follow-up assessment differed from the distribution at baseline. There were fewer severely impaired participants in the follow-up group.
- However, the attrition group had a larger proportion of participants with severe cognitive impairment than the group who returned for follow-up had at baseline.
- Median CD4 counts were significantly higher at the follow-up visit.

5.3.3 Results by hypothesis

Hypothesis 1

- There was no correlation between individual cytokine levels and the degree of cognitive impairment as measured by the GDS category and individual scores.

Likewise, the systemic cytokine profiles at baseline were not related to the degree of cognitive impairment.

- These results were not confounded by participant's ages or their CD4 counts which did not differ significantly across the GDS groups. There were no correlations between the baseline CD4 count and cytokine levels.

Hypothesis 2

- There was a tendency for IL-10, an anti-inflammatory cytokine, to be higher in $\epsilon 4$ carriers. However, this did not quite reach statistical significance.
- There was also a tendency for the concentrations of TGF- β , another anti-inflammatory cytokine, to be lower in the $\epsilon 4$ homozygous group compared with $\epsilon 4$ heterozygous and non- $\epsilon 4$ carriers.
- TGF- β concentrations were significantly higher in $\epsilon 3$ carriers compared with non- $\epsilon 3$ carriers.

Hypothesis 3

- There was no significant association between $\epsilon 4$ carrier status and cognitive impairment. The presence of the $\epsilon 4$ allele was not associated with greater cognitive impairment compared with $\epsilon 2$ and $\epsilon 3$ carriers.

Hypothesis 4

- There was no significant correlation or association between the individual cytokine levels or systemic cytokine profile and the cognitive response to cART.

CHAPTER 6: DISCUSSION

6.1 Summary of rationale for the study

HIV-1 is a major global health concern and South Africa has the highest number of PLWHA in the world (WHO and UNICEF 2011). The HIV-associated neurocognitive disorders affect around 50% of HIV-infected people and can range in severity from mild deficits to a debilitating dementia (Heaton *et al.* 2010; Ellis *et al.* 2009; McArthur *et al.* 1993b). High prevalence rates for HIV-associated dementia (25.3%) and mild neurocognitive disorders (42.2%) have been reported in Cape Town, South Africa. The pathogenesis of HAND is still unclear, but neuroinflammation is believed to play a key role. There is also increasing evidence that repeated acute infections or chronic systemic infections, through signalling from the periphery to the CNS, may enhance the neuroinflammation that ultimately leads to neurodegeneration. Most of our understanding of the disease progression of HIV-1 is, however, based on research findings from countries where subtype (clade) B HIV-infection predominates. These findings may not be generalizable to populations in sub-Saharan Africa where HIV-1 clade C infection is most common (Ellis *et al.* 2009; Kanki *et al.* 1999). There are still limited data available about the neurological complications of HIV-1 clade C. The $\epsilon 4$ allele of APOE has been investigated as a risk factor for the development of HAND. African populations have a higher frequency of the $\epsilon 4$ allele and in South Africa this allele was found to have a high prevalence among isiXhosa speakers (Joska *et al.* 2010a).

A study of the relationships between systemic infection, APOE genotype and cognitive impairment is biologically important and clinically relevant in the South African setting where clade C HIV-infection predominates.

In this study we aimed to understand more about the role of systemic inflammation in CNS neurodegeneration and the pathogenesis of HAND. From a clinical perspective, we hoped to identify risk factors for HIV-associated dementia. We also hoped to identify factors associated with cognitive response to cART. These factors would then enable us to predict which cognitively impaired patients would respond better to cART. We hoped that our findings might help us identify patients at risk of developing HAND who might benefit from earlier initiation of cART.

6.2 Summary of what the study entailed

This retrospective, observational study had 2 components: The cross-sectional component involved 114 participants who were recruited as part of an existing research project. Participants were young HIV-positive, cART-naïve adults from primary care ARV clinics in Cape Town, South Africa. All participants completed baseline assessments where demographic data were collected, a general physical and neurological examination was performed and cognitive function was assessed using a neuropsychological test battery. Blood was also collected for routine laboratory tests and APOE genotyping. Serum was prepared and pro-and anti-inflammatory cytokines were measured in the stored samples. Global deficit scores were calculated from the cognitive tests. These scores were correlated with the cytokines and with APOE data. The longitudinal data of 40 participants who returned for follow-up assessments after at least 9 months on cART were analysed. The GDS at follow-up were compared with GDS at baseline to determine cognitive responses to cART. Correlations were made between baseline cytokine profiles and cognitive responses.

We hypothesised that a predominantly pro-inflammatory cytokine profile in the peripheral blood of HIV-positive participants not yet on cART would correlate with greater cognitive impairment. Conversely, better cognition would be associated with a predominantly anti-inflammatory cytokine profile. Participants with an initial pro-inflammatory cytokine profile would also respond better to cART in terms of cognitive function. We also hypothesised that the presence of the $\epsilon 4$ allele of APOE would be associated with more inflammation and also with greater cognitive impairment.

Next, I shall discuss the results of this study by first looking at the participant characteristics and then at the results per hypothesis. Following this I shall discuss the limitations of this study and recommendations for future research.

6.3 Participant characteristics

90% of the study population was isiXhosa speaking. In South Africa, isiXhosa is the second largest language and is the first language of 24.7% of people in the Western Cape. Cape Town has the second largest population of major cities in South Africa and 38.6% of the population are “black African” (Census 2011). Of the South African population, almost

80% are indigenous African. The demographic profile of my sample was thus representative of that of the South African population.

Participants in the study were mainly female. This is not surprising because it is estimated that 51% of the South African population are female and roughly 17% of women in their reproductive years are HIV-positive. In South Africa the majority of HIV-infected people who attend clinics are female and may present with late-stage disease (Joska *et al.* 2011). Women tend to seek medical attention more readily than men (Cleary *et al.* 1982). The Global AIDS report (2010) indicated that slightly more than half of all PLWHA were female and in sub-Saharan Africa; more women were living with HIV than men (WHO and UNICEF 2011).

The median age of study participants was 30 years. The study specified an age inclusion criterion of 18 to 35 years, partly to exclude the confounding effects of neurodegenerative diseases associated with older age. Advancing age has been associated with the development of HAND. Valcour *et al* found a significantly higher frequency of HAD in the older (50 or more years old) compared to the younger (20-39 years old) groups of the Hawaii Aging with HIV Cohort (Valcour *et al.* 2004b). Neuropathological similarities also exist between Alzheimer's disease and HAND. A study by Esiri *et al* found that the prevalence of Alzheimer plaque formation increased with age in healthy older controls. However, the brains of patients with AIDS showed an increased propensity to Alzheimer plaque formation at a younger age (in their fourth decade) compared with the controls. They proposed that the trigger for plaque formation is the neuroinflammatory response associated with AIDS (Esiri *et al.* 1998). There was no significant age difference between the baseline GDS groups in my study sample and age was therefore not considered to be a confounding factor in the analyses involving cognitive impairment.

A lower level of education may be linked to high-risk behaviour due to less health awareness and less sex education. The lack of adequate sex education and resulting high-risk behaviour can increase the likelihood of people being infected with HIV. In South Africa, people with an education level of less than 10 years of schooling are largely employed in manual labour jobs. The availability of jobs in the manual labour market is scarce, leading to a high unemployment rate in this group of people. Unemployment leads to poor socio-economic conditions, which in turn, are associated with higher risk behaviour. Lower levels of education have been associated with poor cognitive reserve in

Alzheimer's disease studies and also play a role in age-related cognitive decline (Stern 2006; Scarmeas and Stern 2003). Likewise, HAND has been associated with lower levels of education. In my study, the median level of education for the group was 10 years with an IQR of 9 to 11.25. Participants in baseline GDS group 1, the group with no cognitive impairment, had significantly more years of education compared with the other two groups. Findings from the Multicenter AIDS Cohort Study in the US suggested that low education (< 12 years) might be a risk factor that lowers the threshold for neuropsychological abnormalities in HIV-1 infection (Satz *et al.* 1993). Joska *et al.* also found a significant inverse relationship between level of education and level of cognitive impairment in a large cohort of HIV-1-infected patients in South Africa (Joska *et al.* 2010b). These findings are similar to results from other studies in sub-Saharan Africa where lower education levels were associated with lower neuropsychological performances (Kanmogne *et al.* 2010; Lawler *et al.* 2010). It is also possible that lower levels of education could have affected performance in the cognitive tests. In other words, those with less education performed more poorly on cognitive tests, resulting in them being diagnosed as "more impaired". On the other hand, a selection bias has been introduced into the study by excluding people with less than 7 years of education. In doing so we may have excluded a large number of HIV-infected individuals with severe cognitive impairment.

The $\epsilon 3$ allele was found to be most prevalent (60.6%) in my study population and this is in keeping with reported data from all populations (Eichner *et al.* 2002). The frequency of the $\epsilon 4$ allele in the study sample was 0.29, which is high compared with the prevalence of the $\epsilon 4$ allele in populations from European descent, but is comparable to the higher prevalence of $\epsilon 4$ reported in populations of southern Africa (Eichner *et al.* 2002; Zekraoui *et al.* 1997; Farrer *et al.* 1997). The frequency of the homozygous $\epsilon 4$ genotype (8.8%) in this study population is more in keeping with the findings in the Khoi-San (10%) than with Europeans (2-3%) (Sandholzer *et al.* 1995). The participants in my study were predominantly "indigenous African" and it is therefore not unexpected that the prevalence of $\epsilon 4$ is comparable to the rates found in other southern African populations. The proportion of $\epsilon 4$ carriers did not differ significantly amongst the three baseline GDS groups in my study sample. Carrying the $\epsilon 4$ allele did therefore not influence the degree of cognitive impairment. I shall discuss more about APOE genotypes in Hypothesis 3.

There was a high prevalence of mild to moderate cognitive impairment at baseline (37%), while 28% of the participants had severe cognitive impairment. These rates are similar to

the findings of Joska *et al.* who reported the prevalence of MND and HAD in South Africa as 42.4 and 25.4% respectively (Joska *et al.* 2010d). High rates for HIV-associated dementia have also been reported in studies in Uganda (31%) and India (35%) (Wong *et al.* 2007; Riedel *et al.* 2006). However, a much lower prevalence rate of HIV-dementia (only 2%) was reported by the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study in America. They also found lower rates of MND than those in sub-Saharan Africa (Heaton *et al.* 2010).

Why are higher rates of moderate to severe cognitive impairment reported in South Africa and other developing countries? The lower socio-economic status and poorer health care in third world countries may make people more vulnerable to developing cognitive impairment. Poor nutrition has been associated with the development of cognitive impairment, while in South Africa access to cART is still incomplete and people present with late stage HIV-disease (Joska *et al.* 2010d; Robertson *et al.* 2007; González-Gross *et al.* 2001; Guenter *et al.* 1993). We might be seeing the effect of a more neurovirulent and neurotoxic subtype of HIV-1. The HIV epidemic in South Africa and sub-Saharan Africa is largely due to clade C of HIV-1. Although there are varying reports about the neurotoxicity of clade C versus clade B virus, it remains undetermined whether clade C is more likely to cause cognitive impairment.

Could it be that we are overestimating the problem of HAND? PLWHA, especially those who present with more advanced disease, may have other undetected systemic infections which could confound the results of cognitive testing. Many of the cognitive tests have not been fully validated in our population. However, z-scores were calculated from the HIV-negative control data and then used to determine each participant's degree of impairment relative to the means of the control sample. The participants in the control group were age-matched and recruited from the same clinics as the study sample. As previously discussed, it is also possible that those with less education scored lower in the cognitive tests because they were less skilled in the tasks required by the tests and not because they had worse cognitive impairment. Better-validated education-adjusted cognitive tests may need to be developed before further investigating the neuro-cognitive effects of HIV-1 clade C in developing countries. So doing, we would also be able to include HIV-infected individuals with less than 7 years of education.

The median CD4 count of participants at baseline assessment was 177 cells/ μ L. The median baseline CD4 count in GDS group 3, the group with severe cognitive impairment, was lower than the CD4 count in the other two groups, although this was not statistically significant. A low nadir CD4 count has been shown to contribute to the development of HAND (Heaton *et al.* 2010; Childs *et al.* 1999). The lower CD4 count in the HIV-dementia (GDS 3) group was not an unexpected finding since patients with HIV-associated dementia tend to have more advanced HIV infection with lower CD4 counts. Joska *et al.* also found a tendency towards lower CD4 counts in patients with HIV-dementia (Joska *et al.* 2010d). The baseline CD4 counts for this study were collected from patients' clinic records and were assumed to be the nadir count, because all participants were about to enter an ART programme. However, the waiting time to initiation of ART may have varied amongst the participants. In many the CD4 count could have declined further so the true nadir count might have been even lower. The median CD4 counts were significantly higher at the follow-up visit. Increased CD4 count and restored immune function due to cART may benefit the CNS by reducing the number of circulating, activated monocytes, as well as their migration into the brain. The BBB may be less permeable to the influx of inflammatory cells from the systemic circulation when systemic inflammation levels are reduced. This would in turn lead to lower levels of immune activation and neuroinflammation, which would be beneficial to cognitive function (Liner II *et al.* 2008; Sinclair *et al.* 2008; McArthur *et al.* 2004).

At the one-year follow-up cognitive assessment, and after a median of 12 months on cART, there were significantly fewer participants with severe cognitive impairment than at baseline (7.5% compared to 28%). Neurological improvement due to cART remains variable, but the majority of individuals improve after up to a year on treatment (Cross *et al.* 2013; Joska *et al.* 2010c). It is possible that the follow-up GDS groups could include potential selection bias. The very sick or impaired patients did not return for the follow-up visit because they passed away or were too cognitively impaired. In fact, of 38 participants who did not return for a follow-up visit, 37% were in the severely impaired group.

6.4 Results per hypothesis

Hypothesis 1:

There was no association between the individual cytokine concentrations or the cytokine profile measured in the peripheral blood and the degree of cognitive impairment.

The cytokines I selected for analyses are those that have been most studied in the context of HIV-associated cognitive impairment (Matsumura *et al.* 2008; Brabers and Nottet 2006; Zhao *et al.* 2001; Tyor *et al.* 1992). These cytokines were, however, often measured in brain tissue or in the CSF of patients with known cognitive impairment. Some studies have demonstrated an association between elevated cytokine levels in the CSF and HIV-associated cognitive impairment (Yuan *et al.* 2013; Wesselingh *et al.* 1993). The CSF compartment, however, may not shed much light on the pathogenesis of HIV in the brain. The blood compartment may be more informative and is also more readily accessible (McGuire 2009). The peripheral and central cytokine compartments appear to be integrated: cytokines can cross the BBB, and components of the peripheral immune system can therefore initiate an inflammatory process in the brain (Allan and Rothwell 2003; Szelenyi 2001). Few researchers have, however, attempted to correlate cytokines in the peripheral blood and cognitive impairment. A study by Ryan and colleagues failed to show a significant association between the levels of TNF- α in the blood and cognitive impairment in HIV-infected patients. The small numbers of participants in the study by Ryan had late stage HIV-disease and were on cART (Ryan *et al.* 2001). The ART could have lowered the levels of TNF- α in the blood and the numbers might have been too small to detect significant differences between HIV-infected patients with and without cognitive impairment. A smaller study by our group did show significant correlations between systemic cytokines and the degree of cognitive impairment in HAD. Patients with severe cognitive impairment had higher levels of IL-1 β , as measured by ELISA, compared with participants who were mildly impaired or cognitively normal (Mahne 2010). The lack of correlations between the cytokines and the degree of cognitive impairment in my study was disappointing. It is possible that the multi-analyte detection technique did not accurately measure the concentrations of cytokines in the serum. Only a small number of samples elicited a response above that of the control level for IL-1 β , TNF- α , and IL-10. Cytokine concentrations may have been too low and therefore not an accurate reflection of the inflammatory status at baseline. The manner in which the samples were processed

and stored could also have affected the results. Of course, it is possible that the blood was just not the best compartment for measuring inflammatory components that impact on inflammation in the brain. This is, however, contrary to the findings of numerous studies that “link” systemic inflammation and neuroinflammation.

“Pro-inflammatory” cytokines can be neurotoxic, while “anti-inflammatory” cytokines can be neuroprotective. In the correlation of the cytokines with baseline GDS, the “pro-inflammatory” cytokines IL-1 β and TNF- α were significantly positively correlated with each other. This is to be expected because they are both “pro-inflammatory” markers. IL-10, an “anti-inflammatory” cytokine was also positively correlated with IL-1 β and TNF- α . This might have been because IL-10 increased in response to the increase in IL-1 β and TNF- α in an attempt to suppress or dampen the inflammatory processes. IL-10, a normally anti-inflammatory cytokine, could also have been functioning in a “pro-inflammatory” state. Thus, the functions of cytokines overlap significantly. The role of a specific cytokine can also change over time. I thought that the creation of an inflammatory profile might therefore more accurately represent the inflammatory state at any given time. The breakdown in the balance between pro- and anti-inflammatory cytokines in the brain might, after all, lead to neurodegeneration (González-Scarano and Martin-Garcia 2005). The inflammatory profile I attempted demonstrated how the cytokines group together; I could conclude that IL-1 β and TNF- α functioned in a similar manner, presumably as pro-inflammatory cytokines. I did not, however, find any correlation between the inflammatory profile and the degree of cognitive impairment. More sophisticated statistical models may be required to relate multiple cytokine measurements to cognitive function

The CD4 count is a measure of the severity of HIV-disease and therefore probably serves as a proxy marker of disease duration in cART-naïve patients. It could be argued that disease severity might have been a confounding factor in my analyses. For example, the failure to demonstrate an association between cytokine levels and cognition might have been due to the fact that I had a heterogeneous group of patients with varying degrees of disease severity. If numbers were greater, I might have been able to examine the association between cytokines and cognition in smaller groups subdivided according to their CD4 ranges. It could be that the cytokine associations with cognition depended on HIV-disease severity (AIDS staging). There was, however, no correlation between baseline CD4 counts and the cytokine levels or the degree of cognitive impairment.

Median CD4 counts, in fact, did not differ between the three GDS groups. Disease severity therefore did not confound the relationship between cytokine levels and cognition.

Hypothesis 2:

The presence of the $\epsilon 4$ allele of the APOE gene was not associated with increased levels of pro-inflammatory cytokines in the peripheral blood.

The $\epsilon 4$ allele of APOE has been associated with increased levels of pro-inflammatory cytokines in the peripheral blood and CNS of transgenic mice *in vivo* (Lynch *et al.* 2003; Harry *et al.* 2000). ApoE4 has also been found to be less effective at down-regulating the brain's inflammatory response than apoE3 (Lynch *et al.* 2003; Harry *et al.* 2000). There was a tendency of IL-10 to be higher in $\epsilon 4$ carriers, but this was not statistically significant. IL-10 is a predominantly anti-inflammatory cytokine and this finding would appear to contradict what the research has shown. The $\epsilon 4$ allele has been associated with a trend toward lower IL-10 serum levels in a study of patients with coronary artery disease (Tziakas *et al.* 2006). However, IL-10 might have been raised in an attempt to suppress the inflammation initiated by the pro-inflammatory cytokines. It is also possible that the inflammatory processes in general, both pro- and anti-inflammatory, are higher in the presence of the $\epsilon 4$ allele. It has been hypothesised that $\epsilon 4$ allele carriers may show an inflammatory imbalance between pro- and anti-inflammatory cytokines. The role of apoE has been well described in the neuroinflammatory pathogenesis of AD where apoE has both anti- and pro-inflammatory CNS effects. Could it be possible that apoE play a more anti-inflammatory role in the brain with apoE4 being a less effective anti-inflammatory isoform than E2 or E3? Guo and colleagues also examined the effects of apoE3 and apoE4 on IL-1 β in the absence of amyloid- β *in vitro*. Both apoE3 and apoE4 stimulated IL-1 β production in a dose-dependent manner. However, apoE4 displayed significantly more robust pro-inflammatory activity (Guo *et al.* 2004). In my study, the concentrations of TGF- β tended to be lower in the $\epsilon 4$ homozygous group compared with the $\epsilon 4$ heterozygous group and non- $\epsilon 4$ carriers. This finding was not statistically significant, but it would have supported the hypothesis that carriers of the $\epsilon 4$ allele tend to have lower levels of anti-inflammatory cytokines, and are thus more vulnerable to the neurotoxic effects of the pro-inflammatory cytokines, as described in the literature. This susceptibility may be more pronounced in the presence of two $\epsilon 4$ alleles. TGF- β concentrations in this study were significantly higher in $\epsilon 3$ carriers compared with non- $\epsilon 3$ carriers. ApoE3 has been shown

to be neuroprotective (Hayashi *et al.* 2007; Sabo *et al.* 2000; Pedersen *et al.* 2000). Carriers of the APOE ϵ 3 allele have also been shown to be more effective at down-regulating neuroinflammation. Furthermore, it has been reported that lipoproteins containing the apoE3 isoform have higher levels of TGF- β than those containing apoE4. The predominantly anti-inflammatory and neuroprotective properties of TGF- β might contribute to the neuroprotection associated with the ϵ 3 allele (Tesseur *et al.* 2009).

Hypothesis 3:

The presence of the ϵ 4 allele of the APOE gene was not associated with worse cognitive function.

APOE ϵ 4 has been identified as a risk factor for AD. It has also been investigated as a host risk factor for the development of HIV-associated cognitive impairment. There was no significant association between ϵ 4 carrier status and cognitive impairment in this study. Studies investigating the relation between ϵ 4 and HAD have produced conflicting results. Those with positive findings were either studies in non-Clade C countries or the relationship was dependent on advanced age (Valcour *et al.* 2004a; Corder *et al.* 1998). Several studies have failed to demonstrate a relationship between APOE ϵ 4 and HAD. The study by Joska and colleagues was the first in South Africa to evaluate this relationship. They did not find any differences in the allelic frequencies of APOE across the categories of HAND (Joska *et al.* 2010a). Unlike the finding of a significantly lower ϵ 4 allelic frequency in patients with HAD noted in the study by Joska, the percentage of ϵ 4 carriers in the severely impaired group in my study was not significantly different compared with the other two groups. In fact, 50% of the participants in GDS group 3 were carriers of the ϵ 4 allele compared with 55% in the cognitively normal group. The latter does show the high ϵ 4 allelic frequency of HIV-infected patients. Burt *et al.* reported that apoE4 predisposes cells to a significantly higher rate of HIV cell infection *in vitro*. Carrying two ϵ 4 alleles was associated with an accelerated HIV disease course, compared to the ϵ 3 allele that was associated with a slower disease course (Burt *et al.* 2008). ApoE4 affects the dynamics of HIV infectivity and disease progression, and more advanced disease is associated with HAND. The high prevalence of the ϵ 4 allele in my study sample may have made them susceptible to acquiring HIV-infection and may also have predisposed them to develop more advanced disease and cognitive impairment. The association between ϵ 4 and cognitive impairment may, however, only be seen in older HIV-infected individuals

Hypothesis 4:

A predominantly pro-inflammatory cytokine profile at baseline was not associated with greater improvement in cognitive function at follow-up.

More than 50% of the participants who initiated cART and returned for the follow-up assessment had improved cognitive function and more than 80% had a positive treatment effect i.e. they maintained or improved cognitive function. There was also a significant change in GDS group from the baseline to the follow-up visit. This improvement in cognition due to cART is in keeping with numerous reports demonstrating the beneficial effects of cART on preserving or improving cognitive function (Cross *et al.* 2013). In this study, the participants with normal cognition at baseline did not deteriorate. It was less likely for participants with severe cognitive impairment at baseline to improve to normal at follow-up. A degree of cognitive impairment thus remained in these participants despite treatment with cART. This is in keeping with findings from other studies that neuropsychological improvement is not complete. The improvement in cognitive function following cART is variable and some patients still improve after up to a year on cART (Joska *et al.* 2010c). The median duration of cART before the follow-up visit in this study was 12 months. It is therefore possible that some patients might have improved further after the follow-up assessment.

Early improvement in cognitive function due to cART can be due to improved immune function and the suppression of systemic, as well as neuroinflammation. I did not find a correlation between the baseline cytokine profile and the degree of cognitive improvement. Levels of systemic inflammation at baseline did not predict greater improvement in cognitive function due to cART. The reason for this lack of correlation might have been related to technical aspects as discussed in Hypothesis 1. Although the initial response to cART involves suppression of inflammation, it might be that this response is too variable and therefore cannot be accurately predicted by measuring cytokine concentrations in the peripheral blood before cART initiation. It would have been useful to have measurements of the same cytokine concentrations at the follow-up visit to determine whether a difference was present between baseline and follow-up in those patients who improved on cART. There might also be a degree of on-going chronic inflammation in the brain that is not suppressed by cART, which might also explain why a degree of cognitive impairment persists in some.

6.5 Limitations of the study and recommendations for future research

One limitation of my study was its partial retrospective nature. The initial demographic and cognitive data had already been collected prior to my involvement. Incomplete data collection led to the exclusion of a number of participants. Due to the high laboratory costs, I could only use a limited number of cytokines for analysis. My sample size of 114 participants for the cross-sectional analysis was, however, probably adequate to give the study reasonable power. This sample size was also similar to other HAND studies in the developing world such as Uganda, Cameroon and India (Kanmogne *et al.* 2010; Wong *et al.* 2007; Yepthomi *et al.* 2006). However, the attrition rate was high. The loss to follow-up probably related to the poor socio-economic conditions of the study population, financial constraints, lack of transport and inability to leave a job and risk losing their pay for the day. It is also possible that some of the participants who had severe impairment in cognitive function at baseline were lost to follow-up because they deteriorated further or died. Participants were only included in the longitudinal analysis if they had at least 9 months of cART. Some individuals experience a clinical deterioration marked by severe inflammation after initiating cART. This immune reconstitution inflammatory syndrome (IRIS) can occur in patients who have recently started cART and who may have had low CD4 counts that put them at risk for a variety of opportunistic infections. The 9-month treatment criterion limited the chances of IRIS being responsible for clinical findings and thus ensured more accurate assessment. Because of the 9-month treatment criterion, a number of participants had to be excluded from the follow-up group. Having larger follow-up numbers could have produced more significant results in the correlation studies of baseline inflammation and cognitive improvement following cART.

The cytokines in this study were measured using antibody microarray assays, which is considered a reliable method for analysing profiles of inflammatory markers where volumes are limited (Knight *et al.* 2004). The majority of studies correlated the results of the microarray assays and ELISAs, although the degree of correlation varied widely (Elshal and McCoy 2006; Ray *et al.* 2005; Chen *et al.* 1999). Multiplex assays might, however, not accurately measure the actual levels of cytokines and significant variations have been found between the absolute cytokine concentrations as determined by ELISA and the multiplex kits (dupont *et al.* 2005). This might have been the case in my study where measured concentrations of IL-1 β , TNF- α , and IL-10 were generally low. The multi-analyte studies were performed by the CPGR laboratory, and performing ELISAs in our

own lab might have given better results. It was, however, not possible to perform ELISAs due to cost, time, labour and volume of sample limitations. Thawing and re-freezing of the serum samples could also have affected the results.

Another major limitation of this study was the unavailability of CSF due to the lack of approval from the HREC of UCT/GSH for performing lumbar punctures at the time of baseline assessment. As mentioned before, there are sound reasons for measuring inflammation in the blood, but CSF might still have provided me with a more accurate reflection of brain inflammation. CSF would also have been useful in excluding latent or subclinical CNS infections that might have confounded the diagnostic criteria for HAD. The participants did at least have thorough clinical assessments and neuro-imaging at baseline that assisted in excluding other potential causes for cognitive impairment. I think it is still worthwhile to examine the more accessible peripheral circulation for potential markers of inflammation that might correlate with neuroinflammation and cognitive impairment.

An ideal study would involve larger numbers at baseline, and at follow-up. A lumbar puncture should be performed at baseline and CSF cytokine concentrations should be related to serum cytokines at baseline. I would have collected serum for a second blood inflammatory profile at the follow-up visit. A wider range of pro- and anti-inflammatory cytokines should perhaps also be assayed at baseline and follow-up. ELISA technique would be used for cytokine assays instead of multi-analyte detection. Other measurements of inflammation such as TNF α receptor and the CD40 Ligand, a glycoprotein that has been related to cognitive impairment in HIV, may be more useful (Sui *et al.* 2007; Ryan *et al.* 2001). In vivo measurements such as magnetic resonance spectroscopy of the brain could also provide more accurate CNS measurements of inflammation. An older group of HIV-positive participants (> 50 years of age) from the same population will be included for APOE genotyping and correlation with cognitive function.

This study, nevertheless, remains important in that it formed part of the first detailed HAND study in South Africa. I investigated the role of systemic inflammation in HIV-associated cognitive impairment. For the first time in southern Africa we were able to relate peripheral inflammatory markers and APOE genotype to cognitive function. The setting of this study in the primary care ARV clinics of Cape Town also made it valuable and generalizable to

the population of the Western Cape, where there is an enormous burden of people living with HIV/AIDS.

CHAPTER 7: CONCLUSIONS

This study provided information about young HIV-positive patients in South Africa where clade C HIV-1 infection is highly prevalent.

Although no significant correlations were found between systemic inflammation and cognitive impairment, further investigation is warranted. Using alternative cytokine assay techniques and a wider variety of cytokines might yield better results. Finding a potential predictor of HIV-associated cognitive impairment in the blood will be paramount in the resource limited South African setting.

A high prevalence of the $\epsilon 4$ allele was found in the study population. Further investigation of the role of APOE genotype in HIV-associated cognitive impairment is needed. This might involve the inclusion of older HIV-positive participants as a comparison group.

Overall, this study showed that cART on its own results in improvement of cognitive function.

CHAPTER 8: REFERENCES

Achim C, Heyes M, Wiley C. Quantitation of human immunodeficiency virus, immune activation factors, and quinolinic acid in AIDS brains. *J Clin Invest* 1993;91(6):2769.

Albright AV, Soldan SS, González-Scarano F. Pathogenesis of human immunodeficiency virus-induced neurological disease. *J Neurovirol* 2003;9(2):222-227.

Alfahad TB, Nath A. Update on HIV-Associated Neurocognitive Disorders. *Current neurology and neuroscience reports* 2013;13(10):1-8.

Allan SM, Rothwell NJ. Inflammation in central nervous system injury. *Phil Trans R Soc Lond B Biol Sci* 2003;358(1669):1677.

Allavena P, Piemonti L, Longoni D, Bernasconi S, Stoppacciaro A, Ruco L, et al. IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. *Eur J Immunol* 1998 Jan;28(1):359-369.

An SF, Groves M, Gray F, Scaravilli F. Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. *J Neuropathol Exp Neurol* 1999;58(11):1156-1162.

Ancuta P, Kamat A, Kunstman KJ, Kim E, Autissier P, Wurcel Aea. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PloS One* 2008;3(6):2516.

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

Asadullah K, Sterry W, Volk H. Interleukin-10 therapy—review of a new approach. *Pharmacol Rev* 2003;55(2):241-269.

Barber T, Bradshaw D, Hughes D, Leonidou L, Margetts A, Ratcliffe D, et al. Screening for HIV-related neurocognitive impairment in clinical practice: Challenges and opportunities. *AIDS Care* 2013(ahead-of-print):1-9.

Barger SW, Hörster D, Furukawa K, Goodman Y, Kriegstein J, Mattson MP. Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca²⁺ accumulation. *Proceedings of the National Academy of Sciences* 1995;92(20):9328-9332.

Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;220(4599):868-871.

Benedict R. Brief visuospatial memory test-revised. Odessa, FL: Psychological Assessment Resources 1997.

Berger JR, Avison M. The blood brain barrier in HIV infection. *Front Biosci* 2004;9:2680-2685.

Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, et al. CXCR4-activated astrocyte glutamate release via TNF α : amplification by microglia triggers neurotoxicity. *Nat Neurosci* 2001 Jul;4(7):702-710.

Bio-Plex Sandwich immuno assay (image). Taken from: Bio-Plex Pro™ Assays Cytokine, Chemokine and Growth Factors Instruction Manual. Bio-Rad Laboratories, Inc.

Boche D, Cunningham C, Gauldie J, Perry VH. Transforming growth factor-beta 1-mediated neuroprotection against excitotoxic injury in vivo. *J Cereb Blood Flow Metab* 2003 Oct;23(10):1174-1182.

Bogdan C, Paik J, Vodovotz Y, Nathan C. Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-beta and interleukin-10. *J Biol Chem* 1992;267(32):23301-23308.

Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proceedings of the National Academy of Sciences* 1995;92(16):7162-7166.

Bottner M, Kriegstein K, Unsicker K. The transforming growth factor-betas: structure, signaling, and roles in nervous system development and functions. *J Neurochem* 2000 Dec;75(6):2227-2240.

Bouwman FH, Skolasky RL, Hes D, Selnes OA, Glass JD, Nance-Sproson TE, et al. Variable progression of HIV-associated dementia. *Neurology* 1998;50(6):1814-1820.

Brabers NACH, Nottet HSLM. Role of the pro-inflammatory cytokines TNF- α and IL-1 β in HIV-associated dementia. *Eur J Clin Invest* 2006(36):447-458.

Brandt J. The Hopkins Verbal Learning Test: development of a new memory test with six equivalent forms. *Clin Neuropsychol* 1991;5(2):125-142.

Brenchley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? *Nat Immunol* 2006a;7(3):235-239.

Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006b;12(12):1365-1371.

Brew BJ. Evidence for a change in AIDS dementia complex in the era of highly active antiretroviral therapy and the possibility of new forms of AIDS dementia complex. *AIDS* 2004;18:S75-78.

Brew BJ, Rosenblum M, Cronin K, Price RW. AIDS dementia complex and HIV-1 brain infection: clinical-virological correlations. *Ann Neurol* 1995;38(4):563-570.

Brew BJ, Crowe S, Landay A, Cysique LA, Guillemin G. Neurodegeneration and ageing in the cART era. *Journal of Neuroimmune Pharmacology* 2009;4(2):163-174.

Bruno V, Copani A, Besong G, Scoto G, Nicoletti F. Neuroprotective activity of chemokines against N-methyl-D-aspartate or beta-amyloid induced toxicity in culture. *Eur J Pharmacol* 2000;399(2-3):117-121.

Buchwald UK, Geerdes-Fenge HF, Vockler J, Ziege S, Lode H. Interleukin-10: effects on phagocytosis and adhesion molecule expression of granulocytes and monocytes in a comparison with prednisolone. *Eur J Med Res* 1999 Mar 26;4(3):85-94.

Buonaguro L, Tornesello M, Buonaguro F. Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. *J Virol* 2007;81(19):10209-10219.

Burt TD, Agan BK, Marconi VC, He W, Kulkarni H, Mold JE. Apolipoprotein (apo) E4 enhances HIV-1 cell entry in vitro, and the APOE E4/E4 genotype accelerates HIV disease progression. *PNAS* 2008 June 24;105(25):8718-8723.

Butters N, Granholm E, Salmon DP, Grant I, Wolfe J. Episodic and semantic memory: A comparison of amnesic and demented patients. *Journal of Clinical and Experimental Neuropsychology* 1987;9(5):479-497.

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

Carey CL, Woods SP, Gonzalez R, Canover E, Marcotte TD, Grant I. Predictive validity of Global Deficit Scores in detecting neuropsychological impairment in HIV infection. *J Clin Exp Neuropsychol* 2004a;26(3):307-319.

Carey CL, Woods SP, Rippeth JD, Gonzalez R, Moore DJ, Marcotte TD, et al. Initial validation of a screening battery for the detection of HIV-associated cognitive impairment. *Clin Neuropsychol* 2004b May;18(2):234-248.

Census 2011. Available at: www.statssa.gov.za/census2011/default.asp

Chantry D, Turner M, Abney E, Feldmann M. Modulation of cytokine production by transforming growth factor-beta. *Journal of immunology (Baltimore, Md.: 1950)* 1989;142(12):4295.

Chao CC, Hu S, Sheng WS, Bu D, Bukrinsky MI, Peterson PK. Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. *Glia* 1996 Mar;16(3):276-284.

Chelune GJ, Heaton RK, Lehman RA. Neuropsychological and personality correlates of patients' complaints of disability. *Advances in clinical neuropsychology*: Springer; 1986. p. 95-126.

Chen R, Lowe L, Wilson JD, Crowther E, Tzeghai K, Bishop JE, et al. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clin Chem* 1999;45(9):1693-1694.

Cherner M, Masliah E, Ellis RJ, Marcotte TD, Moore DJ, Grant I, et al. Neurocognitive dysfunction predicts postmortem findings of HIV encephalitis. *Neurology* 2002;59(10):1563-1567.

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

Cleary PD, Mechanic D, Greenley JR. Sex differences in medical care utilization: an empirical investigation. *J Health Soc Behav* 1982:106-119.

Clements JE, Zink MC. Molecular biology and pathogenesis of animal lentivirus infections. *Clin Microbiol Rev* 1996;9(1):100-117.

Clifford DB, Mitike MT, Mekonnen Y, Zhang J, Zenebe G, Melaku Z, et al. Neurological evaluation of untreated human immunodeficiency virus infected adults in Ethiopia. *J Neurovirol* 2007;13(1):67-72.

Collins T, Read M, Neish A, Whitley M, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. *The FASEB Journal* 1995;9(10):899-909.

Corder E, H, Robertson K, Lannfelt L, Bogdanovic N, Eggertsen G, Wilkens J. HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. *Nat Med* 1998;4(10):1182-1184.

Corwin J, Bylsma FW. Psychological examination of traumatic encephalopathy. *Clin Neuropsychol* 1993;7(1):3-21.

Cross HM, Combrinck MI, Joska JA. HIV-associated neurocognitive disorders: Antiretroviral regimen, central nervous system penetration effectiveness, and cognitive outcomes. *SAMJ: South African Medical Journal* 2013;103(10):758-762.

Cullen BR. Regulation of HIV-1 gene expression. *FASEB J* 1991;5:2361-2368.

Cupp C, Taylor JP, Khalili K, Amini S. Evidence for stimulation of the transforming growth factor beta 1 promoter by HIV-1 Tat in cells derived from CNS. *Oncogene* 1993 Aug;8(8):2231-2236.

Cutler R, Haughey N, Tammara A, McArthur J, Nath A, Reid R, et al. Dysregulation of sphingolipid and sterol metabolism by ApoE4 in HIV dementia. *Neurology* 2004;63(4):626-630.

Cysique LA, Maruff P, Brew BJ. The neuropsychological profile of symptomatic AIDS and ADC patients in the pre-cART era: a meta-analysis. *J Int Neuropsychol Soc* 2006;12(3):368-382.

Cysique LA, Maruff P, Brew BJ. Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre-and post-highly active antiretroviral therapy eras: a combined study of two cohorts. *J Neurovirol* 2004;10(6):350-357.

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

Davis LE, Hjelle BL, Miller, V.E., Palmer, D.L., Llewellyn AL, Merlin TL, Young SA, et al. Early brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology* 1992;42(9):1736-1739.

Deeks SG. HIV infection, inflammation, immunosenescence and aging. *Annu Rev Med* 2011;62:141-155.

Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. *Top HIV Med* 2009;17(4):118-123.

D'Elia L. Color trails test: Professional manual. : Psychological Assessment Resources; 1996.

Dhar A, Gardner J, Borgmann K, Wu L, Ghorpode A. Novel role of TGF-B in differential astrocyte-TIMP-1 regulation: implications for HIV-1-dementia and neuroinflammation. *J Neurosci Res* 2006;83:1271-1280.

Dore GJ, McDonald A, Li Y, Kaldor JM, Brew BJ, for the National HIV Surveillance Committee. Marked improvement in survival following AIDS dementia complex in the era of highly active antiretroviral therapy. *AIDS* 2003;17(10):1539-1545.

Douek D. HIV disease progression: immune activation, microbes, and a leaky gut. *Top HIV Med* 2007 Aug-Sep;15(4):114-117.

Dunlop O, Goplen AK, Liestol K, Myrvang B, Rootwelt H, Christophersen B, et al. HIV dementia and apolipoprotein E. *Acta Neurol Scand* 1997;95(5):315-318.

dupont NC, Wang K, Wadhwa PD, Culhane JF, Nelson EL. Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants. *J Reprod Immunol* 2005;66(2):175-191.

Eichner JE, Dunn T, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: A HuGE review. *Am J Epidemiol* 2002;155(6):487-496.

Ellis RJ, Callero P, Stockin MD. HIV infection and the central nervous system: a primer. *Neuropsychol Rev* 2009;19:144-151.

Ellis RJ, Deutsch R, Heaton RK, Marcotte TD, McCutchan JA, Nelson JA, et al. Neurocognitive impairment is an independent risk factor for death in HIV infection. *Arch Neurol* 1997;54(4):416-424.

Elshal MF, McCoy JP. Multiplex bead array assays: performance evaluation and comparison of sensitivity to ELISA. *Methods* 2006;38(4):317-323.

Endicott J, Nee J, Harrison W, Blumenthal R. Quality of Life Enjoyment and Satisfaction Questionnaire: a new measure. *Psychopharmacol Bull* 1993.

Erichsen D, Lopez AL, Peng H, Niemann D, Williams C, Bauer M, et al. Neuronal injury regulates fractalkine: relevance for HIV-1 associated dementia. *J Neuroimmunol* 2003 May;138(1-2):144-155.

Esiri MM, Biddolph SC, Morris CS. Prevalence of Alzheimer plaques in AIDS. *Journal of Neurology, Neurosurgery & Psychiatry* 1998;65(1):29-33.

Eugenin EA, Osiecki K, Lopez L, Goldstein H, Calderon TM, Berman JW. CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood-brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. *The Journal of neuroscience* 2006;26(4):1098-1106.

Everall IP, Hansen LA, Masliah E. The shifting patterns of HIV encephalitis neuropathology. *Neurotoxicity research* 2005;8(1-2):51-61.

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. *JAMA* 1997;278(16):1349-1356.

Fiala M, Looney DJ, Stins M, Way DD, Zhang L, Gan X, et al. TNF-alpha opens a paracellular route for HIV-1 invasion across the blood-brain barrier. *Molecular Medicine* 1997;3(8):553.

Fischer-Smith T, Rappaport J. Evolving paradigms in the pathogenesis of HIV-1-associated dementia. *Expert Rev Mol Med* 2005 2 December 2005;7(27).

Fischer-Smith T, Croul S, Adeniyi A, Rybicka K, Morgello S, Khalili K, et al. Macrophage/microglial accumulation and proliferating cell nuclear antigen expression in the central nervous system in human immunodeficiency virus encephalopathy. *The American journal of pathology* 2004;164(6):2089-2099.

Flanders KC, Ren RF, Lippa CF. Transforming growth factor-betas in neurodegenerative disease. *Prog Neurobiol* 1998 Jan;54(1):71-85.

Flow cytometry-based analysis (image). Taken from: <http://www.bio-rad/evportal/en/ZA/LSR/Solutions/LUSM0E8UU/Multiplex-Immunoassays>

Folstein MF, Folstein SE, McHugh PR. Mini-Mental State: a practical method for grading the cognitive state of patients for the clinician. : Pergamon Press; 1975.

Gandhi N, Saiyed Z, Thangavel S, Rodriguez J, Rao K, Nair MP. Differential effects of HIV type 1 clade B and clade C Tat protein on expression of proinflammatory and antiinflammatory cytokines by primary monocytes. *AIDS Res Hum Retroviruses* 2009;25(7):691-699.

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

Gartner S. HIV infection and dementia. *Science* 2000;287(5453):602-604.

Gebicke-Haerter PJ, Van Calker D, Norenberg W, Illes P. Molecular mechanisms of microglial activation. A. Implications for regeneration and neurodegenerative diseases. *Neurochem Int* 1996 Jul;29(1):1-12.

Gelbard HA, Nottet H, Swindells S, Jett M, Dzenko KA, Genis P, et al. Platelet-activating factor: a candidate human immunodeficiency virus type 1-induced neurotoxin. *J Virol* 1994;68(7):4628-4635.

Gelbard HA, Dzenko KA, DiLoreto D, del Cerro C, del Cerro M, Epstein LG. Neurotoxic effects of tumor necrosis factor alpha in primary human neuronal cultures are mediated by activation of the glutamate AMPA receptor subtype: implications for AIDS neuropathogenesis. *Dev Neurosci* 1993;15(6):417-422.

Gendelman HE, Zheng J, Coulter CL, Ghorpade A, Myhanh C, Thylin Mea. Suppression of inflammatory neurotoxins by highly active antiretroviral therapy in human immunodeficiency virus-associated dementia. *J Infect Dis* 1998;178:1000-1007.

Gendelman H, Lipton S, Tardieu M, Bukrinsky M, Nottet H. The neuropathogenesis of HIV-1 infection. *J Leukoc Biol* 1994;56(3):389-398.

Gerdes LU. The common polymorphism of apolipoprotein E: geographical aspects and new pathophysiological relations. *Clinical chemistry and laboratory medicine* 2003;41(5):628-631.

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

Glazner GW, Mattson MP. Differential effects of BDNF, ADNF9, and TNFalpha on levels of NMDA receptor subunits, calcium homeostasis, and neuronal vulnerability to excitotoxicity. *Exp Neurol* 2000 Feb;161(2):442-452.

Gonda MA, Wong-Staal F, Gallo RC, Clements JE, Narayan O, Gildea RV. Sequence homology and morphologic similarity of HTLV-III and visna virus, a pathogenic lentivirus. *FASEB J* 1985;227(4683):173-177.

González-Gross M, Marcos A, Pietrzik K. Nutrition and cognitive impairment in the elderly. *Br J Nutr* 2001;86(03):313-321.

González-Scarano F, Martin-Garcia J. The neuropathogenesis of AIDS. *Nat Rev Immunol* 2005;5(69):81.

Gorman AA, Foley JM, Ettenhofer ML, Hinkin CH, van Gorp WG. Functional consequences of HIV-associated neuropsychological impairment. *Neuropsychol Rev* 2009;19(2):186-203.

Grant I. Neurocognitive disturbances in HIV. *Int Rev Psychiatry* 2008;20(1):33-47.

Grant I, Sacktor N, McArthur J. HIV neurocognitive disorders. In: Gendelman HE, Grant I, Everall IP, Lipton SA, Swindells S, editors. *The neurology of AIDS*. 2nd ed. Oxford: Oxford University Press; 2005. p. 357-373.

Grant I, Atkinson J. Psychiatric aspects of acquired immune deficiency syndrome. *Comprehensive textbook of psychiatry* 1995;2:1644-1669.

Gras G, Kaul M. Review Molecular mechanisms of neuroinvasion by monocytes-macrophages in HIV-1 infection. *Hand* 2010;11(1):8-24.

Green DA, Masliah E, Vinters HV, Beizai P, Moore DJ, Achim CL. Brain deposition of beta-amyloid is a common pathologic feature in HIV-positive patients. *AIDS* 2005;19(4):407-411.

Guenter P, Muurahainen N, Simons G, Kosok A, Cohan GR, Rudenstein R, et al. Relationships among nutritional status, disease progression, and survival in HIV infection. *JAIDS J Acquired Immune Defic Syndromes* 1993;6(10):1130-1138.

Guo L, LaDu MJ, Van Eldik LJ. A dual role for apolipoprotein E in neuroinflammation. *Journal of Molecular Neuroscience* 2004;23(3):205-212.

Haase AT. Pathogenesis of lentivirus infections. *Nature* 1986;322(10):130-136.

Halstead W. *Brain and intelligence*. : University Press; 1947.

Harry GJ, Lefebvre d'Hellencourt C, Bruccoleri A, Schmechel D. Age-dependent cytokine responses: Trimethyltin hippocampal injury in wild-type, APOE knockout, and APOE4 mice. *Brain Behav Immun* 2000;14:288-304.

- Hashimoto M, Ishikawa Y, Yokota S, Goto F, Bando T, Sakakibara Y, et al. Action site of circulating interleukin-1 on the rabbit brain. *Brain Res* 1991;540(1):217-223.
- Haughey NJ, Cutler RG, Tamara A, McArthur JC, Vargas DL, Pardo CA, et al. Perturbation of sphingolipid metabolism and ceramide production in HIV-dementia. *Ann Neurol* 2004;55(2):257-267.
- Hayashi H, Campenot RB, Vance DE, Vance JE. Apolipoprotein E-containing lipoproteins protect neurons from apoptosis via a signaling pathway involving low-density lipoprotein receptor-related protein-1. *The Journal of neuroscience* 2007;27(8):1933-1941.
- Hazleton J, Berman JW, Eugenin EA. Novel mechanisms of central nervous system damage in HIV infection. *HIV/AIDS Res Palliative Care* 2010;2:39-49.
- Heaton RK, Clifford DB, Franklin J, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* 2010;75:2087-2096.
- Heaton RK, Grant I, Butters N, White DA, Kirson D, Atkinson JH, et al. The HNRC 500 - Neuropsychology of HIV infection at different disease stages. *J Int Neuropsychol Soc* 1995;1:231-251.
- Heaton R, Chelune G, Talley JL, Kay G, Curtiss G. Wisconsin card sorting test manual: revised and expanded. Odessa: Psychological Assessment Resources. 1993.
- Heaton RK, Cysique LA, Jin H, Shi C, Yu X, Letendre S, et al. Neurobehavioral effects of human immunodeficiency virus infection among former plasma donors in rural China. *J Neurovirol* 2008;14(6):536-549.
- Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. *AIDS* 2006 Oct 24;20(16):W13-23.
- Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* 1990;31(3):545-548.
- Hosoi T, Okuma Y, Nomura Y. The mechanisms of immune-to-brain communication in inflammation as a drug target. *Current Drug Targets-Inflammation & Allergy* 2002;1(3):257-262.
- Hult B, Chana G, Masliah E, Everall I. Neurobiology of HIV. *Int Rev Psychiatry* 2008;20(1):3-13.

Hurwitz AA, Lyman WD, Berman JW. Tumor necrosis factor alpha and transforming growth factor beta upregulate astrocyte expression of monocyte chemoattractant protein-1. *J Neuroimmunol* 1995 Mar;57(1-2):193-198.

Jana M, Anderson JA, Saha RN, Liu X, Pahan K. Regulation of inducible nitric oxide synthase in proinflammatory cytokine-stimulated human primary astrocytes. *Free Radic Biol Med* 2005 Mar 1;38(5):655-664.

Jannsen RS, Cornblath DR, Epstein LR, McArthur J, Price RW. Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. Report of a Working Group of the American Academy of Neurology AIDS Task Force. *Neurology* 1991;41(6):778-785.

Jayadev S, Garden GA. Host and viral factors influencing the pathogenesis of HIV-associated neurocognitive disorders. *J Neuroimmune Pharmacol* 2009;4(2):175-189.

Johnson MD, Kim P, Tourtellotte W, Federspiel CF. Transforming growth factor beta and monocyte chemotactic protein-1 are elevated in cerebrospinal fluid of immunocompromised patients with HIV-1 infection. *J NeuroAIDS* 2004;2(4):33-43.

Joint United Nations Programme on HIV/AIDS. UNAIDS report on the global AIDS epidemic. 2010.

Jones BN, Teng EL, Folstein MF, Harrison KS. A new bedside test of cognition for patients with HIV infection. *Ann Intern Med* 1993;119(10):1001-1004.

Joseph J, Clifford D, Douglas SD, Fox H, Gendelman HE, Gonzalez-Scarano F, et al. Planning future strategies for domestic and international NeuroAIDS research, July 24–25, 2008. *Journal of Neuroimmune Pharmacology* 2009;4(3):283-297.

Joska JA, Combrinck M, Valcour VG, Hoare J, Leisegang F, Mahne ACea. Association between apolipoprotein E4 genotype and human immunodeficiency virus-associated dementia in younger adults starting antiretroviral therapy in South Africa. *J Neurovirol* 2010a;16(5):377-383.

Joska JA, Fincham DS, Stein DJ, Paul RH, Seedat S. Clinical correlates of HIV-associated neurocognitive disorders in South Africa. *AIDS Behav* 2010b;14:371-378.

Joska JA, Gouse H, Paul RH, Stein DJ, Flisher AJ. Does highly active antiretroviral therapy improve neurocognitive function? A systematic review. *J Neurovirol* 2010c;16:101-114.

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

Joska JA, Westgarth-Taylor J, Myer L, Hoare J, Thomas KGF, Combrinck M, et al. Characterization of HIV-associated neurocognitive disorders among individuals starting antiretroviral therapy in South Africa. *AIDS Behav* 2010d 8 July 2010.

Joska JA, Westgarth-Taylor J, Hoare J, Thomas KG, Paul R, Myer L, et al. Neuropsychological outcomes in adults commencing highly active anti-retroviral treatment in South Africa: a prospective study. *BMC Infectious Diseases* 2012;12(1):39.

Kaloutsis V, Kohlmeyer U, Maschek H, Nafe R, Choritz H, Amor A, et al. Comparison of bone marrow and hematologic findings in patients with human immunodeficiency virus infection and those with myelodysplastic syndromes and infectious diseases. *Am J Clin Pathol* 1994;101(2):123.

Kamboh MI, Sepehrnia B, Ferrel RE. Genetic studies of human apolipoproteins. VI. Common polymorphism of apolipoprotein E in blacks. *Dis Markers* 1989;7(1):49-55.

Kanki PJ, Hamel DJ, Sankalé J, Hsieh C, Thior I, Barin Fea. Human immunodeficiency virus type 1 subtypes differ in disease progression. *J Infect Dis* 1999;179:68-73.

Kanmogne GD, Kuate CT, Cysique LA, Fonsah JY, Eta S, Doh R, et al. HIV-associated neurocognitive disorders in sub-Saharan Africa: a pilot study in Cameroon. *BMC neurology* 2010;10(1):60.

Kaul M, Garden GA, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* 2001 19 April 2001;410:988-994.

Kaul M, Zheng J, Okamoto S, Gendelman H, Lipton S. HIV-1 infection and AIDS: consequences for the central nervous system. *Cell Death & Differentiation* 2005;12:878-892.

Kellar KL, Douglass JP. Multiplexed microsphere-based flow cytometric immunoassays for human cytokines. *J Immunol Methods* 2003;279(1):277-285.

Kelly A, Lynch A, Vereker E, Nolan Y, Queenan P, Whittaker E, et al. The anti-inflammatory cytokine, interleukin (IL)-10, blocks the inhibitory effect of IL-1 beta on long term potentiation. *J Biol Chem* 2001;276:45564-45572.

Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005;62(6):617.

Kim MO, Suh HS, Brosnan CF, Lee SC. Regulation of RANTES/CCL5 expression in human astrocytes by interleukin-1 and interferon-beta. *J Neurochem* 2004 Jul;90(2):297-308.

Klasse PJ, Moore JP. Is there enough gp120 in the body fluids of HIV-1-infected individuals to have biologically significant effects? *Virology* 2004 May 20;323(1):1-8.

Klove H. *Clinical Neuropsychology*. *Med Clin North Am* 1963 Nov;47:1647-1658.

Knight PR, Sreekumar A, Siddiqui J, Laxman B, Copeland S, Chinnaiyan A, et al. Development of a sensitive microarray immunoassay and comparison with standard enzyme-linked immunoassay for cytokine analysis. *Shock* 2004;21(1):26-30.

Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, et al. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* 1986;233(4768):1089-1093.

Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci* 2002;25(3):154-159.

Kraft-Terry SD, Stothert AR, Buch S, Gendelman HE. HIV-1 neuroimmunity in the era of antiretroviral therapy. *Neurobiol Dis* 2010;37:542-548.

Kuhlmann I, Minihane AM, Huebbe P, Nebel A, Rimbach G. Apolipoprotein E genotype and hepatitis C, HIV and herpes simplex disease risk: a literature review. *Lipids in health and disease* 2010;9(1):8.

Kure K, Llana JF, Lyman WD, Soeiro R, Weidenheim KM, Hirano A, et al. Human immunodeficiency virus-1 infection of the nervous system: an autopsy study of 268 adult, pediatric, and fetal brains. *Hum Pathol* 1991;22(7):700-710.

Landmann R, Knopf H, Link S, Sansano S, Schumann R, Zimmerli W. Human monocyte CD14 is upregulated by lipopolysaccharide. *Infect Immun* 1996;64(5):1762-1769.

Langford D, Masliah E. Crosstalk between components of the blood brain barrier and cells of the CNS in microglial activation in AIDS. *Brain Pathol* 2001;11:306-312.

Langford T, Letendre S, Larrea G, Masliah E. Changing patterns in the neuropathogenesis of HIV during the cART era. *Brain pathology* 2003;13(2):195-210.

Laskowitz D, Thekdi A, Thekdi S, Han S, Myers J, Pizzo S, et al. Downregulation of microglial activation by apolipoprotein E and apoE-mimetic peptides. *Exp Neurol* 2001;167(1):74-85.

Lawler K, Mosepele M, Ratcliffe S, Seloilwe E, Steele K, Nthobatsang R, et al. Neurocognitive impairment among HIV-positive individuals in Botswana: a pilot study. *J Int AIDS Soc* 2010 Apr 20;13:15-2652-13-15.

Lawrence DM, Major EO. HIV-1 and the brain: connections between HIV-1-associated dementia, neuropathology and neuroimmunology. *Microbes Infect* 2002;4:301-308.

Letendre SL, McCutchan JA, Childers ME, Woods SP, Lazzaretto D, Heaton RK, et al. Enhancing antiretroviral therapy for human immunodeficiency virus cognitive disorders. *Ann Neurol* 2004;56(3):416-423.

Lien E, Aukrust P, Sundan A, Müller F, Frøland SS, Espevik T. Elevated levels of serum-soluble CD14 in human immunodeficiency virus type 1 (HIV-1) infection: correlation to disease progression and clinical events. *Blood* 1998;92(6):2084-2092.

Liner II KJ, Hall CD, Robertson KR. Effects of antiretroviral therapy on cognitive impairment. *Curr HIV/AIDS Rep* 2008;5:64-71.

Lipton SA. Neuronal injury associated with HIV-1 and potential treatment with calcium-channel and NMDA antagonists. *Dev Neurosci* 1994;16(3-4):145-151.

Liu NQ, Lossinsky AS, Popik W, Li W, Gujuluva C, Kriederman B, et al. Human immunodeficiency virus type 1 enters the brain microvascular endothelia by macropinocytosis dependent on lipid rafts and the mitogen-activated protein kinase signaling pathway. *J Virol* 2002;76(13):6689-6700.

Lotz M, Seth P. TGFB and HIV infection. *Ann NY Acad Sci* 1993;685:501-511.

Lynch JR, Tang W, Wang H, Vitek MP, Bennet ER, Sullivan P, Mea. APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 2003;278(49):48529-48533.

Lynn W, Lightman S. Syphilis and HIV: a dangerous combination. *The Lancet infectious diseases* 2004;4(7):456-466.

Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240(4852):622-630.

Mahley RW, Rall Jr SC. Apolipoprotein E: far more than a lipid transport protein. *Annual review of genomics and human genetics* 2000;1(1):507-537.

Mahne AC. The role of inflammation, oxidative stress and the apolipoprotein E genotype in HIV-associated cognitive impairment: a clinical, biochemical and neuro-imaging study [dissertation]. Cape Town: Univ of Cape Town, 2010.

Mannick JB, Stamler JS, Teng E, Simpson N, Lawrence J, Jordan J, et al. Nitric oxide modulates HIV-1 replication. *J Acquir Immune Defic Syndr* 1999 Sep 1;22(1):1-9.

Mantel PY, Schmidt-Weber CB. Transforming growth factor-beta: recent advances on its role in immune tolerance. *Methods Mol Biol* 2011;677:303-338.

Masliah E, DeTeresa RM, Mallory ME, Hansen LA. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS* 2000;14(1):69-74.

Matsumura S, Shibakusa T, Fujikawa T, Yamada H, Inoue K, Fushiki T. Increase in transforming growth factor- β in the brain during infection is related to fever, not depression of spontaneous motor activity. *Neuroscience* 2007;144(3):1133-1140.

Matsumura S, Shibakusa T, Fujikawa T, Yamada H, Matsumura K, Inoue K, et al. Intracisternal administration of transforming growth factor-beta evokes fever through the induction of cyclooxygenase-2 in brain endothelial cells. *Am J Physiol Regul Integr Comp Physiol* 2008 Jan;294(1):R266-75.

McArthur JC. HIV dementia: an evolving disease. *J Neuroimmunol* 2004;157:3-10.

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

McArthur JC, Haughey N, Gartner S, Conant K, Pardo C, Nath Aea. Human immunodeficiency virus-associated dementia: an evolving disease. *J Neurovirol* 2003;9:205-221.

McArthur JC, Hoover DR, Bacellar H, Miller EN, Cohen BA, Becker JT, et al. Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology* 1993;43(11):2245-2252.

McArthur JC, McDermott MP, McClernon D, Coryse St Hillaire BS, Conant K, Marder K, et al. Attenuated central nervous system infection in advanced HIV/AIDS with combination antiretroviral therapy. *Arch Neurol* 2004;61:1687-1696.

McArthur JC, Steiner J, Sacktor N, Nath A. Human immunodeficiency virus-associated disorders Mind the gap. *Ann Neurol* 2010;67:699-714.

- McCutchan FE. Global epidemiology of HIV. *J Med Virol* 2006;78:S7-S12.
- McDougal JS, Mawle A, Cort SP, Nicholson JKA, Cross GD, Scheppler-Campbell JA, et al. Cellular tropism of the human retrovirus HTLV-III/LAV I. Role of T cell activation and expression of the T4 antigen. *J Immunol* 1985;135(5):3151-3162.
- McElrath MJ, Pruetz JE, Cohn ZA. Mononuclear phagocytes of blood and bone marrow: Comparative roles as viral reservoirs in human immunodeficiency virus type 1 infections. *Proc Natl Acad Sci USA* 1989;86:675-679.
- McGuire D. CSF biomarkers in HIV dementia Through a glass darkly. *Neurology* 2009;73(23):1942-1944.
- Meisel C, Vogt K, Platzer C, Randow F, Liebenthal C, Volk HD. Differential regulation of monocytic tumor necrosis factor-alpha and interleukin-10 expression. *Eur J Immunol* 1996;26:1580-1586.
- Merrill JE, Benveniste EN. Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci* 1996 Aug;19(8):331-338.
- Minghetti L. Role of inflammation in neurodegenerative diseases. *Curr Opin Neurol* 2005;18:315-321.
- Mishra M, Vetrivel S, Siddappa NB, Ranga U, Seth P. Clade-specific differences in neurotoxicity of human immunodeficiency virus-1 B and C Tat of human neurons: significance of dicysteine C30C31 motif. *Ann Neurol* 2008;63(3):366-376.
- Moore DJ, Palmer BW, Patterson TL, Jeste DV. A review of performance-based measures of functional living skills. *J Psychiatr Res* 2007;41(1):97-118.
- Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001;19:683-765.
- Morgan E, Woods S, Letendre S, Franklin D, Bloss C, Goate A, et al. Apolipoprotein E4 genotype does not increase risk of HIV-associated neurocognitive disorders. *J Neurovirol* 2013:1-7.
- Multiplex immunoassay technology (image). Taken from: <http://www.bio-rad/evportal/en/ZA/LSR/Solutions/LUSM0E8UU/Multiplex-Immunoassays>
- Musso T, Espinoza-Delgado I, Pulkki K, Gusella G, Longo D, Varesio L. Transforming growth factor beta downregulates interleukin-1 (IL-1)-induced IL-6 production by human monocytes. *Blood* 1990;76(12):2466-2469.

Myers JK, Weissman MM. Use of a self-report symptom scale to detect depression in a community sample. *Am J Psychiatry* 1980.

Nath A, Berger J. HIV dementia. *Curr Treat Options Neurol* 2004;6:139-151.

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

Nath A, Conant K, Chen P, Scott C, Major EO. Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes A hit and run phenomenon. *J Biol Chem* 1999;274(24):17098-17102.

Navia BA, Jordan BD, Price RWT. The AIDS dementia complex: I. Clinical features. *Ann Neurol* 1986;19(6):517-524.

Navia BA, Price RW. The acquired immunodeficiency syndrome dementia complex as the presenting or sole manifestation of human immunodeficiency virus infection. *Arch Neurol* 1987;44(1):65.

Nguyen MD, Julien J, Rivest S. Innate immunity: the missing link in neuroprotection and neurodegeneration? *Nature Reviews Neuroscience* 2002;3(3):216-227.

Nottet HS, Gendelman HE. Unraveling the neuroimmune mechanisms for the HIV-1-associated cognitive/motor complex. *Immunol Today* 1995 Sep;16(9):441-448.

Nuovo GJ, Alfieri ML. AIDS dementia is associated with massive, activated HIV-1 infection and concomitant expression of several cytokines. *Mol Med* 1996;2(3):358-366.

Oh J, Schwiebert LM, Benveniste EN. Cytokine regulation of CC and CXC chemokine expression by human astrocytes. *J Neurovirol* 1999;5(1):82-94.

Osborne JW, Costello AB. Best practices in exploratory factor analysis: four recommendations for getting the most from your analysis. *Pan* 2009;12(2):131-146.

Pedersen WA, Chan SL, Mattson MP. A Mechanism for the Neuroprotective Effect of Apolipoprotein E. *J Neurochem* 2000;74(4):1426-1433.

Peluso R, Haase A, Stowring L, Edwards M, Ventura P. A Trojan Horse mechanism for the spread of visna virus in monocytes. *Virology* 1985;147(1):231-236.

Perry VH, Newman TA, Cunningham C. The impact of systemic infection on the progression of neurodegenerative disease. *Nature Reviews Neuroscience* 2003;4(2):103-112.

Platzer C, Meisel C, Vogt K, Platzer M, Volk HD. Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. *Int Immunol* 1995;7:517-523.

PlüDdemann A, PlüDdemann A, Flisher AJ, PlüDdemann A, Flisher AJ, Mathews C, et al. Adolescent methamphetamine use and sexual risk behaviour in secondary school students in Cape Town, South Africa. *Drug Alcohol Rev* 2008;27(6):687-692.

Pocernich CB, Sultana R, Hone E, Turchan J, Martins RN, Calabrese V, et al. Effects of apolipoprotein E on the human immunodeficiency virus protein Tat in neuronal cultures and synaptosomes. *J Neurosci Res* 2004;77(4):532-539.

Poirier J, Bertrand P, Kogan S, Gauthier S, Davignon J, Bouthillier D. Apolipoprotein E polymorphism and Alzheimer's disease. *The Lancet* 1993;342(8873):697-699.

Power C, Boisse L, Rourke S, Gill MJ. NeuroAIDS: An evolving epidemic. *Can J Neurol Sci* 2009;36:285-295.

Power C, Selnes OA, Grim JA, McArthur JC. HIV Dementia Scale: a rapid screening test. *JAIDS J Acquired Immune Defic Syndromes* 1995;8(3):273-278.

Prabhakar U, Eirikis E, Davis HM. Simultaneous quantification of proinflammatory cytokines in human plasma using the LabMAP™ assay. *J Immunol Methods* 2002;260(1):207-218.

Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P. The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* 1988;239(4840):586-592.

Pulliam L, Gascon R, Stubblebine M, McGuire D, McGrath MS. Unique monocyte subset in patients with AIDS dementia. *Lancet* 1997;349:692-695.

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

Rappaport J, Joseph J, Croul S, Alexander G, Del Valle L, Amini S, et al. Molecular pathway involved in HIV-1-induced CNS pathology: role of viral regulatory protein, Tat. *J Leukoc Biol* 1999;65(4):458-465.

Rausch DM, Davis MR. HIV in the CNS: pathogenic relationships to systemic HIV disease and other CNS disease. *J Neurovirol* 2001;7:85-96.

Ray CA, Bowsher RR, Smith WC, Devanarayan V, Willey MB, Brandt JT, et al. Development, validation, and implementation of a multiplex immunoassay for the simultaneous determination of five cytokines in human serum. *J Pharm Biomed Anal* 2005;36(5):1037-1044.

Reitan RM, Davison LA. *Clinical neuropsychology: Current status and applications*. : VH Winston & Sons; 1974.

Resnick L, Berger JR, Shapshak P, Tourtellotte WW. Early penetration of the blood-brain-barrier by HIV. *Neurology* 1988;38(1):9-14.

Rey A, Osterrieth P. Translations of excerpts from Andre Rey" s Psychological examination of traumatic encephalopathy and PA Osterrieth" s The Complex Figure Copy Test.. *Clin Neuropsychol* 1993.

Riedel D, Ghatge M, Nene M, Paranjape R, Mehendale S, Bollinger R, et al. Screening for human immunodeficiency virus (HIV) dementia in an HIV clade C-infected population in India. *J Neurovirol* 2006;12(1):34-38.

Roberts AB, Flanders KC, Heine UI, Jakowlew S, Kondaiiah P, Kim SJ, et al. Transforming growth factor-beta: multifunctional regulator of differentiation and development. *Philos Trans R Soc Lond B Biol Sci* 1990;327(1239):145-154.

Robertson KR, Nakasujja N, Wong M, Musisi S, Katabira E, Parsons TD, et al. Pattern of neuropsychological performance among HIV-positive patients in Uganda. *BMC neurology* 2007;7(1):8.

Romanovsky AA. Signaling the brain in the early sickness syndrome: are sensory nerves involved? *Front Biosci* 2004 Jan 1;9:494-504.

Rosenberg ZF, Fauci AS. The immunopathogenesis of HIV infection. *Adv Immunol* 1989;47:377-341.

Ryan LA, Zheng J, Brester M, Bohac D, Hahn F, Anderson J, et al. Plasma levels of soluble CD14 and tumor necrosis factor- α type II receptor correlate with cognitive dysfunction during human immunodeficiency virus type 1 infection. *J Infect Dis* 2001;184(6):699-706.

Sabo T, Lomnitski L, Nyska A, Beni S, Maronpot R, Shohami E, et al. Susceptibility of transgenic mice expressing human apolipoprotein E to closed head injury: the allele E3 is neuroprotective whereas E4 increases fatalities. *Neuroscience* 2000;101(4):879-884.

Sacktor N, McDermott MP, Marder K, Schifitto G, Selnes OA, McArthur JC, et al. HIV-associated cognitive impairment before and after the advent of combination therapy. *J Neurovirol* 2002;8(2):136-142.

Sacktor N, Nakasujja N, Skolasky R. Antiretroviral therapy improves cognitive impairment in HIV+ individuals in sub-Saharan Africa. *Neurology* 2006;67:311.

Sacktor NC, Bacellar H, Hoover DR, Nance-Sproson TE, Selnes OA, Miller EN, et al. Psychomotor slowing in HIV infection: a predictor of dementia, AIDS and death. *J Neurovirol* 1996;2(6):404-410.

Sacktor N, Skolasky R, Tarwater P, McArthur J, Selnes O, Becker J, et al. Response to systemic HIV viral load suppression correlates with psychomotor speed performance. *Neurology* 2003;61(4):567-569.

Sacktor NC, Wong M, Nakasujja N, Skolasky RL, Selnes OA, Musisi S, et al. The International HIV Dementia Scale: a new rapid screening test for HIV dementia. *AIDS* 2005 Sep 2;19(13):1367-1374.

Saha RN, Pahan K. Tumor necrosis factor- α at the crossroads of neuronal life and death during HIV-associated dementia. *J Neurochem* 2003;86(5):1057-1071.

Sandholzer C, Delport R, Vermaak H, Uterman G. High frequency of the apo E4 allele in Khoi San from South Africa. *Hum Genet* 1995;95:46-48.

Santos JRA, Clegg MD. Factor analysis adds new dimension to extension surveys. *Journal of Extension* 1999;37(5):5RIB6.

Satz P, Morgenstern H, Miller EN, SEINES OA, MCARTHUR JC, COHEN BA, et al. Low education as a possible risk factor for cognitive abnormalities in HIV-1: findings from the multicenter AIDS Cohort Study (MACS). *JAIDS J Acquired Immune Defic Syndromes* 1993;6(5):503-511.

Saunders A, Strittmatter W, Schmechel D, George-Hyslop PS, Pericak-Vance M, Joo S, et al. Association of apolipoprotein E allele ϵ 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993a;43(8):1467-1467.

Saunders JB, Aasland OG, Babor TF, Grant M. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction* 1993b;88(6):791-804.

Saura J, Petegnief V, Wu X, Liang Y, Paul SM. Microglial apolipoprotein E and astroglial apolipoprotein J expression in vitro: opposite effects of lipopolysaccharide. *J Neurochem* 2003;85(6):1455-1467.

Sawaya B, Thatikunta P, Denisova L, Brady J, Khalili K, Amini S. Regulation of TNFalpha and TGFbeta-1 gene transcription by HIV-1 Tat in CNS cells. *J Neuroimmunol* 1998;87(1-2):33.

Scarmeas N, Stern Y. Cognitive reserve and lifestyle. *Journal of clinical and experimental neuropsychology* 2003;25(5):625-633.

Schematic representation of an immunoassay sandwich-based assay workflow (image). Taken from:

http://www.biorad.com/evportal/en/ZA/evolutionPortal.portal?_nfpb=true&_pageLabel=SolutionsLandingPage&CatID=LUSMOZMNI

Schnittman SM, Psallidopoulos MC, Lane HC, Thompson L, Baseler M, Massari F, et al. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* 1989;245(4915):305-308.

Schouten J, Cinque P, Gisslan M, Reiss P, Portegies P. HIV-1 infection and cognitive impairment in the cART-era: a review. *AIDS* 2011;25:000-000.

Shapsak P, Kangeane P, Fujimura RK, Commins D, Chiappelli F, Singer E. Editorial NeuroAIDS review. *AIDS* 2011;25:123-141.

Sharpless N, Gilbert D, Vandercam B, Zhou JM, Verdin E, Ronnett G, et al. The restricted nature of HIV-1 tropism for cultured neural cells. *Virology* 1992 Dec;191(2):813-825.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998;59:22-33.

Siawaya JFD, Roberts T, Babb C, Black G, Golakai HJ, Stanley K, et al. An evaluation of commercial fluorescent bead-based luminex cytokine assays. *PLoS One* 2008;3(7):e2535.

Sinclair E, Ronquillo R, Lollo N, Deeks SG, Hunt P, Yiannoutsos CT. Antiretroviral treatment effect on immune activation reduces cerebrospinal fluid HIV-1 infection. *J Acquir Immune Defic Syndr* 2008;47:544-552.

Singh KK, Ellis RJ, Marquie-Beck J, Letendre S, Heaton RK, Grant I, et al. < i> CCR2 polymorphisms affect neuropsychological impairment in HIV-1-infected adults. J Neuroimmunol 2004;157(1):185-192.

Smith JA, Das A, Ray SK, Banik NL. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull 2012;87:10-20.

Smits H, Boven L, Pereira C, Verhoef J, Nottet H. Role of macrophage activation in the pathogenesis of Alzheimer's disease and human immunodeficiency virus type 1-associated dementia. Eur J Clin Invest 2000;30(6):526-535.

Spector SA, Singh KK, Gupta S, Cystique LA, Jin H, Letendre S, et al. 195APOE E4 and MBL-2 O/O genotypes are associated with neurocognitive impairment in HIV-infected plasma donors from Anhui Province, China. AIDS 2010;24(10):1471-1479.

Stern Y. Cognitive reserve and Alzheimer disease. Alzheimer Dis Assoc Disord 2006 Apr-Jun;20(2):112-117.

Stroop JR. Studies of interference in serial verbal reactions 1935.

Sui Z, Sniderhan LF, Schifitto G, Phipps RP, Gelbard HA, Dewhurst S, et al. Functional synergy between CD40 ligand and HIV-1 Tat contributes to inflammation: implications in HIV type 1 dementia. J Immunol 2007 Mar 1;178(5):3226-3236.

Sundar SK, Cierpial MA, Kamaraju LS, Long S, Hsieh S, Lorenz C, et al. Human immunodeficiency virus glycoprotein (gp120) infused into rat brain induces interleukin 1 to elevate pituitary-adrenal activity and decrease peripheral cellular immune responses. Proceedings of the National Academy of Sciences 1991;88(24):11246-11250.

Szelenyi J. Cytokines and the central nervous system. Brain Res Bull 2001 Mar 1;54(4):329-338.

Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. N Engl J Med 2008;358(15):1590-1602.

Tesseur I, Zhang H, Brecht W, Corn J, Gong J, Yanagisawa K, et al. Bioactive TGF- β can associate with lipoproteins and is enriched in those containing apolipoprotein E3. J Neurochem 2009;110(4):1254-1262.

Thieblemont N, Weiss L, Sadeghi HM, Estcourt C, Haeffner-Cavaillon N. CD14^{low}CD16^{high}: A cytokine-producing monocyte subset which expands during human immunodeficiency virus infection. Eur J Immunol 1995;25(12):3418-3424.

Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* 1992 Apr 30;356(6372):768-774.

Titius B, Thiele J, Schaefer H, Kreipe H, Fischer R. Ki-S1 and proliferating cell nuclear antigen expression of bone marrow macrophages. *Acta Haematol* 2009;91(3):144-149.

Toedter G, Hayden K, Wagner C, Brodmerkel C. Simultaneous detection of eight analytes in human serum by two commercially available platforms for multiplex cytokine analysis. *Clinical and Vaccine Immunology* 2008;15(1):42-48.

Torres-Muñoz J, Stockton P, Tacoronte N, Roberts B, Maronpot RR, Petit CK. Detection of HIV-1 gene sequences in hippocampal neurons isolated from postmortem AIDS brains by laser capture microdissection. *Journal of Neuropathology & Experimental Neurology* 2001;60(9):885-892.

Tsunawaki S, Sporn M, Ding A, Nathan C. Deactivation of macrophages by transforming growth factor-beta. *Nature* 1988;334(6179):260-262.

Tulsa O. Electronic Statistics Textbook. WEB: <http://www.statsoft.com/textbook> 2010.

Tyor WR, Glass JD, Griffin JW, Becker PS, McArthur JC, Bezman L, et al. Cytokine expression in the brain during the acquired immunodeficiency syndrome. *Ann Neurol* 1992;31(4):349-360.

Tziakas DN, Chalikias GK, Antonoglou CO, Veletza S, Tentes IK, Kortsaris AX, et al. Apolipoprotein E genotype and circulating interleukin-10 levels in patients with stable and unstable coronary artery disease. *J Am Coll Cardiol* 2006;48(12):2471-2481.

Urosevic N, Martins RN. Infection and Alzheimer's disease: The APOE e4 connection and lipid metabolism. *J Alzheimers Dis* 2008;13:421-435.

Vago L, Bonetto S, Nebuloni M, Duca P, Carsana L, Zerbi P, et al. Pathological findings in the central nervous system of AIDS patients on assumed antiretroviral therapeutic regimens: retrospective study of 1597 autopsies. *AIDS* 2002;16(14):1925-1928.

Valcour V, Shikuma C, Shiramizu B, Watters M, Poff P, Selnes OA, et al. Age, apolipoprotein E4, and the risk of HIV dementia: the Hawaii Aging with HIV cohort. *J Neuroimmunol* 2004a;157:197-202.

Valcour V, Shikuma C, Shiramizu B, Watters M, Poff P, Selnes O, et al. Higher frequency of dementia in older HIV-1 individuals The Hawaii Aging with HIV-1 Cohort. *Neurology* 2004b;63(5):822-827.

Valcour V, Sithinamsuwan P, Letendre S, Ances B. Pathogenesis of HIV in the central nervous system. *Curr HIV/AIDS Rep* 2011 Mar;8(1):54-61.

Vitek MP, Brown CM, Colton CA. APOE genotype-specific differences in the innate immune response. *Neurobiol Aging* 2009;30(9):1350-1360.

Viviani B, Corsini E, Binaglia M, Galli CL, Marinovich M. Reactive oxygen species generated by glia are responsible for neuron death induced by human immunodeficiency virus-glycoprotein 120 in vitro. *Neuroscience* 2001;107(1):51-58.

Wahl SM, Allen JB, McCartney-Francis N, Morganti-Kossmann MC, Kossmann T, Ellingsworth L, et al. Macrophage- and astrocyte-derived transforming growth factor B as a mediator of central nervous system dysfunction in acquired immune deficiency syndrome. *J Exp Med* 1991;173:981-991.

Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, et al. Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. *Proceedings of the National Academy of Sciences* 1987;84(16):5788-5792.

Wang H, Sun J, Goldstein H. Human immunodeficiency virus type 1 infection increases the in vivo capacity of peripheral monocytes to cross the blood-brain barrier into the brain and the in vivo sensitivity of the blood-brain barrier to disruption by lipopolysaccharide. *J Virol* 2008;82(15):7591-7600.

Wang P, Wu P, Siegel MI, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J Biol Chem* 1995;270(16):9558-9563.

Wang T, Rumbaugh JA, Nath A. Viruses and the brain: from inflammation to dementia. *Clin Sci* 2006;110:393-407.

Wärnberg J, Gomez-Martinez S, Romeo J, Díaz L, Marcos A. Nutrition, inflammation, and cognitive function. *Ann NY Acad Sci* 2009;1153:164-175.

Watkins BA, Dorn HH, Kelly WB, Armstrong RC, Potts BJ, Michaels F, et al. Specific tropism of HIV-1 for microglial cells in primary human brain cultures. *Science* 1990;249(4968):549-553.

Watkins LR, Maier SF, Goehler LE. Cytokine-to-brain communication: a review & analysis of alternative mechanisms. *Life Sci* 1995;57(11):1011-1026.

Wechsler D. Wechsler Memory Scale—Third Edition. San Antonio, TX: The Psychological Corporation 1997a.

Wechsler D. WAIS-III: Wechsler adult intelligence scale. : Psychological Corporation San Antonio; 1997b.

Weisgraber KH. Apolipoprotein E: structure-function relationships. *Adv Protein Chem* 1994;45:249-302.

Weiss R, Teich N, Varmus H, Coffin J. RNA tumor viruses. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1985.

Wesselingh SL, Power C, Glass JD, Tyor WR, McArthur JC, Farber JM, et al. Intracerebral cytokine messenger RNA expression in acquired immunodeficiency syndrome dementia. *Ann Neurol* 1993;33(6):576-582.

WHO and UNAIDS. Global HIV/AIDS response: epidemic update and health sector progress towards universal access. Progress report. November 2011.

Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MBA. Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* 1986;83:7089-7093.

Williams K, Alvarez X, Lackner AA. Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. *GLIA* 2001a;36:156-164.

Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, DeBakker C, et al. Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. *J Exp Med* 2001b;193(8):905-915.

Winkler MK, Beveniste EN. Transforming growth factor-beta inhibition of cytokine-induced vascular cell adhesion molecule-1 expression in human astrocytes. *Glia* 1998 Feb;22(2):171-179.

Wojna V, Nath A. Challenges to the diagnosis and management of HIV dementia. *AIDS Read* 2006 Nov;16(11):615-6, 621-4, 626, 629-32.

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

Wong M, Bongiorno P, Gold P, Licinio J. Localization of Interleukin-1 β Converting Enzyme mRNA in Rat Brain Vasculature: Evidence that the Genes Encoding the Interleukin-1 System Are Constitutively Expressed in Brain Blood Vessels. *Neuroimmunomodulation* 1995;2(3):141-148.

Woods SP, Moore DJ, Weber E, Grant I. Cognitive neuropsychology of HIV-associated neurocognitive disorders. *Neuropsychol Rev* 2009;19:152-168.

World Medical Association. Declaration of Helsinki. Ethical principles for medical research involving human subjects. 2009.

Wright E, Brew B, Arayawichanont A, Robertson K, Saminthalapanya K, Kongsangdao S, et al. Neurologic disorders are prevalent in HIV-positive outpatients in the Asia-Pacific region. *Neurology* 2008 Jul 1;71(1):50-56.

Yadav A, Collman RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. *Journal of Neuroimmune Pharmacology* 2009;4(4):430-447.

Yeh MW, Kaul M, Zheng J, Nottet HS, Thylin M, Gendelman HE, et al. Cytokine-stimulated, but not HIV-infected, human monocyte-derived macrophages produce neurotoxic levels of I γ -cysteine. *J Immunol* 2000 Apr 15;164(8):4265-4270.

Yeptom T, Paul R, Vallabhaneni S, Kumarasamy N, Tate DF, Solomon S, et al. Neurocognitive consequences of HIV in southern India: a preliminary study of clade C virus. *J Int Neuropsychol Soc* 2006;12(3):424-430.

Yeung MC, Pulliam L, Lau AS. The HIV envelope protein gp120 is toxic to human brain-cell cultures through the induction of interleukin-6 and tumor necrosis factor-alpha. *AIDS* 1995 Feb;9(2):137-143.

Yoshioka M, Bradley WG, Shapshak P, Nagano I, Stewart RV, Xin K, et al. Role of immune activation and cytokine expression in HIV-1-associated neurologic diseases. *Adv Neuroimmunol* 1995;5:335-358.

Yuan L, Qiao L, Wei F, Yin J, Liu L, Ji Y, et al. Cytokines in CSF correlate with HIV-associated neurocognitive disorders in the post-cART era in China. *J Neurovirol* 2013:1-6.

Zekraoui L, Lagarde J, Raisonnier A, Gerard N, Aouizerate A, Lucotte G. High frequency of the apolipoprotein E* 4 allele in African pygmies and most of the African populations in sub-Saharan Africa. *Human biology* 1997:575-581.

Zhao ML, Kim MO, Morgello S, Lee SC. Expression of inducible nitric oxide synthase, interleukin-1 and caspase-1 in HIV-1 encephalitis. *J Neuroimmunol* 2001 Apr 2;115(1-2):182-191.

Zheng J, Gendelman HE. The HIV-1 associated dementia complex: a metabolic encephalopathy fueled by viral replication in mononuclear phagocytes. *Curr Opin Neurol* 1997;10(4):319-326.

Zhou H, Lapointe BM, Clark SR, Zbytniuk L, Kubes P. A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide. *J Immunol* 2006 Dec 1;177(11):8103-8110.