University of Cape Town

Department of Psychiatry and Mental Health

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THE ROLE OF THE ASTROCYTIC MARKER S100B IN HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS

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ABSTRACT

There are as yet no ideal biomarkers of HIV-associated neurocognitive disorders. As astrocytosis is a feature of HIV encephalitis, the marker S100β may hold promise as a biomarker of HAND. We explored associations between S100β and neurocognition in individuals with HIV in Cape Town, South Africa, before and after antiretroviral therapy (ART) was initiated. The S100β levels in the cerebrospinal fluid (CSF) of forty-six participants with HIV, but not yet on antiretroviral therapy, was quantified using an enzyme-linked immunoassay (ELISA). A battery of cognitive tests was performed and the global deficit score (GDS) was calculated. In twenty of these patients, the S100β analysis and the cognitive tests were repeated approximately six months after the initiation of ART. There was no significant association between cerebrospinal fluid S100β and GDS at baseline (r= -0.070; p= 0.66) or after six months of ART (r= 0.16; p= 0.52). Cerebrospinal fluid S100β levels at baseline did not predict a change in neurocognition on ART (B(SE) = 0.001, (0.001), β=0.025, p=0.85). S100β in the cerebrospinal fluid may not adequately reflect neurocognitive impairment in individuals with HIV. Our results further demonstrate that CSF S100β levels are not affected by ART, indicating persistent neuroinflammation.

ACKNOWLEGEMENTS

I would like to acknowledge:

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b) Mrs. Kareema Poggenpoel for her invaluable assistance with the collection of the data used for this study.
c) The research participants

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ABBREVIATIONS

AAN: American Academy of Neurology

AD: Alzheimer's Dementia

ADC: AIDS Dementia Complex

ADL: Activities of Daily Living

ANI: Asymptomatic neurocognitive impairment

AIDS: Acquired Immune Deficiency Syndrome

ART: Antiretroviral therapy

ARV: Anti-retroviral

BBB: Blood brain barrier

BVMT – R: Brief Visual Memory Test - Revised

CDC: Center for Disease Control

CES-D: Centers for Epidemiologic Studies Depression Scale – Revised

CNS: Central Nervous System

CD40L: CD40 ligand

CHARTER: CNS HIV Antiretroviral Therapy Effects Research

CSF: cerebrospinal fluid

DNA: Deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

ELISA: Enzyme-linked immunoassay

GDS: Global Deficit Score

HAD: HIV-associated dementia

HAND: HIV-associated neurocognitive disorders

HIV: Human Immunodeficiency Virus

HNRC: HIV Neurobehavioral Research Center

HVLT-R: Hopkins Verbal Learning Test - Revised

LP: Lumbar Puncture

MCMD: Minor Cognitive Motor Disorder

MND: Mild Neurocognitive Disorder

MRI: Magnetic Resonance Imaging

PCR: Polymerase Chain Reaction

RNA: Ribonucleic acid

Tat: Transactivator of transcription

TNF: Tumour Necrosis Factor

CHAPTER 1: INTRODUCTION

1. CONTEXT

Since the advent of antiretroviral therapy (ART), the incidence of HIV-associated dementia (HAD) has declined significantly, but the minor HIV-associated neurocognitive disorders (HAND) have become more prevalent (Grant et al., 2014).

This has led to two significant quandaries. Firstly, the question arises why HAND persists even when patients are virally suppressed on ART. Secondly the diagnosis of HAND has become more challenging as individuals with minor cognitive disorders have less severe neurocognitive impairment which may be more difficult to detect in busy clinic settings. The diagnosis therefore relies on neuropsychological test batteries which are time consuming and often difficult to adapt to local settings (Antinori et al., 2007).

Biomarkers of various kinds provide information on different aspects of the neuropathogenesis of HAND. S100 calcium-binding protein β (S100β) is a promising biomarker as it reflects astrocytosis, which may play a role in the neuropathophysiology of HAND. In this study S100β and its relationship with cognitive impairment in HIV-positive patients was explored. If S100β is shown to correlate with cognition, it may be useful in distinguishing HIV-associated effects as opposed to other causes of cognitive impairment in patients with HIV. It may also help to monitor the response on treatment. This may lead to a focus on glial cells such as astrocytes in future neuroprotective strategies.

1.1. HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS

1.1.1. Pathogenesis

HIV is neurovirulent and can affect the human nervous system in numerous ways. A key neuropathological feature is HIV encephalitis, and the clinical correlate: HIV encephalopathy (Cherner et al., 2002). Clinically, HIV-encephalopathy manifests as neurocognitive impairment, which when measured alongside functional impairment, results in disorders such as HIV dementia (McArthur, Brew, & Nath, 2005). HIV dementia is typically a subcortical dementia that includes psychomotor slowing, memory deficits and impairment in attention and executive functioning (McArthur et al., 2005).

The pathogenesis of HIV dementia is not completely understood (Morris, Davis, & Brew, 2010) and it is still unclear why some patients with HIV develop cognitive impairment while others are unaffected (McArthur, 2004)(Brew & Letendre, 2008).

It has been proposed that HIV crosses the blood brain barrier (BBB) and enters the central nervous system (CNS) as passengers in mononuclear cells that are trafficking to the brain – the "Trojan horse theory" (González-Scarano & Martín-García, 2005). Several CNS cell types (astrocytes, perivascular macrophages and microglia) come into contact with these infected cells (González-Scarano & Martín-García, 2005). HIV may become isolated in macrophages in the CNS due to the insufficient penetration of ART into the brain (McArthur et al.,

2005)(González-Scarano & Martín-García, 2005). Uncontrolled HIV replication in the CNS leads to partial breakdown of the BBB, rarefaction of white matter, astrocyte apoptosis, dendritic simplification and neuronal loss (McArthur, 2004)(McArthur et al., 2005)(Morris et al., 2010).

However, it is still unclear how HIV in the CNS contributes to these neurodegenerative changes , especially since neurons are not infected by HIV as they do not express CD4 receptors (González-Scarano & Martín-García, 2005)(Morris et al., 2010).

One theory is that HIV leads to immune activation disproportionate to the amount of virus present in the CNS, and the inflammation eventually leads to neurodegeneration (González-Scarano & Martín-García, 2005). There is a correlation between the presence of neurological deficits and inflammation of the CNS (McArthur, 2004). The inflammatory process leads to changes in the permeability of the BBB and release of pro-chemotactic factors, which allow more monocytes to enter the CNS and to perpetuate the cycle of neuroinflammation and neurotoxicity (González-Scarano & Martín-García, 2005).

The role of astrocytes in HAND

Astrocytes may play an important role in the neuropathogenesis of HAND. Astrocytes are actively involved in many functions of the CNS, including control of extracellular synaptic metabolites, neuronal excitability, syncytial signalling, synaptic plasticity, immune activation, inflammation and maintenance of the BBB (Eugenin & Berman, 2007).

Early studies using in situ polymerase chain reaction (PCR) demonstrated that approximately 1% of astrocytes are infected with HIV in patients with HAD (Takahashi, Wesselingh, & Griffin, 1996). However, a more sensitive PCR detected HIV deoxyribonucleic acid (DNA) in up to 19% of astrocytes in the brains of patients with HAD (Churchill et al., 2009). Most of the cells in the brain are astrocytes and they may therefore act as an important reservoir of HIV in the CNS (Churchill et al., 2009)(Gray et al., 2014)(T. Wang et al., 2009). However, whether astrocytes are susceptible to HIV infection is still questionable since they lack the CD4 receptor which is required for HIV entry into the cell (Gray et al., 2014). Recent evidence from cell cultures with primary human foetal astrocyte cultures suggest that astrocytes trap and internalize HIV-1 virions and engulf fragments of HIV-1-infected macrophages and that astrocytes are therefore unlikely to function as long-term viral reservoirs within the CNS (Russell et al., 2017).

The infection of astrocytes with HIV is correlated with the severity of the neuropathological changes in the brains of patients with HAD (Churchill et al., 2009). Astrocytes are also frequently infected in HIV positive subjects with a clinical dementia but no overt neuropathology. The infection of astrocytes with HIV may therefore be an explanation for HAND in patients who are treated with ART and in whom neuropathology is not evident (Churchill et al., 2009).

Latent infection of astrocytes causes changes in astrocyte gene expression (Churchill et al., 2009)(Z. Wang et al., 2004). The interaction between infected macrophages and astrocytes impede important astrocyte functions such as the uptake of excitatory amino acids including glutamate (González-Scarano & Martín-García, 2005). Activated astrocytes produce tumour necrosis factor (TNF) and other cytokines which intensify glutamate release and decreases the uptake of glutamate by astrocytes (González-Scarano & Martín-García, 2005) . The impaired catabolism of glutamate leads to excitotoxic neuronal death and astrocyte apoptosis which eventually leads to BBB compromise (Churchill et al., 2009) (González-Scarano & Martín-García, 2005)(Gorry, Ong, Thorpe, Bannwarth, & Thompson, 2003). HIV infected astrocytes increase the neurotoxicity of HIV infected microglia (T. Wang et al., 2009). Astrocyte apoptosis occurs commonly and the concentration of apoptotic astrocytes relates with how fast HIV dementia progresses (McArthur et al., 2005).

The activation and apoptosis of astrocytes is a significant cause of BBB compromise, as astrocytes are essential for the maintenance of BBB integrity (Churchill et al., 2009)(Gray et al., 2014) (Z. Wang et al., 2004). Even a small percentage (less than 10%) of HIV-infected astrocytes is sufficient to disrupt the BBB by a gap-junction dependent mechanism (Eugenin & Berman, 2007).

HIV-infected astrocytes may have an active role in monocyte recruitment across the BBB (Churchill et al., 2009) and use gap-junction channels to spread toxic signals to uninfected astrocytes and other cells that support viral replication, such as microglia and macrophages (Eugenin & Berman, 2007)(Gray et al., 2014). The ability of astrocytes to transfer HIV without de novo synthesis of the virus may indicate that astrocytes are able to sequester and protect the virus and hence facilitate viral dissemination in the CNS (Gray et al., 2014).

1.1.2. Classification of HAND

The 1991 American Academy of Neurology (AAN) criteria define two categories of HIV-associated neurologic disease: HAD and minor cognitive motor disorder (MCMD) (Antinori et al., 2007). MCMD is less severe than HAD and consists of impaired cognitive or behavioural function as well as mild impairment in work or activities of daily living (ADL) (Antinori et al., 2007). This set of AAN criteria does not diagnose the persons who have neurocognitive impairment but no functional decline (Antinori et al., 2007).

The HIV Neurobehavioral Research Centre (HNRC) at the University of California, San Diego, proposed updated research criteria which distinguish the following three conditions: asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND) and HAD (Antinori et al., 2007). ANI is the most common form of HAND and is, by definition, impairment in neuropsychological testing with no obvious impairment in daily functioning (Grant et al., 2014) (Antinori et al., 2007). This approach was adopted by the AAN in 2007.

1.1.3. Epidemiology

Together with the sensory neuropathies, HAND remains one of the commonest neurological consequences of HIV (McArthur, 2004)(McArthur et al., 2005). HAND occurs commonly in HIV positive patients, even in those who are on ART (Grant et al., 2014). ART does not completely protect neurons against the harm done by HIV (McArthur, 2004).

With the advent of ART, the incidence of HAD has declined, but there has been an increase in the overall prevalence of HAND (Antinori et al., 2007)(McArthur, 2004)(Morris et al., 2010)(Eugenin & Berman, 2007). In the CNS HIV Antiretroviral Therapy Effects (CHARTER) study, 52% of HIV positive patients on ART had neuropsychiatric impairment (Heaton et al., 2010). 33% of these patients had ANI, 12% had mild neurocognitive disorder and only 3% had HAD.

In South Africa, the prevalence of MND is 42,4% and the prevalence of HAD is 25,4% in people living with HIV who are not receiving ART (Joska et al., 2011).

1.1.4. Consequences

Baseline ANI might be a predictor of the time to symptomatic decline (Grant et al., 2014). In one study, HIV positive patients with ANI developed other symptoms of HIV much sooner than patients without ANI (Grant et al., 2014).

The mild neurocognitive disorders may have a deleterious impact on treatment adherence and driving ability (McArthur, 2004). Patients with mild cognitive impairment have a higher risk to develop AIDS and HIV encephalitis (McArthur, 2004)(McArthur et al., 2005). Mild cognitive impairment can lead to poor adherence to treatment and a non-suppressed viral load (McArthur, 2004)(McArthur et al., 2005). Even mild impairment may be associated with shortened survival and leads to poor employability (McArthur et al., 2005).

Because of these deleterious consequences and the negative impact on treatment adherence, HAND should be identified and treated as soon as possible (McArthur et al., 2005). Prior to universal test-and-treat, it was important to initiate ART in treatment naïve patients. Treated individuals with HAND, who have symptoms (such as adherence difficulties) may require assessment of viral load and CSF sampling. In a small proportion, the presence of compartmentalisation or escape may require a switch of ART regimen or viral genotyping. In those with viral control, adherence support and close follow-up become key. ART can improve the neurocognitive effects of HIV dementia and milder degrees of HAND (McArthur, 2004)(McArthur et al., 2005). It has been demonstrated that cognition continues to improve after even eight weeks on ART and reversal of the neurological deficits may therefore be much slower than expected (McArthur, 2004).

1.1.5. Diagnosis of HAND

For effective treatment to be initiated HAND needs to be diagnosed early and this remains problematic for several reasons:

1) The diagnostic criteria

The 1991 AAN criteria did not recognise patients who have neurocognitive impairment but no functional decline (Antinori et al., 2007). In lifestyles or socio-economic situations which pose fewer cognitive demands, deterioration in cognition may be less likely to result in functional impairment (Antinori et al., 2007). The alternative criteria proposed by the HNRC added an additional category, ANI, which requires mild impairment on neuropsychological testing but not impairment in daily functioning (Antinori et al., 2007)(Grant et al., 2014). However, the criteria for ANI are sensitive but not very specific (Grant et al., 2014). In addition, neither of these sets of criteria showed a strong correlation with the diagnosis of HIV-encephalitis at autopsy (Antinori et al., 2007).

2) Neuropsychological testing

The only way to diagnose HAND, using either set of criteria, is by conducting neuropsychological testing. Neuropsychological test batteries are time-consuming and the performance in these tests is influenced by age, level of education, ethnicity and gender (Antinori et al., 2007). Due to these limitations, neuropsychological tests cannot be used for screening purposes. The performance of neuropsychological test batteries is not realistic in primary health clinics with limited resources and high service demands. Neuropsychological tests are also often not capable of detecting an improvement in function (McArthur, 2004).

3) Confounding factors

Associated conditions, such as depression, substance disorders and hepatitis C infection, contribute to the evolution of ANI (Grant et al., 2014). It can be very difficult to decide whether neurological deficits are a result of HIV dementia or other confounding factors such as diabetes, hepatitis C, cerebrovascular disease or the cognitive effects of old age (McArthur et al., 2005).

1.2. Biomarkers in HAND

A biomarker is an indicator of normal biologic processes, pathophysiological processes or pharmacologic responses to a therapeutic intervention that can be objectively measured (Floyd & Mcshane, 2004).

Biomarkers should be identified for the following reasons:

1) Diagnosis

The use of biomarkers would be useful in assisting with the diagnosis of HAND and could be used as an objective measure for the presence of HAND (Morris et al., 2010)(Brew & Letendre, 2008)(Price et al., 2007). Unlike neuropsychological testing, biomarkers are not influenced by the cultural background and level of education of the patient (Antinori et al., 2007) and may be less time consuming to administer (Yuan et al., 2017).

Biomarkers could be used as additional screening tests for HAND. The Society of Psychiatrists of South Africa has emphasised the need for routine screening of HIV-positive individuals for cognitive impairments (Janse van Rensburg, 2012). Screening instruments for cognitive impairment have been developed but these instruments do not always have a good sensitivity or specificity and can be time consuming (McArthur, 2004). In South Africa, relatively few patients are referred for neuropsychiatric treatment due to inadequate screening of these disorders (Janse van Rensburg, 2012). Primary health care providers are often extremely busy and under immense pressure which could lead to inadequate screening. Biomarkers could be a much more effective way to screen for HIV neurocognitive disorders as it is less time consuming and requires no special training or experience.

A diagnostic challenge when identifying HIV related cognitive disorders is excluding other illnesses which can account for or exacerbate cognitive symptoms (Price et al., 2007). This can be especially challenging in older patients (McArthur, 2004). Biomarkers can help us to distinguish between HIV associated cognitive impairment and cognitive impairment as a result of other causes. A marker for the severity of HAND could provide an objective measure of the proportion of the deficit that was related to HIV, as opposed to the confound (Brew & Letendre, 2008).

2) Monitoring

The fact that ANI increases the risk for symptomatic decline (Grant et al., 2014) is a strong argument for the regular monitoring of these patients. Neuropsychological test batteries have traditionally been used for the monitoring of patients with HAND, but these can be time consuming, do not always detect an improvement in function and are subject to practice effects (McArthur, 2004)(McArthur et al., 2005). Biomarkers could be an objective and effective measure to monitor both symptomatic decline and the response to ART (Price et al., 2007) (Brew & Letendre, 2008).

3) Identification of subtypes

Recent data support the existence of different clinical phenotypes of HAND, including an inactive form (Brew & Letendre, 2008). There is a need for measures that can assess whether HAND is pathologically active or dormant (Price et al., 2007)(Morris et al., 2010). The identification of disease activity is clinically important for two reasons. Firstly, unrecognised inactive disease may mean that the patient is given new antiretroviral drugs needlessly with the consequent increased risk of toxicity (Brew & Letendre, 2008). Secondly, the lack of recognition of disease activity in clinical trials could lead to the inclusion of a large number of patients with inactive disease (Brew & Letendre, 2008).

4) Pathogenesis

Our understanding of the pathogenesis of HAND is limited (Morris et al., 2010). The investigation of biomarkers can help us to gain a better understanding of disease activity (McArthur, 2004).

5) Prognosis

A biomarker that could predict improvement or decline would be extremely helpful (Brew & Letendre, 2008) (Morris et al., 2010). Neither plasma nor CSF HIV ribonucleic acid (RNA) levels are predictive of neurological deterioration and cognitive decline in patients on ART, and this has encouraged the quest for other biomarkers which may better reflect the pathogenic mechanisms underlying HAND (McArthur, 2004).

1.3. S100β

S100β is a calcium-binding protein with two helix-loop-helix (EF-hand) motifs connected by a central hinge region. S100β is produced primarily by astrocytes in the CNS (Donato, 1999) (Donato et al., 2009)(Brew & Letendre, 2008)(Steiner et al., 2007)(Yardan, Erenler, Baydin, Aydin, & Cokluk, 2011).

S100β exerts neurotrophic or neurotoxic effects depending on its concentration (Donato, 1999) (Donato, 2001). At low concentrations, S100β promotes neuritic outgrowth and regeneration, and enhanced survival of neurons (Donato, 1999)(Donato, 2001). However, at high levels it is neurotoxic and causes apoptosis of neuronal and glial cells (Donato, 1999)(Donato, 2001).

S100β levels are elevated in several tumours, the ageing brain and in the brains of patients affected by Alzheimer's disease, Down's syndrome and temporal lobe epilepsy (Donato, 1999)(Donato et al., 2009)(Donato, 2001). Elevated S100β levels have even been implicated in schizophrenia and dyslexia (Donato et al., 2009).The observation that S100β is elevated in these conditions, has led to the idea that S100β might play a role in the pathophysiology of neurodegeneration and brain inflammatory diseases (Donato et al., 2009). S100β has been implicated in the modulation of learning and memory, and in animal models overexpression of S100β was associated with behavioural abnormalities (Donato, 1999)(Donato, 2001).

S100β is secreted by astrocytes which make it an ideal marker of astrocyte activation or astrocytosis (Brew & Letendre, 2008)(Du Pasquier et al., 2013) (Donato et al., 2009). High levels of S100β have been associated with neuronal apoptosis, thus decreasing the neuroprotective effects of astrocytes and the ability of astrocytes to maintain the integrity of the BBB (Du Pasquier et al., 2013) .

S100β could potentially be a biomarker of HIV-associated cognitive impairment for two reasons:

1) S100β is a marker of cognitive impairment in other neurological disorders (Donato, 1999)(Donato, 2001)(Donato et al., 2009) (Yardan et al., 2011).
2) Astrocyte infection plays an important role in the development of HAND, and the activation and apoptosis of astrocytes may directly lead to HIV encephalopathy and cognitive impairment (Churchill et al., 2009). S100β as a marker of astrocytosis may therefore reflect the extent of astrocyte involvement in a patient with HIV.

However, the evidence for astrocytic marker S100β as a biomarker of cognitive impairment in HIV is limited.

Pemberton and Brew (2001) demonstrated that CSF S100β levels correlated with the presence, severity and rapidity of the progression of AIDS dementia complex (ADC) in patients receiving ART. CSF S100β levels were significantly elevated in patients with moderate and severe ADC when compared to patients with no ADC or mild ADC. They showed that CSF S100β levels were a good predictor of rapid progression in patients with ADC. CSF S100β appeared to be a marker of severe dementia in patients with HIV. Yet, in the clinical setting, the milder cognitive disorders create a diagnostic challenge, and it is in those disorders where S100β as a biomarker of cognitive impairment would be most useful and necessary.

Woods et al. (2010) demonstrated that CSF S100β levels were elevated in HIV positive patients with decreased verb generation on neuropsychological testing. S100β was an independent predictor of verb generation in patients who received ART and patients who were not on treatment. S100β was shown to be a marker of executive dysfunction in patients with HIV. S100β levels did not reflect impairment in other cognitive domains.

Abassi et al (2017) showed that HIV positive individuals in Uganda with a CD4 < 200 had elevated CSF S100β levels but, paradoxically, that elevated levels of CSF S100β were associated with decreased odds of developing MND or HAD. However, in Uganda the predominant HIV clade is Clade D unlike South Africa which is Clade C predominant. Clade C may have different cognitive outcomes than Clade D and may be less virulent than other clades (Paul et al., 2014). They also looked at CSF S100β in ART naïve patients only. Yuan et al (2017) showed that CSF S100β levels were higher in cognitively impaired patients than in cognitively normal patients with HIV. In contrast to the Abassi study, over 60% of the patients in this study were on ART and all of these patients were HIV Clade B (Yuan et al., 2017).

Estébanez et al. (2014) looked at S100B levels in a group of HIV patients with cognitive impairment receiving protease inhibitors as triple-drug therapy or as monotherapy and showed no statistical difference in the levels of CSF S100β between the groups. Du Pasquier et al. (2013) compared the CSF concentration of S100β in a group of HIV-infected patients on lopinavir/ritonavir monotherapy with a group on continued therapy. S100β levels were significantly higher in the group receiving monotherapy than in the other group. However, neither of these studies looked at the correlation between S100β levels and cognition.

There are no published data on the response of S100β levels on ART (Brew & Letendre, 2008). According to our knowledge, there are no published South African studies which investigated S100β and its relationship to cognition in HAND. This may be key, as HIV in southern Africa is predominantly Clade C, which have different neuropathological effects to Clade B (Mishra, Vetrivel, Siddappa, Ranga, & Seth, 2008).

In this study, we investigated the relationship between S100B levels and cognition in a South African sample. We also looked at the association between S100B and cognition after a period of ART.

2. ETHICAL CONSIDERATIONS

The larger HAND research project had been approved by the Human Research Committee of the University of Cape Town (HREC 023/2008). The Human Research Committee of the University of Cape Town has also approved the current project and the new analyses (HREC 727/2017).

Participants of the original HAND study signed informed consent for their information and blood and CSF samples to be used for subsequent research. However, the sample is still sensitive in that confidential information is contained in the recorded data and human CSF samples were used. All information was encoded and identifying names were not recorded in the research.

3. AUTHOR GUIDELINES FOR THE JOURNAL OF NEUROVIROLOGY

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CHAPTER 2:

PUBLICATION-READY MANUSCRIPT

THE ROLE OF THE ASTROCYTIC MARKER S100B IN HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS

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Author contributions: John Joska and Marc Combrinck conceptualised the project. Engelina Groenewald collected the data, assisted with the immunological assays and wrote the manuscript. Pieter Naude performed the immunological assays and statistical analysis. John Joska and Pieter Naude critically revised and co-wrote the manuscript.

ABSTRACT

There are as yet no ideal biomarkers of HIV-associated neurocognitive disorders. As astrocytosis is a feature of HIV encephalitis, the marker S100β may hold promise as a biomarker of HAND. We explored associations between S100β and neurocognition in individuals with HIV in Cape Town, South Africa, before and after antiretroviral therapy (ART) was initiated. The S100β levels in the cerebrospinal fluid (CSF) of forty-six participants with HIV, but not yet on antiretroviral therapy, was quantified using an enzyme-linked immunoassay (ELISA). A battery of cognitive tests was performed and the global deficit score (GDS) was calculated. In twenty of these patients, the S100β analysis and the cognitive tests were repeated approximately six months after the initiation of ART. There was no significant association between cerebrospinal fluid S100β and GDS at baseline (r= -0.070; p= 0.66) or after six months of ART (r= 0.16; p= 0.52). Cerebrospinal fluid S100β levels at baseline did not predict a change in neurocognition on ART (B(SE) = 0.001, (0.001), β=0.025, p=.85). S100β in the cerebrospinal fluid may not adequately reflect neurocognitive impairment in individuals with HIV. Our results further demonstrate that CSF S100β levels are not affected by ART, indicating persistent neuroinflammation.

INTRODUCTION

The use of anti-retroviral therapy (ART) has dramatically impacted the neurological sequelae of HIV. The incidence of HIV-associated dementia (HAD) has declined significantly from approximately 20% prior to the use of ART, to less than 5% in the ART era (Janssen, Nwanyanwu, Selik, & Stehr-Green, 1992)(Heaton et al., 2010). However, the minor HIV-associated neurocognitive disorders (HAND) have become more prevalent (Grant et al., 2014)(Joska et al., 2011).

The diagnosis of HAND requires neuropsychological testing, which is not always widely available, or indeed sensitive enough (Antinori et al., 2007). The use of imaging and biofluid markers are held out as essential aids to clinical diagnosis, yet none have proved adequately sensitive or specific to HAND. The value of biomarkers lies in their potential for rapid assay, association with disease progression and/or response to treatment, and in addition are not influenced by the cultural background and level of education of the individual (Antinori et al., 2007)(Morris et al., 2010)(Brew & Letendre, 2008)(Price et al., 2007).

The persistence of HAND in the face of viral suppression on ART continues to vex research efforts. While the pathogenesis of HAND is not completely understood, the latent infection of astrocytes with HIV may be a key to understanding ongoing inflammation and the viral reservoir (Morris et al., 2010)(McArthur, 2004)(Brew & Letendre, 2008)(Churchill et al., 2009). Astrocytes form the majority cell type in the CNS, and nearly 20% are infected with HIV in patients with HIV-associated dementia (Churchill et al., 2009). Chronically activated astrocytes contributes to impaired catabolism of glutamate which results in excitotoxic neuronal death and astrocyte apoptosis (Churchill et al., 2009) (González-Scarano & Martín-García, 2005)(Gorry et al., 2003). The concentration of apoptotic astrocytes may be

associated with the speed of HIV dementia progression (McArthur et al., 2005). The activation and apoptosis of astrocytes is also a significant cause of blood brain barrier compromise as astrocytes are essential for the maintenance of blood brain barrier integrity (Churchill et al., 2009)(Gray et al., 2014)(Z. Wang et al., 2004).

Activated astrocytes are the major source for S100β in the inflamed CNS and is a useful marker of gliosis and brain damage (Brew & Letendre, 2008)(Donato et al., 2009) (Yardan et al., 2011)(Du Pasquier et al., 2013). S100β levels are elevated in several neurodegenerative disorders and may play a role in the pathophysiology of neurodegeneration and brain inflammatory diseases (Donato, 1999)(Donato, 2001)(Donato et al., 2009). High levels of S100β have been associated with neuronal apoptosis and the failure of astrocytes to maintain the integrity of the blood brain barrier (Du Pasquier et al., 2013).

The evidence for S100β as a biomarker of cognitive impairment in HIV is limited. CSF S100β levels may be a marker of severe HAD (Pemberton and Brew (2001)(Yuan et al., 2017). Yet, in the clinical setting, the milder cognitive disorders create a diagnostic challenge, and it is in those disorders where S100β as a biomarker of cognitive impairment would be most useful and necessary. Woods and Iudicello (2011) noted that CSF S100β reflected executive dysfunction in HIV, but they did not investigate impairment in other cognitive domains. Abassi (2017) demonstrated that elevated levels of CSF S100β were associated with decreased odds of developing MND or HAD in ART naïve individuals. Other studies of S100β in HIV explored its association with differing ART regimens, but not neurocognitive impairment (Du Pasquier et al., 2013) (Estébanez et al., 2014).

There are no longitudinal data on S100β pre- and post-ART (Brew & Letendre, 2008). Such longitudinal data may support its use as a marker of astrocyte recovery, as well as change in neurocognition. In addition, there are no published South African studies which investigated S100β and its relationship to cognition in HAND. This may be key, as the HIV epidemic in SA is predominantly Clade C, which exert different neurovirulent effects (Mishra et al., 2008).

AIMS AND OBJECTIVES

1. The primary aim of this study was to determine the association between the levels of CSF S100β and neurocognitive function, as measured by the global deficit score (GDS) of patients with HIV before the initiation of ART, and after six months on ART.
2. The secondary aim was to determine if the introduction of ART had an effect on CSF S100β levels over time.

METHOD

This study forms part of a collaborative research programme into HAND by the Division of Neuropsychiatry at the University of Cape Town and the Department of Psychology and Behavioural Neuroscience at the University of Missouri-St. Louis (Paul et al., 2014). A large database of patient information had been collected and frozen CSF samples were stored at -80°C for further investigations. This research project made use of available stored CSF samples and clinical data.

Participants of the original HAND study signed informed consent for their information and blood and CSF samples to be used for subsequent research. The larger HAND research project as well as the current project was approved by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town.

Participants for the study were recruited from 2011 until 2013 from primary health care HIV clinics in the Western Cape. Patients who were in the pre-treatment counselling phase for ART initiation were identified from the clinical folders. The identified patients were approached and screened for probable inclusion in the study. If patients met the inclusion criteria and agreed to sign informed consent, visits were scheduled at Groote Schuur Hospital. ART was initiated within three months of participation in this study.

Included participants were between 18 and 45 years of age; spoke isiXhosa as primary language; were confirmed HIV seropositive documented by ELISA and confirmed by Western Blot; were stage B or C according to the Centers for Disease Control and Prevention (CDC) criteria; and had at least five years of formal schooling. Participants were excluded from the study if they had a comorbid psychiatric or neurologic disorder that could significantly affect cognition.

At the time of enrolment in the study, a comprehensive medical and demographic history was done, and participants completed the Centers for Epidemiologic Studies Depression Scale - Revised (CES-D). Patients with major depression (a cut-off >18) were excluded from the study. Blood samples were collected from all participants and stored at -80˚C. A viral load and CD4 count were done on these samples. The neuropsychological testing was done by an experienced research technician who is fluent in isiXhosa. Tests were modified to ensure cultural relevancy. The battery included measures of learning: verbal learning was tested using the Hopkins Learning Test – Revised (HVLT – R) (Brandt & Benedict, 2001) and visual learning was assessed with the Brief Visual Memory Test – Revised (Benedict, Schretlen, Groninger, Dobraski, & Shpritz, 1996). The gemstones in the list-learning task of the HVLT-R were replaced with vegetables to ensure cultural relevancy. We assessed executive functioning/ visuospatial abilities using Colour Trails 2 (D'Elia, Satz, Uchiyama, & White, 1996), verbal fluency (fruits and animals) and the Block Design from the WAIS-IV (Wechsler, 1997). Finally, psychomotor speed was tested using Colour Trails 1 (D'Elia et al., 1996), the Grooved Pegboard Test (Klove, 1963), Trailmaking Test A (Reitan, 1955), Digit Symbol (Wechsler, 2008) and Symbol search (Wechsler, 2008). The Global Deficit Score (GDS) was calculated by converting the individual test scores (T-scores) from the

neuropsychological battery to a deficit score ranging from zero (no impairment) to five (severe impairment) (Blackstone et al., 2012). Subjects with a GDS of < 0.5 was classified as neuropsychologically impaired. A GDS cut-off of 0.5 has been demonstrated to provide the best balance between sensitivity and specificity in diagnosing neuropsychological impairment when compared to clinical ratings (Carey, Woods, Gonzalez, et al., 2004).

On completion of the first study visit, participants were called back and invited to return for a CSF sub-study. They signed separate consent to lumbar puncture (LP). Once done, CSF aliquots were stored at -80˚C. Participants were invited to return for a follow-up visit six months after ART was initiated. At this visit, the neuropsychological battery was repeated, serum was taken for viral load and CD4, and another LP was done. The ART regimen that patients were taking at that time, was documented.

Only participants in whom both a CSF sample and the GDS score from the initial assessment were available were included in this analysis. The CSF samples and recorded data of 46 HIV-positive participants were used in this study. In 20 of these, a CSF sample from the follow-up assessment was also available.

EDTA blood samples were collected at the time of enrolment in the study and plasma and cell aliquots were stored at −80°C. RNA was isolated from patient samples using the Abbott Real-time HIV-1 amplification reagent kit, according to the manufacturer's instructions. Viral load was determined using the Abbott m2000sp and the Abbott m2000rt analysers (Abbott laboratories, Abbott Park, Illinois, USA). For CD4 counts, analyses of cells from fresh blood samples were completed on the FACSCalibur flow cytometer in conjunction with the MultiSET V1.1.2 software (BD Biosciences, San Jose, CA, USA).

Baseline and follow-up CSF S100β protein concentrations were quantified with a commercially available ELISA kit (Abnova, KA0037). The analyses were performed according to the manufacturers' guidelines. The intra- and inter-assay coefficients of variation were 7% and 10%, respectively.

Statistical analyses

All analyses were conducted with SPSS version 22.0. Results were considered statistically significant when p-values were <0.05. Baseline and follow-up CSF S100β levels were normally distributed with acceptable skewness and kurtosis.

Exploratory analyses were first performed with Spearman's correlations to determine the associations between
1) baseline CSF S100β levels and cognitive domains at baseline,
2) correlation between baseline CSF S100β with baseline GDS scores and
3) association between follow-up CSF S100β with follow-up GDS scores.
A Bonferroni correction was accounted for the number of cognitive domains tested ($\alpha/n = 0.05/7 = 0.007$) in the associations between baseline CSF S100β levels and cognitive domains at baseline. Separate regression models would subsequently be used for further

analyses with covariates (age, sex, education, nadir CD4 count and viral load) in case significant associations were found. An independent sample t-test was performed to evaluate differences in baseline CSF S100β levels between HIV patients with cognitive impairment vs. without cognitive impairment according to baseline GSD measures. To determine whether baseline S100β was associated with changes in GDS at follow-up, linear regression analyses were performed with follow-up GDS scores as dependent variable and baseline S100β as predictor and baseline GDS as covariate. This was also performed separately for nadir CD4 levels and viral load.

RESULTS

Baseline measures were obtained in n=46 patients. Follow-up measures were obtained approximately six months after initiation of ART and included neuropsychological assessments and S100β levels in n=20 patients.

Table 1 provides a description of the characteristics of the total study sample.

Table 1. Participant characteristics including demographic data, GDS and CSF analysis

	Baseline (n=46)	Follow-up (n=20)
Sex Female n, (%)	44 (95.7)	19 (95.0)
Age, median (IQR)	30.00 (27.00-34.00)	
Education, years, median (IQQ)	11.00 (10.00-11.00)	
GDS, median (IQR)	0.27 (0.09-0.53)	0. 27 (0.00 – 0.17)
CD4, median (IQR)	188.00 (134.50-389.50)	-
Plasma viral load (log10) copies/ml, median (IQR)	4.02 (3.40-4.71)	-
CSF viral load (log10) copies/ml, median (IQR)	1.92 (1.60-3.14)	-
CSF protein (g/l), median (IQR)	0.27 (0.22-0.37)	-
CSF S100β (pg/ml), median (IQR)	146.95 (95.93-199.62)	117.05 (87.26-182.18)

IQR: interquartile range; GDS: Global Deficit Score; CSF: cerebrospinal fluid

The GDS ranged from 0 to 1.6842. In 30.43% (n=14) study participants the GDS was ≥ 0.5 at baseline. CSF S100β levels ranged from 45.7 pg/ml to 233.84 pg/ml at baseline and from 54.14 pg/ml to 194.77 pg/ml at follow-up.

Associations between S100β levels and cognitive measures, and study characteristics

No significant associations were found between baseline CSF S100β levels and GDS at baseline (r= -0.070; p= 0.66) or at follow-up (r= 0.16, p= 0.52). Baseline CSF S100β levels

were also not significantly associated with plasma viral load (r=0.247, p= 0.10) or CD4 counts (r=0.020, p= 0.90).

Table 2. Correlations between CSF S100β levels at baseline and neurocognitive measures, and study characteristics.

Cognitive domain	Tests	Mean (z-scores)	R	P
Verbal	Animal fluency	-0.40 ± 0.92	0.22	0.17
Processing speed	*WAIS-III Digit symbol*	-0.23 ± 0.75	-0.06	0.71
	WAIS-III Symbol search			
	Colour trails I			
	Stroop Colour			
Learning	*HVLT Learning*	-0.11 ± 0.82	0.11	0.48
	BVMT Learning			
Memory Recall	*HVLT Delayed recall*	-0.80 ± 0.54	-0.09	0.58
	BVMT Delayed recall			
Motor Functioning	*Groove peg board Dominant*	0.03 ± 0.59	0.10	0.52
	Groove peg board Non-dominant			
Executive function	*Colour trails II*	-0.20 ± 0.81	0.16	0.32
GDS		0.35 ± 0.34	-0.07	0.66

HVLT: Hopkins Verbal Learning Test; BVMT: Brief Visual Memory Test; GDS: Global Deficit Score

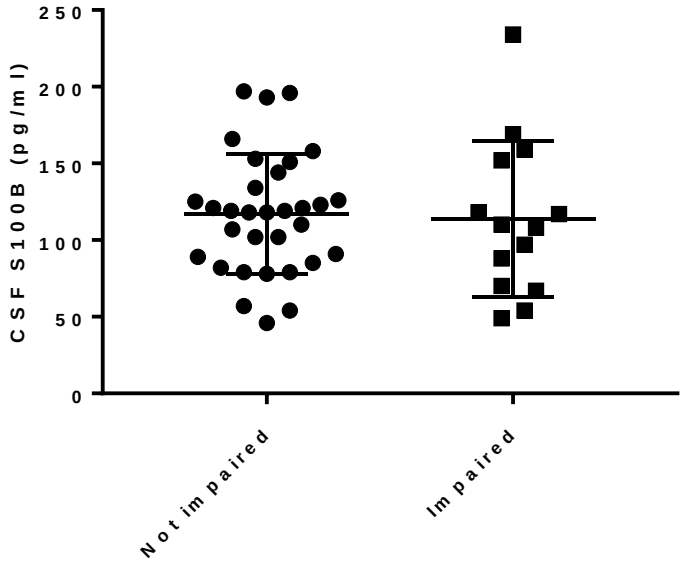

Figure 1. Baseline CSF S100β concentrations in HIV patients without cognitive impairment (n=32) vs. HIV patients with cognitive impairment (n=14). An independent sample t-test showed that there were no significant differences in CSF S100β levels between patients without cognitive impairment vs. cognitive impairment (t=0.102, p=0.919). Not impaired: GDS < 0.5, impaired: GSD ≥ 0.5

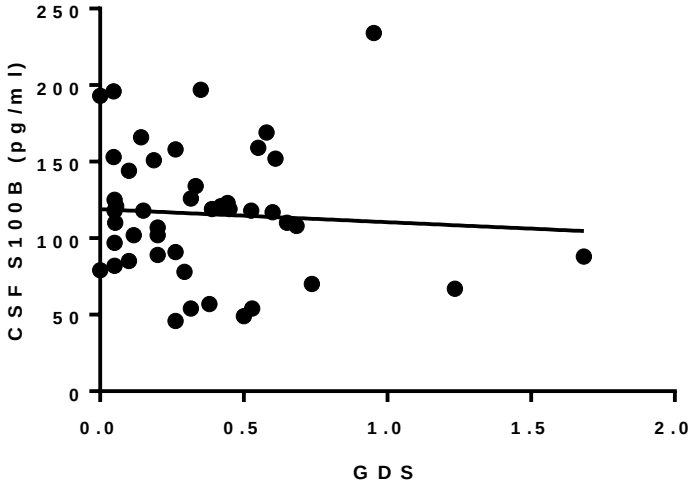

Figure 2. Correlation between baseline CSF S100β with baseline GDS scores, r=-0.07, p=0.66.

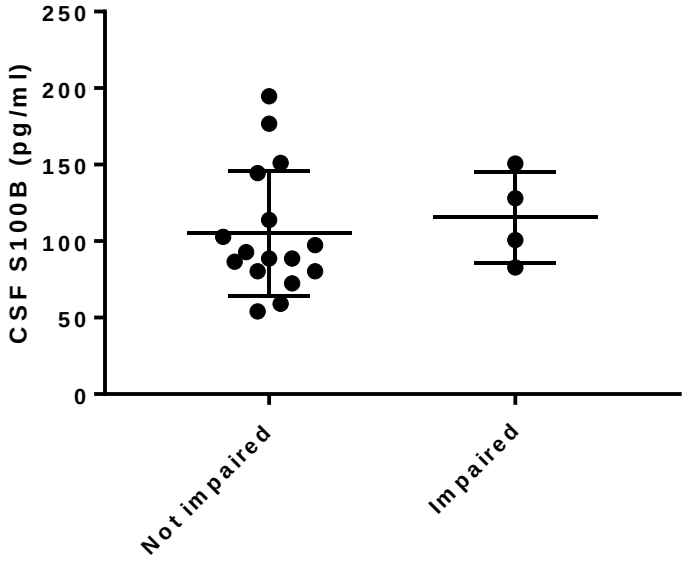

Figure 3. Illustration of follow-up CSF S100β concentrations in HIV patients without cognitive impairment (n=16) vs. HIV patients with cognitive impairment (n=4). Statistical analyses was not performed due to unequal group sizes. Not impaired: GDS < 0.5, impaired: GSD ≥ 0.5.

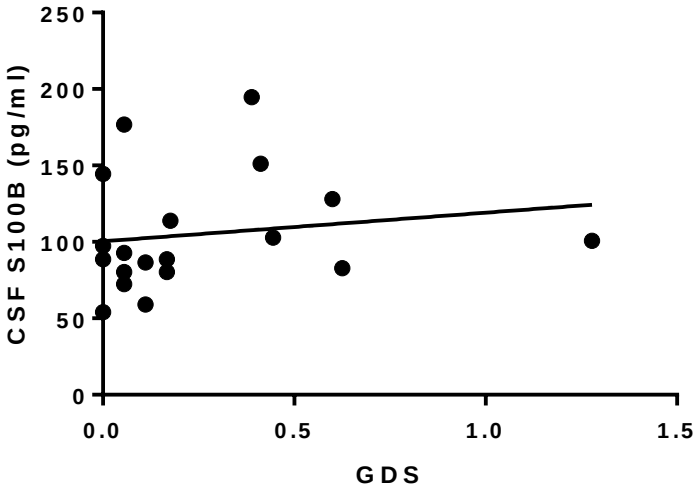

Figure 4: Correlation between follow-up CSF S100β with follow-up GDS scores, r=0.16, p=0.52. Baseline GDS score was significantly correlated with the follow-up GDS score (r=0.81, p<0.001). The follow-up GDS scores declined (mean 0.18 ± 0.26) over this time period. In addition, baseline and follow-up CSF S100β levels were significantly correlated (r=0.73, p<0.001).

Baseline S100β as predictor of changes in Global Deficit Score (GDS)

Because the GDS scores declined over time, we further investigated if baseline CSF S100β levels predicted the change in GDS scores (T2-T1). Baseline CSF S100β did not significantly predict change in GDS, B(SE) = 0.001, (0.001), β= 0.0.025, p= 0.85. In addition, viral load B(SE) = -0.001 (.001), β =-0.21, p=0.133 and nadir CD4 B(SE) = 0.001 (0.001), β =0.24, p=0.111 also did not predict the change in GSD scores over time.

DISCUSSION

In this first longitudinal study of S100β in a pre- and post-ART cohort, we did not observe an association between the marker and cognitive outcomes. We did note that cognition and S100β were significantly correlated at the pre- and post-assessments, confirming reliability of our measurements. Nearly one third of participants (30.43%) met GDS criteria for cognitive impairment using a cut-off of 0.5 (Blackstone et al., 2012). We noted that S100β levels were low at baseline and at follow-up, however these levels were independent of cognition. Our sample was predominantly woman (93.8%), entering care with low CD4 counts. Our findings suggest that S100β may not be useful on its own in monitoring cognition in patients with HIV before ART of after 6 months of ART.

Similar to previous studies (Estébanez et al., 2014)(Woods et al., 2010) we found no correlation between the CSF S100β level and cognitive impairment as measured by GDS at

baseline or at follow-up. This study does not support the findings of Pemberton et al. (2001) or Yuan et al. (2017) that demonstrated that CSF S100β was associated with cognitive impairment in HIV. However, the patients in those studies had more severe cognitive impairment (Pemberton & Brew, 2001), were older (Yuan et al., 2017) and had a lower CD4 (Yuan et al., 2017) than the patients in our study. This study also does not support the findings of Abassi et al.(2017) who demonstrated that elevated CSF S100β were associated with decreased odds of MND or HAD in patients with HIV in Uganda. However, this study did not investigate the response of S100β on ART and looked at patients with Clade D HIV which may have different cognitive outcomes (Paul et al., 2014).

HIV disrupts frontal-subcortical circuits which results in impairment in attention, memory, executive function and psychomotor speed (Carey, Woods, Rippeth, et al., 2004)(Carey, Woods, Gonzalez, et al., 2004). The subcortical deficits are more prominent in untreated disease. The use of a global measure of global cognitive impairment such as the GDS may have been unable to detect isolated deficits in specific cognitive domains (Carey, Woods, Gonzalez, et al., 2004). However, we found no association between S100β levels and the individual domains of executive function, learning and psychomotor speed. This stands in contrast to the study by Woods et al. (2011) in which CSF S100β was found to be associated with impairments in executive dysfunction as measured by action (verb) fluency, a test which was not administered in this study.

Our results show that baseline and follow-up CSF S100β were significantly correlated, which confirms the reliability of the CSF S100β measurements at baseline and at follow-up. It also demonstrates that CSF S100β levels were not affected by the use of ART, a finding which corresponds with other studies (Estébanez et al., 2014)(Pemberton & Brew, 2001).

The mean CSF s100B level at baseline in the current study was 115,61pg/ml. This is much lower than in the Estebanez (2014) study where the mean S100B level of patients receiving triple therapy was 246,8pg/ml and in patients receiving monotherapy 252,4pg/ml. It is also much lower than in the MOST trial where S100β levels were 677pg/ml in the group receiving monotherapy and 313pg/ml in the group receiving triple therapy (Du Pasquier et al., 2013). However, patients in those studies were older, had been on ART for many years and some had a history of neurological diseases. Our cohort was specifically newly treated and included mainly women with lower CD4 cell counts.

There was no association between CSF S100β levels and plasma viral load which confirms that S100β is not a good biomarker of virological control in HIV (Pemberton & Brew, 2001)(Estébanez et al., 2014). There was also no association between S100β levels and plasma CD4 or CSF protein levels. The fact that there was no association between S100β and CD4 is an interesting finding as one would expect a lower nadir CD4 to indicate neuronal or glial injury and therefore elevated S100β levels in the CSF. The finding that there was no association between S100β levels and age is not surprising as the sample was young (mean age 30.68 years) and excluded individuals older than forty-five.

CSF S100β was not a predictor of a change in cognition (as measured by the GDS). The assumption was that patients with high levels of S100β would have astrocyte damage with resultant cognitive impairment which would not respond to ART. The hypothesis in this study was therefore that patients with high levels of S100β would have more cognitive impairment after at least six months of ART. However, this hypothesis was refuted and contradicted the findings of Pemberton and Brew (2001) who demonstrated that S100β levels were higher in individuals who progressed rapidly to death. However, in the Pemberton study, patients were not initiated on ART and most of the subjects in the study had severe HAND. In a 2011 review, Yardan et al (2011) argued that S100β has potential in predicting the efficiency of treatment and prognosis in patients with neurological disorders. We did not find this to be the case in a South African HAND population.

The mean GDS prior to initiation of ART was low. This is clinically relevant as milder forms of cognitive impairment are most prevalent (Joska et al., 2011), the most difficult to diagnose, and the disorder where a biomarker would be the most useful to assist with the diagnosis. We noted, as expected, that significant improvements occurred over time in individuals on ART. As we did not specifically control for practice effects, we cannot conclusively say whether these effects are due to ART only.

The majority of the study population was female which could be explained by the fact that the incidence and prevalence of HIV is much higher among females than males in South Africa (Statistics South Africa, 2015)(Vandormael, Akullian, Dobra, De Oliveira, & Tanser, 2018). However, the ratio of female to male participants in this study is much higher than the national ratio (Statistics South Africa, 2015). The mean age of participants in this study was much lower than in similar studies looking at S100β in the CSF (Estébanez et al., 2014)(Woods et al., 2010). However, the younger age of participants in this study reflects the South African demographic, as HIV is more prevalent among younger individuals in South Africa (Statistics South Africa, 2015) and the mean age of patients who were initiated on ART in primary health care clinics in South Africa, was 29,5 years (Joska et al., 2011). Additionally, only patients between the ages of eighteen and forty-five were included in order to avoid age-related central nervous system abnormalities (Paul et al., 2014).

The low nadir CD4 of study participants could have resulted in chronic neuronal injury which could explain the mild cognitive impairment that was present in many of the study participants. The current guidelines of the National Department of Health in South Africa state that HIV positive patients should be initiated on ART irrespective of their CD4 count (Meintjes et al., 2017). However, at the time that the study was done, South African guidelines recommended that ART should be initiated in HIV positive patients with a CD4 count of less than 350 cells/μl (Cross, Combrinck, & Joska, 2013). This could account for the low CD4 of participants in this study. Another explanation could be that patients often get tested late in the disease and enter care when their CD4 counts are low.

LIMITATIONS OF STUDY

A significant limitation of the study is the small sample size. This study involved the performance of additional tests on CSF samples that were collected in another study. The evidence collected in this study was therefore limited by the information collected and the availability of CSF samples from the original study. The CSF samples of 46 participants were available at baseline, and the CSF samples of 20 participants were available both at baseline and after six months of ART. Yet, to our knowledge it is still the largest longitudinal study of its kind. Other studies tested the CSF S100β levels of a larger number of participants at baseline, but did not repeat these investigations at a later stage or subsequent to the initiation of ART (Woods et al., 2010)(Pemberton & Brew, 2001)(Estébanez et al., 2014)(Abassi et al., 2017)(Yuan et al., 2017).

Certain factors which may affect the association between neurological functioning and S100β, such as other chronic somatic diseases and psychological trauma, were not controlled for in this study.

Another limitation was the fact that the mean GDS of the group was low, and that the majority of patients had mild cognitive impairment. This could be a reason why no statistically significant association was detected between the degree of cognitive impairment and the level of S100β in the CSF. However, the low mean GDS of the sample is clinically beneficial, as most patients have milder degrees of HAND (Joska et al., 2011) and mild cognitive impairment is much more difficult to diagnose.

The large number of female subjects is another limitation, as our sample was not representative of the South African population with HIV.

Due to these limitations, future studies are necessary to determine the effect of CSF S100β on ART in patients with HAND. Such studies should be larger, be more representative of the South African population and investigate the response of CSF S100β over a period of more than six months.

CONCLUSIONS

In conclusion, CSF S100β is not a marker of cognitive impairment in younger mainly female Xhosa-speaking patients with HIV infection in a South African primary health setting. In this group of patients, there was no association between CSF S100β levels and impairment of cognitive domains that are usually affected by HIV. CSF S100β was also not a good predictor of the effect of ART on patients' cognitive functioning. However, the sustained levels of CSF S100β over time indicate that neuroinflammatory processes remain regardless of ART.

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