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Blood and Lumbar Fluid Biomarker Changes in Patients with HIV-Associated Neurocognitive Impairment Treated with Lithium: Analysis from a Randomised Placebo-Controlled Trial

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Contents

Declaration

I, Lindokuhle Thela (Student number THLLIN001), hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgments 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.

Signature: Signed by candidate

Date: 22 September 2022

My role in this project

My role in this project was to conduct a secondary analysis of data that was collected prior and post hoc during a 24-week randomized placebo clinic trial assessing lithium adjuvant therapy in people living with HIV along with a diagnosis of moderate to severe HIV-associated neurocognitive disorder. The study aimed to evaluate the safety of lithium in people living with HIV and the effects of lithium on neurocognition. The study found that lithium therapy is safe for use in people living with HIV. However, the authors could not demonstrate any positive effects/benefits the drug (lithium) had on cognition during the trial.

In this secondary analysis, I evaluate whether lithium conferred neuroprotection by analysing individual biomarker concentration and their changes before and after treatment. Permission to use the data was obtained from the parent study investigators (see acknowledgments and contributions below).

Abstract

HIV-associated neurocognitive disorders (HAND) persist in the era of antiretroviral therapy (ART). Thus, ART does not completely halt or reverse the pathological processes behind HAND. Adjuvant mitigating treatments are therefore prudent. Lithium treatment is known to promote neuronal brain-derived neurotrophic factors (BDNF). Lithium is also an inhibitor of glycogen synthase kinase-3 beta (GSK-3-β). We analyzed biomarkers obtained from participants in a randomized placebo-controlled trial of lithium in ART-treated individuals with moderate or severe HAND. We assayed markers at baseline and 24 weeks across several pathways hypothesized to be affected by HIV, inflammation, or degeneration. Investigated biomarkers included dopamine, BDNF, neurofilament light chain, and CD8+ lymphocyte activation (CD38+ HLADR+). Alzheimer's Disease (AD) biomarkers included soluble amyloid precursor protein alpha and beta (sAPPα/β), Aβ38, 40, 42, and ten other biomarkers validated as predictors of mild cognitive impairment and progression in previous studies. These include apolipoprotein C3, pre-albumin, α1-acid glycoprotein, α1-antitrypsin, PEDF, CC4, ICAM-1, RANTES, clusterin, and cystatin c. We recruited 61 participants (placebo = 31; lithium = 30). The age baseline mean was 40 (±8.35) years and the median CD4+ T-cell count was 498 (IQR: 389 – 651) cells/μL. Biomarker concentrations between groups did not differ at baseline. However, both groups' blood dopamine levels decreased significantly after 24 weeks (adj. p<002). No other marker was significantly different between groups, and we concluded that lithium did not confer neuroprotection following 24 weeks of treatment. However, the study was limited in duration and sample size.

Keywords: HIV. HAND. Biomarkers. Lithium

Acknowledgments and contributions

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List of abbreviations

Aβ	Amyloid-beta
AD	Alzheimer's disease
ANI	Asymptomatic neurocognitive impairment
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BDNF	Brain-derived neurotrophic factor
BBB	Blood-brain barrier
cART	Combination antiretroviral therapy
CAT-Rapid	Cognitive assessment tool-rapid version
CCR5	C-C Chemokine receptor type 5
CPE	CNS penetration effectiveness
CSF	Cerebrospinal fluid
CNS	Central nervous system
DAT	Dopamine transporter

Gp120	Glycoprotein 120
GSK-3-β	Glycogen synthase Kinase-3 beta
HAD	HIV associated dementia
HAND	HIV- associated neurocognitive disorder
HIV	Human immunosuppressive virus
HLA	Human leukocyte antigen
IHDS	International HIV dementia Scale
LP	Lumbar puncture
MCI	Mild cognitive impairment
MND	Mild neurocognitive disorder
MoCA	Montreal cognitive assessment
NfL	Neurofilament light chains
PLWH	People living with HIV
sAPPα	Soluble amyloid precursor protein alpha
sAPP β	Soluble amyloid precursor protein beta
Tat	Transactivator of transcription

Chapter 1. Introduction and literature review

Epidemiology of HIV-Associated Neurocognitive Disorders in the Antiretroviral Era

Despite the widespread use of antiretroviral therapy (ART), HIV-associated neurocognitive disorders (HAND) remain a significant HIV neurological complication (Wang, Y. et al., 2020). There is a 43.9% prevalence of HAND in the era of ART, according to a recent meta-analysis (Wei et al., 2020). In the era of ART, studies have found that most patients with HAND fall in the categories of asymptomatic neurocognitive impairment (ANI) and mild cognitive dysfunction (MND).

While ART has improved the longevity of people living with HIV (PLWH) (Katz & Maughan-Brown, 2017), the rates of HAND have remained significantly high (Wei et al., 2020). HIV-related neurocognitive impairment and advancing age are associated with an increased mortality rate in the ART era (Naveed et al., 2021). Aging and HIV are both associated with chronic inflammation, which has been linked to various diseases, including metabolic disorders and cardiovascular disorders (Nasi et al., 2017). HIV-related inflammation has also been linked to premature and accelerated aging of the central nervous system (Nasi et al., 2017). Thus, it is prudent to explore adjunct treatment that would assist in preventing neuronal injury in PLWH.

Clinical Features of HIV-Associated Neurocognitive Disorders

Although HAND was originally coined for research purposes to describe the spectrum of neurocognitive impairment caused by HIV (Antinori et al., 2007), it is now commonly used interchangeably with neurocognitive disorders due to HIV in the clinical setting. HAND consists of three categories: asymptomatic cognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) (Antinori et al., 2007). An ANI diagnosis is based on a neuropsychological assessment that shows at least two cognitive domain impairments that are at least one standard deviation (SD) below the mean norms on neuropsychological testing. This performance does not interfere with everyday functioning. MND is like ANI, except the impairment must have a mild impact on daily life. HAD is diagnosed when there are deficits in at least two cognitive domains and a 2 SD below the mean norms and interference with day-to-day functioning.

ANI is not clinically significant but is associated with the earlier onset of more severe forms of cognitive impairment compared to PLWH with normal cognitive performance. A longitudinal study from the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) explored the significance of ANI by doing 6 monthly neurocognitive assessments for 45.2 months (Grant et al., 2014). ANI was associated with a shorter time of progression to symptomatic HAND when compared to neurocognitively normal participants. A 2-fold to 6-fold increase in symptomatic neurocognitive disorders was also associated with ANI (Grant et al., 2014).

Before the advent of ART, HAND was typically characterized by the presence of subcortical neurocognitive deficits (Brew & Chan, 2014). The major symptoms are executive, motor dysfunction, and behavioural symptoms such as apathy and irritability (Brew & Chan, 2014). People with disease progression may also develop cortical symptoms (Brew & Chan, 2014). ART use has resulted in the less severe forms of HAND.

A Brief Review on Diagnosing HIV-Associated Neurocognitive Disorders

The diagnosis of HAND is made by combining screening tools, psychometric tests, blood, and neuroimaging investigations. HAND can be detected using several screening tools such as the International HIV dementia scale (IHDS),

the Montreal cognitive assessment (MoCA), the Simioni symptom questionnaire, and the Cognitive Assessment Tool-rapid version (CAT-rapid) (Joska et al., 2016). IHDS is frequently used in South Africa despite a low sensitivity of 45% and specificity of 79% when the cut-off score of less than 10 is used. In this population, it has been shown that a cut-off score of less than 11 may be more useful as it shows a sensitivity of 53% and specificity of 80% (Joska et al., 2011). Another study conducted by Joska et al. (2016), reported that the combination of IHDS and CAT-rapid appears to be better regarding sensitivity (89%) and specificity (82%) when screening for HIV-associated dementia when the cut-off score is less than 16. This combination also showed better results for milder forms of HAND when compared to other screening tools (Joska et al., 2016).

To confirm the diagnosis of HAND, it is necessary to conduct investigations that will confirm HIV as the cause of neurocognitive fallout and exclude potential comorbid disease processes. Plasma HIV viral RNA load and CD4+ T lymphocyte count are the primary markers that can suggest the possibility of HIV-related brain injury (Edén et al., 2016). The reliability of this biomarker declines when ART-treated patients develop cognitive impairment while immunocompetent and suppressed (Edén et al., 2016). It is also important to exclude other potential causes of cognitive impairment that may be present in PLWH. Hepatitis C virus (HCV) (Asnis & Migdal, 2005), syphilis infection, vitamin deficiencies, and other metabolic disorders (Alford & Vera, 2018) are some of the independent causes of neurocognitive impairments that should be investigated. Furthermore, HCV and syphilis infection occur commonly in PLWH, thus when co-existing with HIV may work in synergy towards causing neurocognitive fallout. In addition, it is important to screen for common mental disorders such as depression, anxiety, and substance abuse which occur at high rates in PLWH and could be the potentially reversible cause of neurocognitive fallouts (Nakku, Kinyanda & Hoskins, 2013).

Neuroimaging is also an integral part of assessing HAND. Structural neuroimaging investigations such as computerized tomography (CT) and magnetic resonance imaging (MRI) are sensitive to identifying structural changes in the brain caused by chronic HIV-related degeneration, which may manifest as a loss of brain volume (Ances & Hammoud, 2014). MRI is also helpful for detecting white matter damage (Senocak et al., 2010). With the use of ART, the typical neuropathology found in HAND people is uncommon (Gelman, 2015). Cognitive impairment in this patient is often the result of persistent neuroinflammation, impaired neuron metabolism, and impaired neuron connection (Irollo et al., 2021). Other specialized imaging modalities such as diffusion tensor imaging (DTI) and functional MRI (fMRI) may be useful in detecting abnormalities that are otherwise not visible in structural imaging (Ances & Hammoud, 2014). Table A provides a summary on how to make a diagnosis of HAND.

Table A Approach to diagnosing HAND

Assessment	Specific variables	Rational
Demographic details	Age, level of education, occupation	Age - Older age is associated with AD and vascular dementia Level of education - associated with a low cognitive reserve which increases the risk of neurocognitive fallout. Occupational history: evaluate the level of functional decline because of HAND (Nightingale et al. 2014)
Medical history	Metabolic disorders such as diabetes mellitus and hypertension. Hepatitis C virus (HCV).	Metabolic disorders: linked with small vessel disease which may compound the HIV associated neuronal injury HCV: independently causes a neurocognitive disorder that is like HAND clinically. HIV/HCV confection linked with severe neurocognitive (Alford & Vera. 2018; Asnis & Migdal. 2005)
Psychiatric assessment	Depression	Depression in PLWH has a bidirectional link with HAND. HAND may increase the risk of HAND while patients with HAND may develop depression. Treatment of depression has been linked with improvement of neurocognitive performance. with increased risk of HAND (Nakku, Kinyanda & Hoskins. 2013)
Physical and neurological examination	Motor signs	HAND may lead to extrapyramidal tract signs such as parkinsonism due to the disruption in the basal ganglia (Brew & Chan. 2014).
IHDS, MoCA, CAT-rapid	Dysexecutive cognitive fallout, psychomotor speed slowing, attention, and concentration impairment.	HAND often manifests with subcortical neurocognitive domains impairments which are best screened by the combination of these bedside tools. (Joska et al. 2016)
Blood HIV markers	CD4+ count, viral load	Nadir CD4 count- linked with the development of HAND Persistent Viral load replication - linked with possible continued neuronal injury due to neuroinflammation. (Alford & Vera. 2018)
CSF analysis	CSF viral load	Persisting viral replication is present in some PLWH that are virally suppressed in the plasma. This has been linked with the development or progression of HAND (Edén et al. 2016)
Imaging (CT scan/MRI)	Volume changes and white matter disease.	HAND has been linked with global volume loss. White matter disease is also present in some patients with HAND (Ances & Hammoud., 2014)

Abbreviations: CAT-rapid (Cognitive assessment tool-rapid version), CSF (cerebrospinal fluid), CNS (central nervous system), CT (computerised tomography), HAND (HIV-associated neurocognitive disorders), IHDS (international HIV dementia scale), MoCA (Montreal cognitive assessment), MRI (magnetic resonance imaging).

Pathophysiology of HIV-Associated Neurocognitive Disorders in the Antiretroviral Therapy Era

The pathophysiology of HAND in the era of ART is thought to result from subtle physiological changes in the CNS caused by inflammation and other HIV-related factors that lead to neuronal dysfunction (Irollo et al., 2021). Nonetheless, there is overwhelming evidence suggesting that the cognitive fallout can be attributed to the significant irreversible neuronal injury which occurs before the initiation of ART, known as the "legacy effect" (Nightingale et al., 2014). It has also been demonstrated that despite viral suppression some patients will continue to have HIV-related neuronal injury which is linked to chronic low-grade immune activation (Spudich, 2016). Furthermore, evidence of CNS neuroinflammation in patients on ART is associated with worsened neurocognitive performance (Spudich et al., 2019). The aging process is associated with a state of chronic innate immune activation which can compound the state of immune activation in the aging population living with HIV (Shaw, Goldstein & Montgomery, 2013). Mutevedzi et al. (2013) found that older (+50 years) patients living with HIV had elevated inflammatory markers when compared to the negative age-matched group (Mutevedzi et al., 2013). It is unknown whether HAND in the context of the "legacy effect" and/or chronic immune activation will yield different pathological mechanisms. In any event, the longitudinal course in most patients is stable. In a 5-year longitudinal study (Multicenter AIDS Cohort Study) of virally suppressed patients with

ANI, the course of neurocognitive impairment was described as static in most participants (Sacktor et al., 2016). These findings are like those of a preceding study (CNS HIV Antiviral Therapy Effects Research), which showed that 22% of the participants had worsened neurocognition over 42 months (Heaton et al., 2015).

Fluid Biomarkers in HIV-Associated Neurocognitive Disorder

Biomarkers are defined by the National Institutes of Health as "characteristics that can be measured objectively and evaluated as indicators of a normal biological process, pathogenic process, or therapeutic response" (Atkinson et al., 2001). The evolving clinical phenotype and neuropathology of HAND in the era of ART highlights the need for composite, reliable, sensitive, and specific biomarkers. Since no single biomarker has been identified for HAND in the era of ART, it seems appropriate to combine a range of biomarkers (McLaurin, Booze & Mactutus, 2019). These include HIV factors, immune factors, and neurodegenerative proteins. Figure A illustrates what some of these proteins may indicate in patients with HAND.

Blood Biomarkers In HIV-Associated Neurocognitive Disorders

Blood testing is convenient, and several blood biomarkers can abnormalities that are associated with HIV neuronal neurocognitive impairment. A classic example is the lowest ever $CD4^+$ T lymphocyte count also called nadir CD4 cell count which is a well-established predictor of HAND (Ellis et al., 2011). Plasma viral load has also been shown to correlate with HAND, as a high viral load suggests neuronal injury (Marcotte et al., 2003). High plasma viral load is also a predictor of neurocognitive impairment in PLWH (Marcotte et al., 2003). In the era of HAND, these (current CD4+ T lymphocyte count and viral load) markers have proven less sensitive due to the observation that cognitive deficits persist among PLWH on ART and are virally suppressed. Thus, markers that indicate brain health have been investigated to determine (Bandera et al., 2019). In addition to predicting neuronal injury, these markers can also predict cognitive deficits (worsening and improving) (Bandera et al., 2019). Brain-derived neurotrophic factor (BDNF) is a protein that promotes neuron survival, differentiation, and plasticity (Bathina & Das, 2015). Low plasma BDNF is associated with poor cognitive performance (Levada et al., 2016). HIV depletes BDNF levels by preventing the conversion of passive proBDNF into mature BDNF (Bachis et al., 2012). In a study conducted by Bachis et al. (2012), the addition of gp120 protein prevented proBDNF conversion in rat neurons (Bachis et al., 2012). In the context of HIV neuro infection, dopamine modulates the immune system. Exposure to dopamine irrespective of the amount of virus is linked with a dopamine dose-dependent entry of HIV into the macrophages (Gaskill et al., 2014). Plasma NfL levels in PLWH can be used as reliable biomarkers of HIV-related CNS injury (Gisslén et al., 2016). Furthermore, blood NfL concentrations have been shown to correlate negatively with neurocognitive performance in PLWH (Anderson et al., 2018). Elevated levels are associated with poor cognitive. Plasma markers of immune activity have also been shown to be sensitive markers of neuropathology in PLWH who are on ART. For example, the pathogenesis, progression, and severity of HAND have been linked to HIV-related immune activation. This includes an increased expression of activation of human leukocyte antigen (HLA) DR and CD38+ on CD8+ T-lymphocytes (HLA-DR+CD38+CD8) in PLWH (Liu et al., 1997). The HLA-DR+CD38+CD8 is increased in patients with HAND and correlates with the severity of HAND (Robertson et al., 2020; Ratto-Kim et al., 2018).

Cerebrospinal Fluid Biomarkers In HIV-Associated Neurocognitive Disorder

CSF is the closest tissue to the brain and is the extracellular matrix of the CNS (Weller, 1998). CSF may offer superior insight into HIV-related neuropathology (Weller, 1998). Several proteins have been investigated as potential biomarkers

for HAND, such as markers of neuronal health, HIV CNS compartmentalization, inflammation, and even markers of neurodegenerative processes (Bandera et al., 2019). BDNF concentrations can be used to determine the state of neuronal health, whereas NfL concentrations can determine the state of neuronal injury. CSF BDNF is reduced in patients with PLWH (Bachis et al., 2012). In adults BDNF in the CSF has been shown to correlate with cognitive performance (Li et al., 2009). CSF NfL has also been shown to be sensitive to neuronal injury in PLWH (Abdulle et al., 2007). The CNS system is disrupted during HIV neuro infection. In the initial stages of CNS infection viral proteins like Tat disrupt the dopamine transport (DAT) system resulting in prolonged postsynaptic dopamine neuroexcitation which leads to synaptic damage (Nath et al., 2000). This makes the brain regions that express high dopamine receptors (e.g., the basal ganglia) the primary areas of neuronal injury in HIV. Low levels of CSF dopamine are linked with poor neurocognitive performance in PLWH. Horn et al. (2013) found that people living with HIV appear to express more DAT (10/10) compared to uninfected people. This genotype has been linked with elevated dopamine availability which can exacerbate HIV neuro-infection (Horn et al., 2013). Disruption of CNS dopamine neurotransmission has been implicated in both neuronal injury and poor overall neurocognitive functioning (Nath et al., 2000). In the initial stages of CNS infection viral proteins like Tat disrupt the dopamine transport system resulting in prolonged postsynaptic dopamine neuroexcitation which leads to synaptic damage (Nath et al., 2000). This makes the brain regions that express high dopamine receptors (e.g., the basal ganglia) the primary areas of neuronal injury in HIV.

Amyloid beta-protein pathology is one of the two neuropathological hallmarks of Alzheimer's dementia (AD) (Fan et al. 2019). It is hypothesized that a chronic state of neuroinflammation, microglial activation, and disruption of the blood-brain barrier (BBB) are some of the triggers of amyloid-beta plaques synthesis (Noe et al., 2020). This has resulted in enormous interest in investigating the role of amyloid pathology in HAND since HIV infection also results in the disruption of the BBB and chronic neuroinflammation. Furthermore in vitro studies have found that HIV viral protein Tat has a high affinity for the exterior surface of the amyloid-beta fibrils (Hategan et al., 2017). The interaction of Tat with the external surface promotes amyloid protein synthesis (Hategan et al., 2017). Early studies have also shown that patients infected with HIV have a higher incidence of amyloid-beta deposits in the brain (Green et al., 2005; Esiri, Biddolph & Morris, 1998). Similarly, aging has been identified as a risk factor for amyloid-beta plaques in PLWH. Gisslén et al (2009) found a parallel reduction in the CSF concentration of soluble amyloid precursor protein alpha and beta in patients with AIDS-associated dementia complex (also known as HAD) which reflects amyloid-beta synthesis in the CNS (Gisslén et al. 2009). An increase in the synthesis of amyloid plaques correlates with a reduced concentration of amyloid-beta 42 (Aβ42). Literature reporting on the pattern of Aβ42 clearance in PLWH has conflicting results with some studies demonstrating no change while others report a decrease or an increased clearance (Ortega & Ances, 2014). Nonetheless, amyloid metabolism may be an important biomarker in establishing neuronal health in PLWH the aging population.

Blood Proteomic Biomarkers Which Predict the Conversion of Mild Cognitive Impairment Converting to Alzheimer's Dementia Blood

Hye et al (2014) investigated blood protein correlation with the AD severity and cognitive decline in a cohort of 1148 participants from three multicentre studies by conducting a multiplex analysis. They found that 10 blood proteins were strongly associated with mild cognitive impairment (MCI) progressing to AD with 87% accuracy, 85% sensitivity, and 88% specificity (Hye et al., 2014). The validated plasma proteins to predict AD severity and progression have not been studied in HAND patients. The biomarker panel consisted of the following proteins: Cystatin C, clusterin, A1AT, ICAM, PEDF, RANTES, A1GP, CC4, ApoC,3 and prealbumin (Hye et al., 2014).

Figure A.

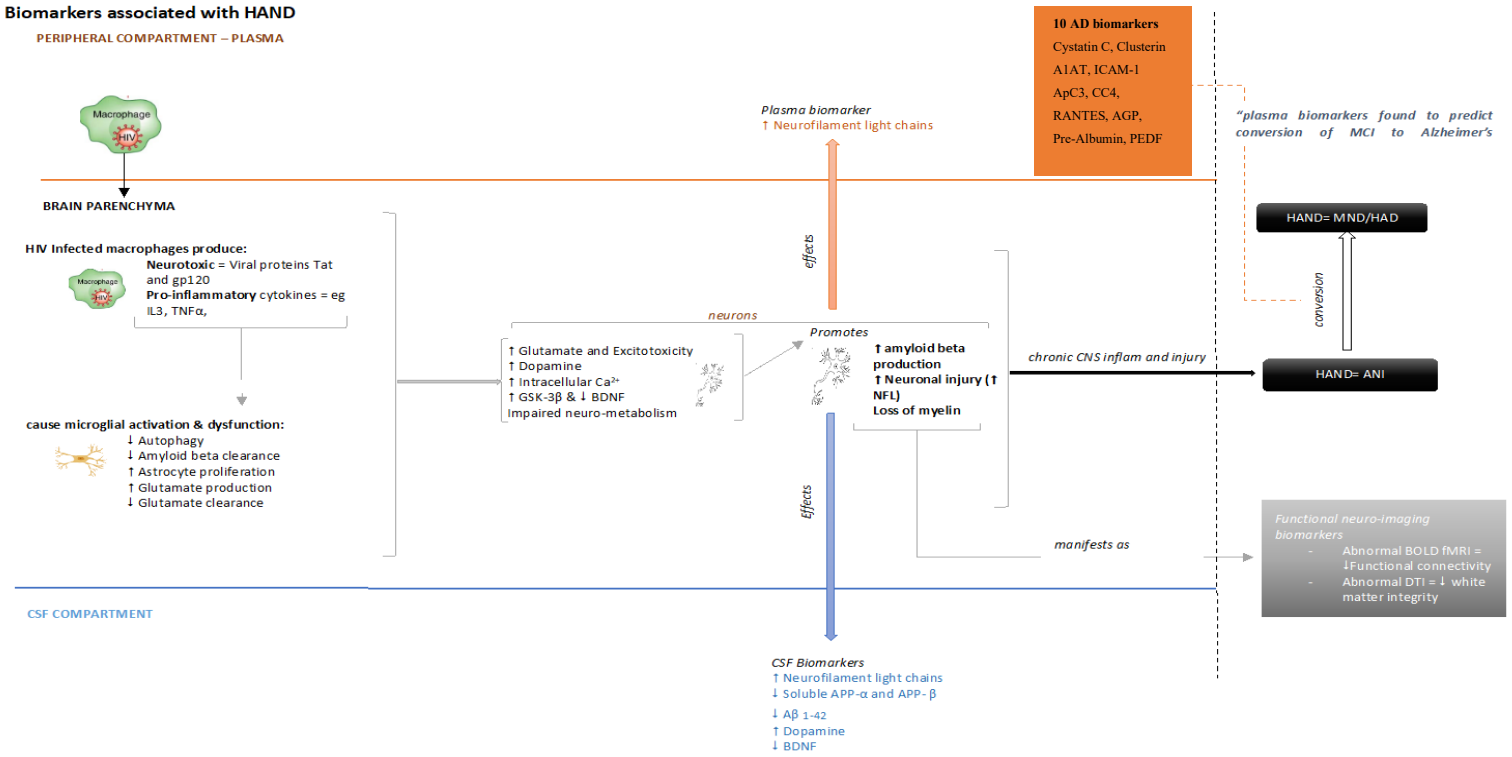

Figure A is a schematic illustration of HIV related neuropathology and potential biomarkers for HIV -associated neurocognitive disorders. The blue solid arrow shows biomarkers that have shown some reliability in the cerebrospinal fluid (CSF). Orange solid arrow – shows one of the biomarkers that has robust evidence in the blood with regards to the presence of neuronal injury in people living with HIV. Dotted orange line demonstrates a list of 10 Alzheimer's disease biomarkers that have been shown highly sensitive and specific in predicting the progression of AD related MCI to dementia.

A Brief Review of Pharmacological Strategies for Treating HIV-Associated Neurocognitive Disorder

HAND is best treated by preventing it from occurring, which can be achieved by early initiation of the ART (Ellis et al., 2011). This is further confirmed by the fact that even in the era of ART a prior or nadir $CD4^+$ T lymphocyte count below 200 is highly predictive of HAND (Ellis et al., 2011). In the prevention and treatment of HAND, the ability of ART to abort viral replication is vital (Ellis et al., 2011). Early initiation of treatment is the best way of preventing HAND, regardless of ART-related factors like penetration of the CNS. The high CNS penetration effectiveness (CPE) score was also shown not to be associated with better neurocognitive outcomes in PLWH (Caniglia et al., 2014). This could in part be explained by a functioning BBB in the early phase of HIV infection which may facilitate better penetration of CNS by ART (Atluri et al., 2015). However, CNS viral replication can persist despite evidence of viral suppression in the plasma raising a hypothesis that a subgroup of PLWH may benefit from ART regimens that target the CNS virus (Omeragic et al., 2020). The addition of a medication that blocks the block CCR5 such as maraviroc is effective in improving global neurocognitive functioning in virally suppressed patients with HAND (Gates et al., 2016).

Pilot studies suggest that cognitive enhancers used in the treatment of AD may improve neurocognitive functioning in people with HAND. A small randomized cross-over pilot study conducted by Simioni et al (2013) treating virally suppressed PLWH with rivastigmine was associated with improved psychomotor speed (Simioni et al., 2013). Enhanced glutamate transmission is implicated in the neuropathology of HAND through neurotoxicity (Ton & Xiong, 2013). HIV protein gp120 reduces the causes of dysfunction of astrocyte ability to clear glutamate at the synapses (Ton & Xiong, 2013). In a study to investigate whether memantine was safe and effective in treating HAND, it was shown that after 16 weeks of treatment there was no improvement in neurocognitive performance, although there was an increase in the N-acetyl aspartate-creatine ratio in the frontal white matter and parietal cortex on MR spectroscopy (Schifitto et al., 2007). Following another 12 weeks of treatment, exposure to memantine led to significant improvements in the mean of 8 neuropsychological tests (NPZ-8) test scores compared with those who received a placebo. Further 48 weeks of exposure led to no further improvement in NPZ-8 scores (Gates et al., 2016).

Other medical conditions need to be excluded in the diagnosis of HAND (confounding diseases), but this should be reconsidered, since these conditions may compound HIV's effects on the brain. Having hepatitis C, for example, increases the risk of developing and having a severe form of HAND (Asnis & Migdal, 2005). The role of chronic metabolic disorders and certain lifestyles that predispose to vasculopathy has also been emphasized (Alford and Vera JH. 2018). Diabetes for example is associated with an increased risk of HAND because of impaired BBB (Rom et al., 2020). Table B provides a summary of potential adjuvant treatment of HAND.

Table B. Potential adjuvant treatments of HAND (intervention studies)

Drug	Type of study and number of participants	Outcome	Authors and year (Reference)
Memantine	16-week randomized double-blind placebo-controlled study of 140 participants with mild to severe ADC	There was a significant increase in the N-acetyl aspartate to creatine ratio, in the frontal white matter (P = 0.040) and parietal cortex (P = 0.023) (memantine group) No improvements in cognitive performance.	Schifitto et al., 2007
Lithium	A ten-week open-label study of 15 cognitively impaired participants.	There was a reduction in the glutamate+glutamine (Glx) peak in the frontal gray matter, increased fractional anisotropy, decreased mean diffusivity in several brain areas, and changes in brain activation patterns. No cognitive improvement	Schifitto et al., 2009
Minocycline	24-week double-blind placebo-controlled (107 participants with HIV cognitive impairment).	There was no cognitive improvement based on the NPZ-8.	Sacktor et al., 2011
Rivastigmine	20-week double-blind placebo-controlled crossover study of 17 participants with HAND.	There was no improvement in the primary outcome (ADAS-Cog) however the speed of information processing improved.	Simioni et al., 2013.
Maraviroc	A 12-month prospective double-blinded pilot randomized controlled trial with HAND (17 participants).	No treatment-related changes were detected in H-MRS metabolites or cerebrospinal fluid biomarkers. Medium to large effect sizes in favour of improved global neurocognitive performance in the maraviroc arm over time.	Gates et al., 2016
Lithium	A 24-week randomized placebo-controlled study in patients with moderate to severe HAND (66 participants).	There was no improvement in neurocognitive performance. There were no differences in the H-MRS brain metabolite differences between the placebo and lithium group.	Decloedt et al., 2016
Paroxetine and fluconazole	A 24-week randomized double-blind, placebo-controlled two by two factorial design study in patients with HAND (45 participants).	Biomarkers of cellular stress, inflammation, and neuronal damage were not affected by paroxetine. HIV+ individuals receiving fluconazole did not show a benefit in cognition and showed an increase in multiple markers of cellular stress compared to the no fluconazole arms.	Sacktor et al., 2017

Abbreviations: ADAS-cog (Alzheimer's Disease Assessment Scale–Cognitive subscale; ADC (AIDS dementia complex); HAND (HIV-associated neurocognitive disorder); NPZ-8 (8 mean neuropsychological test composite z score), H-MRS (Proton magnetic resonance spectroscopy).

Neuroprotective Effects of Lithium as Adjuvant Therapy for HIV-Associated Neurocognitive Disorders

Lithium is a well-established treatment of bipolar mood disorders (Malhi & Outhred, 2016). Besides the ability to treat and prevent relapses in bipolar mood disorders, lithium can protect neurons from inflammation and neurotoxicity. Lithium is known to work by inhibiting the GSK-3-β and regulates neurotransmitters such as dopamine and glutamate (Malhi & Outhred, 2016). Lithium is also able to promote the expression of BDNF in the neurons (Malhi & Outhred, 2016). GSK-3-β is a serine protein kinase that promotes cellular apoptosis (Thornton et al., 2017). Increased expression of GSK-3-β in the CNS has been linked with neurodegeneration in disorders such as AD. GSK-3-β induces dynamin-related protein-1-Ser616 phosphorylation which leads to dysfunctional mitochondrial alternation in fission (Thornton et al., 2017). In experimental mouse neurons, it was shown that failure to inactivate the GSK-3-β was associated with neuronal cell death in certain regions of the hippocampus and the cortex (Thornton et al., 2017).

Several drugs available in clinical practice such as lithium, valproic acid, and lithium have been shown to have a mechanism of action that involves the inhibition of the GSK-3-β in the neurons (Yang et al., 2017). Lithium inhibits the GSK-3-β by reducing its fission with the mitochondria and cell death through the downregulation of the dynamin-related protein-1 in retinal ganglion -5 cells (Yang et al., 2017). The GSK-3-β expression is increased in the CNS tissue during

HIV infection. Maggirwar et al. (1999) conducted a study to demonstrate the effects of HIV tat protein in the expression of the GSK-3-β in rat neurons (Maggirwar et al., 1999). They found that Tat was associated with an enhanced expression of GSK-3-β which resulted in neurotoxicity. When the neurons were treated with lithium the activity of GSK-3-β was reduced which resulted in the reversal of neurotoxicity (Maggirwar et al., 1999).

Neuroprotection is also facilitated by lithium's ability to regulate neurotransmission and prevent neurotoxicity (Malhi et al., 2013). HIV causes neurotoxicity (and neuronal damage) by disrupting dopamine and glutamate neurotransmission. Gp120 protein has been shown to disrupt the function of dopamine transporter (DAT). A post-mortem case-control study of people who had HIV versus negative controls found a significant reduction in dopamine levels in the putamen, caudate and substantia nigra (Kumar et al., 2009). A dopaminergic system imaging study early study showed that PLWH diagnosed with HAD showed reduced DAT availability in the putamen and ventral striatum when compared to HIV negative controls (Wang et al., 2004). DAT dysfunction may result in poor re-uptake of the dopamine from the synaptic terminal thus causing neuronal toxicity which may lead to dopaminergic neurons terminals injury (Wang et al., 2004). Lithium treatment has been shown to cause an upregulation of neural BDNF. BDNF is a major player in the neuron's ability to adapt, survive, learn, and neuroplasticity (Quiroz et al., 2010). Lithium regulates the activity of adenyl cyclase enzymes and the production of cyclic adenosine monophosphate (cAMP). cAMP enhances the activity of protein kinase A which leads to activation of transcription factors such as cAMP response element-binding protein which increases the production of BDNF (Quiroz et al., 2010). In HIV-infected brains the neuronal BDNF is lower in patients that are infected with HIV. This has been linked with the ability of gp120 protein to inhibit the conversion of proBDNF to BDNF (Bachis et al., 2012).

Hypothesis

Lithium is the treatment of choice for treatment and prevention of mania in people with bipolar disorder. Lithium has a broad mechanism of action that has been shown to be neuroprotective against various neurotoxic pathways. Our hypothesis is that patients with moderate to severe HAND have peristent active neuropathology that can altered or corrected by adding adjuvanct lithium. Hypothetically the neuroprotective effects of lithium should cause changes in fluid biomarkers following treatment.

Rationale for the study

This is a secondary study that will report on fluid biomarkers' data collected from a 24-week randomized placebo-controlled clinical trail of lithim in patients on stable antiretroviral therapy diagnosed with moderate to severe HAND. The primary objective of the primary study was to determine whether lithium could have a positive impact on the neurocognitive perfomance in the participants. Neither lithium nor placebo had an effect on neurocognitive performance (Decloedt et al., 2016). In a post-doc analysis (unpublished) both treatment groups were found to have a noticeable improvement in neurocognitive performance. This improveent was atributaed to repeated neurocognitive testing " practice effect".

Aims

In this study, we aim to determine whether exposure to lithium affects the expression or concentration of fluid (blood and LP (CSF)) biomarkers of HAND and AD in antiretroviral treated patients with moderate to severe HAND.

Study Objectives

1. To determine if adjunctive lithium therapy could modify fluid HAND and AD biomarkers in a cohort of patients with moderate to severe HAND who are treated with ART.
2. To determine the magnitude of change in the concentrations of fluid HAND and AD biomarkers at the end of intervention (24 weeks).

Study design

This is a secondary exploratory analysis of data that was collected from a randomised placebo-controlled clinical trial of lithium in participants with moderate to severe HAND on stable antiretroviral therapy.

Sample consideration

We chose to enrol a total of 54 participants for each treatment arm to account for about 10% loss to attrition. The global deficit score (GDS) has been shown to improve by a mean of approximately 0.13 in patients with the mild to moderate (>0.25 to <0.75) and 0.6 in patients in the severe HAND (>0.75) in the population we studied (Joska e al. 2012). In a previous comparable study, it was found that 12 weeks of adjuvant lithium treatment in stable ART treated patients improved GDS by a mean of approximately 0.3 (Letendre et al. 2006). We recruited participants with a similar profile to this study. However, our object was more conservative, and we aimed for a GDS difference of 0.25 (vs 0.3). For a power of 90% and alpha of 0.05 we needed a sample size of at least 49 participants per arm.

Methods

We report secondary findings of the previously completed randomised placebo-controlled clinical trial of lithium in participants with moderate to severe HAND treated with antiretroviral therapy (Identifier: PACTR201310000635418). We did an exploratory analysis of the biomarkers that were obtained before the interventions and at the need of the interventions. My role in this study was to collate and clean all biomarker datasets. A comprehensive report on the parent study design, methodology, and participant recruitment is included in the supplementary text. The blood/plasma and lumbar puncture biomarkers that were selected for this study include BDNF, NfL, dopamine, sAPPα, sAPPβ, Aβ38, 40, and 42, Cystatin C, clusterin, A1AT, ICAM-1, PEDF, RANTES, A1GP, CC4, ApoC3, and prealbumin. Detailed information on the biomarkers in suplementary text.

Ethics consideration

This study was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 772/2020). The parent study was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (071/2013) and Stellenbosch University (M13/07/027). The clinical trial was registered on the Pan African Clinical Trials Registry (Identifier: PACTR201310000635418). Consent was obtained in the parent study and therefore there was no consent obtained in this secondary analysis

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Chapter 2: Publication ready manuscript prepared for the Journal of Neurovirology

Blood and Cerebrospinal Fluid Biomarker Changes in Patients with HIV-Associated Neurocognitive Impairment Treated with Lithium: Analysis from a Randomised Placebo-Controlled Trial

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Abstract

HIV-associated neurocognitive disorders (HAND) persist in the era of antiretroviral therapy (ART). Thus, ART does not completely halt or reverse the pathological processes behind HAND. Adjuvant mitigating treatments are therefore prudent. Lithium treatment is known to promote neuronal brain-derived neurotrophic factors (BDNF). Lithium is also an inhibitor of glycogen synthase kinase-3 beta (GSK-3-β). We analyzed biomarkers obtained from participants in a randomized placebo-controlled trial of lithium in ART-treated individuals with moderate or severe HAND. We assayed markers at baseline and 24 weeks across several pathways hypothesized to be affected by HIV, inflammation, or degeneration. Investigated biomarkers included dopamine, BDNF, neurofilament light chain, and CD8+ lymphocyte activation (CD38+ HLADR+). Alzheimer's Disease (AD) biomarkers included soluble amyloid precursor protein alpha and beta (sAPPα/β), Aβ38, 40, 42, and ten other biomarkers validated as predictors of mild cognitive impairment and progression in previous studies. These include apolipoprotein C3, pre-albumin, α1-acid glycoprotein, α1-antitrypsin, PEDF, CC4, ICAM-1, RANTES, clusterin, and cystatin c. We recruited 61 participants (placebo = 31; lithium = 30). The age baseline mean was 40 (±8.35) years and the median CD4+ T-cell count was 498 (IQR: 389 – 651) cells/μL. Biomarker concentrations between groups did not differ at baseline. However, both groups' blood dopamine levels decreased significantly after 24 weeks (adj. p<002). No other marker was significantly different between groups, and we concluded

that lithium did not confer neuroprotection following 24 weeks of treatment. However, the study was limited in duration and sample size.

Keywords: HIV. HAND. Biomarkers. Lithium

Introduction

Since the advent of antiretroviral therapy (ART), HIV management has improved dramatically, resulting in a decrease in early mortality in people with HIV (PWH) (Trickey et al. 2017). Despite this, HIV-associated neurocognitive disorders (HAND) continue to affect a significant proportion of PWH who are adequately treated (Heaton et al. 2010). Furthermore, HAND is an independent prognostic marker of mortality (Naveed et al. 2021). Globally 44.9% of PWH meet the Frascati criteria for HAND. Asymptomatic neurocognitive impairment (ANI) accounts for 26.5% of this number, whereas mild neurocognitive disorder contributes 8.5%, and HIV-associated dementia contributes 2.1%. Though ANI has no clinical significance, the presence of ANI is associated with a 4-6-fold risk of developing symptomatic HAND (Grant et al. 2014).

The high reported rates of ANI in PWH are, however, controversial. There is growing concern that relying solely on neuropsychological performance can lead to false positives of up to 20% (Gisslen et al. 2011). Further, many sociodemographic factors may account for low cognitive performance in PWH. This may lead to an overestimation of the prevalence of ANI. Some PWH with ANI may also perform within a spectrum of normality during neuropsychological assessments (Nightingale et al. 2021). Accordingly, to validate ANI, biomarkers should be developed to distinguish individuals with subtle neuropathology in the brain from those who perform within normal limits.

The pathophysiology of HAND is complex and multifactorial. The viral pathway involves neuronal dysfunction and irreversible neuronal injury, which often correlates with the level of virus circulating in the plasma (Marcotte et al. 2003). The presence of HAND during adequate viral suppression could result from pre-treatment injury called legacy effect (Qu et al. 2022) and persistent viral replication in the CNS compartment, as in the case of CSF viral scape (Nightingale et al. 2014). Chronic compartmentalized neuroinflammation persists even during viral suppression (Ulfhammer et al. 2018). Eden et al. (2018) found that during adequate ART treatment, neopterin levels (a marker of CNS immunoactivity) are higher in those with ANI compared to those with normal cognition (Eden et al. 2016). Similarly, a study by Yuan et al. (2013) also found a strong correlation between HAND and CSF inflammation during adequate viral suppression (Yuan et al. 2013).

Preliminary results from observational and experimental studies suggest that this form of chronic neuronal injury may set off neurodegeneration. In addition, there has been a steady rise in the number of PWH living beyond the age of 50 years (Autenrieth et al. 2018). Therefore, identifying potential neuroprotective compounds or therapies has become even more pressing. An ideal therapy should rectify the pathways implicated in the pathophysiology of HAND (Lindl et al. 2010; Rumbaugh et al. 2008; Turchan et al. 2003). Drugs tested in previous clinical trials include memantine, minocycline and paroxetine (Sacktor et al. 2017; Simioni et al. 2013; Schifitto et al. 2007). Two smaller pilot trials investigated the efficacy of lithium in HAND, finding small imaging and clinical evidence of effect (Decloedt et al. 2016; Schifitto et al. 2009). Lithium is a well-established treatment for bipolar mood disorders (Malhi and Outhred 2016). Besides the ability to treat and prevent relapses in bipolar mood disorders, lithium can protect neurons from

inflammation and neurotoxicity. Lithium is known to work by inhibiting the GSK-3-β and regulates neurotransmitters such as dopamine and glutamate. Lithium also promotes the expression of BDNF in the neurons.

GSK-3-β is a serine protein kinase that promotes cellular apoptosis (Thornton et al. 2017). Increased expression of GSK-3-β in the CNS is associated with neurodegeneration in disorders such as AD. GSK-3-β induces DRP1-Ser616 phosphorylation, resulting in a dysfunctional mitochondrial alternation in fission. In an experiment with mouse neurons, GSK-3-β hyperactivity resulted in neuronal cell death in some areas of the hippocampus and the cortex (Thornton et al. 2017). There is an increase in CNS tissue GSK-3-β activity during HIV infection. Maggirwar et al. (1999) experiment on the activity of rat neurons GSK-3-β after exposure to Tat showed that Tat was associated with an enhanced expression of GSK-3-β, resulting in neurotoxicity (Maggirwar et al. 1999). Treating the neurons with lithium caused a reduction in the GSK-3-β and the attenuation of neurotoxicity (Maggirwar et al. 1999). GSK-3-β is a promotor of neuroinflammation, another primary pathway of neuronal injury in patients with HAND.

Various surrogate biomarkers can be analyzed from plasma and CSF to assess the effectiveness of treatments against HAND. Some of the biomarkers have been studied extensively in HAND and found to be highly sensitive and specific for neuronal injuries, such as the neurofilament light chains (NfL) (Gisslén et al. 2016). Blood and CSF concentrations of NfL positively correlate with the severity of the neuronal injury and cognitive impairment in PWH (Gisslén et al. 2016; Anderson et al. 2018). Low plasma and CSF BDNF are associated with poor cognitive function (Levada et al. 2016). HIV decreases BDNF levels by preventing the conversion of passive proBDNF into mature BDNF (Bachis et al. 2012). Peripheral (blood) and central dopamine (DA) pathways are both involved in the pathogenesis and severity of HAND. Peripheral DA is linked with a dose-dependent entry of HIV into the macrophages, which facilitates HIV neuro-invasion (Gaskill et al. 2014). There is a correlation between a reduction in central DA levels and the severity of neurocognitive impairment in PWH. In PWH, an increase in the expression of human leukocyte antigen (HLA) DR and CD38+ on CD8+ T-lymphocytes (HLA-DR+CD38+CD8) is associated with the continuing neuronal injury and progression of HAND (Liu et al. 1997; Robertson et al. 2020; Ratto-Kim et al. 2018).

Because of similarities between AD and HAD, such as the chronic inflammatory state, research has been conducted on CSF amyloid and tau protein metabolism in patients with HAND. Aβ plaque synthesis may be triggered by chronic inflammation, microglial activation, and disruption of the blood-brain barrier (BBB) (Noe et al. 2020), which is also seen in HIV. Furthermore, in vitro studies have found that HIV viral protein Tat has a high affinity for the surface of Aβ fibrils (Hategan et al. 2017). Tat interacts with this surface, promoting Aβ plaques synthesis (Hategan et al. 2017). HIV-infected patients also have a higher incidence of Aβ deposits in the brain (Green et al. 2005; Esiri et al. 1998). Clifford et al. (2009) found reduced CSF $A\beta_{1\text{-}42}$ in PWH diagnosed with HAND compared to healthy matched controls. The concentration of CSF $A_{\beta 1\text{-}42}$ was not different to that of patients with mild Alzheimer's type dementia (Clifford et al 2009). However, CSF tau concentration was higher in the Alzheimer's type dementia participants when compared to that of the control and HAND participants.

Similarly, in a study by Gisslen et al. (2009), the CSF $A\beta_{1\text{-}42}$ was lower in participants with AIDS dementia complex (ADC) compared to cognitively normal PWH (CNP) participants. While the high CSF tau protein metabolite was higher in ADC participants, it was not significantly different from that of the control and CNP participants (Gisslen et al. 2009). These findings suggest that HAND neuronal injury does not show the pattern of neuronal injury observed in AD. In recent years several AD predictive biomarkers were validated as having high accuracy (87%), sensitivity (85%),

and specificity (88%) in detecting the progression of mild cognitive impairment of the AD type (Hye et al. 2014). These markers are primarily inflammatory markers that are easily detectable in plasma. In addition to amyloid and tau, these markers may serve as potential surrogates for the HAND.

Lithium confers neuroprotection by modulating neurotransmission and preventing neurotoxicity (Malhi et al. 2013). In Neuro-HIV, viral proteins, namely Tat and gp120 interfere with dopamine and glutamate, resulting in neurotoxicity. For example, gp120 inhibits the dopamine transporter (DAT) reuptake of dopamine, causing prolonged postsynaptic neurostimulation. The result is the loss of neurons with a high density of dopamine receptors, such as in the basal ganglia. Many studies have shown that treatment with lithium enhances the expression of neuronal BDNF. BDNF promotes neuronal survival, growth, neuroplasticity, and learning (Quiroz et al. 2010). A reduction in the expression of neuronal BDNF occurs during HIV neuro-infection. The viral protein gp120 inhibits the conversion of proBDNF to BDNF by binding to the C-C chemokine receptor type 5, leading to a higher proBDNF/BDNF ratio which correlates with HAND severity.

In this study, we aimed to determine whether lithium can halt or reverse the injury caused by HIV in patients diagnosed with moderate to severe HAND who are on stable ART treatment. This study is the first to use biomarkers across several pathways hypothesized to be involved in HAND and AD.

Methods

Study design, participants, and setting

This study reports a secondary analysis of data collected prospectively during a 24-week randomized placebo-controlled clinical trial of lithium in patients with severe to moderate HAND who are on ART. This manuscript describes the changes observed in large blood and cerebrospinal fluid (CSF) biomarker data (Decloedt et al. 2016). According to the preliminary study, neither lithium nor placebo affected cognitive performance. However, in both treatment arms, there was an improvement in the global deficit score (GDS), likely attributable to the practice effect because of repeated neurocognitive testing.

Methods of the parent study

Study participants were recruited from the Nolungile Site C clinic in Khayelitsha and followed up at Groote Schuur Hospital. The inclusion criteria were: ≥18 and ≤70 years, cognitive impairment defined by the GDS of ≥ 0.5, uninterrupted ART treatment for at least six months a plasma HIV RNA <400 copies/ml. The exclusion criteria were participants taking lithium within 30 days of entering the study, acquired immune deficiency syndrome, history of substance use including benzodiazepines, presence of neurosyphilis, vitamin B12 deficiency, abnormal brain imaging results, and the presence of traumatic brain injury. In the end, the study recruited 66 patients. The participants were randomly assigned to a placebo arm = 34 (31 completed) and a lithium arm = 32 (30 completed). Demographic data and other outcome measures such as the GDS and blood and CSF biomarkers were collected during the first visit and at the end of the clinical trial. The clinical trial received ethical approval from the universities of Cape Town (071/2013) and Stellenbosch (M13/07/027). The registration number of the trial is PACTR201310000635418.

Biomarker consideration

The biomarkers that were selected in the priori were the fluid biomarkers that were the primary outcome objectives of the parent study. These biomarkers were blood CD8+ T lymphocyte activation and blood and CSF dopamine and BDNF. The post hoc biomarkers were the neurofilament light chain and Alzheimer's disease-related biomarkers. All analytes (individual biomarkers or protein) were processed in single batches or one group using the same equipment, reagent, and technician.

Statistical considerations

Sample size

We enrolled 54 participants for each treatment arm to account for about a 10% loss to attrition. The GDS has been shown to improve by a mean of approximately 0.13 in patients with mild to moderate (>0.25 to <0.75) and 0.6 in patients with severe HAND (>0.75) in the population we studied (Joska et al. 2012). In a previous comparable study, it was found that 12 weeks of adjuvant lithium treatment in stable ART-treated patients improved GDS by a mean of approximately 0.3 (Letendre et al. 2006). We recruited participants with a similar profile to this study. However, our object was more conservative, and we aimed for a GDS difference of 0.25 (versus 0.3). For a power of 90% and alpha of 0.05, we needed a sample size of at least 49 participants per arm. GDS is an alternative method for determining cognitive impairment in HIV-positive individuals (Blackstone et al. 2012). The GDS was intended to be a user-friendly, automated approach highlighting performance deficits. The method considers the severity and the number of deficits in performance throughout the test battery while assigning less weight to performances within the normal range. GDS is preferred over the clinical rating scales when the aim is to identify severe levels of cognitive impairment.

Data analysis

The data were analyzed with GraphPad Prism 9. Categorical variables are presented as counts and or percentages. Continuous variables are presented as means (standard deviations) or medians (interquartile range), depending on the distribution. Bivariate and group comparisons were conducted with Chi-square or Fisher's exact tests, or t-tests or Wilcoxon signed-rank tests, depending on the type and distribution of the variables. Paired tests were used when appropriate. A *p-value* of less than 0.05 was statistically significant. When there was statistically significant, we conducted a post hoc analysis using the Bonferroni test to correct for false discovery rate (FDR) because of multiple comparisons. Both arms were subjected to two analyses. First, at baseline and again at week 24, unpaired tests were used to compare biomarker levels between the placebo and lithium arms. Second, paired tests assessed each biomarker for longitudinal changes from before the intervention (week 0) to the end (24 weeks).

Blood CD8+ T lymphocyte activation measurement

Peripheral blood mononuclear cell (PBMC) was resuspended in PBS containing 0.5% BSA and stained with commercially available antibodies (CD8-FITC, CD38-PE, CD3-PerCPCy5, HLADR-APC, all from BD Biosciences) and analyzed by flow cytometry using a FACSCalibur flow cytometer (BD Biosciences). After gating the lymphocyte population in the FSC/SSC-plot, the following cell populations were defined: T-cells (CD3+), CD8+ T-cells (CD3+/CD8+) and activated CD8+ T cells (CD3+/CD8+/CD38+/HLADR+). PBMC were collected at baseline (week 0) and week 24 and stained with anti-CD3/CD8 to define CD8+ T cells and counterstained with anti-CD38/HLADR to detect the frequency of activated CD8+ T cells.

Plasma and CSF BDNF and DA concentrations

Plasma (all patients) and CSF (n = 35 at baseline and n = 18 at week 24) samples were collected at weeks 0 and 24 and subjected to BDNF enzyme-linked immunosorbent assay (ELISA). Plasma and CSF samples were inactivated at 56°C for 30 minutes. BDNF (Abcam) and DA (Abnova) concentrations were determined by commercially available ELISA kits according to the manufacturers' instructions. Briefly, for the BDNF ELISA, plasma samples were diluted at a ratio of 1:4 before ELISA, and CSF samples were analyzed undiluted. For the DA ELISA, CSF and plasma samples were analyzed undiluted. All ELISA DA experiments were run in one round on the same day using a single plate and reagents (one batch). Three experiments were conducted for the BDNF ELISA. All CSF BDNF ELISA experiments (before and post-intervention) were conducted in one batch. The plasma BDNF ELISA experiments were conducted in two batches at different points (before and post-intervention).

Plasma biomarkers of mild cognitive impairment to AD progression

The following plasma proteins were measured using multiplex bead assays (Luminex xMAP): acid glycoprotein (AGP), apolipoprotein C3 (ApoC3), pre-albumin, alpha-1 antitrypsin (A1AT), pigment epithelium-derived factor (PEDF), complement component 4 (CC4), intercellular adhesion molecules-1 (ICAM-1), regulated on activation, normal T cell expressed and secreted (RANTES), clusterin and cystatin c. Median fluorescent intensity (MFI) of the xMAP assays were measured using the xPONENT 3.1 (Luminex Corporation) The MFI were exported into Sigma Plot (Systat Software; Version 12.5) for estimation of protein concentrations using a 5-parameter logistic fit.

Plasma and CSF Neurofilament light chains

CSF NfL concentration was measured using a commercially available sandwich ELISA according to the kit manufacturer's instructions (UmanDiagnostics, Umeå, Sweden). Plasma NfL concentration was measured using the commercially available Single molecule array (Simoa) NF-Light assay (Quanterix, Billerica, MA), according to a protocol previously described in detail (Gisslén et al., 2016). All ELISA experiments (before and post intervention) were conducted in a single batch.

CSF amyloid proteins

sAPPα/β concentrations were measured in CSF with a duplex immunoassay and electrochemiluminescence detection (Meso Scale Discovery, Rockville, MD). CSF Aβ38, Aβ40, and Aβ42 concentrations were measured using a triplex immunoassay with electrochemiluminescence detection (Meso Scale Discovery, Rockville, MD). The measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Intra-assay coefficients of variation were below 10%.

The study protocol and approval

University of Cape Town Faculty of Health Sciences Human Research Ethics Committee approved this study (HREC REF: 772/2020).

Results

Participant baseline characteristics before randomisation

Participants in the two treatment arms did not differ in terms of age (p=0.48), gender (p=0.26), years of education (p = 0.81), CD4+ T lymphocyte counts (p = 0.80), GDS (p = 0.79) or time on ART (p=0.64).

Table 1 Characteristics of participants at enrolment

Characteristic	Treatment groups		
	Placebo n = 34	Lithium n = 32	p-value
[a]Age (mean ± SD)	40.53± 8.71	39.03±8.09	0.48
[b]Gender			
Female	28 (82%)	30 (94%)	0.26
Males	6 (18%)	2 (6%)	
[c]Years of education			
< 10 years	16 (47%)	14 (44%)	
≥ 10 years	18 (53%)	18 (56%)	0.81
[c]CD4 count: median (IQR)	498 (379 – 665)	502 (391 – 649)	0.80
[c]GDS: median (IQR)	1.12 (0.82 – 1.53)	1.10 (0.8 – 1.5)	0.79
Antiretroviral therapy			0.33
NNRTI-based	30(88%)	26(81%)	
PI-based	4(12%)	6(19%)	
[c]Time on treatment(months): median (IQR)	40 (25 – 73)	51 (22 – 77)	0.64

[a]Unpaired t-test, [b]Fischer exact test, [c]Mann-Whitney test, SD: Standard deviation, IQR: Interquartile range, NNRTI: Non-nucleoside reverse transcriptase inhibitors, PI: Protease inhibitors

Blood biomarkers

No differences were observed between the two arms concerning individual biomarker concentrations. Biomarkers in the plasma of both treatments did not indicate any possible neuronal injury or dysfunction at baseline. In comparison to normal sociodemographic ranges, the expressions were as follows: 1) normal: NfL, DA, PEDF; 2) elevated: BDNF, CC4, cystatin c and 3) low: pre-albumin, RANTES, ApoC3, AGP, A1AT and ICAM-1.

Blood CD8+ T lymphocytes (CD8+ HLADR+ CD38+) activation

In week 24, the placebo arm median (IQR) was 4.2 (3 – 6.1) % compared with the lithium arm median (IQR) of 4.2 (3 – 6.1) %, p = 0.54. Treatment exposure did not result in significant changes in either treatment arm (Supplementary Table 9 and 11)

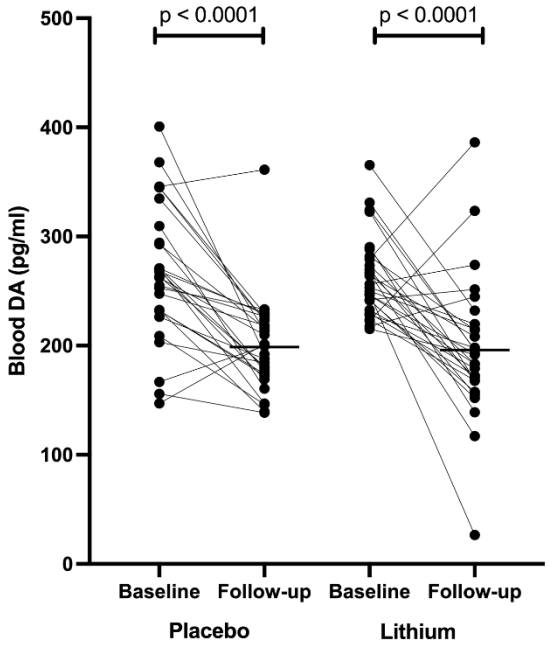
Plasma dopamine

Both treatment arms showed statistically significant (p < 0.0001) changes in dopamine (DA) concentration at the end of the intervention (24 weeks). In both groups, the changes remained statistically significant (p < 0.002) after post hoc FDR (Figure 1, Supplementary Table 9 and 11). In the placebo group, the median (IQR) dopamine concentration was reduced from the median (IQR) of 262.3 (231.4 – 301.6) to 199.5 (173.3 – 225.4) pg/ml, a median difference of -62.8 pg/ml. In the lithium group, the median (IQR) DA concentration was reduced from 249.9 (229.5 – 279.2) to 191.8 (168.0 – 217.5) pg/ml, a median difference of -58.1 pg/ml.

Plasma BDNF

A nearly significant (p = 0.05) change in the lithium group was observed in the concentration of BDNF at the end of the intervention (Supplementary Table 11). The median (IQR) concentration of BDNF dropped from a median of 36.51 (27 – 54) ng/ml to 29.63 (22 – 52) ng/ml, a median difference of -5.059 (-15 – 4).

Fig 1



Changes in plasma or blood DA after 24 weeks of exposure. Baseline indicates before the intervention, and follow-up indicates after the intervention (week 24). Statistically significant changes are seen in both treatment arms (Mann-Whitney U-test for paired analysis of the nonparametric distribution of values).

CSF Biomarkers

Biomarkers in the CSF of both treatments did not indicate any possible neuronal injury or dysfunction at baseline. In comparisons to normal sociodemographic ranges, the expressions were as follows: 1) normal: sAPPα, dopamine and BDNF; 2) elevated: sAPPβ and 3) low: NfL Aβ38, Aβ40, Aβ42.

The concentrations of sAPPα ($p=0.01$; adjusted $p=0.05$) and sAPPβ ($p=0.05$, adjusted $p=0.05$) differed significantly between the two arms at 24 weeks. The sAPPα and sAPPβ were higher in the placebo group with median differences of 174.5 pg/ml, and 380.5 pg/ml, respectively. There was a significant change in the sAPPα concentration in the placebo arm, with a median increase of 38.50 (25 - 53) pg/ml and $p=0.03$. Post hoc FDR correction removed this effect with adjusted $p=0.15$ (Supplementary Table 3). No changes in the concentration of sAPPα were observed in the lithium arm.

Discussion

The present study presents a secondary analysis of a large dataset of biomarkers analyzed from the blood/plasma and CSF during a 24-week randomized placebo-controlled clinical trial of patients with HAND. We conducted multiple biomarker comparisons, some of which are validated as biomarkers of HAND and others from Alzheimer's dementia. We hypothesized that the AD biomarkers could help determine the effects of neuroprotective agents because of the similarities in the pathogenesis of HAND and AD. Plasma DA was significantly changed following treatment with lithium. The reduction in the plasma DA was also reduced in the placebo arm with a similar statistically significant of $p=0.002$.

These findings could not support the neuroprotective benefits of lithium in moderate to severe HAND. Contrary, a previous study showed some evidence of lithium neuroprotection against HIV. In a small pilot study of PWH with HAND, lithium treatment for ten weeks normalized neuronal metabolism and integrity (Schifitto et al. 2009). Evidence of neuroprotection was determined as an increase in neuronal glutamate/glutamine peak, an increase in white matter fractional anisotropy, and a reduction in mean diffusivity. There is also evidence that lithium treatment reverses HIV-related injuries in animal studies. Dou et al. (2005) found that lithium restored microtubule-associated protein 2 neurites and synaptic density in murine HIV encephalitis models (Dou et al. 2005). In addition, lithium reduced the activity of GSK-3-β. In another laboratory experiment, Tat infection of murine neurons resulted in a GSK-3-β activity increase. However, lithium treatment of the infected neurons resulted in attenuating the GSK-3-β activity (Maggirwar et al. 1999). Lithium is also well known for its ability to promote BDNF production (Yasuda et al. 2009).

It is also relevant to note that despite the attempt to use a broader approach to neuropathogenesis, the selected biomarkers are not directly linked with the putative pathway of lithium mechanism of action except for BDNF. A reduction in the BDNF occurs in various neuropsychiatric disorders and neurodegenerative disorders (Benussi et al. 2017). Changes in BDNF levels are also apparent in the blood of subjects with CNS diseases (Ventriglia et al. 2013). The plasma and CSF levels of BDNF have been used as markers to show cognitive status, and a correlation between plasma BDNF and brain levels has been suggested in animal and human studies (Balietti et al. 2018). In accordance, less BDNF was found in the brains of HIV-infected persons with HIV dementia than those without, possibly due to an impaired ratio of proBDNF to mature BDNF (Bachis et al. 2012).

Lithium response is associated with the Val66Met functional polymorphism of the BDNF gene located on chromosome 11p13 (Rybakowski 2014; Dmitrzak-Weglarz et al. 2008), and it was suggested that the therapeutic effects of lithium might be in part via modulation of BDNF (Castrén and Kojima 2016). However, this was not confirmed in other

populations except Caucasians (Michelon et al. 2006). In our study, BDNF levels were reduced in the plasma of HIV-infected individuals on ART following lithium treatment, indicating a possible cognitive decline in the future in these individuals. The reasons for reduced serum BDNF levels in individuals receiving lithium remain unknown. It is possible that our patients were inadequate lithium responders as lithium did not recover GDS, and it is known that excellent lithium responders are associated with normal blood BDNF levels (Rybakowski 2014). In another study with euthymic adolescents with bipolar disorder, a lower BDNF level was detected in their blood after taking lithium (Cevher et al. 2016). The variations in the BDNF gene promoter region affect the expression of BDNF and its role in various neuropsychiatric disorders. For example, in an experiment with mice neurons, the antimanic effects of lithium were linked with BDNF modulation, which was not the case with the antidepressant effects (Gideons et al. 2017). These findings suggest that lithium's action can be influenced not only by the neuropsychiatric status of patients but also by variations in the BDNF gene's promoter region which affects the expression of BDNF (Hing et al. 2012). BDNF was included in our analysis, but no changes were observed following treatment with lithium.

Elevated DA results in activation of GSK-3-β, and lithium antagonizes this effect due to inhibition of GSK-3-β (Beaulieu et al. 2004). Lithium has been shown to regulate altered DA function (Malhi and Outhred 2016). HIV infection is associated with increased DA concentrations in CSF (Scheller et al. 2010). In PWH with the DAT10/10-repeat allele (Horn et al. 2013), we expected to find reduced DA levels. In our study, we could not see an effect of lithium on CSF DA concentrations due to the small number of patients with available CSF, and we did not check for polymorphisms in our population. However, we found a statistically significant reduction in plasma DA in both arms after the intervention. DA in plasma is classified as a hormone rather than a neurotransmitter. Three systems modulate peripheral DA: the sympathetic branch of the autonomous nervous system, the autocrine/paracrine DA system and the adrenomedullary hormonal system, producing large amounts of catecholamines in response to acute stress or elevated arousal (Laverty 1978; Tank and Wong 2015). In our study, both treatment arms showed significant changes in DA levels after the intervention, suggesting that the observed DA reduction is independent of lithium pharmacotherapy. We can postulate that this reduction in DA concentration is because participants were more anxious during the examination at the beginning of the study (baseline) than at the follow-up visit. Unfortunately, we do not have blood pressure measurements to support our conjecture and the only study we know of that assessed a link between plasma catecholamine levels and anxiety and found no statistically significant changes used visual anxiety stressors (Gutierrrez-Martin et al. 2022) and not acute passive intrinsic stress as we might have in our study. Further, the participants in this study were under care. They received continuous support and financial compensation, which could all lead to normalisation or reduction of dopamine compared to the previous studies.

Because of the similarities between HAND and AD pathophysiology, we explored possible changes in the AD biomarkers at the end of the intervention (24 weeks). However, lithium did not change any of these biomarkers. In experimental animal models of AD, lithium's ability to prevent or reduce AD pathology has been demonstrated. Lithium was shown to reduce amyloid-beta synthesis in drosophila models of AD (Sofola-Adesakin et al. 2014). In a traumatic brain injury mouse model, lithium was also shown to reduce the synthesis of amyloid-beta and Tau protein phosphorylation (Yu et al. 2012). There is a possibility that the lack of lithium evidence of neuroprotection may be due to some factors that distinguish AD from HAND. The AD biomarkers of disease progression came from an older population than the cohort (Hye et al. 2014). The means (SD) age of participants in our study was 7.8 years younger than that of Hye et al. (2014), (mean (SD) 39 years vs. 76 years). While Hye et al. (2014) biomarkers demonstrated high sensitivity and specificity for

predicting progress from MCI to AD, HAND has been described as a stable neurocognitive disorder during viral suppression (Sacktor et al. 2016). Furthermore, if a severe form of neuropathology is present in patients with moderate to severe HAND, the utility of these biomarkers may be compromised.

It is important to note that some of the biomarkers were within normal ranges, indicating no evidence of ongoing injury or neuronal dysfunction in both treatment arms. In addition, participants showed improvement in the GDS, likely secondary to the practice effect (Decloedt et al. 2016). One of the robust reasons for the HAND in this study that would not be mitigated by lithium is the legacy effect (Nightingale et al. 2014). Therefore, in this study, the presence of HAND was not necessarily indicative of persistent viral neuropathogenesis. Furthermore, the inclusion criteria included viral suppression and adequate ART. Even though CSF viral replication can continue even when there is evidence of viral suppression in the plasma, participants with CSF viral escape would be expected to demonstrate progressive neuropathology, which should manifest with clinical signs. In this study, participants did not show evidence of progressive neurological fallout (Decloedt et al. 2016).

Limitations

The study results should be interpreted considering various limitations. The participants in our study are homogeneous in terms of ethnicity and gender. Participants are mainly middle-aged females. We also analyzed many biomarkers from a small study sample size which may result in false-positive findings. Despite improving the duration of treatment compared with previous studies, we cannot rule out that prolonged exposure to lithium may cause some changes in the expression of biomarkers. Moreover, most of all, the expression of the biomarkers in both treatment groups indicated no active neuronal injury or dysfunction before the interventions.

Conclusions

There was no evidence of lithium neuroprotection through surrogate biomarkers in this study. At baseline, neither plasma nor CSF concentrations indicated neuronal injury, which may explain the negative findings. This is, therefore, a potential confounder for this study. Future studies with participants with evidence of ongoing neuronal injury should be conducted to determine whether lithium provides neuroprotection

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Conflicts of Interest

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, reMYND, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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Supplementary text

Tables of analysis

Table 1: Blood HAND biomarkers concentration differences between arms at week 0

Biomarker	Placebo arm	Lithium arm	P-value
NfL (pg/ml)[1]			
Number	34	31	
Mean	5.9 (2.8)	5.5 (2.5)	
Median (IQR)	4.9 (2.8)	4.9 (3.2)	0.5
DA (ng/ml)[1]			
Number	33	31	
Mean (SD)	15.9 (3.5)	15.6 (2.3)	
Median (IQR)	15.7 (4.2)	15.0 (2.9)	0.6
95% CI	14.6 – 17.1	14.8 – 16.4	
BDNF (ng/ml)[1]			
Number	17	16	
Mean	37.0 (26.2)	42.4 (21.2)	
Median	33.4 (23.9)	38.4 (31.8)	0.2
95% CI	27.6 – 46.5)	34.4 – 50.3	

[1]Mann-Whitney test, [2]Unpaired t-test
HAND: HIV associated neurocognitive disorder., A: Dopamine, NfL: Neurofilament light chains, SD = standard deviation,
IQR = expressed as the difference in the range

Table 2: Blood HAND biomarkers concentration differences between arms at week 24

Biomarker	Placebo arm	Lithium arm	P-value
NfL (pg/ml)[1]			
Number	30	29	
Mean	6.716(4.673)	5.524(2.968)	
Median (IQR)	5.024(3.523)	4.777(2.446)	0.287
95% CI	4.971 – 8.461	4.395 – 6.653	
DA (ng/ml)[1]			
Number	29	29	
Mean (SD)	12.42(3.704)[1]	11.76(3.831)[1]	
Median (IQR)	11.97(3.12)	11.51(2.97)	0.479
95% CI	11.01 – 13.83	10.31 – 13.22	
BDNF (ng/ml)[1]			
Number	29	29	
Mean	36.35(25.07)	35.55(19.06)	
Median	28.15(28.26)	30.57(29.6)	0.761
95% CI	26.82 – 45.89	28.30 – 42.80	

[1]Mann-Whitney test, [2]Unpaired t-test
HAND: HIV associated neurocognitive disorder., A: Dopamine, NfL: Neurofilament light chains, SD = standard deviation,
IQR = expressed as the difference in the range

Table 3. Blood AD biomarkers concentration differences between arms at week 0

Biomarker	Placebo arm	Lithium arm	P-value
ApoC3 (mcg/ml)[1]			
Number	34	31	
Mean (SD)	4.1 (2.5)	4 (2)	0.7
Median (IQR)	3.2 (2.5)	3.6 (2.1)	
95% CI	3.2 – 5.0	3.3 – 4.5	
Prealbumin (mg/dL)[1]			
Number	34	31	
Mean (SD)	6.8 (3.3)	6.9 (3.4)	0.9
Median (IQR)	6.2 (3.9)	5.9 (5.1)	
95% CI	5.7– 7.9	5.7 – 8.2	
AGP[1] (mcg/ml)[1]			
Number	32	28	
Mean (SD)	254. (151.7)	207.6 (162.2)	
Median (IQR)	222.9 (151.1)	171.9 (140.7)	0.1
95% Cl	199.9 – 309.3	144.7 – 270.5	
A1AT (mcg/ml)[1]			
Number	29	25	
Mean (SD)	48.4 (5.6)	48.8 (48.5)	0.8
Median (IQR)	49.1 (8.6)	48.5 (8.7)	
95% CI	46.3 – 50.6[2]	46.3 – 51.4	
PEDF (mcg/ml)[2]			
Number	33	30	
Mean (SD)	5.0 (1.3)	5.2 (1.1)	
Median (IQR)	5.2 (1.9)	4.9 (1.5)	0.5
95% Cl	4.6 – 5.5	4.8 – 5.6	
CC3 (mcg/ml)[1]			
Number	32	30	
Mean (SD)	137.6 (27.7)	158.4 (43.3)	
Median (IQR)	141.0 (44.5)	150.5 (49.5)	0.1
95% CI	127.6 – 147.6	142.2 – 174.5	
ICAM-1 (ng/ml)[1]			
Number	34	31	
Mean (SD)	1997 (738.0)	2093 (1075)	
Median (IQR)	2188 (1 086)	1985 (1 082)	0.9
95% CI	1739 - 2254	1698 - 2487	
RANTES ng/ml)[1]			
Number	34	31	
Mean (SD)	483.6 (177.0)	483.8 (249.6)	
Median (IQR)	482.2 (243.8)	446.2 (226.4)	0.7
95% CI	421.9 – 545.4	392.2 – 575.4	
Clusterin (mcg/ml)[2]			
Number	34	31	
Mean (SD)	1236 (302.7)	1294 (375.5)	
Median (IQR)	1245 (369)	1308 (602.2)	0.5
95% CI	1131 - 1342	1156 - 1432	
Cystatin (mcg/ml)[2]			
Number	8	7	
Mean (SD)	1.2 (0.2)	1.4 (0.3)	
Median (IQR)	1.2 (0.3)	1.4 (0.4)	0.1
95% CI	1.1 – 1.3	1.2 – 1.7	

[1]Unpaired t-test, [2]Mann- Whitney test, CSF: Cerebrospinal fluid, NfL: Neurofilament light chains,
BDNF: Brain-derived neurotrophic factor, SD = standard deviation,
IQR = Interquartile range (expressed as the difference in the rang

Table 4. Blood AD biomarkers concentration differences between arms at week 24

Biomarker	Placebo arm	Lithium arm	P-value
ApoC3 (mcg/ml)[1]			
Number	30	29	
Mean (SD)	3.953(2.188)	4.247(2.018)	
Median (IQR)	3.340(1.457)	3.680(1.78)	0.458
95% CI	3.136 – 4.770	3.479 – 5.014	
Prealbumin (mg/dL)[1]			
Number	30	29	
Mean (SD)	7.002(2.980)	7.340(3.641)	0.943
Median (IQR)	6.635(3.543)	6.130(4.47)	
95% CI	5.889 – 8.115	5.955 -- 8.725	
AGP[1] (mcg/ml)[1]			
Number	29	27	
Mean (SD)	233.1(136.4)	203.6(204.0)	
Median (IQR)	192.9(176.7)	204.0(136.4)	0.603
95% CI	181.2 - 285	160.6 - 246	
A1AT (mcg/ml)[1]			
Number	28	28	
Mean (SD)	49.94(5.366)	49.67(6.557)	
Median (IQR)	48.79(6.07)	49.83(9.14)	0.823
95% CI	47.85 – 52.02	47.13 – 52.22[1]	
PEDF (mcg/ml)[2]			
Number	28	28	
Mean (SD)	5.230(1.384)	5.219(0.951)	
Median (IQR)	5.070(2.11)	5.255(1.18)	0.974
95% CI	4.693 – 5.766	4.850 – 5.588	
CC3 (mcg/ml)[1]			
Number	28	27	
Mean (SD)	150.6	157.3	
Median (IQR)	140.7(54)	159.0(42.5)	0.237
95% CI	131.2 – 169.9	143.1 – 171.4	
ICAM-1 (ng/ml)[1]			
Number	30	29	
Mean (SD)	1868(656.1)	2055(1695)	
Median (IQR)	1782(783)	1907(1 219)	0.758
95% CI	1623 - 2114	1410 - 2700	
RANTES ng/ml)[1]			
Number	30	29	
Mean (SD)	471.6(417.3)	488.2(252.6)[1]	
Median (IQR)	378.8(222.6)	438.9(191.3)	0.144
95% CI	315.8 – 627.4	392.2 – 584.3	
Clusterin (mcg/ml)[2]			
Number	30	29	
Mean (SD)	1179(345.8)	1143(270.8)	
Median (IQR)	1168(683.1)	1118(373.2)	0.661
95% CI	1050 - 1308	1040 - 1246	
Cystatin (mcg/ml)[2]			
Number	6	3	
Mean (SD)	1.279(0.1645)	1.330(0.163)	
Median (IQR)	1.238(0.197)	1.383(0.312)	0.669
95% CI	1.106 – 1.451	0.9266 – 1.734	

[1]Mann-Whitney test,[2]Unpaired t-test
AD: Alzheimer's dementia, SD = Standard deviation,
IQR = Interquartile range (expressed as the difference in the range)

Table 5. CSF HAND biomarker concentration differences between arms at week 0

Biomarker	Placebo arm	Lithium arm	P-value
NfL (pg/ml)[1]			
Number	16	16	
Mean (SD)	421.7 (133.2)	443.1 (213.5)	0.7
Median (IQR)	398.0 (184.8)	472.5 (373.7)	
95% CI	350.7 – 492.7	329.3 – 556.9	
BDNF (ng/ml)[2]			
Number	20	16	
Mean (SD)	1.2 (0.8)	1.5 (0.8)	
Median (IQR)	0.9(1.1)	1.3 (0.7)	0.1
95% CI	0.8 – 1.6	1.1 – 1.9	
Dopamine (ng/ml)[1]			
Number	15	17	
Mean (SD)	2.0 (1.8)	2.9 (2.3)	
Median (IQR)	1.3 (2.3)	2.4 (3.3)	0.4
95% CI	1.0 – 3.0	1.7 – 4.0	

[1]Unpaired t-test, [2]Mann- Whitney test, CSF: Cerebrospinal fluid, NfL: Neurofilament light chains,
BDNF: Brain-derived neurotrophic factor, SD = standard deviation, IQR = Interquartile range (expressed as the difference in the range)

Table 6. CSF HAND biomarker concentration differences between arms at week 24

Biomarker	Placebo arm	Lithium arm	P-value
NfL (pg/ml)[1]			
Number	8	8	
Mean (SD)	406.8(127.1)1	331.3(151.3	0.2981
Median (IQR)	341.0(239.3)	309.0(192.8)	
95% CI	300.5 – 513.0	204.8 – 457.7	
BDNF (ng/ml)[2]			
Number	7	8	
Mean (SD)	1.984(1.435)	1.213(0.3319)	
Median (IQR)	1.309(1.5)	1.309(0.539)	0.463
95% CI	0.658 – 3.311	0.935 – 1.490	
Dopamine (ng/ml)[1]			
Number	7	6	
Mean (SD)	2.176(2.271)	1.647(1.339)	
Median (IQR)	1.031(2.721)	1.730(2.556)	0.836
95% CI	0.076-4.276	0.242 – 3.051	

[1]Unpaired t-test, [2]Mann- Whitney test, CSF: Cerebrospinal fluid, NfL: Neurofilament light chains,
BDNF: Brain-derived neurotrophic factor, SD = standard deviation, IQR = Interquartile range (expressed as the difference in the range)

Table 7. CSF AD biomarker's concentration differences between arms at week 0

Biomarker	Placebo arm	Lithium arm	P-value
sAPPα (pg/ml)[2]			
Number	17	17	
Mean (SD)	245.5 (134.0)	169.6 (106.5)	0.03*
Median (IQR)	244.0 (153.5)	140.0 (141.5)	0.15#
95% CI	176.6 – 314.4	114.8 – 224.3	
sAPPβ (pg/ml)[2]			
Number	17	17	
Mean (SD)	568.6 (286.7)	431.1 (224.9)	
Median (IQR)	550.0 (320.5)	389.0 (312.5)	0.10
95% Cl	421.3 – 716.0	315.4 – 546.7	
Aβ38 (pg/ml)[2]			
Number	17	17	
Mean (SD)	1968 (939.2)	1493 (610.2)	0.12
Median (IQR)	1915 (1008)	1442 (1139)	
95% CI	1485 - 2451	1180 - 1807	
Aβ40 (pg/ml)[1]			
Number	16	17	
Mean (SD)	4892(1642)	4187 (1750)	
Median (IQR)	4879(2 998)	4035 (3402)	0.24
95% CI	4016 - 5767	3287 - 5087	
Aβ42 (pg/ml)[1]			
Number	17	17	
Mean (SD)	467.9 (207.1)	369.9 (173.6)	
Median (IQR)	460.0 (342.5)	360.0 (306)	0.14
95% CI	361.4 – 574.3	280.7 – 459.2	

[1]Unpaired t-test, [2]Mann- Whitney test, * statistically significant, #adjusted p-value
CSF: Cerebrospinal fluid, SD = Standard deviation,
IQR = Interquartile range (expressed as the difference in the range)

Table 8. CSF AD biomarker's concentration differences between arms at week 24

Biomarker	Placebo arm	Lithium arm	P-value
sAPPα (pg/ml)[1]			
Number	8	8	
Mean (SD)	246.4 (94.08)	130.3 (64.60)	0.01*
Median (IQR)	293.5 (161.8)	119.0 (131.05)	0.05#
95% CI	167.7 – 325.0	76.24 – 184.3	
sAPPβ (pg/ml)[2]			
Number	8	8	
Mean (SD)	572.6 (222.5)	330.5 (147.0)	0.05#
Median (IQR)	700.0 (365.5)	319.5 (290)	0.02*
95% Cl	386.6 – 758.6	207.6 – 453.4	
Aβ38 (pg/ml)[2]			
Number	8	8	
Mean (SD)	2010 (865.7)[1]	1443 (719.7)	
Median (IQR)	1986 (1 122)	1377 (1 426.7)	0.18
95% CI	1286 - 2734	841.7 - 2045	
Aβ40 (pg/ml)[1]			
Number	8	8	
Mean (SD)	5105 (1948)	3892 (1860)	
Median (IQR)	4965 (3 162)	3742 (3 560)	0.22
95% CI	3476 - 6734	2337 - 5446	
Aβ42 (pg/ml)[1]			
Number	8	8	
Mean (SD)	448.5 (187.6)	348.6 (183.4)	
Median (IQR)	395.5 (275)	315.5 (331.2)	0.29
95% CI	291.6 – 605.4	195.3 – 502.0	

[1]Unpaired t-test, [2]Mann- Whitney test, * statistically significant, #adjusted p-value
CSF: Cerebrospinal fluid, SD = Standard deviation,
IQR = Interquartile range (expressed as the difference in the range)

Table 9. Placebo arm Blood HAND biomarkers changes from week 0 to week 24

Biomarker	Week 0	Week 24	Differences	P-value
CD8+ Activation[1](%)				
Number	26	26		
Mean (SD)	4.7 (3.5)	5.1 (3.6)	0.34 (3.1)	
Median (IQR)	4.1 (2.1 – 5.5)	4.2 (3 – 6.1)	0.53 (-1 – 2.1)	0.30
95%CI	3.3 – 6.2	3.62 – 6.5	-0.9 – 1.6	
DA[1] (ng/ml)				
Number	28	28		
Mean (SD)	16.01 (3.73)	11.93 (2.61)	-4.09 (3.54)	<0.0001*
Median (IQR)	15.9 (15 – 18)	11.75 (10.38 – 13.42)	-3.67 (-6.87 – -1.71)	0.002#
95% CI	14.57 – 17.46	10.91 – 12.94	-5.46 –2.71	
BDNF[1] (ng/ml)				
Number	28	28		
Mean (SD)	37.73 (27.16)	36.66 (25.47)	-1.062 (21.88)	0.49
Median (IQR)	35.6 (22 – 46)	28.6 (19.2 – 50)	-4.809 (-20 - 11)	
95% CI	27.20 – 48.26	26.79 – 46.54	-9.547 – 7.423	
NfL[1] (pg/ml)				
Number	30	30		
Mean (SD)	6.07 (2.90)	6.72 (4.67)	0.64 (2.97)	
Median (IQR)	5 (4.4 – 7.8)	5.02 (4 – 7.6)	-0.14 (-0.97 – 1.8)	0.78
95%CI	4.99 – 7.16	4.97 – 8.46	-0.74 - 1.11	

[1]Wilcoxon test,[2]Parametric paired t-test, [3]Unpaired Man Whitney Test, * statistically significant, #adjusted p-value
DS: Descriptive statistics, HAND: HIV- associated neurocognitive disorder, AD: Alzheimer's dementia,
DA: Dopamine, NfL: Neurofilament light chains, SD: standard déviation, IQR: Interquartile range

Table 10. Placebo arm: Blood AD biomarkers changes from week 0 to week 24

Biomarker	Week 0	Week 24	Differences	P-value
ApoC3[1] (mcg/ml)				
Number	30	30		
Mean (SD)	3.91 (2.22)	3.95 (2.19)	0.042 (2.541)	0.74
Median (IQR)	3.17 (2.39 – 4.49)	3.34 (2.8 – 4.26)	0.040 (-0.62 – 1.05)	
95%CI	3.08 – 7.74	3.136 – 4.770	-0.905 – 0.992	
Pre-Albumin[1] (mg/dL)				
Number	30	30		
Mean (SD)	6.59 (2.9)	7 (2.98)	0.41 (3.1)	0.45
Median (IQR)	6.18 (4.5 – 7.8)	6.64 (4.9 – 8.4)	0.9 (-1.6 – 2.3)	
95% CI	5.5 – 7.7	5.9 – 8.1	-0.75 – 1.59	
AGP[1] (mcg/ml)				
Number	27	27		
Mean (SD)	263.4 (161.9)	225.5 (138.3)	-37.88 (196.20)	0.22
Median (IQR)	229.5 (165.2 – 333.5)	179.5 (140.9 – 297.9)	-53.83 (-161.9 – 40.61)	
95% CI	199.3 – 327.5	170.8 – 280.20	-115.50 – 39.73	
A1AT[1] (mcg/ml)				
Number	27	27		
Mean (SD)	48.89 (5.729)	56.69 (24.36)	8.096 (25.70)	0.43
Median (IQR)	49.08 (44.85 – 53.22)	48.92 (46.84 – 58.02)	-2.00 (-4.59 – 13.56)	
95% CI	46.33 – 50.86	47.05 – 66.33	-2.072 – 18.26	
PEDF[1] (mcg/ml)				
Number	30	30		
Mean (SD)	5.19 (1.52)	6.72 (6.17)	1.52 (6.65)	0.66
Median (IQR)	5.22 (4.13 -6.21)	5.17 (4.21 – 6.94)	0.16 (-1.02 -1.55)	
95% CI	4.63 – 5.77	4.42 – 9.03	-0.96 – 4.00	
CC4[1] (mcg/ml)				
Number	28	28		0.43
Mean (SD)	138.4 (28.22)	150.6 (49.98)	12.14 (51.38)	
Median (IQR)	142.6 (109.9 – 156.2)	140.7 (113.3 –167.3)	1.76 (-8.29 –17.61)	
95% CI	127.5 – 149.4	131.2 – 169.9	-7.778 – 32.07	
ICAM-1[2] (ng/ml)				
Number	30	30		
Mean (SD)	1967 (736.4)	1868 (656.1)	-98.29 (1005)	0.59
Median (IQR)	2188 (1365 – 2439)	1782 (1448 – 2231)	-331.9 (-969 –982)	
95% CI	1668 – 2242	1623 – 2114	-473.7 – 277.1	
RANTES[1] (ng/ml)				
Number	30	30		
Mean (SD)	474.1 (182.5)	471.6 (417.3)	-2.480 (371.7)	0.12
Median (IQR)	441.3 (340.5 – 581.9)	378.8 (283 – 506.0)	-9.9(-167– 68.44)	
95% CI	405.91 – 542.2	315.8 – 627.4	-141.3 – 136.3	
Clusterin[2] (mcg/ml)				
Number	30	30		
Mean (SD)	1233 (288.8)	1179 (345.8)	-53.94 (410.5)	0.48
Median (IQR)	1245 (1065 – 1423)	1168 (876 –1559)	-87.88 (-281.9 – 206.6)	
95% CI	1125 – 1341	1050 – 1308 [2]	-207.2 – 99.35	
Cystatin[3] (mcg/ml)				
Number	8	6		
Mean (SD)	1.23 (0.15)	1.28 (0.17)	-----	0.38
Median (IQR)	1.18 (1.13 – 1.38)	1.24 (1.18 – 1.38)		
95% CI	1.35 – 1.35	1.45 – 1.45		

[1]Wilcoxon test,[2]Parametric paired t-test, [3] Unpaired Man Whitney Test, * statistically significant
DS: Descriptive statistics, HAND: HIV- associated neurocognitive disorder, AD: Alzheimer's dementia, DA: Dopamine, NfL: Neurofilament light chains, SD: standard deviation, IQR: Interquartile range

Table 11. Lithium arm: HAND blood biomarkers change from week 0 to week 24

Biomarker	Week 0	Week 24	Differences	P-value
CD8+ Activation[1](%)				
Number	24	24		
Mean (SD)	6.5 (6.1)	6.1 (5.1)	-0.37 (4.9)	
Median (IQR)	4.9 (2.8 – 7)	4.7 (2.7 – 8.7)	-0.69 (-2.1 – 2.3)	0.88
95%CI	3.9 – 9	3.9 – 8.3	-2.4 – 1.7	
DA[1] (ng/ml)				
Number	29	29		
Mean (SD)	15.76 (2.21)	11.76 (3.83)	-4.00 (4.42)	<0.0001*
Median (IQR)	15.21 (14 – 17)	11.51 (10 – 13)	-3.80 (-7 – -2)	0.002#
95% CI	14.92 – 16.60	10.31 – 13.22	-5.69 – 2.32	
BDNF[1] (ng/ml)				
Number	28	28	28	
Mean (SD)	40.26 (20.27)	34.96 (19.14)	-5.306 (14.80)	0.05
Median (IQR)	36.51 (27 – 54)	29.63 (22 – 52)	-5.059 (-15 – 4)	
95% CI	32.40 – 48.12	27.54 – 42.38	-11.04 – 0.43	
NfL[1] (pg/ml)				
Number	29	29		
Mean (SD)	5.38 (2.31)	5.52 (2.97)	0.14 (2.56)	
Median (IQR)	4.91 (3.6 – 6.5)	4.78 (3.9 – 6.3)	-0.18 (-0.9 – 0.33)	0.37
95%CI	4.50 – 6.26	4.39 – 6.65	-0.68 – 0.31	

[1]Wilcoxon test, [2]Parametric paired t-test, [3] Unpaired Man Whitney Test, * statistically significant, #adjusted p-value
HAND: HIV-associated neurocognitive disorder, AD: Alzheimer's dementia, NfL: Neurofilament light chains
DA: Dopamine, SD: Standard deviation, IQR: Interquartile range

Table 12. Lithium arm: AD blood biomarkers change from week 0 to week 24

Biomarker	Week 0	Week 24	Differences	P-value
ApoC3[1] (mcg/ml)				
Number	29	29		
Mean (SD)	3.87 (1.63)	4.25 (2.02)	0.38 (2.19)	0.49
Median (IQR)	3.49 (2.74 – 4.9)	3.68 (2.98 – 4.8)	-0.01 (-0.7 – 1.3)	
95%CI	3.25 – 4.49	3.48 – 5.01	-0.45 – 1.21	
Pre-Albumin[1] (mg/dL)				
Number	29	29		
Mean (SD)	6.67 (3.35)	7.34 (3.64)	0.67 (2.79)	0.45
Median (IQR)	5.78 (4.32 – 9)	6.13 (5 – 9)	0.47 (-1.4 – 2.3)	
95% CI	5.39 – 7.95	5.96 – 8.73	-0.39 – 1.73	
AGP[1] (mcg/ml)				
Number	24	24		
Mean (SD)	214.6 (171.60)	208.1 (113.5)	-6.52 (188.1)	0.68
Median (IQR)	183 (137 – 269)	207 (125 – 261)	-15.19 (-93 – 74)	
95% CI	142.1 – 287.10	160.2 – 256.0	-85.95 – 72.91	
A1AT[2] (mcg/ml)				
Number	24	24		
Mean (SD)	48.66 (6.33)	49.29 (6.68)	0.64 (8.35)	0.71
Median (IQR)	48.07 (45 – 54)	49.77 (45 – 54)	-2.02 (-4.1 – 9)	
95% CI	45.98 – 51.33	46.48 – 52.11	-2.89 – 4.16	
PEDF[1] (mcg/ml)				
Number	28	28		
Mean (SD)	5.49 (1.65)	5.22 (0.95)	-0.28 (1.65)	0.67
Median (IQR)	5.09 (4.3 – 6.2)	5.26 (4.4 – 5.6)	-0.27 (-1 – 0.7)	
95% CI	4.86 – 6.14	4.85 – 5.59	-0.92 – 0.36	
CC4[1] (mcg/ml)				
Number	27	27		0.56
Mean (SD)	160.8 (44.99)	157.3 (35.81)	-3.59 (41.60)	
Median (IQR)	151 (124 – 190)	159 (133 – 175)	-3.020(-13 – 5)	
95% CI	143.11 – 178.6	143.1 – 171.4	-20.05 – 12.86	
ICAM-1[1] (ng/ml)				
Number	29	29		
Mean (SD)	2105 (1111)	2055 (1695)	-49.76 (1194)	0.62
Median (IQR)	1985 (1383 – 2542)	1907 (1176 – 2395)	-15.13 (-852 – 282)	
95% CI	1682 – 2525	1410 – 2700	-503.2 – 404.3	
RANTES[1] (ng/ml)				
Number	29	29		
Mean (SD)	481.1 (258.1)	488.2 (252.6)	7.14 (305.9)	0.70
Median (IQR)	429(340 – 572)	439 (360 – 551)	-6.7 (-151 – 214)	
95% CI	382.9 – 579.3	392.2 – 584.3	-109.2 – 123.5	
Clusterin[2] (mcg/ml)				
Number	29	29		
Mean (SD)	1289 (379.7)	1143 (270.8)	-145.9 (436.2)	0.08
Median (IQR)	1308 (968 – 1565)	1118 (919 – 1292)	-91.3 (-485 – 191)	
95% CI	1145 - 1433	1040 - 1246	-311.8 – 20.01	
Cystatin[3] (mcg/ml)				
Number	7	3		
Mean (SD)	1.40 (0.27)	1.33 (0.16)	------	0.83
Median (IQR)	1.35 (1.2 – 1.6)	1.38 (1.15 – 1.5)		
95% CI	1.16 – 1.65	0.93 – 1.73		

[1]Wilcoxon test, [2]Parametric paired t-test, [3] Unpaired Man Whitney Test, * statistically significant
HAND: HIV-associated neurocognitive disorder, AD: Alzheimer's dementia, NfL: Neurofilament light chains
DA: Dopamine, SD: Standard deviation, IQR: Interquartile range

Table 13. Placebo arm: HAND CSF biomarkers change from week 0 to week 24

Biomarker	Week 0	Week 24	Difference	P-value
DA[2] (ng/ml)				
Number	4	4		
Mean (SD)	1.95 (1.06)	1.18 (0.61)	-0.77 (1.04)	
Median (IQR)	2.28 (0.8 – 2.7)	0.97 (0.8 – 1.8)	-0.89 (-1.7 – 0.28)	0.38
95% CI	0.27 – 3.63	0.21 – 2.15	-2.43 – 0.89	
BDNF[2] (ng/ml)				
Number	5	5		
Mean (SD)	0.93 (0.65)	1.52 (0.65)	0.59 (0.75)	
Median (IQR)	0.54 (0.44 – 1.6)	1.31 (1 – 2.2)	0.62 (-0.06 – 1.2)	0.19
95% CI	0.12 – 1.73	0.72 – 2.33	-0.33 – 1.53	
NfL[2] (pg/ml)				
Number	6	6		
Mean (SD)	384.0 (88.69)	392.3 (124.0)	8.333 (149.2)	>0.99
Median (IQR)	361.5 (306 – 480)	341.0 (303 – 535)	-8.50 (-90.5 – 103)	
95% CI	290.9 – 477.1	262.2 – 522.4	-148.3 – 164.9	

[1]Paired student-test,[2]Wilcoxon, * statistically significant,
CSF: Cerebrospinal fluid, HAND: HIV-associated neurocognitive disorders, AD: Alzheimer's dementia, NfL: Neurofilament light chains,
BDNF: Brain-derived neurotrophic factor, DA: Dopamine, SD: Standard deviations, IQR: Interquartile range, LP: lumbar puncture

Table 14. Placebo arm: AD CSF biomarkers change from week 0 to week 24

Biomarker	Week 0	Week 24	Difference	P-value
Aβ38[2] (pg/ml)				
Number	6	6		
Mean (SD)	1794 (538.5)	1873 (464.1)	79.33 (392.6)	0.56
Median (IQR)	2036 (1302 – 2186)	1986 (1441 – 2221)	235.0 (-223 – 324)	
95% CI	1229 – 2359	1386 – 2360	-332.7 – 491.3	
Aβ40[1] (pg/ml)				
Number	6	6		
Mean (SD)	5055 (1569)	4919 (1296)	-136.2 (145)	
Median (IQR)	5199 (3754 – 6357)	4965 (3768 – 6152)	359.0 (-834 – 751)	0.83
95% CI	3408 – 6701	3558 – 6279	-1659 – 1389	
Aβ42[1] (pg/ml)				
Number	6	6		
Mean (SD)	456.3 (167.4)	418.8 (112)	-37.50 (151.2)	
Median (IQR)	468.5 (331 - 602)	395.5 (353 – 510)	28.50 (-212 – 76)	0.57
95% CI	280.6 – 632.0	301.7 – 535.9	-196.2 – 121.2	
sAPPα[2] (pg/ml)				
Number	6	6		
Mean (SD)	225.8 (63.65)	265.8 (75.13)	40.00 (21.79)	0.03*
Median (IQR)	247.5 (177 – 270)	298.5 (193 – 323)	38.50 (25 – 53)	0.15#
95% CI	159.0 – 292.6	187.0 – 344.7	17.13 – 62.87	
sAPPβ[2] (pg/ml)				
Number	6	6		
Mean (SD)	527.0 (152.1)	607.7 (192.5)	80.67 (92.82)	
Median (IQR)	526.5 (406 – 686)	700 (371 – 739)	84.5(9 – 163)	0.16
95% CI	367.4 – 686.6	405.6 – 809.7	-16.74 – 178.1	

[1]Paired student-test,[2]Wilcoxon, * statistically significant, #adjusted p value
CSF: Cerebrospinal fluid, HAND: HIV-associated neurocognitive disorders, AD: Alzheimer's dementia, NfL: Neurofilament light chains,
BDNF: Brain-derived neurotrophic factors, DA: Dopamine, SD: Standard deviations, IQR: Interquartile range, LP: lumbar puncture

Table 15. Lithium arm: HAND CSF biomarkers change from week 0 to week 24

Biomarker	Week 0	Week 24	Difference	P-value
DA[2] (ng/ml)				
Number	2	2	N/A	
Mean (SD)	2.90 (3.18)	0.95 (0.89)		
Median (IQR)	2.90 (0.65 – 5.2)	0.95 (0.32 – 1.58)		Few pairs
95% CI	-25.67 – 31.48	-7.02 – 8.91		
BDNF[2] (ng/ml)				
Number	4	4		
Mean (SD)	1.41 (0.48)	1.18 (0.28)	-0.22 (0.35)	
Median (IQR)	1.424 (0.94 – 1.85)	1.23 (0.89 – 1.42)	-0.29 (-0.51 – 0.13)	0.38
95% CI	0.642 – 2.17	0.739 – 1.63	-0.79 – 0.34	
NfL[1] (pg/ml)				
Number	6	6		
Mean (SD)	362.8 (177.0)	338.7 (178.1)	-24.17 (40.44)	
Median (IQR)	352.0 (206 – 519)	302.5 (182 – 516)	-11.50 (-51 – 3)	0.20
95% CI	177 – 548.6	151.8 – 525.6	-66.61 – 18.28	

[1]Paired student-test,[2]Wilcoxon, * statistically significant
CSF: Cerebrospinal fluid, HAND: HIV-associated neurocognitive disorders, AD: Alzheimer's dementia, NfL: Neurofilament light chains, BDNF: Brain-derived neurotrophic factor, DA: Dopamine, SD: Standard deviations, IQR: Interquartile range, LP: lumbar puncture

Table 16. Lithium arm: AD CSF biomarkers change from week 0 to week 24

Biomarker	Week 0	Week 24	Difference	P-value
Aβ38[1] (pg/ml)				
Number	6	6		
Mean (SD)	1126 (570.1)	1125 (486.1)	-0.833 (146.8)	
Median (IQR)	1045 (615 – 1722)	999.0 (659 – 1717)	-0.5 (-130 - 108)	0.99
95% CI	527.9 – 1724	615.2 – 1635	-154.9 – 153.2	
Aβ40[1] (pg/ml)				
Number	6	6		
Mean (SD)	3134 (1748)	3114 (1374)	-19.33 (470.3)	
Median (IQR)	3016 (1544 – 4591)	2803 (1905 – 4527)	-20.00 (-354 – 361)	0.92
95% CI	1299 – 4968	1672 – 4556	-512.9 – 474.2	
Aβ42[1] (pg/ml)				
Number	6	6		
Mean (SD)	260.7 (161.7)	267.5 (118.8)	6.833 (50.69)	
Median (IQR)	248.0 (114 – 396)	251.0 (160 – 375)	15.50 (-41 – 46)	0.76
95% CI	91.02 – 430.3	142.8 – 392.2	-46.37 – 60.03	
sAPPα[2] (pg/ml)				
Number	6	6		
Mean (SD)	114.7 (63.96)	119.2 (72.05)	4.500 (16.24)	0.56
Median (IQR)	81.50 (71 – 191)	81.00 (68 – 211)	8.5(-11 – 16)	
95% CI	47.55 – 181.8	43.56 – 194.8	-12.55 – 21.55	
sAPPβ[2] (pg/ml)				
Number	6	6		
Mean (SD)	400.2 (297.9)	298.5 (157.8)	-101.7 (291.3)	
Median (IQR)	320.0 (182 – 603)	235.5 (165 – 487)	18.50 (-202 – 37)	0.43
95% CI	87.59 – 712.7	132.9 – 464.1	-407.4 – 204.0	

[1]Paired student t-test,[2]Wilcoxon, * statistically significant
CSF: Cerebrospinal fluid, HAND: HIV-associated neurocognitive disorders, AD: Alzheimer's dementia, NfL: Neurofilament light chains, BDNF: Brain-derived neurotrophic factors, DA: Dopamine, SD: Standard deviations, IQR: Interquartile range, LP: lumbar puncture

Parent study methodology

The information contained in this document is directly (word for word) taken from the protocol as well as the published article. This is not the original work of the candidate but additional information to satisfy the recommendations from the examiners.

1. A randomized controlled trial of lithium carbonate in individuals with HIV clade C-associated neurocognitive impairment: a phase IIb proof of principle study (Study protocol) (Eric Decloedt et al)
2. Moderate to severe HIV-associated neurocognitive impairment: A randomized placebo-controlled trial of lithium: (Decloedt et al., 2016)
3. A randomized trial of lithium in HIV-associated neurocognitive disorders: neuromodulatory effects using a multi-modal approach (Decloedt et al – unpublished).

Participant recruitment and methodology of the parent study

The participants were enrolled mainly from the Nolungile Site C clinic in Khayelitsha and followed up at Groote Schuur Hospital, University of Cape Town. A total of 66 patients with moderate to severe HAND were enrolled in the study. Participants were assigned to either lithium or a placebo. On the first visit, demographic information, global deficit score (GDS), and biomarkers were collected. The above baseline data sets were collected again after 24 weeks (the end of taking lithium or a placebo). The study was approved by the University of Cape Town (071/2013) and the University of Stellenbosch (M13/07/027). Its registration number or identifier is PACTR201310000635418.

Inclusion criteria

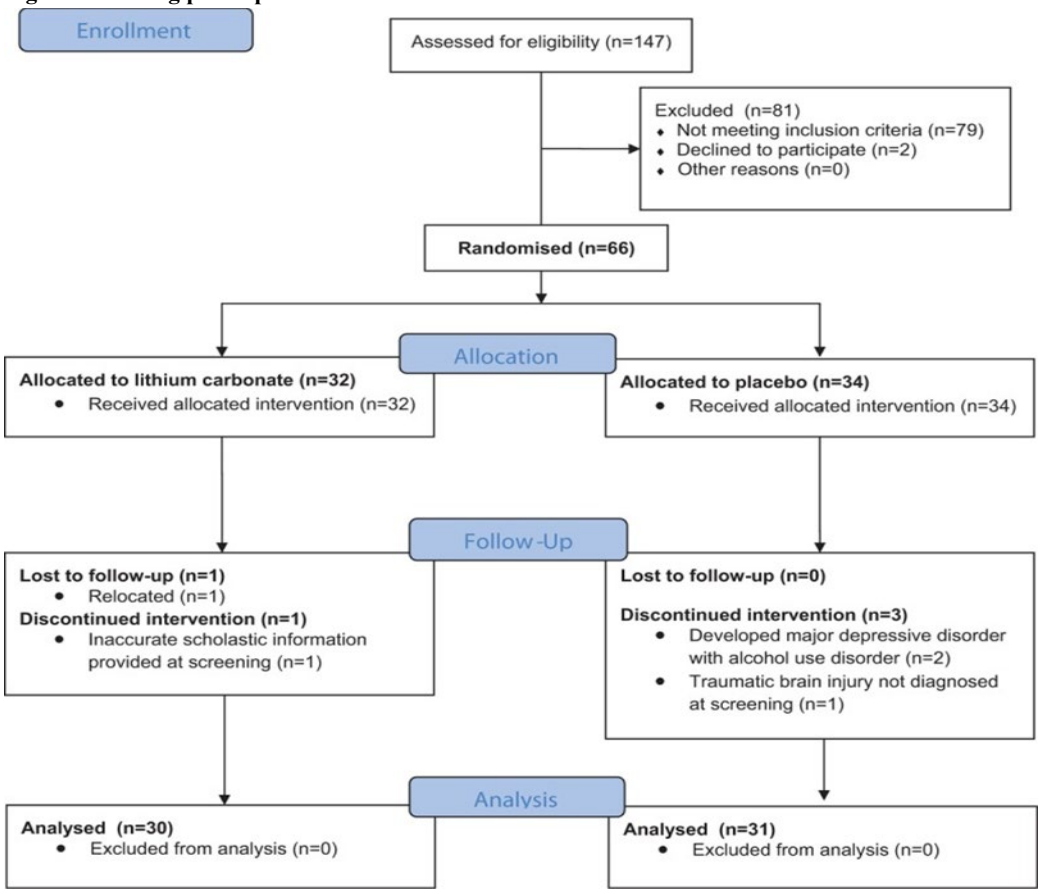
- Participants were HIV-infected adults (≥18 and ≤70 years)
- ART for at least 6 months,
- Suppressed viral load (blood HIV RNA < 400 copies/ml)

- Cognitive impairment as defined by a GDS ≥ 0.5

Exclusion criteria

- Participants on Lithium within 30 days
- Active acquired immune deficiency syndrome (AIDS)-defining opportunistic infection, history of drug or alcohol abuse within 3 months before screening, had a positive urine drug screen for drugs of abuse (amphetamine, benzodiazepine, cannabis, cocaine, opiate),
- Confirmed neurosyphilis or vitamin B12 deficiency,
- Imaging structural abnormalities,
- Significant head injury or severe mental illness.

Figure 1 showing participant recruitment and treatment arm allocation



Ethical considerations

Each participant's caretaker was also required to sign an informed consent form as it was anticipated that the ability of participants to provide consent may vary (participants may understand their need for ART and impaired memory, but they may not recall all aspects of the study procedures and risks). Every study visit was accompanied by a caregiver.

Minimizing the adverse effects of the investigational drugs

- Electrocardiogram (ECG) QTc of less than 450ms for males and less than 470ms for females
- We excluded participants at increased risk of lithium toxicity such as the presence of thyroid disease, renal impairment (as defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min) and those with chronic diarrhea Hydration state were

Statistical methods

For a power of 90% at alpha 0.05, we required 49 participants per arm to detect an absolute change in GDS of 0.25. We sought to enrol 54 participants in each arm to account for a 10% loss to follow-up or withdrawal. Previous research has shown that ART alone improved

the GDS by a mean of 0.13 and 0.6 in patients with a GDS in the mild to moderate (>0.25 to <0.75) and severe (>0.75) ranges, respectively. Twelve-week adjunctive lithium therapy in patients stable on ART improved the GDS by 0.3 and we opted to detect a more conservative GDS difference of 0.25 with a standard deviation of 0.375, which was calculated using the range in the published studies divided by 4. We conducted an intention-to-treat and per-protocol analysis for the primary endpoint. For the intention-to-treat analysis, we carried over the last data points when the week 24 endpoints were missing, example for missing GDS at week 24 we used GDS at enrolment.

Selection of biomarkers

CSF at baseline and at the final visit markers

- Viral load.
- Cells.
- Protein (serum/CSF albumin ratio).
- Lithium carbonate concentrations (last visit);
- Putative biomarkers such as amyloid protein, neopterin, dopamine, BDNF, and clusterin;
- Dopamine and dopamine metabolites.
- Inflammatory markers such as IL6/2 and TNF alpha.

Plasma baseline and at the final visit markers

- GSK-3-ß in PBMC.
- Expression of dopamine receptor protein and gene expression of sub-types 2 and 5 in PBMC;
- BDNF.

BDNF and DA measurements

Plasma (all patients) and CSF (n=35 at baseline and n=18 at Week 24) samples were collected at baseline and at week 24 and subjected to BDNF-ELISA. Plasma and CSF samples were inactivated at 56°C for 30 minutes. BDNF (Abcam) and DA (Abnova) concentrations were determined by commercially available ELISA kits according to the instructions of the manufacturers. Briefly, for the BDNF ELISA, plasma samples were diluted at a ratio of 1:4 prior to ELISA, CSF samples were analyzed undiluted. For the DA ELISA, plasma and CSF samples were analyzed undiluted. Data was analyzed using GraphPad prism software (Prism 6.0 for MacOSX). D'Agostino Pearson test was used for normal distributed data. We used a student's t-test to compare the means of two data sets or one-way ANOVA to compare the means of multiple data sets for normal-distributed data. For non-normal distributed values, we used the Mann-Whitney U-test for two data sets or the Kruskal-Wallis test for multiple data sets. Dependent values were analyzed using a paired student's t-test (for normal-distributed values) or a Wilcoxon-test (for non-normal-distributed values). Potential correlations of values were analyzed by linear regression. Differences or correlations with an associated p-value < 0.05 were regarded as statistically significant.

Plasma proteins associated with AD severity

We selected 10 plasma proteins to measure using multiplex bead assays (Luminex xMAP): acid glycoprotein (A1AcidG), apoliproteinC3 (ApoC3), prealbumin, alpha-1 antitrypsin (A1AT), pigment epithelium-derived factor (PEDF), complement component 4 (CC4), intercellular adhesion molecules (ICAMs), regulated on activation, normal T cell expressed and secreted (RANTES) and clusterin. Median fluorescent intensity (MFI) of the xMAP assays was measured using the xPONENT 3.1 (Luminex Corporation) The MFI were exported into Sigma Plot (Systat Software; Version 12.5) for estimation of protein concentrations using a 5-parameter logistic fit. Data quality control checks were performed for each xMAP assay. Data quality was assessed using the following parameters: proportion of missing data points, intra-assay performance (% coefficient of variation [CV] of the duplicate measures of the samples), inter-assay performance (% CV of the optical densities of standards 1-4) and quality of the standard curve. Univariate statistical analysis was performed in SPSS 22 (IBM). All protein concentration values were log10 transformed to achieve normal distribution of the data. The association of the covariates age, gender, assay variability with the proteomic data was examined. The majority of the proteins were affected by assay variability. Therefore, a generalized linear regression model (GLM) was performed to adjust the data for assay variabilty, and all subsequent analyses were performed using the GLM adjusted data. The impact of assay variability with Luminex

data was sufficiently corrected by GLM. T-test was performed to investigate changes in group analysis and Pearson R correlation used to observe associations between proteins and cognitive scores.

Schedule of measures during the study (Protocol: Li in HAND RCT- 28 August 2014 version 4)

Week	-4 to 0			1	2	4	6	8	10	12	14	16	18	20	22	23	24
	Visit 1 (Screening)	Visit 2 (MRS visit)	Visit 3 (Dispensing visit)	Visit 4	Visit 5	Visit 6		Visit 7		Visit 8		Visit 9		Visit 10		Visit 11 (MRS visit)	Visit 12 (Final)
Informed consent	x																
Medical history	x																
Physical exam	x					x		x		x		x		x			x
Height	x																
Weight	x					x		x		x		x		x			x
Lithium plasma concentration				x	x	x		x		x		x		x			x
Haematology[1]	x																x
Chemistry[2]	x					x		x		x		x		x			x
HIV viral load	x																x
β-HCG	x																x
Drug screen[3]	x																
ECG[4]	x					x		x		x		x		x			x
CD4+ count	x																x
Neuropsychological battery[5]	x																x
CSF sampling[6]			x														x
Genotyping[7]	x																x
Tremor measurement[8]	x			x	x	x		x		x		x		x			x
Plasma sampling for exploratory markers[9]			x														x
MRS		x														x	
Investigational drug dispensing			x			x		x		x		x		x			
Adverse events	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→

List of biomarkers collected, and quantity analysed in the secondary study.

Biomarker	Placebo group Baseline	Placebo group week 24 (participants)	Lithium group baseline (participants)	Lithium group week 24 (participants)
Cerebrospinal fluid				
Neurofilament light chains	16	08	16	08
sAPPα	17	08	17	08
sAPPβ	17	08	17	08
Aβ38	17	08	17	08
Aβ40	17	08	17	08
Aβ42	07	08	17	08
CSF Dopamine	17	06	16	08
CSF BDNF	20	07	16	08
Blood				
Neurofilament light chains	34	30	31	29
ApoC3	34	30	31	29
Pre-albumin	34	30	31	30
AGP-1	33	29	28	28
A1AT	29	30	25	28
PEDF	34	30	31	28
CC4	C	28	30	27
ICAM-4	34	30	31	29
RANTES	34	30	31	29
Clusterin	34	30	31	29
Cystatin	08	06	07	03
Plasma Dopamine	33	29	31	29
Plasma BDNF	32	29	30	29