**The neurological phenotype of patients with HIV associated neurocognitive disorder (HAND) on antiretroviral therapy in South Africa**

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# Abstract

**Background**

The neurologic manifestations of HIV include a spectrum of HIV associated neurocognitive disorders (HAND), as well as a cluster of neurologic symptoms and signs. The combined phenotype has been modified but not eradicated by antiretroviral therapy (ART). The effects of clade on neurocognitive impairment have been explored in some cohorts, but there are no data from South Africa where clade C predominates. We aimed to describe neurological manifestations in HIV-C infected patients with HAND established on ART and correlate with neurocognitive impairment. In addition, we explored associations between efavirenz (EFV) and its metabolite (8-hydroxy efavirenz [OH-8-EFV]) with neurological manifestations.

**Methods**

We conducted cross sectional analyses of the neurological examination findings of participants with well-characterised HAND. Neurocognitive function was summarized using the Global Deficit Score (GDS). Neurological examination data was grouped into clusters made up of clinically related neurological signs. Multiple linear regression models were used to explore associations between neurological clusters and neurocognitive function, as well as plasma and cerebrospinal EFV and OH-8-EFV concentrations.

**Results**

We included 80 participants. 90% were female with a median age of 35 (interquartile-range (IQR) 32 – 42) and a median GDS of 0.94 (IQR 0.63-1.36). The patients were established on ART for a median of 40 months and at entry had a median CD4+ T-cell count of 498 cell/mm3. We found statistical significant associations between HIV-associated neurocognitive impairment severity and neurological manifestations: gait [slow walking speed (p=.03; R2=.06), gait ataxia (p<.01; R2=.21), abnormal gait appearance (p<.01; R2=.18)]; coordination [upper extremity bradykinesia (p<.01; R2=.10) and lower extremity bradykinesia (p=.01; R2=.10)]; primitive reflexes [jaw jerk (p=.04; R2=.05) and palmo-mental response (p=.03; R2=.06)]; smooth pursuit (p=.01; R2=.09) and saccades (p<.01; R2=.15). No significant associations were found between plasma and cerebrospinal EFV or OH-8-EFV concentrations and any neurological sign.

**Conclusion**

We found the neurological sign clusters of gait, coordination, primitive reflexes, smooth pursuits and saccades were associated with GDS and could be used in clinical practice to guide the assessment of HAND severity.

# 1. Introduction

Mild forms of HIV-associated neurocognitive disorder (HAND) remain prevalent in combination antiretroviral therapy (cART) experienced patients with rates reported to be as high as 45%.1 These mild forms include asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND). ANI may not interfere with everyday functioning, while MND, and the most severe HAND- HIV-dementia (HIV-D) cause mild to severe interference with day-to-day functioning. The cornerstone of diagnosis is neuropsychological testing. Performance below 1 SD from the norm in at least 2 domains, is indicative of impairment. 2

However, the diagnosis of HAND has various challenges. The high prevalence of HAND has been proposed by some to result from the administration of detailed batteries, minimizing above-average performance, and using summative approaches which increase sensitivity, yet risks a high false-positive rate.3The use of a 1 SD below mean cut-off has been suggested to be too liberal, therefore contributing to a high false-positive rate.4–6 In addition, detailed neuropsychological (NP) assessments are the gold standard for HAND diagnosis that are not always practical in the clinical setting.2,7 Therefore clinicians initially rely on various screening tools, functional assessments and/or limited NP tests for diagnosis.7 The screening tests, including the International HIV dementia scale (IHDS), are limited in their ability to detect milder forms of HAND with a high false-negative rate.8

Neurological signs found on clinical examination are not currently included in HAND definitions2, although abnormalities in gait, coordination, frontal release signs as well as ocular signs in the form of impaired smooth pursuit and saccades have been associated with HAND.9–16 It has been previously suggested to include primitive reflexes, which can be assessed during the clinical examination, in the case definition of HAND.16 The inclusion of neurologic signs in case definitions may bolster clinical assessment when NP testing availability is low.

Clade differences may influence HAND presentation.17 Pre-clinical data suggested that a substitution in HIV-C vs HIV-B reduced neuro-virulence.18 Clinical and imaging studies in South Africa confirm that the tat substitution conferred similar neuro-toxicity.19 The neurological phenotype, however, has only been described in a clade B predominant setting and not in the predominant clade C HIV infected sub-Saharan Africa. 9–16

An additional theoretical driver of persistent HAND, is ART-associated neurotoxicity. Efavirenz and its major metabolite, 8-hydroxy-efavirenz (8-OH-EFV) in particular have been implicated in neurotoxicity and may contribute to the phenotype of HAND.20,21 In a randomised controlled trial (RCT), patients starting efavirenz-based cART rather than protease inhibitors or all-nucleoside reverse transcriptase inhibitor regimens had less improvement in neurocognitive function scores after 48 weeks.22 Patients from the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) cohort who received efavirenz performed worse in several cognitive domains compared with protease inhibitor users after more than a year of ART.23 Additionally, a recent case series report of 20 participants describes late onset efavirenz-induced ataxia and encephalopathy secondary to supra-therapeutic concentrations of efavirenz in underweight and presumed genetic slow metabolizers.24

The primary objective of our study was to investigate associations between neurological sign and symptom clusters and the degree of neurocognitive impairment in HIV-infected South Africans with a predominant clade C HIV infection. Our secondary objective was to explore the pharmacokinetic relationship of CSF and plasma efavirenz and 8-OH-EFV with neurological sign and symptom clusters.

# 2. Methods

**Participants:**

We included adults (≥ 18 and ≤ 70 years) who were screened and/or participated in a RCT (PACTR201310000635418) investigating lithium as adjunctive therapy in patients with HAND.25 Participants were established on ART for at least 6 months prior to the study with suppressed viral loads (HIV PCR <400copies/mL).

**Neurological assessment**

Participants received a full neurological examination by medical practitioners who had received additional training in the neurological evaluation. All neurological assessments were reviewed by the onsite study neuropsychiatrist. Each neurological sign and symptom was assessed according to a standardised neurological assessment and quantified based on a previously defined tool.26,27 The neurological examination included the evaluation of certain neurological categories that included peripheral neuropathy (visual analogue scale, vibration perception and ankle reflexes), motor system (involuntary movement, muscle bulk, tone, reflexes and power), gait (appearance, coordination, timed gait test), limb coordination, reflexes (deep tendon reflexes, primitive reflexes (jaw jerk, snout, grasp, palmo-mental, glabellar tap and plantar response)) and other (neck stiffness, facial strength and ocular signs (saccades and smooth pursuit)). A score of 0 = sign absent and 1 = sign present with further ranking depending on the assessment (**Supplementary material 1**).26

**Neuropsychiatric assessment**

The Global deficit score (GDS), a summative neuropsychology test battery score adjusted for age, education, gender, and ethnicity was calculated for each participant.28 The domains and tests utilized included attention (Mental Alternation Test, Digit Span, Paced Auditory Serial Addition Test), learning and memory (the Hopkins Verbal Learning Test), motor speed (Finger Tapping Dominant Hand, Finger Tapping Non-Dominant Hand, Grooved Pegboard Test Dominant Hand, Grooved Pegboard. Test Non-Dominant Hand), psychomotor speed (Trail Making Test A, Color Trails Test 1, Digit Symbol-Coding), executive function (Color Trails Test 2, Stroop Color-Word Test, Wisconsin Card-Sorting Test), visual learning and memory (Rey Complex Figure), and verbal fluency (Animals and Fruit and Vegetables). Symptoms of depression were also screened for using the Center for Epidemiologic Studies Depression (CES-D) scale.

**Pharmacokinetic assessment**

Forty-six participants consented to lumbar punctures. CSF microbiology studies were reported on 36 participants while efavirenz and 8-OH-EFV pharmacokinetic data was available for all 46 participants.

***Pharmacokinetic sampling:*** Paired plasma and CSF samples for efavirenz and its metabolites were collected. Participants recorded time of efavirenz dosing the night before and were admitted the following morning for pharmacokinetic sampling. Mid-dosing lumbar punctures were performed. Whole blood was collected within 45 minutes of CSF sampling, centrifuged within 1 hour of collection, aliquoted and stored at -80°C until analysis. CSF was aliquoted and stored at -80°C until analysis.

***Efavirenz and metabolites:*** Drug assays were performed at 2 laboratories. The analytical laboratory in the Division of Clinical Pharmacology at the University of Cape Town quantified total efavirenz in plasma and CSF using validated liquid chromatography tandem mass spectrometry (LC/MS-MS) assays. The lower limit of quantification (LLOQ) for plasma efavirenz was 19.5 ng/ml. For CSF the LLOQ for total efavirenz was 0.5 ng/ml. The Bioanalytical Facility, Department of Molecular and Clinical Pharmacology at the University of Liverpool quantified total CSF 8-OH-EFV, plasma 8-OH-EFV and 7-OH-EFV in plasma and CSF samples using validated LC/MS-MS assays.29 We could not quantify CSF 7-OH-EFV. The LLOQ for CSF 8-OH-EFV, plasma 8-OH-EFV and plasma 7-OH-EFV was 3.125 ng/ml, 5.0 ng/ml and 5.0 ng/ml, respectively. Concentrations below the limit of quantification (BLQ) were analyzed as missing data.

## 2.3 Ethics

All participants had entered the trial voluntarily and gave written informed consent. The study was approved by the Stellenbosch University Health Research Ethics Committee (0578/2017) and University of Cape Town Human Research Ethics Committee (071/2013).

## 2.4 Data management

The originally collected neurological data had to be re-organized and re-coded in order to allow for effective statistical analysis. To do this a number of steps were taken. (**Figure 1**)

First, the neurological data was divided into the 6 original neurological categories (peripheral neuropathy, motor, gait, coordination, reflexes and other). Under each neurological category neurological signs and symptoms were clustered into groups of clinical relatedness forming ‘neurological sign and symptom clusters’. (**Refer to Table 1)**

Second, the individual signs and symptoms were re-coded by classifying each sign or symptom as either absent (0) or present (1). Exceptions to the re-coding rule included the signs and symptoms of peripheral neuropathy (visual analogue scale, vibration perception and ankle reflexes) and primitive reflexes (jaw jerk, snout, grasp, palmo-mental, glabellar tap and plantar response) where ranking according to severity was allowed. An example of a neurological sign and symptom cluster was the primitive reflex sign and symptom cluster which included the individual clinical signs of jaw jerk, snout reflex, grasp reflex, palmo-mental reflex, glabellar tap and the plantar response. (**Supplementary material 2**)

Third, with the data clustered and recoded, a cumulative score was calculated for each neurological sign and symptom cluster by adding their constituent sign and symptoms scores together. For example, the lower motor neuron (LMN) cluster’s cumulative score was calculated by adding the individual signs of muscle bulk, motor tone, strength, deep tendon reflexes and absent plantar response. The higher the score the more severely affected the cluster. (**Supplementary material 2**)

Four, the cumulative score was used to categorize neurological sign and symptom clusters as either normal (=0) or impaired (=1) by comparing the cumulative score to established cut-off values. If the cumulative score was below the cut-off value pathology was absent. If the cumulative score was more or equal to the cut-off value pathology was regarded as present. (**Table 2**) A cut-off ≥1 was selected for all clusters except the objective peripheral neuropathy (≥2) and primitive reflex cluster (≥2). In both of these clusters, a score of 1 would have increased the false positive rate due to broader clinical interpretation.

To be labeled with pathology in the objective peripheral neuropathy cluster the patient had to fulfill one of the three scenarios: have sufficiently decreased vibration perception score in one or both legs; have absent reflexes ­in one or both legs or have a combination of a decreased vibration perception score and hypoactive reflexes. For the primitive reflex cluster the presence of ≥2 primitive reflexes have a greater clinical significance in predicting abnormality than a solitary primitive reflex.13,16,30 For this reason the number of individual primitive reflexes, including their severity, were taken into consideration when calculating the cumulative score.

***Statistical analysis:***

Categorical data was described using proportions and depending on the number of variables, Chi-square or Fisher exact tests were used in hypothesis testing where appropriate. Numerical data distribution was assessed for normality and data were further described using means and standard deviations or medians and interquartile ranges where appropriate. (**Table 3**)

For the primary outcome, associations between GDS and the neurological sign and symptom clusters were determined using a linear regression model. (**Table 4**) Exceptions were for the Ocular sign and Deep tendon reflex clusters. Instead, associations between the cluster’s constituent signs (i.e. increased and decreased deep tendon reflexes for the Deep tendon reflex cluster; smooth pursuits and saccades for the Ocular sign cluster) and GDS were determined. Additionally, selected signs from the primitive reflex cluster (jaw jerk, snout, grasp, palmo-mental, glabellar tap and plantar response) and their association with GDS were also determined. These selected signs had been shown to correlate with HIV-associated neurocognitive impairment in the pre-ART era. A p-value of <0.05 was considered significant.

Secondary outcomes explored associations between efavirenz and 8-OH-EFV plasma and CSF concentrations, and their respective plasma: CSF ratios with neurological clusters. Linear regression models were used with a p-value of <0.05 for statistical significance.

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# 3. Results

Eighty participants were included in the study. All participants were Black Africans, with the majority being both Xhosa speaking (n=73, 91%) and female (n=72, 90%). The median GDS was 0.94 (Interquartile range, IQR 0.63 – 1.36). The baseline characteristics are further presented in **Table 3**.

We found significant associations with gait in the form of slow walking speed (p=.03; R2=.06), gait ataxia (p<.01; R2=.21) and abnormal gait appearance (p<.01; R2=.18) as well as with coordination in the form of upper extremity bradykinesia and ataxia (p<.01; R2=.10) and lower extremity bradykinesia and ataxia (p=.01; R2=.10). When looking at selected neurological signs we found significant associations in the primitive reflex cluster in the form of jaw jerk (p=.04; R2=.05) and palmo-mental response (p=.03; R2=.06) as well as in the ocular sign cluster in the form of smooth pursuits (p=.01; R2=.09) and saccades (p<.01; R2=.15). A negative association was found with the palmo-mental response. All other associations were found to be positive.

No significant associations were found with the neurological sign and symptom clusters of lower motor neuron signs, upper motor neuron: pyramidal signs, upper motor neuron: extra-pyramidal signs, deep tendon reflexes, primitive reflexes (including the individual signs of snout reflex, grasp reflex, glabellar tap and Babinski response), neck stiffness and facial strength. (**Table 4**)

Many participants experienced symptoms, and had signs suggestive of peripheral neuropathy (39%). However, in linear regression analysis both subjective and objective peripheral neuropathy were not found to be significantly associated with GDS. (**Table 4**)

CSF parameters, which included CSF -cell count, -culture, -chemistry, -albumin and CSF: serum albumin ratios showed no abnormalities when compared with the National Health Laboratory Service (NHLS) reference ranges (data not shown).

The pharmacokinetics of efavirenz and its metabolites in plasma and CSF has been previously described (manuscript currently under peer review).In summary, plasma efavirenz median (IQR) was 1960 (1390 – 3200) ng/ml, range 55 – 18100 ng/ml; CSF efavirenz median (IQR) 17.25 (10.7 – 19.9) ng/ml, range 1.73 – 119 ng/ml.

Plasma 8-OH-EFV median (IQR) was 1808 (1325.5 – 2498.7) ng/ml, range 68.8 – 4887.5 ng/ml; CSF 8-OH-EFV median (IQR) 4.17 (3.80 – 5.79) ng/ml, range 3.15 – 9.56 ng/ml;

We found no statistically significant associations between any of the neurological clusters and efavirenz and 8-OH-EFV plasma and CSF concentrations or their respective plasma-to-CSF ratios (data not shown).

# 4. Discussion

To our knowledge this is the first study to look at potential relationships that exist between neurological signs and the degree of HAND in a clade C predominant sample. We found that certain neurological signs are associated with more severe forms of HAND, especially relating to abnormalities in gait (slowed walking speed, gait ataxia and abnormal gait appearance), coordination (bradykinesia and ataxia of the lower and upper limbs), the re-appearance of primitive reflexes (the jaw jerk) as well as ocular signs in the form of impaired smooth pursuits and saccades. We found that plasma and CSF efavirenz and 8-OH-EFV concentrations were not associated with neurological symptoms and signs.

Our findings replicated that of others who associated extrapyramidal motor signs (including slowness of hand movements, body bradykinesia and gait abnormalities) with neurocogntive decline in the post-ART era15 and the signs of extrapyramidal motor signs (including bradykinesia, gait abnormalities), frontal release signs and ocular signs (smooth pursuit abnormalities and impaired saccades) with neurocogntive decline in the pre-ART era.9–14,16

The study done in the post-ART era described extrapyramidal motor signs using a summative Unified Parkinson’s Disease Rating Scale (UPDRS) motor score finding associations with both HAND severity and age.15 Other neurological signs were not evaluated. Additional extrapyramidal signs found in the motor portion of the UPDRS, including hypomimia and postural/action tremor, were not replicated in our study. Most research on the neurological manifestations has been done during the pre-ART era.9–14,16 Navia et al. reviewed clinical records of HAD participants retrospectively describing both early and late neurological manifestations. Early neurological manifestations included loss of balance and ataxia (being the most common), leg weakness, pyramidal tract signs and tremor. Late neurological features included worsening ataxia accompanied by hypertonia, incontinence, leg weakness, frontal release signs (predominantly the grasp reflex), tremor, myoclonus and seizures. Peripheral neuropathy of variable severity was present in 48% of participants.9 A subsequent study followed HIV-seropositive participants over 4.5 years where they were shown to be more susceptible to abnormalities in rapid alternating movement, frontal release signs and extrapyramidal signs (including bradykinesia, gait abnormalities and tremor) over time compared to their HIV-negative counterparts.10 The association with frontal release signs has been replicated in other studies.12,13,16Ocular abnormalities, especially in smooth pursuit have also been described.14 Pyramidal tract signs, tremor, myoclonus, incontinence and seizure found in the pre-ART studies were not replicated in our study. We found that the palmo-mental reflex had a negative association with the GDS which is counter intuitive.

Current studies describing the neurological phenotype of HAND are based on a male and Clade B predominant setting.9–16 The majority of these studies were done on populations in a pre-ART era where participants had more extensive HIV-associated immunosuppression and/or AIDS with co-morbid opportunistic diseases. 9–14,16 There are limited studies describing the neurological phenotype in the post-ART era.15 Clade C is the most prevalent HIV-1 subtype at the epicenter of the AIDS pandemic of sub-Saharan Africa and clade C is predominant (89%) in our study setting (Cape Town).31 Our study is the first to describe the neurological phenotype in a Clade C predominant and African female population (90%) with both high CD4 counts and supressed viral loads on ART. South Africa sees a high proportion of women presenting for HIV care.32 Similarities in our study findings to both pre- and post-ART studies could suggest similarities in the neurological phenotype between genders, although further studies are needed to clarify this relationship. Due to a limited number of males in this study (n=10%) statistical differences could not be shown. HIV-positive women may have clinically significant cognitive vulnerabilities compared to HIV-positive men in the development of HAND, particularly in psychomotor speed, attention and motor skills.33–35 The differences in our findings could be explained by clade differences which have been suggested to influence HAND presentation.17 Compared to pre-ART studies, our patients received ART resulting in less severe disease with both higher CD4 counts and suppressed viral loads which could explain the absence of more severe signs and symptoms.

Efavirenz is known to initially cause neuropsychiatric effects with symptoms of headache, dizziness, impaired concentration, abnormal dreams and anxiety being the most common. This is usually transient in nature and resolves over time. Efavirenz, along with its metabolite 8-OH-EFV, has been shown to be neurotoxic through a range of different mechanisms even at therapeutic concentrations.36 A recent case series report of 20 participants describes efavirenz toxicity as a reversible clinical syndrome of ataxia (truncal and/or limb) and encephalopathy (impaired consciousness, psychosis or delirium) secondary to supra-therapeutic concentrations of efavirenz in underweight and presumed genetic slow metabolizers on long term- ART.24 No patients in our study had efavirenz toxicity which may explain the lack of associations with neurological signs.

Our study has a number of limitations. First, the primary RCT on which our study is based was not originally designed to look at associations between neurological manifestations and the GDS.25 Our study was observational in nature and not powered to allow for multiple linear regression. We could not perform a cross-variable analysis to examine how certain variables may have been related. Without matched HIV seronegative controls the true predictive value of certain neurological signs and symptoms could not be reliably estimated. Additionally, available data did not provide the precise ART duration and nadir CD4 count which may have influenced results. Second, examining doctors were only blinded to the degree of neurocognitive impairment and not to the participant’s HIV-status during neurological examination. For this reason, examination bias may have been introduced. Third, no measure of functional ability was assessed in this population. This limited our ability to reliably assess the effect of neurological signs on activities of daily living (ADL). Last, patients with severe forms of HAND generally have a longer standing systemic disease and therefore patients with higher GDS may have worse neurological symptoms and signs due effects on the peripheral, rather than the central nervous system (e.g. in the form of peripheral neuropathy). This study did not show significant associations with peripheral neuropathy, although underlying peripheral neuropathy could have contributed to lower limb ataxia and abnormal gait.

Current guidelines include the addition of daily functioning impairment to its criteria when differentiating between symptomatic and asymptomatic neurocognitive impairment. Patients with ANI have at least 1 SD deficit in at least 2 ability domains of neurocognitive testing in the absence of any impairment of daily functioning. In contrast, symptomatic HAND, which includes (mild neurocognitive disorder) MND and HAD, require daily functional impairment leveled at different severities.2 Self-reported ADL, being the most convenient, time and cost-effective method used in clinical practice, can be influenced by a multitude of factors including bias, depression and the level of cognitive dysfunction. This can therefore increase the risk for diagnostic errors, especially when over-reliance on self-reported functional status is given.37,38 The prevalence of ANI changed from 76% using Self-Report alone to 59% using a combination of Self-Report and Performance based approaches, thus demonstrating potential inflation of asymptomatic over symptomatic HAND when relying on patient self-report only. Other studies have suggested that current criteria itself allows for an unacceptable high false-positive rate4–6 and that the high prevalence of HAND does not necessarily correlate with clinical experience.3 Labeling a patient with symptomatic neurocognitive impairment can hold certain prognostic value especially regarding the progression to further neurocognitive decline. Although current emphasis is placed on the neurocognitive deficits affecting daily functioning, one should also be mindful on how neurological impairments could contribute. Neurological impairment may provide information relating to functional impairment and potentially be a proxy for self-reported ADL. Therefore, an emphasis on the importance of assessing the neurological system in a more fine-grained manner in patients suffering from HAND disorders seems prudent. It will not only allow for a better understanding of the potential factors contributing to a patient’s overall functional impairment (e.g. abnormalities in gait and coordination) but it will also emphasize their role as biomarkers for disease progression. Current definitions of HAND, although recognizing that extrapyramidal abnormalities occur commonly in HAND, opted to exclude these in proposed classification criteria due to insufficient evidence showing reliable associations with neurocognitive impairments and because the cause of such symptoms can be difficult to establish (i.e secondary to HIV infection instead of a comorbid condition).2 The inclusion of these neurological signs in the definition of HAND should be re-considered.

In summary, we found that certain neurological signs can be indicative of worsening degrees of HIV-associated neurocognitive impairment. We found that a clade C neurological phenotype of HAND showed similarities to previous studies but differences can be attributed to clade differences, gender and the degree of HIV-associated immunosuppression in patients not receiving ART-therapy.

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