Title:

The neurological phenotype of South African patients with HIV associated neurocognitive disorder.

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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 neurological manifestations have been modified but not eradicated by antiretroviral therapy (ART). We describe the neurological phenotype in South African black HAND patients with predominant HIV-1 subtype C infection on ART and its association with neurocognitive impairment and efavirenz and 8-hydroxy-efavirenz concentrations.

Methods

We conducted a cross-sectional analyses of the neurological examination findings of HIV+ patients with neurocognitive impairment and used multiple linear regression to explore associations with neurocognitive impairment, efavirenz and 8-hydroxy-efavirenz pharmacokinetics (plasma and CSF).

Results

We included 80 participants established on ART (median 40 months), 72 (90%) were female, aged 35 (32 – 42) years, median [interquartile-range (IQR)], with a median GDS 0.94 (IQR 0.63-1.36). We found statistical significant associations with neurocognitive impairment and neurological signs: gait [slow walking speed ($p=0.03$; $R^2=0.06$), gait ataxia ($p<0.01$; $R^2=0.21$), abnormal gait appearance ($p<0.01$; $R^2=0.18$)]; coordination [upper extremity bradykinesia ($p<0.01$; $R^2=0.10$) and lower extremity bradykinesia ($p=0.01$; $R^2=0.10$)]; reflexes [jaw jerk ($p=0.04$; $R^2=0.05$),

palmo-mental response (p=0.03; R^2=0.06)]; ocular signs [smooth pursuit (p=0.01; R^2=0.09), saccades (p<0.01; R^2=0.15)] and motor signs [spasticity (p=<0.01; R^2=0.15), muscle weakness (p=0.01; R^2=0.08)]. No significant associations were found between plasma and cerebrospinal efavirenz or 8-hydroxy efavirenz concentrations and any neurological sign.

Conclusion

We found that individual neurological signs were associated with neurocognitive impairment in South African black HIV+ patients with predominant HIV-1 subtype C infection on ART and could be used in clinical practice to assess severity.

Introduction

Mild forms of HIV-associated neurocognitive disorder (HAND) remain prevalent in antiretroviral experienced patients (up to 45%).[1] HAND is diagnosed using neuropsychological testing with patients performance below 1 standard deviation from the norm in at least 2 cognitive domains, however such detailed neuropsychological assessments are not always practical in the clinical setting.[2,3] Clinicians therefore rely on various screening tools, functional assessments and limited neuropsychological tests for diagnosis.[2] Unfortunately, screening tools, including the International HIV dementia Scale, are limited in their ability to detect milder forms of HAND with a high false-negative rate.[4] Currently, neurological signs are not included in HAND definitions[3], although abnormalities in gait, coordination, frontal release signs as well as ocular signs (impaired smooth pursuit and saccades) have been associated with HAND in patients with predominately HIV-1 clade B infection.[5–13] The inclusion of primitive reflexes in the case definition of HAND has been suggested.[12] Moreover, HAND neurological manifestations have not been described in the clade C predominant HIV infected sub-Saharan Africa.[5–13] Clade variances demonstrate different degrees of neuronal toxicity *in vitro* with reduced neuro-virulence of HIV clade C versus clade B, although supporting clinical data is lacking.[14–17] Additionally, efavirenz and its metabolite 8-hydroxy-efavirenz may be an additional contributor to neurocognitive impairment.[18–21]

Our primary objective is to describe the neurological phenotype in South African black HIV+ patients with neurocognitive impairment and with predominant HIV-1 subtype C on ART. Our secondary objective was to assess the neurological

phenotype association with neurocognitive impairment and efavirenz and 8-hydroxy-efavirenz concentrations (plasma and CSF).

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 neurocognitive impairment.[22] Participants were established on ART for at least 6 months prior to the study with suppressed viral loads (HIV PCR <400copies/mL). All participants 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).

Neurological assessment: Participants received a full neurological examination by medical practitioners who received additional training in the neurological evaluation. All neurological assessments were reviewed by a neuropsychiatrist. Each neurological sign and symptom was assessed according to a standardized neurological assessment and quantified based on a previously defined tool.[23,24] The neurological examination included peripheral neuropathy (visual analogue scale, vibration perception and ankle reflexes), motor system (involuntary movement, muscle bulk, tone and power), gait (appearance, coordination , timed gait test), limb coordination, reflexes (deep tendon reflexes and the primitive reflexes snout, grasp, palmo-mental, glabellar tap and plantar response) neck stiffness, facial strength and ocular signs (saccades and smooth pursuit). A binary score was assigned to indicate absent and present neurological signs to allow for statistical analysis.[23]

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.[25] The domains (tests utilized) were 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-hydroxy-efavirenz pharmacokinetic data were 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 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-hydroxy-efavirenz and plasma 8-hydroxy-efavirenz and plasma using validated LC/MS-MS assays.[26] The LLOQ for CSF 8-hydroxy-efavirenz and plasma 8-OH-EFV was 3.125 ng/ml and 5.0 ng/ml respectively. Concentrations below the limit of quantification were analyzed as missing data.

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. For the primary outcome, associations between GDS and the individual neurological signs and symptoms were determined using a linear regression model. A p-value of <0.05 was considered significant. The primary outcome was further explored using a multivariate linear regression model and variables with either poor variation and/or too few events were excluded from the model. A p-value of <0.05 was considered significant. Secondary outcomes explored associations between efavirenz and 8-hydroxy-efavirenz plasma and CSF concentrations, and their respective plasma:CSF ratios with neurological signs and symptoms. Linear regression models were used with a p-value of <0.05 for statistical significance.

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 presented in **Table 1**. Many participants experienced symptoms, and had signs, suggestive of peripheral neuropathy (n=32, 40%). However, in linear regression analysis both subjective and objective peripheral neuropathy indicators were not found to be significantly associated with GDS (**Table 2**). A number of neurological signs were associated with GDS (**Table 2**). Statistical significant associations for motor signs were spasticity ($p<0.01$; $r^2=0.15$) and muscle weakness ($p=0.01$; $r^2=0.08$); abnormalities in gait in the form of slowed walking speed ($p=0.03$; $r^2=0.06$), gait ataxia ($p<0.01$; $r^2=0.21$) and abnormal gait appearance ($p<0.01$; $r^2=0.18$); abnormalities in limb coordination in the form of upper extremity bradykinesia ($p<0.01$; $r^2=0.10$) and lower extremity bradykinesia ($p=0.01$; $r^2=0.10$); brisk deep tendon reflexes in the form of jaw jerk ($p=0.04$; $r^2=0.05$); primitive reflexes in the form of palmo-mental reflex ($p=0.03$; $r^2=0.06$); and lastly ocular signs including impaired smooth pursuits ($p=0.01$; $r^2=0.09$) and impaired saccades ($p<0.01$; $r^2=0.15$). All associations were found to be positive, except for the palmo-mental response and snout reflex which had a negative association. When included in a multivariate linear regression model certain neurological signs and their association with GDS remained statistically significant (**Table 3**). Additional variables found to be associated with GDS, not established in univariate analysis, were vibration perception score as a measure of peripheral neuropathy ($p<0.01$), the grasp reflex ($p=0.03$) as well as gender ($p=0.05$). CSF parameters, which included CSF -cell count, -culture, -chemistry, -albumin and CSF: serum albumin ratios were normal (data not shown). We previously described the pharmacokinetics of

efavirenz and its metabolites in plasma and CSF.[27] 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-hydroxy-efavirenz median (IQR) was 1808 (1325.5 – 2498.7) ng/ml, range 68.8 – 4887.5 ng/ml and CSF 8-hydroxy-efavirenz 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 signs and symptoms and efavirenz or 8-hydroxy-efavirenz in plasma or CSF or their respective plasma-to-CSF ratios (data not shown).

Discussion

To our knowledge this is the first study that examined the association between neurological signs and HAND severity in a black African population with a predominant clade C HIV-infection. We found that certain neurological signs are associated with more severe forms of HAND, especially abnormalities in gait (slowed walking speed, gait ataxia and abnormal gait appearance), coordination (bradykinesia of the lower and upper limbs), motor signs (spasticity, decreased muscle strength, hyperreflexia), reflexes (palmo-mental reflex) as well as ocular signs (impaired smooth pursuits and saccades). Multivariate analysis confirmed the association of motor signs (decreased muscle strength); abnormalities in gait (slowed walking sleep); primitive reflexes (palmo-mental reflex) and ocular signs (impaired smooth pursuits) with GDS. Neurological symptoms and signs were not associated with plasma or CSF efavirenz or 8-hydroxy-efavirenz concentrations.

Our findings replicate that of others who associated abnormalities in gait, coordination (body bradykinesia, abnormalities in rapid alternating movement),

motor signs (spasticity, decreased muscle strength, hyperreflexia), frontal release signs and ocular signs (impaired smooth pursuits) with HAND.[6,8–13,28,29] We found that the palmo-mental reflex had a negative association with the GDS which is counter intuitive. The neurological phenotype of HAND has been best described during the pre-ART era.[6,8–10,12,28,29] The first description in 1986 described a wide range of deficits from mild weakness and coordination disturbances to seizures, incontinence and quadriparesis.[5] Subsequent studies described abnormalities in rapid alternating movement, frontal release signs, extrapyramidal signs and abnormalities in eye movements demonstrating mostly abnormal smooth pursuits.[6,8–10,12,29] Neurocognitive impairment in the post-ART era is associated with extrapyramidal signs and soft neurological signs described in the Heidelberg Scale.[11,13] Neurological phenotype of HAND data is predominantly representative of a male HIV-1 clade B infected population with data lacking in females and HIV-1 subtype C infected individuals. Clade C is the most prevalent HIV-1 subtype of sub-Saharan Africa and is predominant (89%) in our study setting (Cape Town).[30] Our findings contribute to the growing body of clinical evidence that neuropathology is similar in both Clade C and B HIV-1 subtypes.[16,17] South Africa sees a high proportion of women presenting for HIV care.[31] HIV positive women have an increased susceptibility of cognitive impairment particularly in psychomotor speed, attention and motor skills while no sex differences were demonstrated in HIV negative participants.[32,33] These differences reflect the proposed influences of 'sexual dimorphism on immune function, pathogenesis and antiretroviral pharmacokinetics', but also differences in mental health prevalence and sociodemographic patterns between sexes.[32,33] Our study population consisted of a predominant female population and we found similarities in the neurological

phenotype compared with a male predominant setting. However, appropriately powered studies designed to confirm this finding is required.

Efavirenz is known to initially cause neuropsychiatric symptoms such as headache, dizziness, impaired concentration, abnormal dreams and anxiety.[34,35] This is usually transient in nature and resolves over time. Efavirenz, along with its metabolite 8-hydroxy-efavirenz, has been shown to be neurotoxic through a range of different mechanisms even at therapeutic concentrations.[18,19,36] Patients starting efavirenz-based ART rather than protease inhibitors or all-nucleoside reverse transcriptase inhibitor regimens had less improvement in neurocognitive function scores after 48 weeks in a randomised controlled trial (RCT).[37] 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.[21] A recent case series report of 20 participants describes efavirenz toxicity as a reversible clinical syndrome of ataxia and encephalopathy in underweight and presumed genetic slow metabolizers on long term- ART.[38] Efavirenz and 8-hydroxy-efavirenz was therefore an important confounding factor to consider in our population. No patients in our study had efavirenz toxicity which may explain the lack of association between efavirenz and 8-hydroxy-efavirenz 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.[22] Second, the primary RCT included participants with moderate to severe neurocognitive impairment (GDS ≥ 0.5) and our distribution of

impairment was skewed, therefore limiting our ability to describe associations with milder forms of neurocognitive impairment. Third, our study was observational in nature and not appropriately powered to allow for an accurate multivariate linear regression model. Without matched HIV seronegative controls, the true predictive value of certain neurological signs and symptoms could not be reliably estimated. Fourth, 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. Fifth, 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 or to be able to define HAND stage. Last, it is possible that some of our findings are due to peripheral neuropathy rather than the central nervous system disease.

Current guidelines include the addition of daily functioning impairment to its criteria when differentiating between symptomatic and asymptomatic neurocognitive impairment.[3] The prevalence of asymptomatic neurocognitive impairment decreased from 76% to 59% when combining self-report and performance based approaches.[39] Self-reported activities of daily living is the most convenient method to assess daily functioning but may be influenced by self-report bias, depression and the severity of cognitive dysfunction and can therefore increase the risk for HAND false-positive errors.[39,40] Neurological impairment could potentially be used as a tool to objectively assess certain aspects of daily function (i.e. coordination and gait) and can be used together with self-report to further delineate functional impairment. Further research is however needed to better define the neurological phenotype and its relationship to functional impairment. Additionally, neurological signs could be used as a pragmatic

and easy to determine marker of neurocognitive decline, especially in a busy clinic setting where it could augment the clinical assessment of HAND especially when the availability of neuropsychological testing is limited. However, the true predictive value of the neurological phenotype for the severity of HAND needs to be determined.

In summary, we found that certain neurological signs are associated with more severe forms of HAND, especially abnormalities in gait, coordination, motor signs, reflexes as well as ocular signs in South African black HAND patients with predominant HIV-1 subtype C on ART. Our findings do not suggest a difference in neurological phenotype between HIV clade C and B or between males and females. Neurological symptoms and signs were not associated with plasma or CSF efavirenz or 8-hydroxy-efavirenz concentrations.

Appendix 1

Name	Location	Role	Contribution
Sean G. Anderson, MBChB	University of Stellenbosch, Cape Town, South Africa	Author	Analyzed the data; interpreted the data; drafted the manuscript for intellectual content.
Michael Mccaul, MSc	University of Stellenbosch, Cape Town, South Africa	Author	Analyzed the data; revised the manuscript for intellectual content.
Saye Khoo, MBBS, MD	University of Liverpool, Liverpool, UK	Author	Acquisition of pharmacokinetic data; revised the manuscript for intellectual content.
Lubbe Wiesner, PhD	University of Cape Town, Cape Town, South Africa	Author	Acquisition of pharmacokinetic data; revised the manuscript for intellectual content.
Ned Sacktor, MD, PhD	Johns Hopkins University, Baltimore	Author	Interpreted the data; revised the manuscript for intellectual content.
John A. Joska, MBChB, PhD	University of Cape Town, Cape Town, South Africa	Author	Major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content.
Eric H. Decloedt, MBChB, PhD	University of Stellenbosch, Cape Town, South Africa	Author	Designed and conceptualized the study; major role in the acquisition of data; interpreted the data; revised the manuscript for intellectual content.

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