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HIV-associated Neuropathy and Autonomic Dysfunction in South Africans on established ART impacts daily living

For the degree: Master of Medicine (MMed) in Medicine

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Declaration

I, Meagan Taryn Dudley, hereby declare that this research report is completely my own (except where acknowledgements indicate otherwise). No part of it has ever been published in any format, or submitted for another degree in this or any other university. I empower the University of Cape Town to reproduce for the purpose of research either the whole or any portion of my work in any manner that suits the institution. I intend to submit the results of my research in a published format to a journal of my choice at a later date

Signed on this 20th day of April 2019 at Plumstead

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Abstract

Introduction

A common complication of Human Immunodeficiency Virus (HIV) and anti-retroviral therapy (ART) is distal sensory polyneuropathy (DSP). Older age and previous TB are risk factors for DSP among HIV-infected Africans before and shortly after ART initiation. Little is known about autonomic dysfunction in Africans on long-term ART and the impact of DSP and autonomic impairment on their quality of life. Our aim was to describe the frequency, characteristics and functional consequences of DSP and autonomic dysfunction in a healthy HIV-infected community-based cohort after at least 5 years of ART.

Methods

HIV-infected South Africans on the government-sponsored ART program for at least 5 years were included in this cross-sectional analysis. Each consenting participant underwent a focussed neurological assessment using the Brief Peripheral Neuropathy Screen (BPNS) and a reduced version of the Total Neuropathy Score (rTNS). DSP was defined as the presence of at least 2 neuropathic signs in a distal and symmetrical distribution, and symptomatic DSP (SDSP) when accompanied by neuropathic symptoms. Heart rate variability and orthostatic hypotension were measured as described by the Ewing classic battery, and the Survey of Autonomic Symptoms (SAS) questionnaire assessed the presence and severity of autonomic symptoms. We used a modified version of the Lower Extremity Functional Scale (LEFS) to assess lower limb physical ability.

Results

The 67 participants had a median age of 41 years (interquartile range (IQR) 36-46) and 61 (91 %) were women. The median duration of ART was 7 years (IQR 6-10). DSP criteria were met in 54 (80.6%) and 24 (44.4%) had symptomatic DSP. Comparing participants with DSP to those without DSP, there was no difference in sex (P=0.39), age (P=0.79), current CD4 (P=0.69), viral suppression (P=0.34), ART duration (P=0.22) or previous tuberculosis (TB) (P=0.72) in those with DSP. Similar outcomes were obtained for SDSP. Abnormal autonomic tests were present in 60%. Those with SDSP had more severe autonomic symptoms than those with asymptomatic DSP (*P*=0.0008). We found that those with DSP and SDSP had significantly lower LEFS percentage scores than those without (P=0.039 and P=0.013 respectively).

Conclusion

DSP remains a common complication of HIV in the modern era of ART and can lead to significant functional impairment. Autonomic dysfunction is prevalent in SDSP.

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Abbreviations

ADSP Asymptomatic Distal Sensory Polyneuropathy

ART Antiretroviral Therapy

ASP Autonomic Symptom Profile

BPNS Brief Peripheral Neuropathy Screen

DBP Diastolic Blood Pressure

dNRTI or **D-drugs** Dideoxynucleoside Reverse Transcriptase Inhibitors

DSP Distal Sensory Polyneuropathy

D4T Stavudine

CASr Reduced Composite Autonomic Score

CASS Composite Autonomic Severity Score

HDL High Density Lipoprotein

HIV Human Immunodeficiency Virus

hsCRP Highly Sensitive C-Reactive Protein

LDL Low Density Lipoprotein

LEFS Lower Extremity Functional Scale

SAS Survey of Autonomic Symptoms

SDSP Symptomatic Distal Sensory Polyneuropathy

SBP Systolic Blood Pressure

TDF Tenofovir Disoproxil Fumarate

TIS Total Impact Score

TNS Total Neuropathy Score

TNSr reduced Total Neuropathy Score

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Chapter 1

Introduction and Literature Review

HIV-associated distal sensory polyneuropathy (DSP) is a common complication of HIV and its treatment [1–5]. In 2016, an estimated 6.1 million South Africans were living with HIV, 56% of whom were on antiretroviral therapy (ART)[6]. This number is expected to increase since the national implementation of the 'Universal Test and Treat' policy in South Africa in September 2016[7]: ART is now available to all HIV-infected individuals regardless of CD4 count or staging. People with HIV are living longer and clinicians are now facing an aging HIV-positive population who are presenting with non-communicable illnesses that may be related to long-term ART, chronic inflammation or immune activation [8]. Some have shown that HIV-infected patients are more at risk of developing DSP before or within the first 3 to 6 months of ART [5,8,9]. However, HIV-associated DSP, in the setting of long-term ART in African populations, is a topic that is poorly studied.

HIV-associated DSP occurs in two clinical contexts: as a direct consequence of HIV infection or as an adverse effect of ART, also termed antiretroviral toxic neuropathy [1,8,10,11]. Common symptoms of DSP are numbness, burning sensation, and stabbing pain in the feet and lower extremities [1]. Painful DSP can result in psychological distress and physical disability; thereby adversely affecting quality of life[12,13]. Furthermore, neuropathic pain is usually not alleviated by regular analgesia[10,14,15]. A South African-based cross-sectional study by Maritz *et al* demonstrated that the frequency of symptomatic DSP was 23% in ART-naïve individuals compared to 40% in the ART- and 36% in the stavudine (D4T)-exposed patients with more advanced HIV disease (Maritz et al., 2010). However, results recently obtained in a South African cohort followed from pre-ART initiation to 2 years on D4T- or tenofovir (TDF)-based regimens showed that neuropathic symptom prevalence did not differ significantly between the two ART regimens and in fact largely improved on ART in both groups [5].

Older age is frequently cited as a risk factor for DSP amongst the ART-exposed [1–3,5,10,14–18]. With an increased lifespan on effective ART, one must anticipate that the aging South African HIV positive population will be faced with this troubling complication. Female sex and previous tuberculosis (TB) are also mentioned as risk factors for DSP in the ART-exposed [1,2,5,10]. Mitochondrial toxicity is implicated in the pathophysiology of antiretroviral toxic neuropathy [8,11]. The dideoxynucleoside reverse transcriptase inhibitors (dNRTIs) - stavudine and didanosine – were considered the chief culprits [2,3,17,19], both of which were used in Sub-Saharan Africa until 2010 when national ART guidelines changed first line ART from

D4T to TDF-based regimens[20]. It remains to be seen whether this change has been truly beneficial to those at risk of DSP.

Functional Impairment in HIV-infected subjects with DSP

As the incidence of opportunistic diseases and mortality continue to decline in the era of ART, quality of daily living must remain an important goal of therapy in this aging HIV-positive population. There is little data about functional impairment and quality of life of those who are affected by HIV-associated DSP, particularly in Sub-Saharan Africans. In Rwanda, 185 HIV-infected participants (mean age of 38.7 years; SD ± 9.6) were asked whether DSP affects quality of life [13]. Physical and psychological quality of life was found to be significantly lower in those with DSP than those without DSP. This cohort consisted of both ART-exposed (duration unknown) and ART-naïve patients making it difficult to apply these outcomes to our population on long-term ART. An American Study by Galantino *et al* found that participants with HIV-associated DSP had significantly lower scores on lower limb function questionnaires and quality of life screening tools than HIV-positive patients without DSP [15]. Additionally, 80% of those with DSP were likely to seek medical treatment for pain compared to the 11% without DSP supporting the notion that symptomatic DSP negatively affects daily living. All of these participants were receiving ART, had relatively preserved CD4 counts and had similar clinical and demographic characteristics. The mean age of those with DSP was 42 years. The scoring questionnaire used by Galantino *et al* - the Lower Extremity Functional Scale (LEFS, explained later) - was found to be highly predictive of physical and mental quality of life in patients with HIV-associated DSP.

Consequently, it is imperative for clinicians to understand how DSP can impact the lives of patients so that early identification and intervention can occur.

Autonomic Dysfunction in HIV-infected subjects with DSP

Early studies showed autonomic dysfunction in African and non-African patients with advanced HIV or untreated disease[21–23]. However, the presence of autonomic dysfunction in African medically stable patients established on long-term ART has been scarcely described. In the last decade Robinson-Papp et al found autonomic dysfunction in 61% of HIV-positive American patients with a median CD4 count of 439 cells/µL, most (95%) of who were on ART with suppressed viral loads, yet there was no association between autonomic dysfunction and ART in this group [24]. Features of autonomic dysfunction include orthostatic hypotension, decreased heart rate variability, sleep dysfunction, altered sweating, constipation or diarrhoea, nausea and vomiting, impotence and bladder dysfunction. These symptoms

have the potential to be debilitating. Moreover, there is an association between cardio-autonomic dysfunction and increased mortality in diabetic subjects[25,26]. These deleterious characteristics of autonomic dysfunction highlight the importance of exploring this topic in the context of HIV patients who are now living longer on ART. Two European studies assessed autonomic dysfunction in HIV-positive outpatients on ART compared to HIV negative controls and found an increased resting heart rate and decreased heart rate variability demonstrating impaired parasympathetic tone in the HIV-positive group[27,28]. Both studies evaluated patients with median CD4 counts above 500 and on ART for a median of 7 years, most (> 90%) of whom had suppressed viral loads. These characteristics and the comparison to age- and sex-matched controls make both studies decidedly relevant in discerning autonomic dysfunction in the setting of long-term ART. Limitations were the small sample size and the cross-sectional nature of both studies. McIntosh *et al* reinforced the concept of autonomic dysfunction in the era of ART in a meta-analysis describing decreased heart rate variability in HIV-positive individuals on ART compared to HIV-negative controls [29]. With the exception of Robinson-Papp *et al*, all of the aforementioned studies excluded diabetic patients in order to limit confounding factors. Except for a small sub-group of studies within the meta-analysis, those on anti-hypertensive medication were also excluded. Most studies tend to focus on cardio-autonomic testing with little or no attention to the other autonomic domains. One study used the Autonomic Symptom Profile (ASP) – a 169-item questionnaire of 11 autonomic domains – and found that 40% of HIV-positive virologically suppressed patients had symptoms of autonomic dysfunction compared to 9% of HIV-negative controls [30]. This group classified this finding as clinically significant autonomic neuropathy. However, the completion of a 169-item questionnaire is time-consuming and not practical in a South African setting. The authors believe that the addition of simple autonomic symptom questionnaire, encompassing all domains, may provide a simple, yet comprehensive evaluation of autonomic dysfunction in the healthy, ART-exposed population.

It is well known that peripheral neuropathy, particularly small fiber neuropathy, can be accompanied by autonomic neuropathy, as is the case with diabetic and alcoholic peripheral neuropathy. There is limited data on the relationship between autonomic neuropathy and HIV-associated DSP. Rogstad *et al* did not find any correlation between autonomic dysfunction and HIV-associated neurological disease in ART-naïve African patients [22]. However, the previously mentioned study by Robinson-Papp *et al* found those with severe DSP (Total Neuropathy Score (TNS) ≥15) were 3-times more likely to have autonomic symptoms and/or signs compared to those with little or no DSP (TNS<4) [24]. Another study also found significantly lower sweat volumes (as defined by the quantitative sudomotor axon reflex test) in ART-

exposed patients, a majority of whom were virologically suppressed, with neuropathy compared to those without neuropathy [31]. Further research about the presence of autonomic symptoms in ART-exposed patients, their severity and their impact on quality of life is needed. It would therefore be important to explore autonomic neuropathy in the ART-treated population so that screening and treatment strategies can be instituted.

In this cross-sectional study we aimed to assess the frequency and severity of neuropathy and autonomic dysfunction, as well as the impact on daily function in healthy HIV-infected Africans after at least 5 years of ART.

Chapter 2

Methods

Study Design and Population

Ambulant, community-based HIV-positive patients were recruited from a community health care center in Crossroads, Cape Town. Subjects were eligible for inclusion if they were HIV-positive, ≥18 years, on ART for at least 5 years and were clinically well. Subjects were excluded if they had an active opportunistic infection, current anti-tuberculosis therapy of less than 1 month duration, liver or renal failure, or active alcohol or substance misuse. All participants provided written informed consent prior to assessment [32]. This study was approved by the University of Cape Town Research Ethics Committee (HREC REF 221/2008 and HREC REF 200/2017).

Participant Assessment

Participant assessment was conducted over 2 consecutive days. A trained Xhosa speaking fieldworker was present during these assessments as Xhosa is the predominant language of the study participants. Patient demographics, height and weight were recorded. A comprehensive medical history of participant habits, co-morbid disease, pregnancy and medication (including ART) usage was taken. Recent viral load (taken within 3 months of the study visit) and CD4 count (taken within 6 months of the study visit) were obtained from the national lab results system. Each participant had the following blood tests in a fasting state: oral glucose tolerance test (OGTT), fasting lipogram, serum creatinine and highly sensitive C - reactive protein (hsCRP).

A focussed neuropathy examination was performed by a clinician (SM, CD, MB or MD) under the direct supervision of a neurologist (JMH). A medical examination was performed by a specialist physician (MB) to exclude any intercurrent illness. Cardio-autonomic testing (conducted by MB) occurred on the second day and included RR interval change (which reflects heart rate variability) and orthostatic hypotension.

The neuropathy assessment comprised the Brief Peripheral Neuropathy Screen (BPNS) and reduced Total Neuropathy Score (TNSr) as previously described by Maritz *et al*[10]. The performance characteristics of these tools has been discussed elsewhere[18,21,33,34]. All questionnaires were in English and Xhosa [10].

The sensory symptoms evaluated by the BPNS are symmetrical neuropathic pain, numbness and "pins-and-needles". These symptoms are graded on a numerical rating scale (NRS) from 1 (mild) to 10 (severe). The total BPNS sensory severity grade is the single highest NRS value of any of the 3 symptoms. Vibration at the terminal hallux (using a 128 Hz tuning fork) and ankle reflexes are the two examination domains evaluated by the BPNS. Vibration was scored from 0 (vibration felt for >10 second, or normal) to 3 (no feeling of vibration). Ankle reflexes were scored from 0 (clonus) to 4 (absent) with a middle score of 2 indicating normal reflexes.

The TNSr assesses sensory symptoms, pin sensibility, vibration sensibility, deep tendon reflexes and muscle strength in a quantitative fashion. It aims to describe the extent of limb involvement from 0 (no abnormality) to 4 (symptom/sign extending above knee/elbow or complete paralysis). Proprioception (i.e. joint position sense) is not part of either of these neuropathy scoring systems, but was included in our assessment of these patients. Proprioception measured at the terminal hallux was assessed in a series of 10 trials and then defined as either normal, reduced (>2/10 mistakes) or absent. All abnormal scores required bilateral limb involvement to be classified as DSP.

For the purposes of this study, DSP is defined as the presence of at least 2 of the following neuropathic signs in a distal and symmetrical distribution: decreased vibration, or altered proprioception, or decreased deep tendon reflexes, or weakness in the ankle and toes, or decreased pin sensibility. Symptomatic DSP is defined as 2 signs plus any 1 neuropathic symptom, viz. paraesthesia, numbness or pain. Patients are classified as asymptomatic if they have 2 signs without any symptoms.

The Lower Extremity Functional Scale (LEFS) comprises 20 questions that centers on day-to-day lower limb physical ability. This was originally developed to assess disability in patients with neuromuscular disorders [15] in higher income countries. We used a version of the LEFS that has been validated in an African setting and modified to suit the activities of daily living of patients from lower income

countries[35]. Each activity is subjectively ranked from 0 (extreme difficulty) to 4 (no difficulty) to attain a total out of 80. During data analysis, we used a percentage score with 100% reflecting no difficulty in activity.

The Survey of Autonomic Symptoms (SAS) is a valid, easily administered questionnaire that can be used in clinical trials assessing autonomic symptoms [36]. It was found to have a greater sensitivity and specificity than the ASP for autonomic symptoms in early diabetic neuropathy[36]. The SAS consists of 11 questions for women and 12 for men. It focuses on the presence and degree of severity of autonomic symptoms of the following domains: orthostatic, secretomotor, vasomotor, gastrointestinal, urinary, and male sexual dysfunction. Each item is graded on a severity score ranging from 1 (absent) to 5 (severe). The severity scores for each symptom are added up to give a total impact score. Zilliox *et al* applied the SAS in diabetics with neuropathy and age-matched healthy controls, and found >3 symptoms would provide > 90% specificity and 65% sensitivity in determining autonomic dysfunction, and a total impact score (TIS) of > 7 would provide > 90% specificity and > 60% sensitivity in determining autonomic symptom severity [36]. These cut-offs were applied to the participants of this study.

We developed a reduced Composite Autonomic Score (CASr) (figure 1), inspired by the Composite Autonomic Severity Score (CASS) defined in Low *et al* [24], to describe autonomic dysfunction in our study population. It comprised of 3 parameters each contributing 1 point if present: abnormal heart rate variability, orthostatic hypotension, and a significant SAS (> 3 symptoms) or TIS (> 7). All participants were instructed to fast for ≥8 hours prior to testing and thus would have no coffee or medication on the morning of the 2nd day of assessment. Heart rate variability and orthostatic hypotension were measured as described by the Ewing classic battery [37]. Heart rate response to moving from a lying to standing was assessed by measuring the the beat to beat (R–R) interval change during this change in position. An R-R interval ratio of ≤ 1.04 was abnormal. Orthostatic hypotension was measured and defined in the conventional method: drop in SBP of ≥ 20mmHg or DBP ≥10mmHg when moving from lying position to standing after 2 mins. We also enquired about symptoms of dizziness and palpitations while these measurements were taken.

1

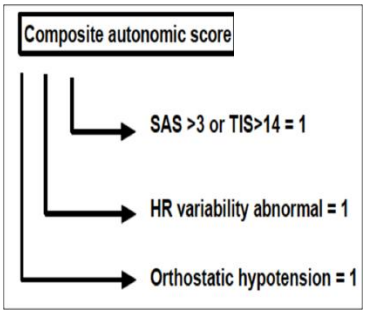


Figure 1 The Reduced Composite Autonomic Score

Statistical Analysis

Statistical analysis was performed using Excel, Graph Pad (version 7), OpenEpi (www.openepi.com) and STATA 14.1 (Stata Corp, College Station, Texas). Variables were reported based on their normality; means (±SD) and medians (IQR) - normal and skewed data respectively. The difference between proportions was tested using the Z-test for proportions. The Student T-test was used for continuous, normally distributed variables, e.g. amongst participants with DSP versus those without DSP (see table 1). The non-parametric Mann-Whitney U test was used for group comparisons in skewed continuous variables. Graphical presentation of the different scores was done using Graph Pad. A p-value of ≤0.05 was used as the threshold for statistical significance, while p-values up to 0.10 were considered as borderline significant.

Chapter 3

Results

Study Population

Sixty-nine patients were enrolled into this study from August 2014 to December 2016. Two were excluded-; one had defaulted ART and was intoxicated with alcohol on the second day of examination, and another had only been on ART for 2 years. Of the 67 remaining participants one participant did not attend the second day of testing and had missing autonomic data, fasting blood tests and information about the ART regimen.

The characteristics of the study cohort are shown in Table 1. Of the 67 participants, 91% were female with a mean age of 41.6 years (standard deviation (±SD) 8.6). All participants were black Africans. The median time on ART was 7 years (IQR 6-10) and the mean CD4 count was 560 cells/µL (±SD 236.5). Fifty-

six (84%) of the participants were virologically suppressed, defined as < 400 copies/ml by the HIV Research Network [38]. Thirty-nine (58%) had a prior history of TB. Fifty (75%) patients took tenofovir-based regimens; 13 (19%) were on zidovudine-based regimens, and 3 (4%) were on stavudine-based regimens. No patients were taking didanosine. Fifty-seven (85%) participants had had a change in their ART regimen since initiating ART. The reasons supplied for the change included virological failure (13), drug side-effects (10), pregnancy (1) and for convenience (29) with a switch from 3 drugs to a fixed-dose combination. Four participants could not recall why they were switched. Two patients were using cotrimoxazole (Bactrim) prohylaxis. Fourteen were using isoniazid prophylaxis but only 5 of these patients were taking vitamin B6 supplementation. Eight patients were taking anti-hypertensive agents: six were on a single agent; two were on 2 agents.

Table 1 Characteristics of participants with DSP (2 neuropathic signs) and without DSP

Characteristic	Total N=67	DSP 2-Signs N=54	DSP-free N=13	*P*-value
Women, N (%)	61 (91)	50 (92.6)	11(84.6)	0.37*
Age (years), mean (±SD)	41.6 (8.6)	41.8 (8.7)	41.1(8.5)	0.79ᵗ
Previous TB, N (%)	39 (58.2)	32 (59.3)	7 (53.9)	0.72*
Weight (kg), mean (±SD)	71.3 (16.3)	70.3 (15.5)	75.7 (19.3)	0.28ᵗ
Height (m), median (IQR)	1.59 (1.55-1.63)	1.60 (1.55-1.63)	1.56 (1.55-1.62)	0.42ʏ
Current CD4 (cells/uL), mean (±SD)	560.2 (236.5)	566.7 (247.1)	536.8 (200.2)	0.69ᵗ
Viral suppression, N (%)	56 (83.6)	44 (81.5)	12 (92.3)	0.34*
Viral load <20 copies/ml , N %	50 (74.6)	38 (70.4)	12 (92.3)	0.10*
ART duration (years), median (IQR)	7 (6-10)	7.5 (6-10)	7 (5-9)	0.22ʏ
D-Drugs, N (%)	3 (4.5)	3 (5.6)	0	0.38*
Efavirenz, N (%)	34 (50.8)	30 (55.6)	4 (30.8)	0.11*
Isoniazid, N (%)	14 (20.9)	9 (16.7)	5 (38.5)	0.08*

DSP, distal sensory polyneuropathy; SD, standard deviation; TB, tuberculosis; IQR, interquartile range; viral suppression refers to <400 copies/ml; ART, anti-retro viral therapy; D-Drugs, dideoxynucleoside reverse transcriptase inhibitors e.g. Stavudine (D4T) or didanosine (DDI); *P*-values derived by *z-test, ᵗt-test, ʏMann-Whitney

Frequency and Characteristics of DSP

Most (81%) of the participants met criteria for DSP arbitrarily defined as the presence of 2 neuropathic signs. Indeed, 66 of the 67 participants had at least one symmetrical neuropathic sign. The

characteristics of patients with and without DSP are shown in Table 1. The median time on ART was 7.5 years (IQR 6-10). The mean age of those with DSP was 41.8 years (±SD 8.7) and the median CD4 count was 567 cell s/µL (±SD 247.1). Most (81%) of those with DSP were virologically suppressed. Compared with participants without DSP, there was no difference in: sex (*P*=0.37), age (*P*=0.79), current CD4 (*P*=0.69), viral suppression (*P*=0.34), ART duration (*P*=0.22), previous TB (*P*=0.72) and hsCRP (*P*= 0.44**).** Of the 9 Isoniazid-users who were not on Vitamin B6 supplements, the majority (67%) had DSP but this was not statistically significant (*P*=0.83). There was a trend towards an association between current isoniazid use and DSP (*P*=0.08). None of the metabolic or anthropometric parameters, including body mass index (BMI), were associated with the presence of DSP (see supplementary table 1). As the frequency of DSP was so high based on an arbitrary definition of 2 neuropathic signs, we also used a more stringent definition for DSP of ≥3 signs to determine if this would significantly alter our results. With the exception of previous TB, these differences did not alter when we used this alternative definition (see supplementary table 2). Previous TB tended to be more common in those with DSP-3 signs (68 vs. 47%, *P*=0.08).

Frequency and Characteristics of Neuropathic Symptoms and SDSP

Amongst the total cohort, the most frequent symptom was numbness (31 %) followed by pain, aching and burning (30%) and then "pins-and-needles" (28%) (see table 2). Figure 2 shows the range of symptom severity according to the BPNS. Most participants described moderate to moderately severe symptoms (NRS ranging from 4 to 8). Despite this only 4 patients were prescribed medication for relief of their neuropathic pain: 3 used amitriptyline and one paracetamol. The most common neuropathic signs in the total cohort were: decreased pin sensibility and deep tendon reflexes (79%). Impaired vibration (49%) and proprioception sense (40%) were also prevalent. There was only 1 patient who had mild decreased power at the ankle and toes. This patient also had impaired vibration sense, deep tendon reflexes, pin sensibility and had a high BPNS total sensory severity grade of 4 (NRS between 9-10). Most patients were asymptomtomatic with a NRS of 0 (see figure 2). Figure 3 shows the TNSr score spread across the entire cohort, with a median score of 6 (IQR 4-8) out of a total of 20 implying that the anatomical extent of disability was limited to the distal regions.

SDSP was present in 24 (44%) of those with DSP (see supplementary table 3). The frequency of neuropathic signs were: impaired deep tendon reflexes (100%) followed by altered pin sensibility (92%), proprioception (63%), and vibration (58%) (see table 2). Comparing participants with SDSP and ADSP, there was no difference in: sex (*P*=0.20), age (*P*=0.61); current CD4 (*P*=0.27); viral suppression (*P*=0.70),

ART duration (*P*=0.85), previous TB (*P*=0.32), hsCRP (*P*= 0.26), or the use of efavirenz (*P*=0.75) and isoniazid (P=0.46). None of the metabolic or anthropometric parameters were associated with the presence of SDSP.

We measured serum vitamin B12 in 26 of the 27 patients with impaired proprioception (one specimen was misplaced): none had values below the normal laboratory reference range of 138 pmol/L. The mean serum vitamin B12 amongst those with impaired proprioception was 365 pmol/L (SD± 127.3).

Table 2 Comparative frequency and severity of neuropathic clinical features

	Total Cohort N=67	Symptomatic DSP N= 24		Asymptomatic DSP N=30	*P*-value
Neuropathic Feature	n (%)	n (%)	Severity* median (IQR)	n (%)	
Pain, aching, burning	20 (29.9)	16 (66.7)	5 (3.8-6.3)	-	
"Pins-and-Needles"	19 (28.4)	14 (58.3)	4 (3-6.8)	-	
Numbness	21 (31.3)	17 (70.8)	4 (3-6)	-	
Impaired Vibration	33 (49.3)	14 (58.3)	-	19 (63.3)	0.71
Reduced/absent Reflexes	53 (79.1)	24 (100)	-	30 (100)	1.00
Decreased Pin Sensibility	53 (79.1)	22 (91.7)	-	27 (90)	0.83
Impaired Proprioception	27 (40.3)	15 (62.5)	-	12 (40)	0.10
Decreased Distal Power	1 (1.5)	1 (4.2)	-	0	0.26

SDSP, symptomatic distal sensory polyneuropathy; ADSP, asymptomatic distal sensory polyneuropathy; IQR, interquartile range ; *Severity according to Brief Peripheral Neuropathy Screen (BPNS) numerical rating scale of 1 (mild) to 10 (severe). *P*-values derived by z-test.

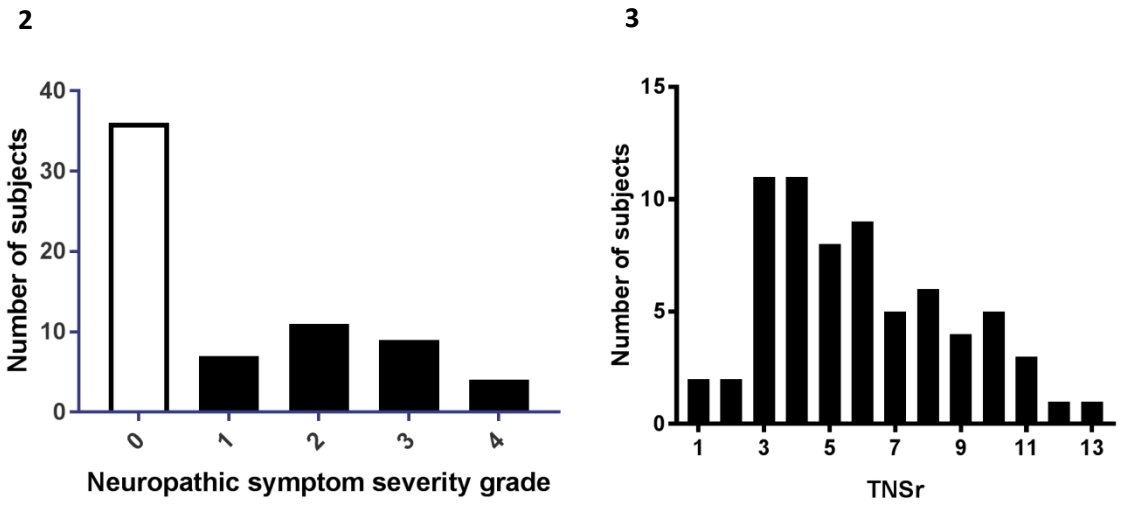


Figure 2 Distribution of the Brief Peripheral Neuropathy Screen (BPNS) total sensory severity grade amongst the total cohort. The total grade is determined by the highest points assigned to each symptom on a numerical rating scale (NRS) of 1 (mild) to 10 (severe). Grade 0 refers to no symptoms; Grade 1 refers to NRS 2-3; Grade 2 refers to NRS 4-6; Grade 3 refers to NRS 7-8; Grade 4 refers to NRS 9-10.

Figure 3 Distribution of the reduced Total Neuropathy Score (TNSr) for the entire cohort. The total score (out of 20) represents the anatomical extent of the 5 symptoms and signs. Each symptom and sign is graded from 1 (only in toes/fingers or mild weakness) to 4 (up till knees/elbow or paralysis).

Autonomic Dysfunction

Figure 4 illustrates the frequency of abnormal CASr in the total cohort. A majority (60%) of the cohort attained a positive CASr (i.e. at least 1 out of 3 points). Half of these participants scored significantly in the SAS or TIS only; 25% had only cardio-autonomic signs (i.e. abnormal heart rate variability and orthostatic hypotension) and the other 25% had both cardio-autonomic and significant scores for SAS or TIS. Two participants had diabetes and only one of these had a positive CASr. Of those who had a positive CASr, 5 (13%) participants achieved the maximum score of 3, 6 (15%) achieved a score of 2, and 29 (73%) achieved a score of 1.

Ten patients exhibited a postural drop in blood pressure; half of these patients had associated symptoms. The most common symptoms reported were light-headedness (49%) and cold feet (39%) (table 3). The least common symptoms were erectile dysfunction (5%), persistent diarrhea (8%) and increased sweating in hands (9%). None of the participants had more than 7 (out of the 12) autonomic symptoms present (see figure 5). Most participants had either 0, 2 or 4 symptoms present. The severity scores were relatively low amongst the cohort, with most of the symptomatic patients scoring a TIS of 2

(out 60 for men and 55 for women) (see figure 6). Anticholinergics, diuretics, beta blockers and alpha blockers can potentially affect autonomic tests (Robinson-Papp, Sharma, Simpson, & Morgello, 2013). It is important to note that 2 of the patients with postural hypotension were taking anti-hypertensive medication, and another 2 were on tricyclic agents. A third patient taking a tricyclic agent also had significant SAS scores. The remaining patients were not taking any of these medications.

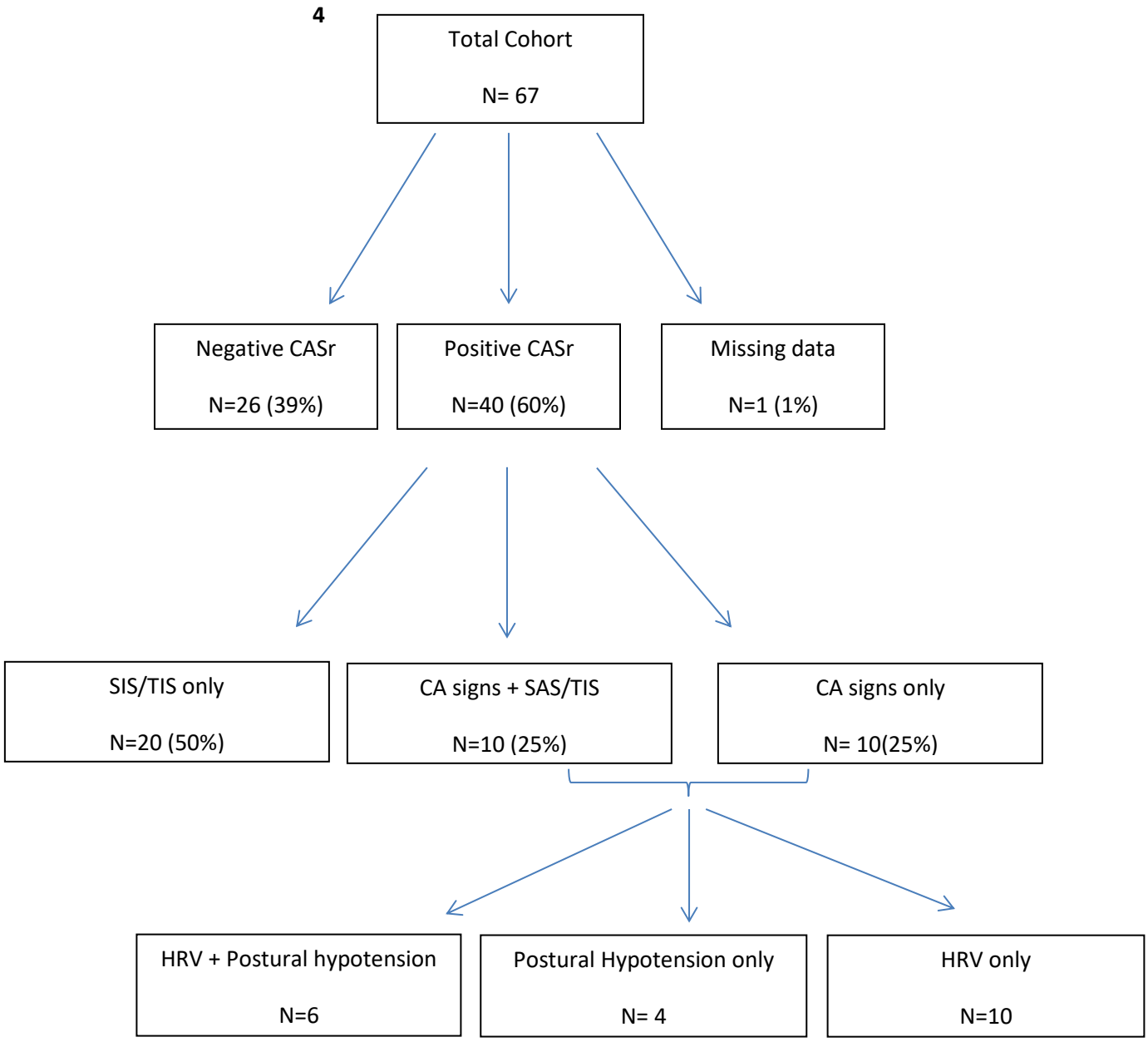
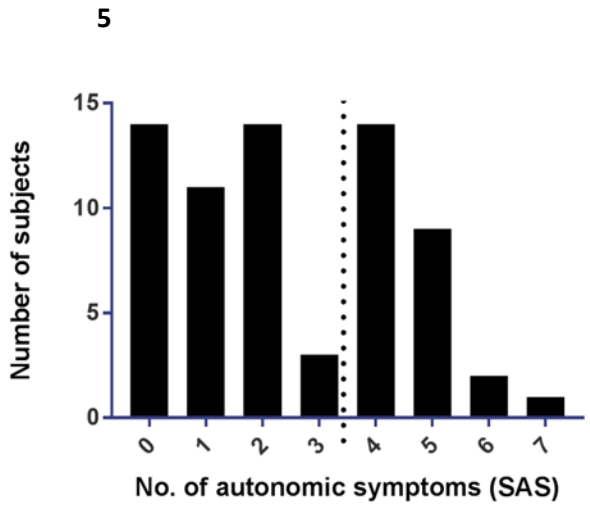


Figure 4 Distribution of the reduced Composite Autonomic Score (CASr) according to Survey of Autonomic Symptoms (SAS) or Total Impact Score, and cardio-autonomic (CA) signs amongst the total cohort. Positive CASr= 1 to 3 points. Negative CASr= 0 points. SAS>3 points ;TIS>7 points; HRV, heart rate variability

Table 3 Frequency and severity of each autonomic symptom amongst the total cohort. Each symptom is scored on severity scale from 1 (absent) to 5(severe).

Autonomic Symptom	Symptom frequency, n(%)	Symptom severity, median (IQR)
Light headedness	33 (49.3)	3 (2-3)
Dry mouth/dry eyes	17 (25.4)	2 (1-2)
Blue or pale feet	12 (17.9)	3 (2-4)
Colder feet	26 (38.8)	4 (2-5)
Decreased sweating in feet	14 (20.9)	2 (1-2)
Decreased/absent sweating in feet after exercise	13 (19.4)	2 (1-2)
Increased sweating in hands	6 (9)	2 (1-2)
Nausea, vomiting, bloating after small meal	14 (20.9)	2 (2-3)
Persistent diarrhoea	5 (7.5)	2 (1-3)
Persistent constipation	16 (23.9)	2 (1-4)
Urinary incontinence	9 (13.4)	2 (1-3)
Erectile Dysfunction*	3 (4.5)	4 (3-5)

IQR, interquartile range ; *For males only



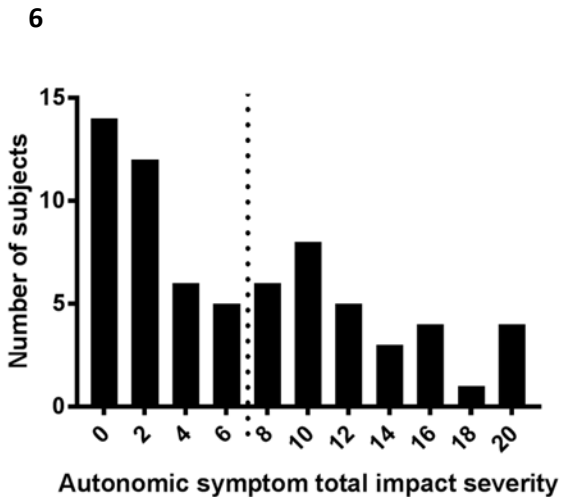


Figure 5 Number of autonomic symptoms that were present amongst the total cohort. The dotted line represents the median*. SAS, survey of autonomic symptoms

Figure 6 Frequency of the severity scores (TIS, total impact score) amongst the total cohort. The dotted line represents the median*.

Table 4 Autonomic symptoms and signs by presence of neuropathy

Characteristic	Total n=67	DSP-2 signs n=54	No DSP-2signs n=13	*P*- value
Symptoms				
SAS score, median (IQR)	2 (1-4)	2 (1-4)	1 (0-4)	0.22¥
SAS total impact score, median (IQR)	6 (1-10.5)	6.5 (2-10.8)	2 (0-8)	0.18¥
Signs				
Abnormal Heart rate variability, N (%)	16 (23.9)	14 (25.9)	2 (15.4)	0.42*
Postural hypotension, N (%)	10 (14.9)	10 (18.5)	0	0.09*
CAS, N (%)	38 (56.7)	32 (59.3)	6 (46.2)	0.4*

DSP, distal sensory polyneuropathy; SAS, survey of autonomic symptoms; IQR, interquartile range; CAS, composite autonomic score; *P*-values derived by *z-test, ¥Mann-Whitney

Table 4 shows that there was no correlation between DSP-2signs and the presence of autonomic symptoms (*P*= ≥ 0.18) or abnormal heart rate variability (*P*= 0.42), but there was a trend towards an association with postural hypotension (*P*=0.09). Those with SDSP showed a trend towards having more autonomic symptoms (median 4; IQR 1-5) compared to those with ADSP (median 2; IQR 1-3; *P*=0.063) (see supplementary table S3). Those with SDSP also had significantly higher TIS than those with ADSP (median score 10; IQR 2.8-15 vs median score 3; IQR 2-7.8; *P*=0.0008). There was a moderate correlation between ART duration and the SAS which was statistically significant (r=0.34, *P*=0.002) (see supplementary figure 3). Thus, those with autonomic symptoms were more likely to have SDSP and longer exposure to ART.

Functional Impairment associated with DSP and Autonomic Dysfunction

In order to assess whether the presence of DSP is functionally relevant in a patient's daily life, we asked each participant to complete the LEFS questionnaire. Three participants did not complete the LEFS. Only

one participant obtained a LEFS score of less than 50%. This participant, who had an LEFS score of 47.5%, also had SDSP and a high CASr score of 3. Table 5 demonstrates the LEFS activities that were most frequently impaired amongst the cohort. Overall, we found that those with DSP had significantly lower LEFS percentage scores than those without DSP (93.8% vs 100%; IQR 94.4-100; *P*=0.039) (see figure S4), and those with SDSP had lower scores than ADSP (88.8% vs 96.3%; IQR 90.0-98.8; *P*=0.04) (see table S3).

There was a moderate negative correlation between LEFS and CASr (r=-0.36; *P*=0.0035) (see figure S5), but a stronger negative correlation between autonomic severity scores and LEFS (r=-0.59; *P*=<0.001) (see figure S6). Interestingly, the LEFS correlated stronger with the BPNS symptom severity (r=-0.46; *P*<0.001) (see figure S7) compared to the TNSr (r=-0.24; *P*=0.05) (see figure S8).

Table 5 Frequency of impaired LEFS activities amongst the total cohort, DSP 2-signs and SDSP

Difficult Activity	Total Cohort N=67	DSP-2 signs N=54	SDSP N=24
Occupation or school work	13 (19.4)	12 (22.2)	6 (25)
Hobbies e.g. going to church	5 (7.5)	5 (9.3)	4 (16.7)
Taking a bath	3 (4.5)	3 (5.6)	2 (8.3)
Walking from room to room	4 (6)	3 (5.6)	2 (8.3)
Putting on shoes or socks	6 (9)	6 (11.1)	4 (16.7)
Squatting	11 (16.4)	10 (18.5)	6 (25)
Lifting object from floor	12 (17.9)	9 (16.7)	3 (12.5)
Light housework	3 (4.5)	3 (5.6)	2 (8.3)
Heavy activities	22 (32.8)	19 (35.2)	11 (45.8)
Getting into/out of car/taxi	7 (10.4)	6 (9)	4 (16.7)
Walking from your home to neighbours'	7 (10.4)	6 (11.1)	4 (16.7)
Walking to shop or church	14 (20.9)	12 (22.2)	8 (33.3)
Going up/down 10 stairs or steep hill	29 (43.3)	24 (44.4)	13 (54.2)
Standing for 1 hour	22 (32.8)	20 (37)	13 (54.2)
Sitting for 1 hour	10 (14.9)	9 (16.7)	7 (29.2)
Fast walking on even ground	20 (29.9)	19 (35.2)	9 (37.5)
Fast walking on uneven ground	29 (43.3)	25 (46.3)	11 (45.8)
Making sharp turns while walking/running	22 (32.8)	21 (38.9)	12 (50)
Standing up fast from squatting	21 (31.3)	18 (33.3)	11 (45.8)
Turning in bed	8 (11.9)	8 (14.8)	6 (25)

Figures represented as N(%); LEFS, lower extremity functional scale; DSP, distal sensory polyneuropathy; SDSP, symptomatic distal sensory polyneuropathy

Chapter 4

Discussion

In this African cohort of 67 healthy HIV-infected patients on ART for at least 5 years, most of whom were virologically suppressed and not using dNRTI, DSP remains common. Using a stringent definition of DSP

that includes 2 neuropathic signs, 81% of participants had DSP. This is considerably higher than the prevalence of 60% in a similar South African D4T-exposed cohort [10] with DSP defined as 2 neuropathic signs on ART for a median of only 14 months. Most (59%) of those with DSP had previous TB which can serve as marker of advanced HIV before or shortly after ART initiation. This apparent severity of HIV at the start of ART may explain the high prevalence of DSP in our cohort.

Age was not associated with the presence of DSP or SDSP in our study population. Our small sample size, narrow age range (SD 8.6 years) and prevalent neuropathy status may account for this. Previous TB had only a marginal association with DSP when the definition of 3 neuropathic signs was used. This may suggest that only the more severe neuropathy cases were associated with previous TB. Despite the significant number of participants on Isoniazid without Vitamin B6, there was no association between the lack of this supplement and DSP. However, the total number of INH-users was small making this observation inconsequential. BMI, mean SBP and high triglyceride levels were previously found to be associated with DSP in pre-ART South African populations [5,10] but this was not reproduced in this cohort who had been on effective ART for more than 6 years.

Almost half (44%) of those with DSP were symptomatic. Interestingly, only 2 patients with SDSP used analgesia, viz. amitriptyline. This may indicate that SDSP is not well recognized by clinicians treating these healthy ART-experienced patients, and is thus under-treated. This prospect is quite disconcerting in view of our finding that LEFS scores are lower in SDSP than those without neuropathic symptoms, indicating that these symptoms do impact daily living.

Our study found proprioception dysfunction in 40% after 7 years of ART. In a similar African cohort, Center *et al* found that the frequency of altered toe proprioception was 2% (in 102 individuals) after 2 years of ART. The reason for this substantial difference is unclear. Vitamin B12 deficiency was excluded as a confounder in our cohort. Interestingly, Centner *et al* also found that the prevalence of other large fiber signs (reflexes and vibration) increased after 2 years [5]. Proprioception is not usually assessed in HIV neuropathy studies and our result suggests that it may be important to use this modality to assess large fiber dysfunction in patients on long term ART.

Abnormal autonomic tests, particularly the SAS, were quite prevalent in this relatively young ART-exposed group. It is important to note that most of these patients were not taking medications that could affect the results of these autonomic tests. High prevalence of autonomic dysfunction has also been found in other ART-exposed, healthy populations [24,30]²⁴²⁴. Although,

admittedly, one of these populations consisted of slightly older individuals many of whom suffered from metabolic comorbidities and substance abuse [24]. Clinical examination excluded dehydration, cardiac failure or any acute illness that could account for the postural hypotension found in 15% of the population. Adrenal insufficiency was not excluded as a possible confounder. We did not exclude those being treated for hypertension and diabetes as this could have biased our study results towards individuals free from chronic diseases of lifestyle – a population that is not representative of South African society at large. However, the notion of confounding is not absolutely certain because of the small number of hypertensives (8) and diabetics (2) within our cohort. Furthermore, only 2 out of the 10 with the postural hypotension were taking anti-hypertensive treatment. The SAS was useful in that it explored the other domains of autonomic function that our limited cardio-autonomic testing did not. Zilliox *et al* found an association between an increased SAS score or TIS and a reduced quantitative sudomotor axon reflex test (QSART) sweat volume [36], thus further validating the SAS as an important screening tool. Our most common autonomic symptoms were light headedness and cold feet. Robinson-Papp *et al* found similar results despite their age and sex difference [24].

Our study found that heart rate variability was the predominant autonomic sign. The characteristics of the HIV-positive groups with decreased heart rate variability in the studies by Askgaard *et al* and Lebech *et al* were strikingly comparable to our own cohort: median CD4 of 552 cells/µL and on ART for a median of 7.2 years, 99% of whom had suppressed viral loads[27,28]. And similar to these studies, there was no association between autonomic parameters and CD4 count and ART duration in our cohort. Interestingly, although there was no association between autonomic dysfunction and DSP, those with SDSP were more likely to have autonomic dysfunction than those without. The association between CASr and SDSP would suggest a common pathogenesis for this shared small fiber neuropathy. Whether the nature of this insult is immune regulated or as a result of direct ART toxicity is not clear.

This study confirms that DSP and SDSP impair daily living. Although the median LEFS percentages in those with DSP and SDSP were relatively high implying that our cohort did not have marked functional impairment overall, these scores were significantly lower than those who did not have DSP or SDSP. In the American study by Galantino, participants with DSP obtained a median LEFS raw score of 39 (48.8%) which is considerably lower than our cohort with DSP[15]. This may be indicative of the resilience of our patients who come from a low socio-economic background. Higher TIS scores also seemed to adversely affect LEFS percentages in this cohort, although this inverse relationship was only moderately strong.

We believe our simple approach to diagnosing DSP and autonomic dysfunction by using bedside skills and tools will assist clinicians in screening for this common complication amongst HIV-infected patients attending resource-restricted community health care centers in South Africa. This will hopefully lead to prompt and appropriate treatment of symptomatic DSP and autonomic dysfunction thereby diminishing its negative affect on function and quality of life.

Recommendations and Limitations

Given the small sample size, it may be difficult to extrapolate these results to the wider ART-exposed population. Furthermore, under the current "Universal Test and Treat" policy, asymptomatic HIV-infected patients with preserved CD4 counts are now receiving ART - a population that has not been extensively studied. Our cohort, however, is representative of a South African population that initiated ART during the advanced stages of HIV under the previous government-sponsored ART roll-out program. Clinicians must keep this difference in mind when dealing with the former group.

A longitudinal analysis would have been useful to ascertain whether DSP or autonomic dysfunction was present prior to ART initiation, and if the prevalence and characteristics of these complications altered on long-term ART. We only enquired about current alcohol abuse; not previous alcohol abuse. We acknowledge that previous alcohol abuse may have been a confounder in those with DSP and autonomic dysfunction.

The investigators did not use formal neurophysiological testing to confirm the diagnosis of DSP or autonomic dysfunction. However, these tests assess large fiber function and are expected to be normal in small fiber neuropathies such as HIV-associated DSP. The validated neuropathy screening tools, BPNS and rTNS, can be used as guides for screening for DSP in the ART-exposed population.

Conclusion

DSP is prevalent in this community-based, HIV-infected cohort on ART ≥5years and can lead to functional impairment in some individuals. Autonomic dysfunction was associated with SDSP. DSP and SDSP impaired daily living. Further research on autonomic dysfunction and functional impairment in HIV-associated DSP is needed. Attention to pain management must remain a priority.

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Supplementary Tables

Table S1: Fasting Metabolic Syndrome values by DSP-2 signs

Characteristic	Total N=67	DSP 2-Signs N=54	DSP-free N=13	p-value
BMI (kg/m²), mean (±SD)	28 (6.3)	27.6 (5.7)	29.9 (8.3)	0.23ᵗ
SBP (mmHg), mean (±SD)	125.2 (20.2)	125.3 (20.5)	124.9 (19.5)	0.95ᵗ
DBP (mmHg) mean (±SD)	78.3 (14.4)	78.5 (14.7)	77.2 (13.4)	0.77ᵗ
hsCRP (mg/L), median (IQR)	4.5 (2.2-7.8)	4.6 (2.4-7.8)	3.6 (1.1-7.2)	0.44ʸ
Fasting blood glucose (mmol/L), median (IQR)	4.9 (4.5-5.4)	4.9 (4.5-5.4)	5.0 (4.6-5.3)	0.95ʸ
Total cholesterol (mmol/L), median (IQR)	4.5 (3.8-5.0)	4.5 (3.8-5.0)	4.6 (3.8-5.0)	0.99ʸ
Triglycerides (mmol/L), median (IQR)	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.94 (0.7-1.0)	0.59ʸ
HDL (mmol/L), median (IQR)	1.5 (1.2-1.9)	1.4 (1.1-1.9)	1.7 (1.6-2.0)	0.09ʸ
LDL (mmol/L), median (IQR)	2.4 (1.9-2.9)	2.4 (2.0-2.9)	2.5 (1.9-2.9)	0.70ʸ

DSP, distal sensory polyneuropathy; BMI, Body mass index; SD, standard deviation; SBP, systolic blood pressure; DBP, systolic blood pressure; hsCRP, highly-sensitive C-reactive protein; IQR, interquartile range; HDL, high density lipoprotein; LDL, low density lipoprotein

Table S2 Characteristics of participants with DSP (3signs) and without DSP (3 signs)

Characteristic	Total N=67	DSP (3 signs) N=37	DSP-free (3signs) N=30	p-value
Age (years), mean (±SD)	41.6 (8.6)	42.8 (36-47)	40.3 (8.5)	0.24ᵗ
LEFS %, median (IQR)	95 (88.1-100)	93.8 (83.8-98.8)	97.5 (91.3-100)	0.15ʸ
Current CD4 (cells/uL), mean (±SD)	560.2 (236.5)	590.1 (264.2)	528.2 (202.5)	0.32ᵗ
Viral suppression (<400 copies/ml), N (%)	56 (83.6)	30 (81.1)	26 (86.7)	0.54
Viral load <20 copies/ml , N %	50 (74.6)	26 (70.3)	24 (80)	0.36
Previous TB, N (%)	39 (58.2)	25 (67.6)	14 (46.7)	0.085

DSP, distal sensory polyneuropathy; SD, standard deviation; LEFS, lower extremity functional scale; TB, tuberculosis; *P*-values derived by *z-test, ᵗt-test, ʸMann-Whitney

Table S3 Characteristics of participants with SDSP and ADSP

Characteristic	SDSP N= 24	ADSP N= 30	*P*-value
Women, N (%)	21 (87.5)	29 (96.7)	0.20*
Age (years), mean (±SD)	42.5 (10.5)	41.2 (7.1)	0.61ᵗ
LEFS % , median (IQR)	88.8 (81.3-97.5)	96.3 (90.0-98.8)	0.04ᵞ
SAS score, median (IQR)	4 (1-5)	2 (1-3.8)	0.063
SAS total impact score, median (IQR)	10 (2.8-15)	3 (2-7.8)	0.0008
Weight (kg), mean (±SD)	70 (15.4)	70.5 (15.9)	0.92ᵗ
Height (m), median (IQR)	1.62 (1.55-1.65)	1.59 (1.55-1.62)	0.44 ᵞ
BMI (kg/m²), mean (±SD)	27.5 (6.6)	27.6 (5.1)	0.94ᵗ
SBP (mmHg), mean (±SD)	127.5(20.8)	123.6 (20.5)	0.50ᵗ
DBP (mmHg) mean (±SD)	80.9 (15.5)	76.6 (14.0)	0.29ᵗ
Current CD4 (cells/uL), mean (±SD)	517 (373-581)	602.9 (271.5)	0.27ᵗ
Viral suppression (<400 copies/ml), N (%)	19 (79.2)	25 (83.3)	0.70*
Viral load <20 copies/ml , N %	18 (75)	20 (66.7)	0.51*
ART duration (years), median (IQR)	6.5 (5.75-10.5)	8 (6.0-9.0)	0.85ᵞ
D-Drugs, N (%)	2 (8.3)	1 (3.3)	0.43*
Efavirenz, N (%)	13 (54.17)	17 (56.67)	0.75*
Isoniazid, N (%)	5 (20.83)	4 (13.33)	0.46*
Previous TB, N (%)	16 (66.67)	16 (53.33)	0.32*
hsCRP (mg/L), median (IQR)	6.3 (3.23-10.83)	4.2 (2.2-6.9)	0.26 ᵞ
Fasting blood glucose (mmol/L), median (IQR)	4.9 (4.5-5.18)	5.1 (4.6-5.4)	0.48ᵞ
Total cholesterol (mmol/L), median (IQR)	4.6 (3.7- 5.15)	4.3 (3.9-4.7)	0.73ᵞ
Triglycerides (mmol/L), median (IQR)	0.87 (0.7- 1.2)	0.9 (0.8-1.1)	0.75ᵞ
HDL (mmol/L), median (IQR)	1.44 (1.2-1.9)	1.4 (1.1-2.0)	0.73ᵞ
LDL (mmol/L), median (IQR)	2.38 (2-3.2)	2.4 (2.0-2.8)	0.42ᵞ

SDSP, symptomatic distal sensory polyneuropathy; ADSP, asymptomatic distal sensory polyneuropathy; LEFS, lower extremity functional scale; SAS, survey of autonomic symptoms; IQR, interquartile range; SD, standard deviation; BMI, Body mass index; SBP, systolic blood pressure; DBP, systolic blood pressure; ART, anti-retro viral therapy; D-Drugs, Stavudine (D4T) or didanosine (DDI); hsCRP, highly-sensitive C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein ; *P*-values derived by *z-test, ᵗt-test, ᵞMann-Whitney

Table S4 LEFS and Autonomic characteristics of the total cohort, DSP 2-signs and no DSP

Characteristic	Total Cohort N=67	DSP 2-signs N=54	No DSP N-13	*P*-Value
LEFS % , median (IQR)	95 (88.1 -100)	93.8 (85.6-98.8)	100 (94.4-100)	**0.039**[ɣ]
CASr, N(%)	38 (56.7)	32 (59.3)	6 (46.2)	0.4
SAS score, median (IQR)	2 (1-4)	2 (1-4)	1 (0-4)	0.22
SAS total impact score, median (IQR)	6 (1-10.5)	6.5 (2-10.8)	2 (0-8)	0.18

DSP, distal sensory polyneuropathy; LEFS, lower extremity functional scale; IQR, interquartile range;

CASr, reduced composite autonomic score; SAS, survey of autonomic symptoms

Supplementary Figures

S1

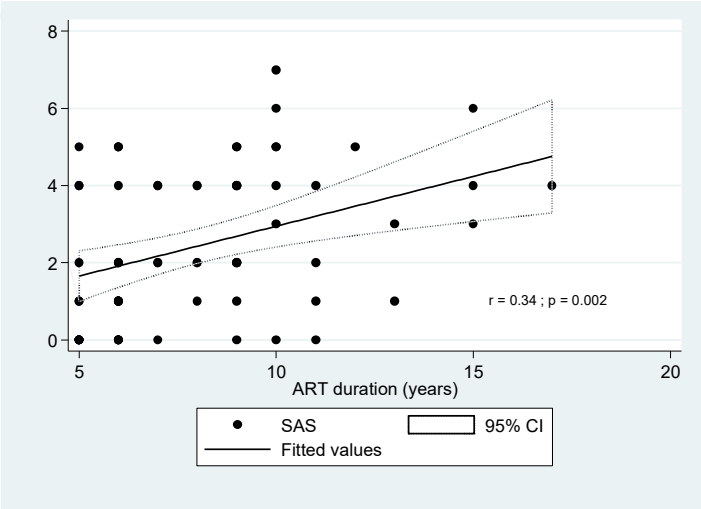

Figure S1 Correlation between ART (anti-retroviral therapy) duration and SAS (survey of autonomic symptoms).

S2

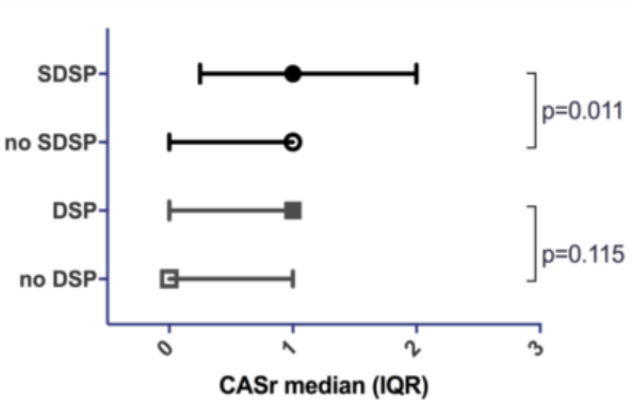

Figure S2 Line graph depicting the association between DSP, SDSP and CASr. The "no SDSP" classification does not only consist of asymptomatic DSP but includes all patients who did not meet criteria for DSP or SDSP. DSP, distal sensory polyneuropathy ; SDSP, symptomatic distal sensory polyneuropathy; CASr, reduced composite autonomic score; IQR, interquartile range

S3

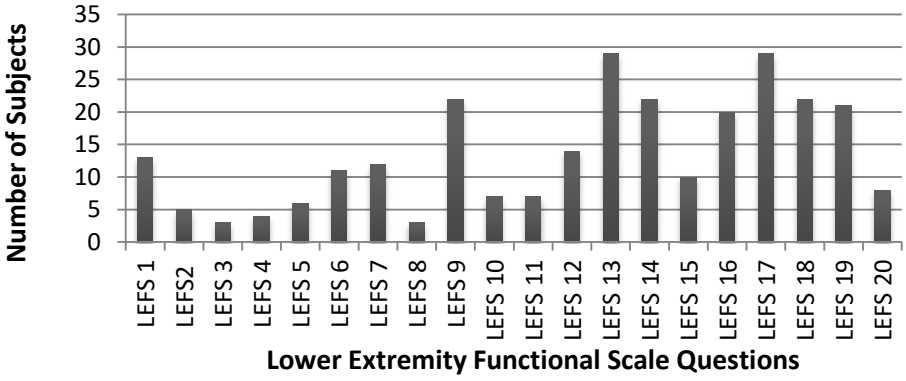

Figure S3 Frequency of impaired LEFS (lower extremity functional scale) activities in the total cohort

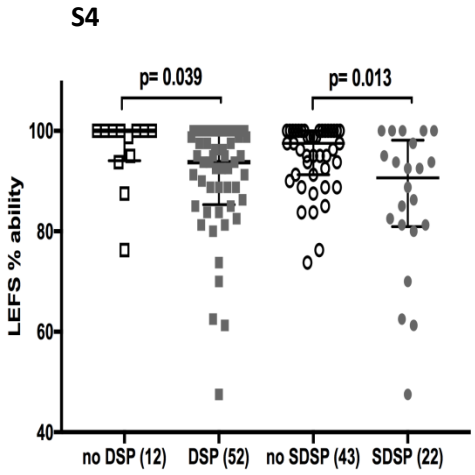

Figure S4 Scatter plot depicting the association between DSP, SDSP and LEFS. The "no SDSP" classification does not only consist of asymptomatic DSP but includes all patients who did not meet criteria for DSP or SDSP. DSP, distal sensory polyneuropathy ; SDSP, symptomatic distal sensory polyneuropathy; LEFS, lower extremity functional scale

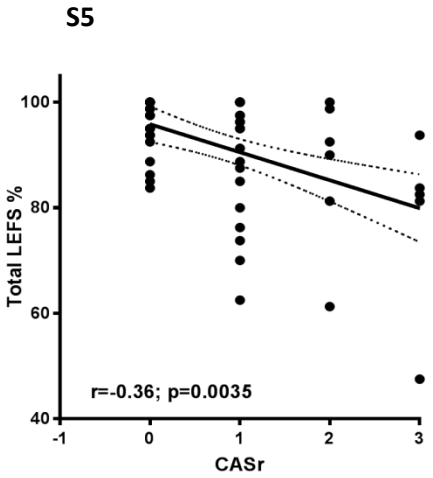

Figure S5 Correlation between LEFS (Lower Extremity Functional Scale) and CASr (reduced composite autonomic score)

S6

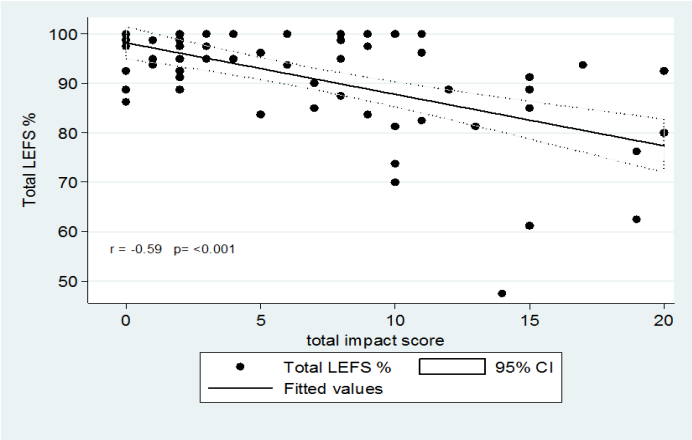

Figure S6 Correlation between LEFS% (lower extremity functional scale) and TIS (total impact score)

S7

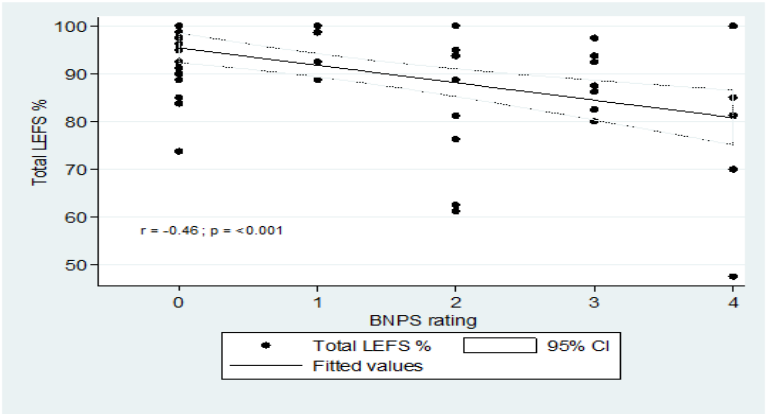

Figure S7 Correlation between LEFS% (lower extremity functional scale) and BPNS (brief peripheral neuropathy screen) Symptom Severity Grade

S8

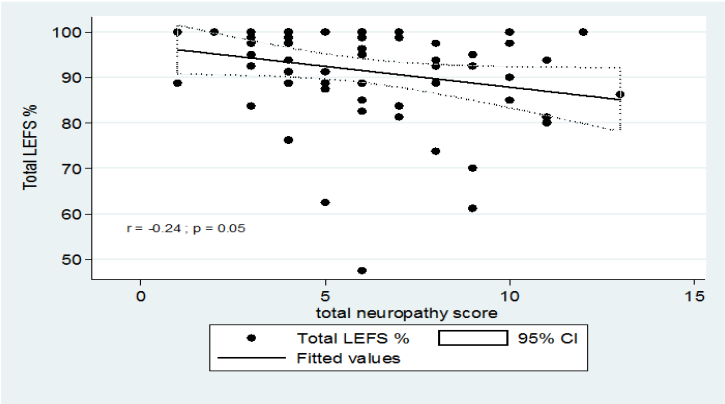

Figure S8 Correlation between LEFS% (lower extremity functional scale) and TNSr (reduced total neuropathy score)

Appendix A

Patient information and consent document: Understanding the role of inflammation and the metabolic syndrome in aging in a South Africans with HIV-infection on ART.

Researchers: Dr M Borkum, Dr S Mazondwa, Dr M Dudley

Supervisors: Dr N Wearne, Prof J Heckmann, Dr J Dave, Prof M Combrinck

You were previously part of a research project studying the effects of antiretroviral therapy (ART) on your body's glands, nerves, blood sugar and cholesterol levels. That study occurred over a 2 year period and you attended at least one of those visits. Here we are asking you whether you would participate in one 5-year follow-up study visit. If we do obtain funding we may contact you again in 5 years' time for a 10 year study visit. Here we would like to give you information about the previous study results as well as what we plan to do in this 5 year visit. Please remember that you do not have to participate in the research and you may talk to anyone you feel comfortable with about the research before deciding.

WHAT HAVE WE LEARNT FROM THE FIRST 2 YEARS (McHAART STUDY)

As you know, ART cannot cure HIV/AIDS but it helps fight the human immunodeficiency virus and has helped patients feel a lot better. ART has made HIV-infection a chronic disease and we anticipate that most people will become older and remain on ART for many years. However, ART also has side-effects. Some of these side-effects include the development of diabetes, cholesterol problems, changes in body shape (usually either increased or decreased fat in certain areas of the body) and damage to the nerves in your feet. Very little is known about these problems in HIV-positive individuals in South Africa. In our initial study we found very few patients developed diabetes or cholesterol problems despite the persons gaining weight. Also, during the 2 years of study we found the blood pressure measurements remained normal. Many people who had pain in their feet before ART did very well on ART and in some the pain went away completely. However, a small proportion developed numbness in the feet as time went by.

In this study we would like to see what happens 5 years after starting ART. This would help the doctors and patients to understand the side-effects of ART after a longer treatment period, how commonly these side effects occur and how they can best be treated.

We will focus less on body fat and more on the heart, brain and kidneys. The study involves two mornings of testing in E7 and G13, New Groote Schuur Hospital (3 hours of testing in total). This will include a heart ECG test and blood pressure test lying and standing. We will also put a 24-hour blood pressure machine on your arm which we would like you to return the next morning.
We will again be studying the blood sugar and cholesterol tests although bloods will only be taken at 2 time points. We will also ask for a urine specimen on 2 mornings- the morning of the tests and the morning when the blood pressure machine is returned.

We will perform the nerve testing (as previously) and a slightly longer version of memory and brain function testing (20-30 minutes). We will ask some questions about your general health and how you copy in your day to day life.

The study visit will extend over 2 mornings; one short visit of 60 minutes (urine and memory tests) and one longer visit of 2.5 hours (blood sampling, heart, nerve and urine tests.

WHAT WILL HAPPEN IF I AGREE TO PARTICIPATE?

The same as previously: We will ask you not to eat or drink anything from 10 pm the night before your appointment. At your appointment the doctor will examine you (your blood pressure, ECG heart tests, nerve test to look for any damage to the nerves in your feet) and will insert a drip cannula (a small plastic device that stays inside the vein allowing all further blood samples to be taken from it thus avoiding any further need for needle pricks). Using a syringe a sample of blood (~ 25 ml, 1 tablespoon) will be taken from the cannula. After taking the blood sample, you will be asked to drink a glass of water with glucose, a form of sugar. A further blood sample will be taken at 2 hours (120 min) after completing this drink. The blood will be sent to a laboratory and tested for glucose, insulin and cholesterol. Some of the blood will be frozen and kept for testing at a later stage. Whilst waiting for your blood samples to be taken the study staff will do the following: a) using a tape measure they will measure your body waist and your height b) take blood pressure measurements c) measure your body weight using a scale.

While you are waiting you will be asked to complete a questionnaire about symptoms and about how you are able to function in your everyday life.

Following this will assess the nerves in your feet and perform the 3 minute brain function test testing memory, rapid finger and hand movements.

New assessments: A neuropsychologist (under supervision of Prof Combrinck), will perform another set of brain function tests in a quiet room for about 20-30 minutes. This will include reading red and green cards (Stroop test), linking numbers or letters with a

pencil (trail-making), putting little pegs/pins into tiny holes (testing fine movements) and memory. This test will be after you have eaten (either day one or two).

A 24 hour ambulatory BP cuff will be placed on your arm and will record your BP while you are walking and sleeping. It will be removed the next morning. You will give a urine sample in the morning before you are free to leave.

All transport costs to the hospital will be covered. On the first day you will receive a lunch packet with a sandwich and juice. We will provide each person R250 for participating in this research to cover your time at the hospital and transport home. After the first day we will provide you with R100 and after you return the second day with the BP machine we will give you the remaining R150.

The study staff will also review all of your previous clinic records including all of your previous blood test results (including HIV status, viral load, CD4 count, liver function, kidney function, etc)

WHAT ARE THE POSSIBLE DISCOMFORTS OF PARTICIPATING IN THE STUDY?

Having blood taken will be the only discomfort in this study. Risk of infection will be minimized by using sterile procedures, and all blood samples will be taken by suitably qualified persons.

WHAT ARE THE POSSIBLE BENEFITS?

If you are found to have any of side-effects of ART then arrangements will be made for you to be seen at a hospital where doctors specially trained in looking after these problems will look after you. This study will help doctors find these problems early therefore the required investigation and treatment can be done before any further problems arise.

DO I HAVE TO PARTICIPATE?

You do not have to participate in this study. Your participation is voluntary and if you agree to participate then you will be required to sign a form. You can withdraw from the study at anytime and this will in no way affect your treatment in the future.

WILL THE INFORMATION REMAIN CONFIDENTIAL?

Your records will only be viewed by your doctors and people involved in this study. Your details will not be made available to anybody not involved in this study. Although absolute confidentiality cannot be guaranteed the staff involved in this study will strive to keep your records as confidential as possible.

CONTACT DETAILS OF STUDY STAFF

Should you have any questions then please contact Dr Megan Borkum or Dr Sim Mazondwa

Ph: 072 246 5633

Principal Investigators: Wearne and Heckmann

Organisation: University of Cape Town

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant _____

Signature of Participant _____

Date_____

Name of Doctor taking consent _____

Signature of Doctor _____

Date_____

Appendix B

Revised V3 May 2015

SA BRIEF PERIPHERAL NEUROPATHY SCREENING/EXAM (UCT REC 221/2008)

Patient Number: _____ Date of Visit (DD/MM/YYYY) ____/____/20____

We are going to ask you a few questions about sensation in your legs. We will also briefly examine the nerves in your arms and legs. We may advise the clinic doctor on treatment.

BPNS : INSTRUCTIONS FOR RECORDING SYMPTOMS: *Ask subject to rate the severity of each symptom in 1a to 1c on a scale of 0 (absent) to 10 (most severe) for right and left feet, legs- worst in last week.* Enter the score for each symptom in the block marked Severity. Enter extent of symptoms eg Soles of feet/ toes (TNS=1); up to ankle (TNS=2); up to knee (TNS= 3) or above (TNS=4) on the TNS score overleaf.

1a. Pain, aching, burning in feet or legs. Ingaba iinyawo zakho zibuhlungu, ziyaqagamba, ziyatshisa kangangee-veki ezimbini?

Normal	Mild	→	→	→	→	→	→	→	→	Severe
0	1	2	3	4	5	6	7	8	9	10
Andinantlungu! →		→		→		→	Ndineentlungu ezigqithisileyo!			

Nn1 Score 1a ☐

1b. "Pins-and-Needles" in feet or legs. Ingaba iinhawo zakho zineenaliti noonotaka kangangee-veki ezimbini?

Normal	Mild	→	→	→	→	→	→	→	→	Severe
0	1	2	3	4	5	6	7	8	9	**10**
Andinantlungu! →		→		→		Ndineentlungu ezigqithisileyo!				

Nn2 score 1b ☐

1c. Numbness (lack of feeling) in feet or legs. Ingaba iinyawo zakho zinobundindisholo kangangee-veki ezimbini?

Abukho ubundindisholo		→	→	→	→	→	→	→	→	Andiva nto
0	1	2	3	4	5	6	7	8	9	**10**

Nn3 Score 1c ☐

TOTAL SENSORY PRESENCE/SEVERITY SCORE: Obtain the single highest severity score from 1-10 in 1(a - c) above:

0 = Grade 0 1-3 = Grade 1 4- 6 = Grade 2 7-8 = Grade 3 9-10 = Grade 4

Total sensory severity GRADE ____/4 nn4

1e. Do you have any other unpleasant symptoms in you legs such as cramps? Yes (1) No (0) nn6

1f. Has anything helped for the pain (medicine or other) ? _____ Yes (1) No (0) nn7

1i. If you have any of the abovementioned unpleasant symptoms in your feet, when did it start and for how long?
_____ N/A (0) ____ years nn10

2. INSTRUCTIONS FOR EVALUATING PERCEPTION OF VIBRATION:
Press the 2 ends together of a 128 Hz tuning fork, and release suddenly; place the vibrating tuning fork on the subject's clavicle; can they recognise the vibration or "buzzing" (ngcungcazela) of the tuning fork? Repeat and immediately place the vibrating tuning fork firmly on the interphalangeal bone (not nail) of one great toe and begin counting the seconds. Subject to tell you when the "buzzing" stops. Repeat on the other side.

Vibration Perception
(Take highest score but both R & L must be abnormal)

0- Vibration felt for >10 seconds (normal)
1- Vibration felt for 6-10 seconds (mild loss)
2- Vibration felt for 5 seconds or less (moderate loss)
3- No feeling of vibration (severe loss)

	Right	Left	
Great toe interphalangeal bone	___	___	(2) use highest value_____/3 nn11

Revised V3 May 2015

3. INSTRUCTIONS FOR EVALUATING DEEP TENDON REFLEXES:

With the subject seated, the examiner uses one hand to press upward on the ball of the foot, dorsiflexing the subject's ankle to 90 degrees. Using a reflex hammer (long-handled), the examiner strikes the Achilles tendon.

Reflexes
- 4- Absent
- 3- Reduced (difficult to elicit)
- 2- Normal deep tendon reflexes
- 1- Hyperactive deep tendon reflexes
- 0- Clonus

	Right	Left		
Ankle Reflexes: (Take highest score but both R & L must be abnormal)	_____	_____	(3) use highest value____/4	nn12

Final score for BPNS (1+2+3) ____/11 nn13

Reduced TNS score: tick in the box :

	0	1	2	3	4	
a. Sensory symptoms from 1a,b,c: Pain, burning pins or numbness	*none*	*Only in toes or soles of feet*	*Symptoms extend to ankle or wrist*	*Symptoms extend to knee or elbow*	*Symptoms knee or elbow or functionally disabling*	nn14
b. Pin sensibility	*normal*	*Reduced in fingers /toes*	*Reduced up to wrist/ ankles*	*Reduced up to elbow/ knee*	*Reduced above elbow/ knee*	nn15
c. Vibration sensibility (use normal as for BPNS)	*normal*	*Reduced in fingers /toes*	*Reduced up to wrist/ ankles*	*Reduced up to elbow/ knee*	*Reduced above elbow/ knee*	nn16
d. Deep tendon jerks	normal	Ankle reflexes reduced	Ankle reflexes absent	Ankle reflexes absent, other reduced	All reflexes absent	nn17
e. Strength- ankle and toes plantar & dorsi-flexion	normal	Mild weakness (MRC 4)	Moderate weakness (MRC 3)	Severe weakness (MRC2)	Paralysis (MRC 0-1)	nn18

Final score for rTNS (a+b+c+d+e) _____/20 nn19

Symptomatic DSP: a ≥1 and b≥1 or c≥1 or d≥1 yes 1/ no 0 _____ nn20

Asymptomatic DSP: a =0 yes 1/ no 0 _____ nn21

Proprioception in both toes Normal=0 reduced=1 absent=2 _____ nn25

Additional

f. Number of autonomic symptoms	*none*	*1 symptom*	2 symptoms	3 symptoms	4-5 symptoms	nn26

g. Autonomic symptom impact score	0		nn27

Do you fall easily? yes 1/ no 0 _____ nn28

Appendix C

LEFS-Modified (Journal of Social Aspects of HIV/AIDS vol 11 (1), 2014)

Study number_____ Date :
___/___/201__

Sinomdla wokwazi ukuba ingaba ufumana ubunzima ekwenzeni lemisebenzi ikhankanywe ngezantsi ngenxa yengxaki ngelungu elingezantsi lomzimba wakho. Sekilisha inombolo kumgca ngamnye ongqamelana/ongqinelana nempendulo yakho efanelekileyo.

Nceda unike impendulo kumsebenzi ngamnye. Namhlanje, ingaba unobunzima okanye ungaba nobunzima:

Umsebenzi	Awukwazi ukuwenza umsebenzi	Unzima kakhulu	Unzinyana	Unzima kancinci	Awukho nzima
1. Bokwenza eminye yemisebenzi yakho odla ngokuyenza (umz. Umsebenzi okungenisela imali okanye nokuba ngowuphi na umsebenzi owenzayo), umsebenzi wasekhaya okanye umsebenzi wesikolo	0	1	2	3	4
2. Bokwenza izinto odla ngothanda ukuzenza, izinto ezikonwabisayo okanye imidlalo, umz. ukuya emitshatweni, ecaweni okanye ukutyelela abahlobo	0	1	2	3	4
3. Bokuya/bokuphuma kwigumbi lokuhlambela okanye lokuhlamba	0	1	2	3	4
4. Bokuhamba phakathi kwamagumbi (njengokusuka kwigumbi lakho lokulala usiya kwigumbi langasese, ekhitshini, njalo njalo.)	0	1	2	3	4
5. Bokunxiba nokuba sesiphina isihlangu okanye iikawusi, kuquka nezilipasi okanye izihlangu ezivulekileyo	0	1	2	3	4
6. Bokuchopha (umz. Ukuchopha ethoyilethi okanye ukuchopha nokuba kuphi)	0	1	2	3	4
7. Bokuphakamisa into, enjengengxowa yegrosari okanye imbhombhozi encinci yeelitha eziyi-5 ezele ngamanzi, ibhaskethi yeetapile, njl njl., ephantsi	0	1	2	3	4
8. Bokwenza imisebenzi engekho nzima ekhayeni lakho (enjengokupheka, ukucoca indlu, ukondlula ibhedi okanye nokuba ngowuphi na umsebenzi olula ekhaya)	0	1	2	3	4
9. Bokwenza imisebenzi enzima ekhaya (enjengokomba, ukuphakamisa ingxowa enzima yeetapile, imbhombhozi yamanzi yeelitha	0	1	2	3	4
10. Bokungena okanye bokuhlika emotweni okanye eteksini	0	1	2	3	4

11. Bokusuka endlwini yakho usiya kwindlu kammelwane wakho okanye ukuhamba iimitha eziyi-100	0	1	2	3	4
12. Bokuhamba ikhilomitha, umzekelo ukuya emarikeni/evenkileni, ecaweni okanye nakweyiphi na indawo	0	1	2	3	4
13. Bokunyuka usehla kwizitezi eziyilishumi okanye ukuhamba kwindlela enyukayo okanye kumhlaba ongagudanga okanye ongekho tyaba	0	1	2	3	4
14. Bokuma iyure	0	1	2	3	4
15. Bokuhlala iyure, njengaxa usecaweni, eteksini okanye ezintlanganisweni	0	1	2	3	4
16. Bokuhamba ngokukhawuleza kumhlaba othe tyaba	0	1	2	3	4
17. Bokuhamba okanye bokubaleka kumhlaba ongagudanga/ongathanga tyaba	0	1	2	3	4

18. Bokujika kwidolo [turn] elibukhali ngelixa uhambayo/bokubaleka ngamendu	0	1	2	3	4
19. Bokuma ngokukhawuleza eva kokuchopha	0	1	2	3	4
20. Bokujika ebhedini	0	1	2	3	4

Activity	Unable to perform activity	Quite a bit of difficulty	Moderate difficulty	A little bit of difficulty	No difficulty
1. Any of your usual work, (e.g. work that earns you income or any other work you do) housework or school activities	0	1	2	3	4
2. Your usual hobbies, recreational or sporting activities, e.g. attending weddings, church or visiting friends	0	1	2	3	4
3. Getting into or out of the bath/taking bath	0	1	2	3	4
4. Walking between rooms (such as walking from your room to toilet, bathroom, kitchen, etc)	0	1	2	3	4
5. Putting on any kind shoes or socks, including slippers or open shoes, if applicable	0	1	2	3	4
6. Squatting (e.g. squatting on pit latrine/doing any squatting activity)	0	1	2	3	4
7. Lifting an object, like a bag of groceries or a small container like a 5-litre container full of water, basket of potatoes, etc., from floor	0	1	2	3	4
8. Performing light activities around your home (such as preparing a meal, cleaning a house, making a bed or any other light activity at home)	0	1	2	3	4

9. Performing heavy activities around your home (digging, lifting a heavy bag of potatoes, 20-litre gerrican of water, shifting a big items, etc.	0	1	2	3	4
10. Getting into or out of a car/taxi	0	1	2	3	4
11. Walking across from your home to a neighbour's or walk about 100m across	0	1	2	3	4
12. Walking a km, such as going to market, church or any other place	0	1	2	3	4
13. Going up or down 10 stairs (about 1 flight of stairs) or walking up a steep and irregular ground	0	1	2	3	4
14. Standing for 1 hour	0	1	2	3	4
15. Sitting for 1 hour, like when in church, taxi or meetings	0	1	2	3	4
16. Fast walking on even ground	0	1	2	3	4
17. Fast walking/running on uneven ground	0	1	2	3	4
18. Making sharp turns while walking/running very fast	0	1	2	3	4
19. Standing up fast from squatting	0	1	2	3	4
20. Turning in bed	0	1	2	3	4

Appendix D

Survey of Autonomic Symptoms

Study number:_____ Date: ___/____/201__

Uluhlu 1. Uphononongo ngeempawu (okanye izigulo) ezizenzekalayo[a]						
	Q1a. Ingaba wakha wanazo na ezinye zezimpawu zempilo zilandelayo kwezinyanga zintandathu zidlulileyo? 1 = Ewe; 0 = Hayi	Q1b. Ukuba uphendule u-Ewe kuMbuzo 1a, ungathi ezimpawu zikukhathaza kangakanani. 1 =Nakanye; 2= Kancinci; 3 = Ngamanye amaxesha; 4 = Manqapha- nqapha; 5 = Kakhulu				
Iimpawu/iingxaki zempilo						
1.Ingaba unesiyezi?	1 = Ewe 0 = Hayi	1	2	3	4	5
2.Ingaba unomlomo owomileyo okanye amehlo omileyo?	1 0	1	2	3	4	5
3. Ingaba iinyawo zakho zimpatsha-mpatsha okanye zibhlowu?	1 0	1	2	3	4	5
4. Ingaba iinyawo zakho ziyabanda kunomzimba wakho wonke?	1 0	1	2	3	4	5
5. Ingaba kwehlile na ukubila okusezinyaweni zakho xa ukuthelekisa nokubila okusemzimbeni wakho?	1 0	1	2	3	4	5
6. Ingaba kwehlile okanye abukho na ukubila ezinyaweni zakho (umzekelo, emva kokuzilolonga okanye emva kwemozulu eshushu)?	1 0	1	2	3	4	5
7. Ingaba kwandile na ukubila ezandleni zakho xa ukuthelekisa nokubila okusemzimbeni wakho uwonke?	1 0	1	2	3	4	5
8.Ingaba unesicaphucaphu, uyagabha, okanye uyaqunjelwa emva kokutya ukutya okuncinci?	1 0	1	2	3	4	5
9. Ingaba unokuhambisa okungapheliyo (uhambisa amatyeli angaphezu kwesithathu)?	1 0	1	2	3	4	5
10. Ingaba unokuqhingwa okungapheliyo (utawa kanye ngemini)?	1 0	1	2	3	4	5
11. Ingaba uyathontsiza xa ugqiba ukuchama?	1 0	1	2	3	4	5
12. Ingaba unengxaki yokuvukelwa (oku kuphathelele kumadoda)?	1 0	1	2	3	4	5

Total number of symptoms _____ total severity of symptoms_____

Survey of Autonomic Symptoms[a]		
	Q1a. Have you had any	Q1b. If you answered yes

	of the following health symptoms during the past 6 months? (1 = Yes; 0 = No)	in Q1a, how much would you say the symptom bothers you? (1 = Not at all; 2 = A little; 3 = Some; 4 = A moderate amount; 5 = A lot)				
Symptom/health problem						
1. Do you have lightheadedness?	1 0	1	2	3	4	5
2. Do you have a dry mouth or dry eyes?	1 0	1	2	3	4	5
3. Are your feet pale or blue?	1 0	1	2	3	4	5
4. Are your feet colder than the rest of your body?	1 0	1	2	3	4	5
5. Is sweating in your feet decreased compared to the rest of your body?	1 0	1	2	3	4	5
6. Is sweating in your feet decreased or absent (for example, after exercise or during hot weather)?	1 0	1	2	3	4	5
7. Is sweating in your hands increased compared to the rest of your body?	1 0	1	2	3	4	5
8. Do you have nausea, vomiting, or bloating after eating a small meal?	1 0	1	2	3	4	5
9. Do you have persistent diarrhea (more than 3 loose bowel movements per day)?	1 0	1	2	3	4	5
10. Do you have persistent constipation (less than 1 bowel movement every other day)?	1 0	1	2	3	4	5
11. Do you have leaking of urine?	1 0	1	2	3	4	5
12. Do you have difficulty obtaining an erection (men)?	1 0	1	2	3	4	5

a Number of symptoms reported: ____(sum of column A, 0-12 for men and 0-11 for women); total symptom impact score: ___(sum of column B, 0-60 for men and 0-55 for women).

Appendix E

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

24 April 2017

HREC REF: 200/2017

Prof J Heckmann
Neurology
E8-74
NGSH

Dear Prof Heckmann

PROJECT TITLE: HIV-ASSOCIATED NEUROPATHY AND AUTONOMIC DYSFUNCTION IN A SOUTH AFRICAN COMMUNITY-BASED COHORT ON ESTABLISHED ART (MMed-candidate-Dr M Dudley) sub-study-221/2008

Thank you for submitting your response letter to the Faculty of Health Sciences Human Research Ethics Committee dated 19 April 2017.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 April 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that the student, Dr Meagan Dudley will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 200/2017

This serves to confirm that the University of Cape Town Human Research Ethics Committee compiles to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

0FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/08/19
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	14/8/2018

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	7 August 2018		
HREC REF Number	200/2017	Current Ethics Approval was granted until	April 2018
Protocol title	**HIV-associated Neuropathy and Autonomic Dysfunction in a South African community-based cohort on established ART**		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	√ Yes ☐ No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.	HREC/REF 221/2008 –valid and renewed until March 2019		
Principal Investigator	JM Heckmann		
Department / Office Internal Mail Address	E8-74 Neurology, UCT		

1.1 Does this protocol receive US Federal funding?	☐ Yes	√ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	√ No
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget	☐ Yes	√ No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)