HIV-ASSOCIATED SENSORY NEUROPATHY IN THE ERA OF TENOFOVIR-BASED HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

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DECLARATION

I, Prinisha Pillay, declare that this thesis is my own work, except where otherwise specified. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree of examination at this or any other University.

_____

_____ day of _____ 2018

DEDICATION

In loving memory of my dearest Dad

Pragalathan Aaroomugam Pillay

1962 - 2015

You left me halfway through this journey, I thank you for your support up until your last days

RESEARCH OUTPUTS

1. Peer reviewed original research publications

Pillay P, Wadley AL, Cherry CL, Karstaedt AS, and Kamerman PR (2015) Pharmacological treatment of painful HIV-associated sensory neuropathy. *S Afr Med J.* **105** (9): 769-772.

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2. Local conference presentations

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3. International conference presentations

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ABSTRACT

HIV-associated sensory neuropathy (HIV-SN) is a common and often painful neurological condition associated with HIV-infection and its treatment with neurotoxic antiretroviral drugs such as stavudine. antiretroviral drugs. The prevalence of HIV infection remains high in sub-Saharan Africa and is expected to stay high in the anticipated future. This high prevalence will persist, irrespective of the reduction in infection rates, due to the improved supply and accessibility of ARV therapies, which significantly improve survival rates. Longer survival rates for people living with HIV/AIDS (PLWHA) will lead to an accumulation of chronic complications associated with HIV infection and its treatment in the population. Furthermore, with the removal of the neurotoxic antiretroviral drug stavudine in all first-line therapies in South Africa and it's replacement with the supposedly less neurotoxic drug tenofovir, it is anticipated that the incidence of conditions such as HIV-SN will decrease. In my thesis I have focused on HIV-SN, a chronic comorbid and often painful condition that can significantly affect quality of life.

In the first aspect of my thesis, I investigated the six-month incidence of HIV-SN in ARV naïve individuals initiating updated cART, which consists of the single fixed dose combination (FDC) pill that contains the ARV tenofovir. This single fixed dose conimantion pill contained tenofovir and efavirenz either given with lamivudine (3TC) or emtricitabine (FTC) and is the standard first-line therapy given to all pateints inititating ARV therapy for the first time in South Africa. I also investigated what clinical factors may be associated with the development of HIV-SN in this population. This is important as there is a need to re-examine risk factors for the development of HIV-SN in the setting where stavudine is no longer prescribed. This thesis presents the first longitudinal study to assess the incidence of HIV-SN in a cohort of individuals of 100% black African ancestry, that were universally exposed to TDF-based cART, and the clinical factors associated with the development of HIV-SN.

I recruited 120 ARV naïve individuals initiating TDF-based cART at the Lenasia South Local Community Hospital, Johannesburg and assessed HIV-SN using the AIDS Clincal Trials Group (ACTG) Brief Peripheral Neuropathy Screen. Painful HIV-SN was defined as present if the participant had at least one symptom (pain) and at least two signs (reduced vibration sense in the great toe, absent ankle reflexes or decreased pin-prick sensitivity in the feet). Non-painful HIV-SN was defined as present if the participant had at least two signs (as described above). A total of 83 individuals completed the study, and the cumulative incidence of neuropathy at the end of six-months was 200 cases per 1000 patients (95% CI: 120-290). The incidence rate was 0.47 (95% CI: 0.3-0.67) per person year. Eleven (13%) individuals developed non-painful HIV-SN and nine (11%) developed painful HIV-SN. Asymptomatic SN had a slower onset (>3 months) compared to symptomatic SN, which developed rapidly (within

the first 3-month period). The presence of pain was a significant risk factor for the development of HIV-SN ($p< 0.001$). Increasing height and receiving treatment for a current tuberculosis (TB) infection were independently associated with the risk of HIV-SN. However, other established risk factors for HIV-SN such as increasing age, current CD4 T-cell count and sex were not associated with the development of HIV-SN in our cohort. Our finding of a six-month cumulative incidence of 200 cases per 1000 patients on TDF-based cART who had never previously been exposed to any form of cART indicates that HIV-SN is certainly not a thing of the past, and that the number of new cases is still high. Our finding also contradicts the belief that updated cART therapies, which do not include drugs associated with neurotoxicity, will eliminate new cases of HIV-SN.

Participants also donated blood samples across the six-month study period (0, 2, 4, and 6 months) for the extraction of plasma. We used a total of n=119 plasma samples (approximately 30 samples at each time-point), to investigate the possible role of chemokines in the development of HIV-SN. Based on reports in the literature, we selected four chemokines, CCL5 (RANTES), CCL2 (MCP-1), CX3CL1 (Fractalkine) and CXCL12 (SDF-1). We measured the levels of these chemokines at the four time-points using Luminex pre-mixed magnetic bead assays, to assess whether they associated with the development of HIV-SN. Based on the observed concentration values from the assay for the chemokine (CCL5) RANTES, we opted to exclude CCL5 from the chemokine analysis as most values were far outside of the upper limit of the standard curve, indicating that the analyte had not be diluted enough for the assay. We did not find any significant associations between levels of chemokines measured and the development of HIV-SN across the six-month period. The lack of association observed between the levels of chemokines measured and the development of neuropathy, may well be due to the wide and often irregular sampling intervals we faced during collection of patient blood samples.

In the third aspect of my thesis, I explored the role of psychological factors including depression, anxiety and catastrophizing in individuals who developed a painful or non-painful HIV-SN. I investigated whether these psychological factors associated with having a painful HIV-SN and whether pain and psychological factors affected quality of life in this South African population. As a secondary objective, I also completed a sub-group analysis of the painful HIV-SN group to determine whether psychological factors predicted the intensity of their neuropathic pain. This study is the first, globally, to assess the relationship between these psychological factors and painful and non-painful HIV-SN.

I recruited 197 participants from the Greenhouse Pharmacy, at the Chris-Hani Baragwanath Hospital, Soweto. All participants had a confirmed HIV infection and were currently on ARV therapy. We assessed HIV-SN using the AIDS Clinical Trials Group (ACTG) Brief Peripheral Neuropathy Screen. Painful HIV-SN was defined as present if the participant had at least one symptom (pain) and two signs (reduced vibration sense in the great toe, absent ankle reflexes or decreased pin-prick sensitivity in the feet). Non-painful HIV-SN was defined as present if the participant had at least two signs. One hundred and twenty-five (125/197) participants had painful HIV-SN and seventy-two (72/197) participants had non-painful HIV-SN. Among participants with painful HIV-SN, 85% (106/125) reported their pain as “moderate” to “severe” in intensity, with a median pain intensity of 7 (IQR 1-10). We found that depression, anxiety and pain catastrophizing did not associate with the presence of painful HIV-SN in our cohort. We did find, however, that increased depressive symptoms and the presence (but not intensity) of pain independently associated with reduced quality of life. Furthermore, greater depressive symptoms independently associated with increased pain intensity in patients with painful HIV-SN. Painful HIV-SN is difficult to treat pharmacologically so focusing on improving quality of life and function could be another angle to take therapeutically. Furthermore, placing a greater emphasis on assessing and treating depressive symptoms in patients with SN could be advantageous and may result in a reduction in pain intensities reported.

My study is the first in sub-Saharan Africa to report on the present incidence of HIV-SN in the era of TDF-based cART and factors associated with the development of SN in this African population on this regimen. My findings highlight the substantial burden of symptomatic HIV-SN, and their detrimental impact on quality of life. My findings further emphasize the need for assessing and treating the high burden of depressive symptoms observed in our population.

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LIST OF ABBREVIATIONS

ACTG	Aids Clinical Trial Group
AIDS	Acquired immune deficiency syndrome
ARV	Antiretroviral
ATN	Antiretroviral toxic neuropathy
ATP	Adenosine triphosphate
AZT	Zidovudine
BMDM	Bone marrow derived macrophage
BMI	Body Mass Index
BPNS	Brief Peripheral Neuropathy Screen
cART	Combination antiretroviral therapy
CCL2	C-C motif ligand 2
CCL5	C-C motif ligand 5
CCR2	C-C chemokine receptor 2
CCR5	C-C chemokine receptor 5
CHC	Community Health Hospital
CMV	Cytomegalovirus
CNS	Central nervous system
CX3CL1	C-X3-C motif chemokine ligand 1
CX3CR1	CX3C chemokine receptor 1
CXCL11	C-X-C motif chemokine ligand 11
CXCL12	C-X-C motif chemokine ligand 12
CXCR4	C-X-C chemokine receptor 4
d4t	Stavudine
ddi	Didanosine
ddc	Zalcitabine
DFNS	German research network on neuropathic pain
DN4	Douleur Neuropathique 4
DRG	Dorsal root ganglion
EDTA	Ethylene-Diamine-Tetra-Acetic acid
EFV	Efavirenz
EI	Entry inhibitor
EQ-5D-3L	3 Level version of EuroQol EQ-5D
FTC	Emtricitabine
gp 120	Envelope glycoprotein 120
HAART	Highly active antiretroviral therapy
HbA1c	Glycated haemoglobin (A1c)
HIV	Human immune virus
HIV-DSP	HIV-associated distal sensory neuropathy
HIV-SN	HIV-associated sensory neuropathy
HRQOL	Health related quality of life
HSCL-25	Hopkins symptoms checklist-25
IENFD	Intra epidermal nerve fibre density
IFNγ	Interferon-gamma
IL-10	Interleukin-10

IL-1a	Interleukin-1 alpha
IL-1b	Interleukin-1 beta
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
INH	Isoniazid
IQR	Inter quartile range
IRIS	Immune reconstitution inflammatory syndrome
LANSS	Leeds assessment of neuropathic symptoms and signs
LED	Light-emitting diodes
MCP-1	Monocyte chemotactic protein 1
MMSE	Mini mental state examination
mRNA	Messenger ribonucleic acid
mtDNA	mitochondrial DNA
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
PCS	Pain catastrophizing scale
PI	Protease inhibitor
PLWHA	people living with HIV/AIDS
QOL	Quality of life
QST	Quantitative sensory testing
RANTES	CCL5
RNA	Ribo nucleic acid
SD	Standard deviation
SDF-1	CXCL12
siRNA	small interfering RNA
SN	Sensory neuropathy
TB	Tuberculosis infection
TCSS	Toronto clinical scoring system
TDF	tenofovir
TNFα	Tumour necrosis factor alpha
TNFR	Tumour necrosis factor receptor
TNSr	Total neuropathy score
TRPv1	Transient receptor potential vanilloid 1
UENS	Utah early neuropathy scale
VAS	Visual analogue scale
WBPQ	Wisconsin brief pain questionnaire
WHO	World health organisation

Chapter 1

Introduction

1.1 HIV-related pain

1.1.1 Characteristics of HIV-related pain

Pain is highly prevalent in people living with HIV/AIDS (PLWHA) (between 75% to 91%) (Narasimooloo *et al.,* 2011; Parker *et al.,* 2014) and this pain is often undertreated in PLWHA (Bouhassira *et al.,* 1999; Forna *et al.,* 2007; Parker *et al.,* 2014; Turk *et al.*, 2010). There are various causes of pain experienced by PLWHA (Kamerman & Mitchell 2011; Parker *et al.,* 2014) which may encompass nociceptive and neuropathic components (Collins & Harding 2007). Possible causes for the pain experienced by these individuals include: a direct effect of HIV on the peripheral or central nervous system, opportunistic infections that may arise, presence of tumours or cancer, exposure to neurotoxic antiretroviral therapies (stavudine, didanosine and zalcitabine), and other iatrogenic causes such as chemotherapeutic agents and procedural pain. Also, pre-existing co-morbid disease may contribute to the burden of this pain (Dobalian *et al.,* 2004; Keswani *et al.*, 2002; Parker *et al.,* 2014). A recent systematic review of pain in PLWHA found that these individuals often rate their pain as 'moderate to severe' in intensity (Parker *et al.,* 2014). This finding has huge implications for patient's quality of life and may be the basis for further psychological distress in these individuals.

1.1.2 Prevalence of HIV-related pain at different anatomical sites in PLWHA

There are numerous pain sites reported by PLWHA and these data are consistent across African populations but differ slightly when compared to those from abroad. A summary of the various anatomical sites where pain has been reported as more prevalent in PLWHA is provided in Table 1.1. Wahab and colleagues (2011) found that of the 40% of individuals in their cohort who reported moderate to severe pain, lower limbs (40.9%), head and neck region (31.8%), and abdomen (31.8%) were the major regions affected by pain. Congruently, Mphalele and colleagues (2012) who investigated pain in ambulatory HIV-positive individuals in both rural and urban settings also found that the head region (26% in rural vs. 39% in urban) and abdomen (30% in rural vs. 20% in urban) were frequent pain sites reported by individuals in their cohort. Other pain sites reported by Mphalele and colleagues (2012) include chest pain (19% in rural vs. 30% in urban), genital (15% only in rural group) and pain in the feet (33% only present in urban group) and these sites are not unique to African populations only (Del Borgo et al. 2001; Nair et al. 2009). The consistency of these particular pain sites in HIV-infected individuals is seen in the Indian population as well, as Nair and colleagues (2009) also found in their South Indian cohort that headache (28.8%) and the soles of the feet (25%) were common sites affected by pain. Indeed, it is also important to note that studies conducted prior to the 1999 period (i.e. Breitbart *et al.,* 1996, Larue *et al.,* 1997 and Hewitt *et al.,* 1997) may well describe individuals who were either untreated or did not receive the ARV drug

stavudine. Therefore, the possibility exists that the mechanisms and thus the risk factors involved here could be very different and it is important to consider this fact when comparing risk factors across these different cohorts.

1.1.3 Factors associated with HIV-related pain

1.1.3.1 Pain and HIV disease markers

Several studies have assessed the relationship between CD4 T-cell counts and the prevalence of pain (Aouizerat *et al.* 2010; Van As *et al*. 2009; Martin *et al.* 1999; Peltzer *et al.* 2008; Powers *et al.* 2007; Richardson *et al.* 2009; Rosen *et al.* 2008). In their study on pain in ambulatory HIV-positive individuals with and without intravenous drug use, Martin and colleagues (1999) reported a significant correlation between number of pain sites and CD4 T-cell counts in those individuals not using intravenous drugs (Martin et al. 1999). This infers that as CD4 T-cell counts decrease symptom burden increases. Aouizerat (2010) also reported a significant correlation between decreased CD4 T-cell counts and increased pain prevalence. In contrast, Ellis and colleagues (2010) reported a higher nadir CD4 T-cell count as well as previous, but not current, exposure to neurotoxic ARVs as risk factors for painful HIV-associated sensory neuropathy (HIV-SN) in their cohort (Ellis et al. 2010). The latter finding has previously been reported by another study (Cherry et al. 2003) In their review of HIV-related pain and its management, Kamerman and Mitchell (2012) interrogated the association between CD4 T-cell counts and pain prevalence in HIV-positive individuals and concluded that pain prevalence in HIV is not associated with CD4 T-cell counts (Kamerman & Mitchell 2011). Also, spurious associations between pain prevalence and increased CD4 counts may be evident in successful cART treatment, where there is a distinctive pattern of increases in CD4 T-cell counts and simultaneous decreases in viral loads.

Several sub-Saharan African studies also reported no consistent correlation between CD4 T-cell counts and measures of pain (Peltzer & Phaswana-Mafuya 2008; Powers et al. 2007; Rosen et al. 2008; Van As et al. 2009). In addition, with successful cART, an increase in CD4 T-cell count should be paralleled by a decrease in pain in these patients, which is not the case in many studies (Breitbart et al. 1996; Del Borgo et al. 2001; Wahab & Salami 2011) and possibly the reason behind the persisting high pain prevalence. Indeed, only in a study by Cervia and colleagues (2010), which was limited to a small sample size of 41 patients receiving cART, was a reduction in pain prevalence and pain intensity reported. Certainly, there is a need for further studies to be conducted to support this premise (Cervia et al. 2010). The relationship between presence of pain and disease staging has also been investigated by

several studies (Aouizerat et al. 2010; Breitbart et al. 1996; Del Borgo et al. 2001; Martin et al. 1999; Wahab & Salami 2011). A few of these studies report no relationship between stage of disease and the presence of pain in their cohorts (Breitbart et al. 1996; Del Borgo et al. 2001; Wahab & Salami 2011). This was supported by Kamerman and Mitchell (2012) who concluded that disease staging does not affect pain while patients are ambulatory. However, it is plausible that pain prevalence may increase at later disease stages (progression to stages III and IV). Indeed, two studies have reported strong associations between the prevalence of pain and disease stages III and IV (Nair et al. 2009; Martin et al. 1999).

Table 1.1 Prevalence of pain at different anatomical sites in PLWHA.

Authors	Number of patients studied	Pain distribution
(Larue *et al*., 1997)	116: out patient 118: in patient 56: ambulatory patients	Figure 1.1 (a)
(Del Borgo et al. 2001)	152	Figure 1.1 (b)
(Nair et al. 2009)	140	Figure 1.1 (c)
(Parker et al., 2017)	229	Figure 1.1 (d)
(Maree et al., 2013)	500	Figure 1.1 (e)
(Hewitt et al. 1997)	151	Figure 1.1 (f)
(Wahab & Salami 2011)	79	Figure 1.1 (g)
(Mphahlele et al., 2012)	Rural: 125 Metropolitan: 396	Rural cohort: Figure 1.1 (h) Metropolitan cohort: Figure 1.1 (i)

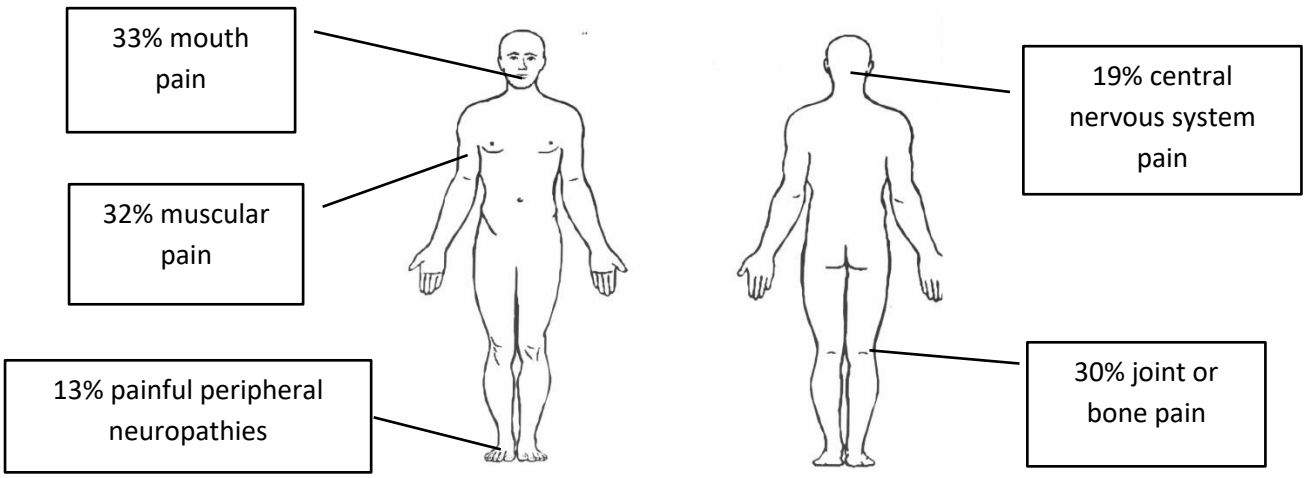


Figure 1.1 (a) Pain distribution in (Larue et al.,1997).

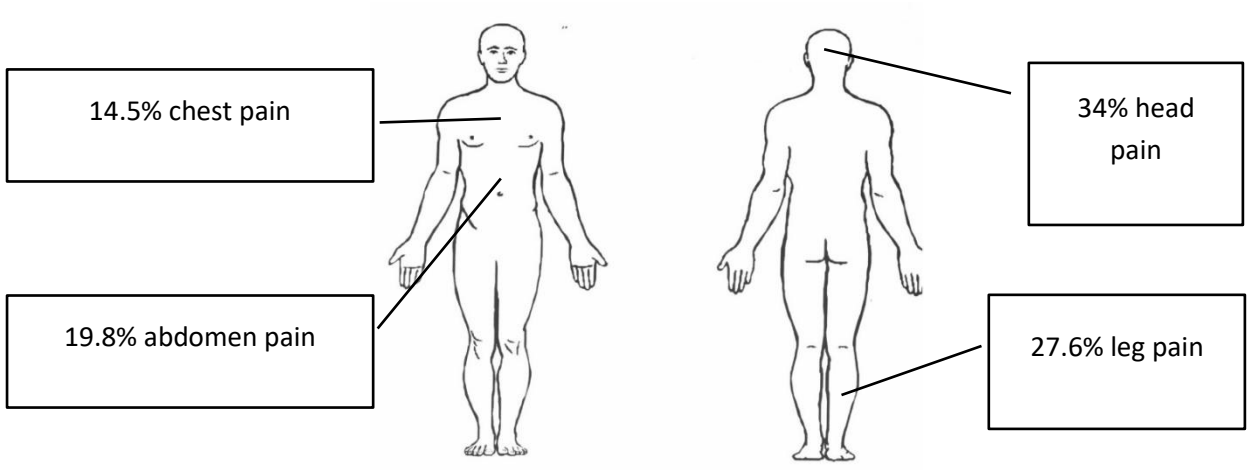


Figure 1.1 (b) Pain distribution in (Del Borgo et al. 2001).

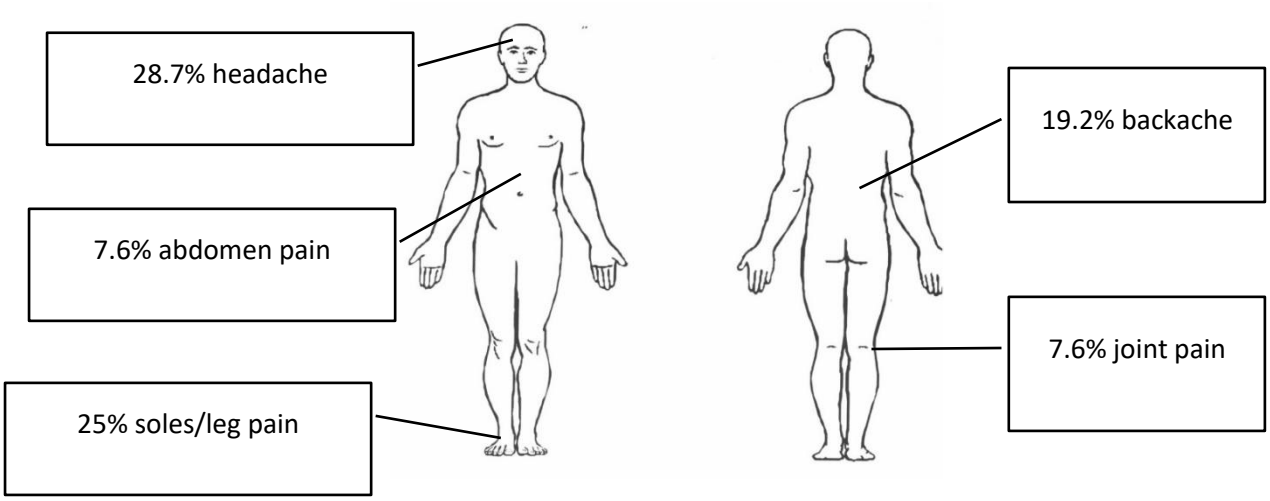


Figure 1.1 (c) Pain distribution in (Nair et al. 2009).

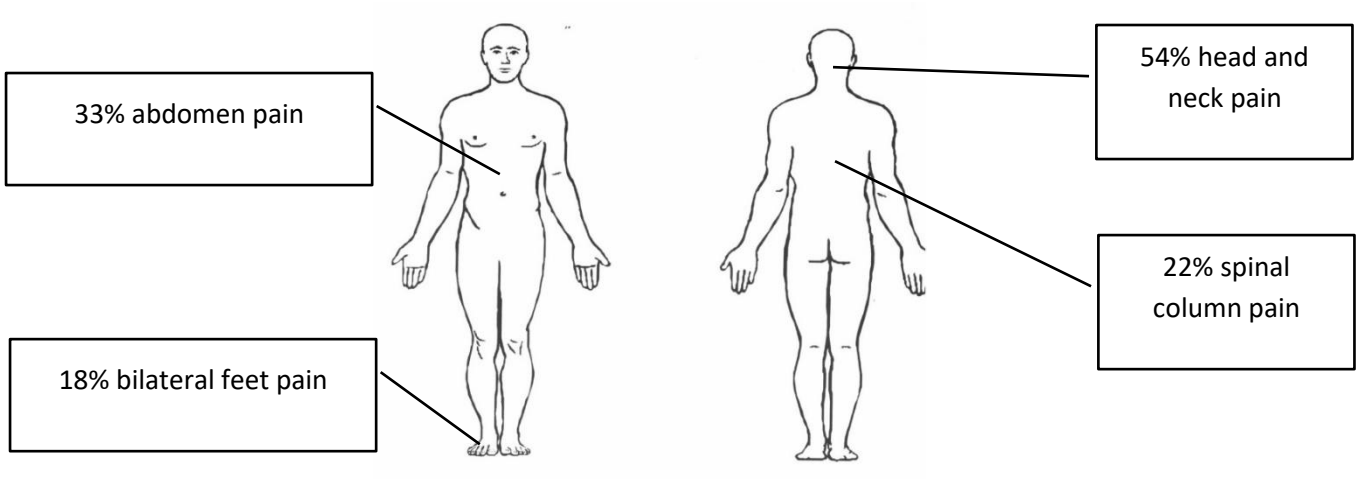


Figure 1.1 (d) Pain distribution in (Parker et al. 2017)

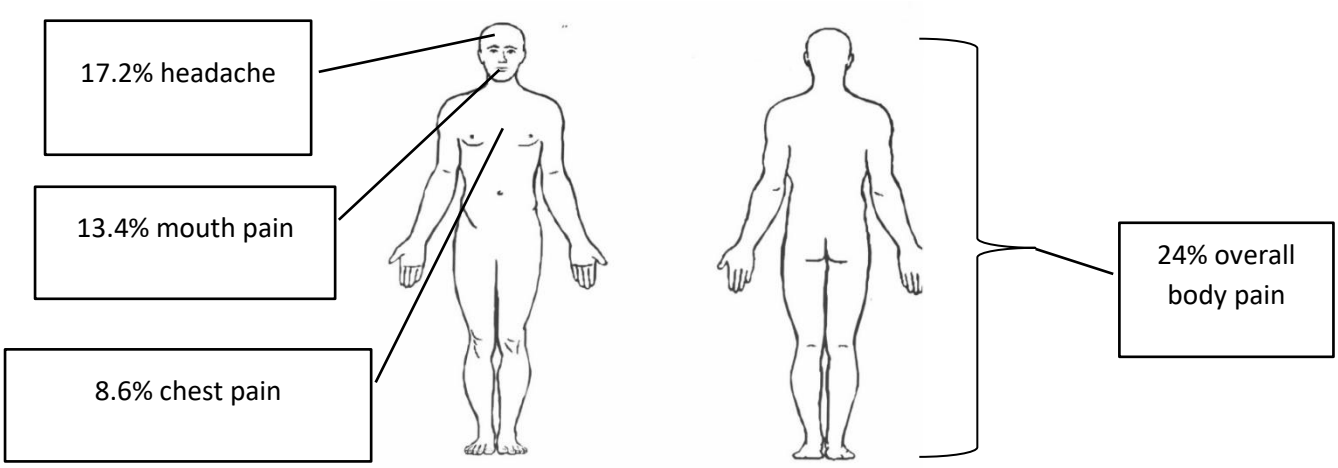


Figure 1.1 (e) Pain distribution in (Maree et al., 2013).

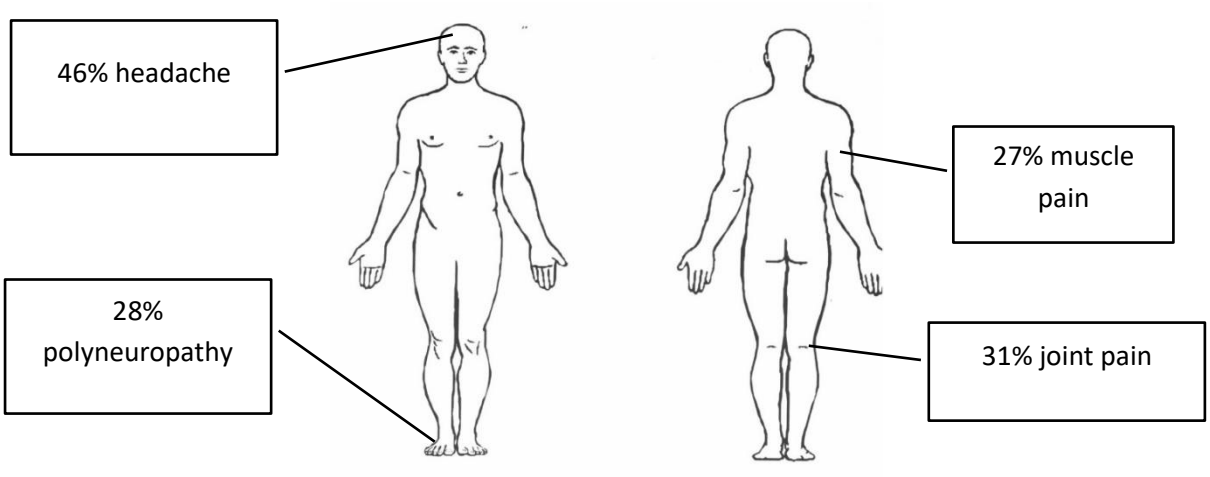


Figure 1.1 (f) Pain distribution in (Hewitt et al. 1997).

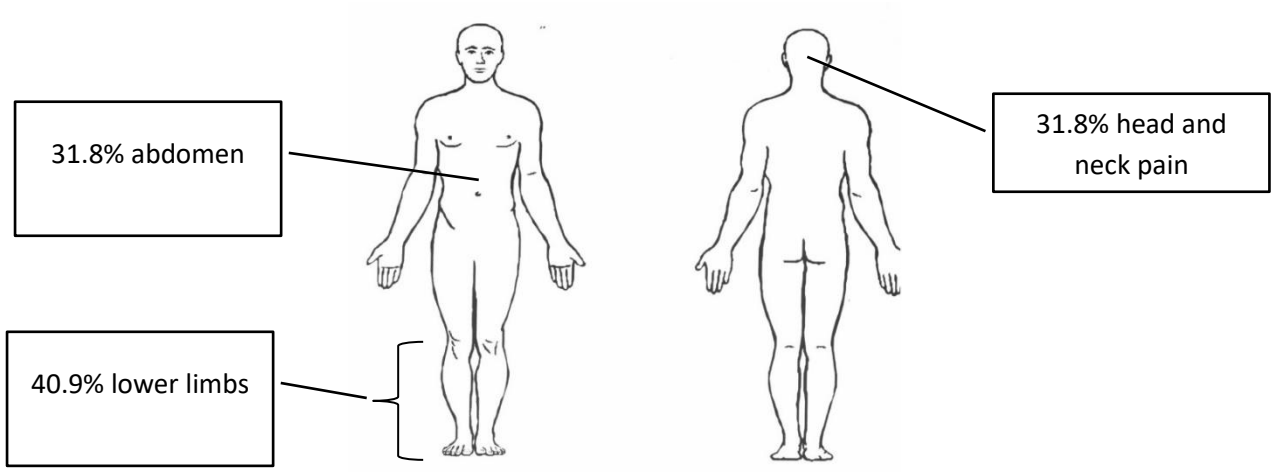


Figure 1.1 (g) Pain distribution in (Wahab & Salami 2011).

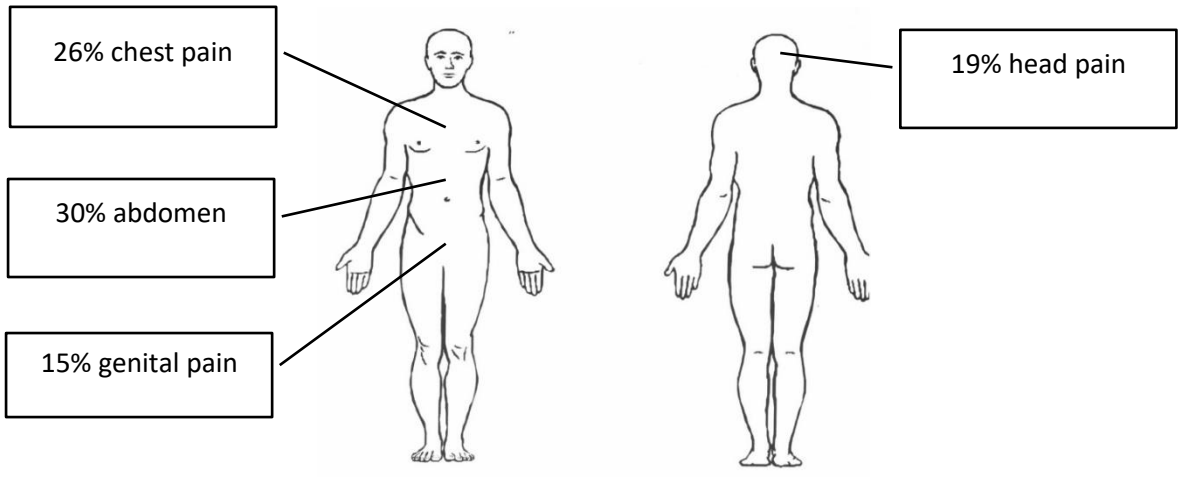


Figure 1.1 (h) Pain distribution in rural cohort (Mphahlele et al., 2012)

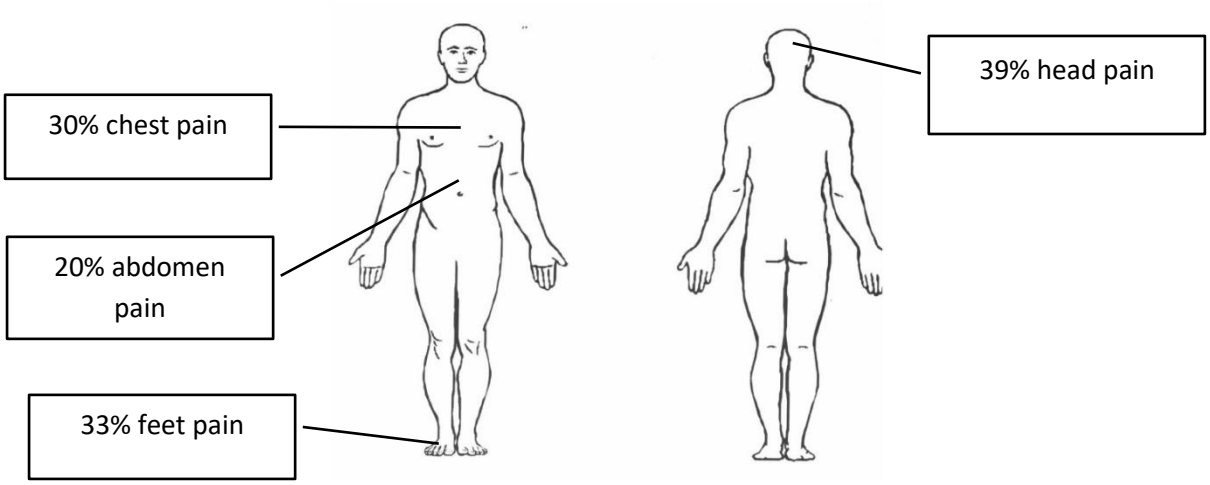


Figure 1.1 (i) Pain distribution in metropolitan cohort (Mphahlele et al., 2012).

1.2 Overview of HIV-associated sensory neuropathy:

HIV-associated distal sensory polyneuropathy (HIV-SN) is a symmetrical, predominantly sensory, polyneuropathy which is characterised by a "dying back" pattern of axonal degeneration (McArthur, Brew & Nath 2005; Holland et al. 1997; Polydefkis 2002). This polyneuropathy is a frequent and often painful complication of both HIV infection and treatment with specific dideoxynucleoside nucleoside analogue antiretrovirals (ARVs) such as stavudine, zalcitabine and didanosine (Cherry et al., 2003; Cornblath & Hoke, 2006; Moyle & Sadler, 1998). Morbidities such as HIV-SN are highly prevalent in both developed and developing countries (Ellis et al. 2010; Smyth et al. 2007). The prevalence of HIV-SN lies between 30% to 57%in ambulatory HIV-positive individuals (Wester *et al.*, 2005; Skopelitis *et al.*, 2007; Maritz *et al.*, 2010b; Wadley *et al.*, 2011), with 38% to 90% of these individuals experiencing pain ranging from mild to severe in intensity (Evans et al. 2011; Ellis et al. 2010; Smyth et al. 2007). However, with the worldwide removal of the neurotoxic drug stavudine from all first-line therapies and it's subsequent replacement with tenofovir, it is anticipated that the incidence of HIV-SN and particularly painful HIV-SN may reduce signficantly. Therefore, we may perhaps see a reduction in the development and reporting of pain in PLWHA who initiate updated cART.

1.2.1 Definition of HIV-SN

A common problem associated with reviewing data and literature on HIV-SN is the obvious lack of a 'gold standard' definition for the neurological condition. Several studies have relied on assessing signs and symptoms in their definition of HIV-SN (Forna et al. 2007; Wadley et al. 2011a; Shurie & Deribew 2010; Sacktor et al. 2009; Maritz et al. 2010b; Conradie et al. 2014; Centner et al. 2014). However, others have relied solely on the use of symptoms

(Beadles et al. 2009; Menezes et al. 2011; Kiwuwa-muyingo et al. 2014; Hawkins et al. 2007; Jamisse et al. 2007).

In order to give a complete representation of HIV-SN, the use of both signs and symptoms in the definition of the neurological condition is probably the best choice (Cherry et al. 2005). In the clinic setting, the presence of symptoms such as pain and burning in PLWHA who are either ARV naive or on ARV treatment often alludes to possible neurological deficit and prompts an assessment. But pain is not a universal symptom of HIV-SN, with around 20-40% of PLWHA with HIV-SN presenting with symptoms other than pain e.g. paraesthesia's and/or numbness (Arenas-Pinto et al. 2015; Ellis et al. 2010; Cherry et al. 2012; Phillips et al. 2014). Alternatively, other conditions that may present with foot pain (e.g. plantar fasciitis) may be interpreted as a SN, inflating prevalence estimates. Therefore, relying solely on the presence of symptoms and not signs of nerve deficits may largely underestimate the actual prevalence of the neuropathy and leave many cases undetected. Furthermore, neuropathy-free individuals and those who presented with symptoms of neuropathy but no signs, have shown similar findings on Intraepidermal nerve fibre density (IENFD) and Quantitative sensory testing (QST) assessments (Cherry et al. 2005), therefore indicating that symptoms alone may not provide an accurate definition. Conversely, relying solely on signs of neuropathy may also not be ideal and lacks clinical relevance (Simpson et al. 2002; Polydefkis et al. 2002; Zhou et al. 2007; Martina et al. 2003; Phillips et al. 2014).

1.2.2 Pain in individuals with HIV-SN

Neuropathic pain associated with HIV-SN is often debilitating and has been shown to significantly reduce health related quality of life (HRQOL), frequently affecting sleep and various aspects of physical and emotional functioning in infected individuals (Cocito et al. 2006; Ellis et al. 2010). Certainly, pain is an extremely common reason for individuals with HIV-SN to seek assistance (Smyth et al. 2007; Maritz et al. 2010b), however not all these individuals will experience pain (Amruth et al. 2014) and not all will report their pain (Parker, et al., 2014). Therefore, the study by Ellis and colleagues (2010) attempted to identify the reason behind some individuals in their cohort remaining asymptomatic, and others reporting pain. This study formed part of the CHARTER study group and found that despite exposure to updated cART therapies, both neuropathic pain and HIV-SN neuropathy remain prevalent in the cART era. Indeed, they also noted that clinical correlates of HIV-SN have indeed changed in the cART era and that further research is required to determine HIV-SN pathogenesis in the era of TDF-based cART. Ceratinly, this information is vital for clinicians treating individuals who present with HIV-SN, and directly affects management strategies for

painful HIV-SN. Studies that have been conducted on nerve pathology in HIV-SN show only minor differences in the somatosensory nerve function between painful HIV-SN and non-painful HIV-SN (Martina et al. 2003). Furthermore, there are also no clear differences in skin biopsy data between painful HIV-SN and non-painful HIV-SN (Mountford et al. 2017; Bouhassira et al. 1999; Martina et al. 2003). Indeed, data on IENFD from skin biopsy samples in HIV-SN are limited and not as well documented as in diabetic neuropathy (Alam et al. 2017). The lack of clear differences in nerve pathology and skin biopsy data for painful and non-painful HIV-SN makes it difficult to discern the probable cause of painful HIV-SN, and warrants further investigation of factors such as chemokines, cytokines and genetic factors which may highlight the susceptibility of particular individuals for developing painful HIV-SN. Furthemore, since pain is a subjective experience, we must note that measurements of tissue damage i.e. reduction in IENFD may not correlate with objective measurements of pain. Indeed, a recent study by Mountford and colleagues (2017) investigated the expression of chemokine receptors on cells surrounding cutaneous nerves in PLWHA and found a significant association between the presence of HIV-SN and an upregulation of chemokine receptors (CX3CR1, CCR2 and CCR5) in $CD3^+$ and $CD14^+$ cells in PLWHA. These findings are novel and suggest that these molecules may well be the targets of future therapies in HIV-SN.

1.2.3 Pathogenesis of HIV associated sensory neuropathy (HIV-SN):

The pathogenesis of sensory neuropathy is not entirely understood, however there are a few hypothesised mechanisms. HIV-SN may be a direct consequence of HIV-infection i.e. the human immunodeficiency virus itself, the immune system response to the virus or exposure to neurotoxic antiretroviral therapy which includes nucleoside reverse transcriptase inhibitors (NRTIs) (Kamerman et al. 2012; Evans et al. 2011; Menezes et al. 2011). It is possibly these three factors acting in combination or alone that are the most significant mediators of HIV-SN (Kamerman et al. 2012). When the primary insult in the peripheral nervous system is caused by the virus or the immune response to the virus, the neuropathy is classified as an HIV-associated distal symmetrical polyneuropathy (HIV-DSP), and where the insult is caused due to the exposure of some classes of dideoxynucleoside drugs, such as stavudine and didanosine, an antiretroviral toxic neuropathy (ATN) develops (Ferrari et al. 2006; Gonzalez-Duarte, Robinson-Papp & Simpson 2008). The extent to which viral or immune-mediated damage has occurred or ARV exposure triggering clinically overt neuropathy is unclear, and hence these definitions are probably arbitrary.

1.2.3.1 Virus-mediated neurotoxicity in HIV-SN

HIV-associated sensory neuropathy is characterised by axonal degeneration of long axons in distal regions of the body i.e. the lower limbs and feet. This axonal degeneration follows a "dying back" pattern (Figure 1.1), where the degeneration of each axon starts at the distal end and moves retrogradely (Holland et al. 1997; Polydefkis et al. 2002; Wallace et al. 2007a; Wallace et al. 2007b). The degeneration is accompanied by reduced IENFD, along the peripheral nerve trunk, and a loss of neurones in the dorsal root ganglion (DRG) (Kamerman et al. 2012; Polydefkis 2002; Holland et al. 1997). Post-mortem and neuro-anatomical study findings in individuals who presented with late-stage HIV disease, have confirmed that even when clinical features of HIV-associated sensory neuropathy were absent, immune cell infiltration and inflammatory mediator release was still observed in the peripheral nerve trunk and DRG (Rizzuto et al. 1995; Nagano et al. 1996; Yoshioka et al. 1994).

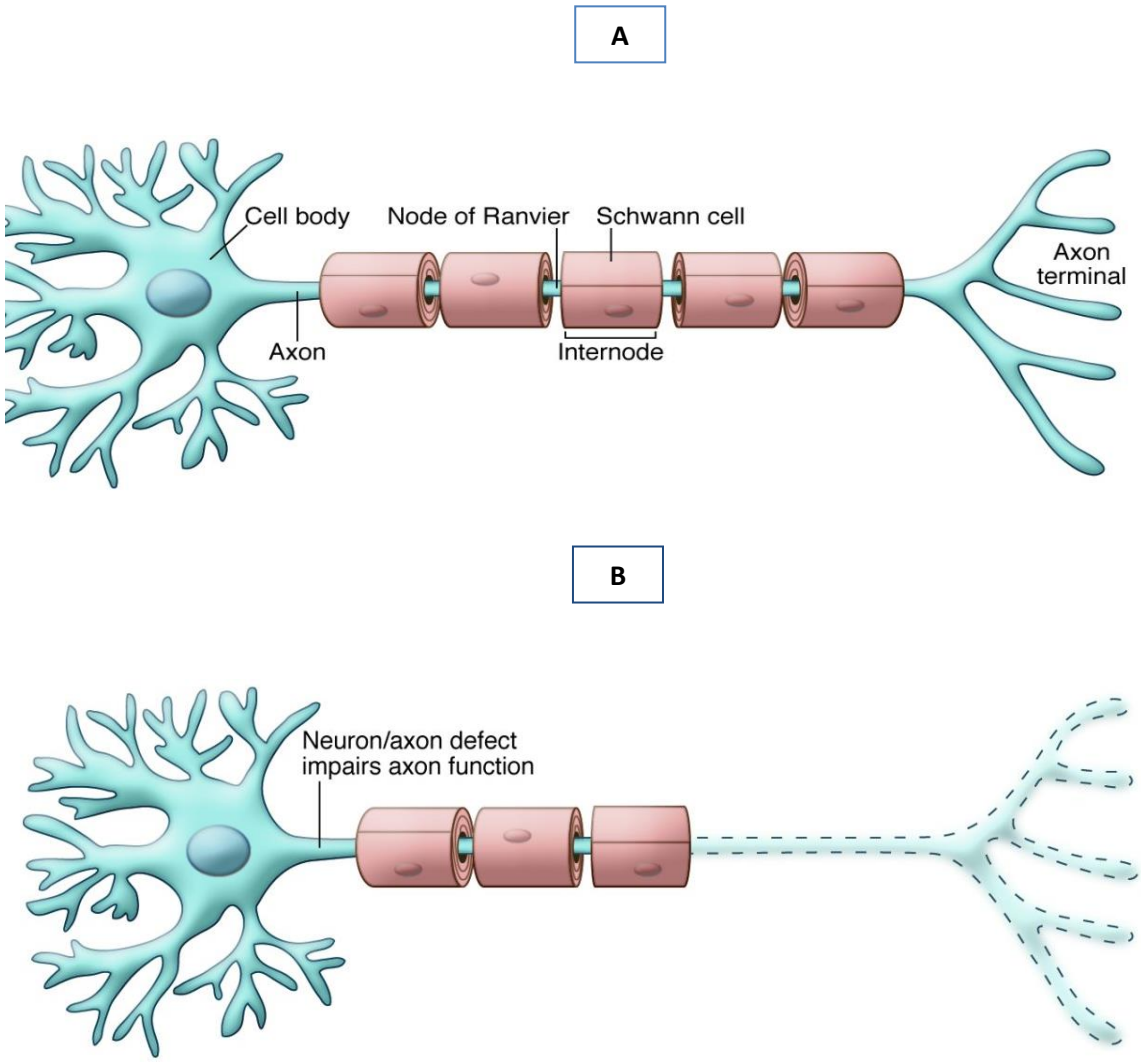


Figure 1.1 Diagram showing a healthy peripheral neuron (A) and axonal degeneration following a “dying back” pattern, where the degeneration of each axon starts at the distal end and moves retrogradely (B). Adapted from (Scherer 2011).

Neurones do not express CD4 receptors (which are required for viral entry into a cell) thus it is not probable that nerve damage in HIV-infected individuals is a direct result of active infection of neurones themselves. Instead, the primary viral protein, HIV-1 gp120, a coat protein that mediates binding and transmission of HIV through contact with CD4 proteins and chemokine receptors CCR5 and CXCR4, has been implicated (Keswani et al. 2002). Gp120 has been used in *in vivo* and *in vitro* studies to recreate models of viral neurotoxicity (For review: Kamerman et al. 2012; Moss et al. 2015), with the neurotoxic effects of gp120 being reported in several studies (Oh et al. 2001; Keswani et al. 2003; Melli et al. 2006; Wallace et al. 2007; Wallace et al. 2007b).

Wallace and colleagues (2007b) exposed rat nerves to the gp120 protein (via acute perineural application) and reported after two-weeks of application, macrophage infiltration significantly increased in the nerve trunk area. They also found macrophage infiltration, satellite cell activation and inflammatory mediator production in the DRG together with a 'dying' back pattern of distal nerve fibre endings in the epidermis (Wallace et al. 2007b). Similarly, Herzberg and Sagen (2001) also found axonal swelling and increased expression of TNFα in the nerve trunk area where gp120 was applied (Herzberg & Sagen 2001). At the level of the spinal cord, Zheng and colleagues (2011a) investigated the effects of gp120 application in exposed rat nerves, and reported both axonal swelling and increased expression of TNFα in the DRG and in glial cells located in the dorsal horn of the spinal cord (Zheng et al. 2011b).

The viral coat protein gp120 may act directly on neurones (as described above), via activation of neuronal receptors to cause apoptosis or via an indirect mechanism, via stimulation of macrophages, microglia and astrocytes. The indirect mechanism of gp120 neurotoxicity involves activation of chemokine receptors on the surface of neurons and Schwann cells (Keswani et al. 2003; Melli et al. 2006; Zheng et al. 2011). When co-cultures of rat DRG neurones and Schwann cells were exposed to gp120, a dose-dependent neutrite degeneration occurred together with neuronal apoptosis which involved a caspase-3 mechanism. In addition, mitochondrial-dependent neuronal apoptosis also occurred via involvement of inositol triphosphate-dependent calcium release from the endoplasmic reticulum (Keswani et al. 2003b; Höke et al. 2009). These findings highlight the indirect neurotoxicity of gp120, which affects the neuronal calcium homeostasis, and ultimately causes apoptosis. Keswani and colleagues (2003b) findings support data on the indirect mechanism of gp120 neurotoxicity involving Schwann cell chemokine receptors, where the viral protein gp120 binds to CXCR4 receptors, which are at times located on Schwann cells, and this binding then stimulates release of the chemokine CCL5. This chemokine release activates CCR5 receptors (found on DRG neuronal cells) and ultimately induces TNFα release from these neuronal cells. Subsequently, either via paracrine or autocrine mechanisms, there is an increase in the expression of TNFR receptors which ultimately leads to cell death via apoptosis (Keswani et al. 2003b; Melli et al. 2006) (Figure 1.2).

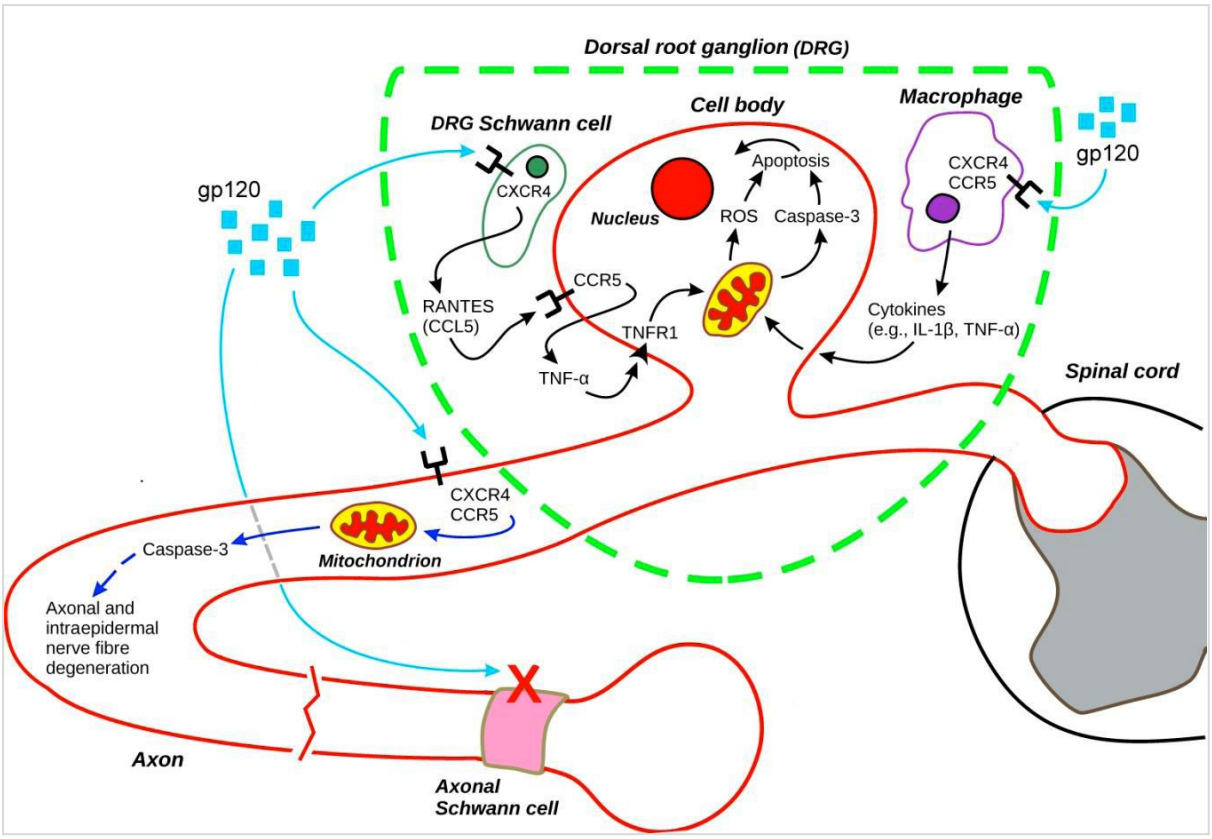


Figure 1.2 Pathogenesis of neurotoxicity produced by exposure to the HIV viral protein envelope gp120. Diagram taken from (Kamerman et al. 2012).

The study by Melli and colleagues (2006) used a 'compartmentalized cell culture system' to investigate the axonal degeneration that underlies HIV-associated sensory neuropathy (Melli et al. 2006). Their findings indicate that the HIV viral envelope protein gp120 causes neuronal apoptosis and axonal degeneration via two independent mechanisms: i) indirectly, where in the presence of Schwann cells, a lesion to cell bodies of the DRG results in neuronal apoptotic death leading to axonal degeneration and ii) directly, via local axonal toxicity, where binding of gp120 to axonal cell surface receptors (CCR5 and CXCR4) activates an apoptotic caspase pathway within the axon (See figure 1.2). Melli and colleagues (2006) also suggested a possible protective effect of axonal Schwann cells, as in the absence of Schwann cells there was direct gp120-mediated neutrite degeneration. However, when Schwann cells were present, the effects of gp120 were reduced, thus alluding to a possible shielding mechanism via the axonal Schwann cell (This is represented by the '**X**' marking on the axon in Figure 1.2).

The recent study by Moss and colleagues (2015) used rat DRG cultures to assess gp120 neurotoxicity and rat bone marrow derived macrophage (BMDM) cultures to assess gp120-associated gene expression changes. They found that neutrite degeneration induced by gp120, occurred up until 24hours after application. In addition, the interaction between gp120

and neurones was observed within one hour of application in around 10% of DRG neurones, and gp120 also significantly increased the expression of 25 cytokine-related genes in primary BMDMs, including IL-1α, IL-1β, TNFα, CXCL11, CCL2 and several more. Interestingly, they also found the chemokine receptor (CCR5) antagonist, maraviroc, inhibited gp120-induced TNFα gene expression in a dose-dependent manner (Moss et al. 2015). This indicates that the abovementioned effects occurred via gp120 activation of the chemokine receptor (CCR5).

1.2.3.2 Chronic immune activation and responses to HIV-infection

One of the major characteristics of chronic HIV-infection is the persistent immune system activation (Appay & Sauce 2008; Rajasuriara et al. 2015). HIV-infection is characterised by chronic viral replication and an ongoing immunodeficiency where there is a loss of CD4 T-cells. Immune system responses to infection include the innate immune response, where the response is immediate and the adaptive immune response, where the response is a delayed antigen-specific response (Appay and Sauce, 2008; Alison et al., 2011). The adaptive response is made up of both cell-mediated (cytotoxic T-cells) and antibody-mediated (humoral) responses (Alison et al., 2011; Paiardini and Müller-Trutwin, 2013).

The development of HIV-DSP may occur following activation and infiltration of macrophages into the nerve fibres and dorsal root ganglion as well as the release of pro-inflammatory cytokines around the DRG (Yoshioka et al. 1994; Nagano et al. 1996; Rizzuto et al. 1995; Moss et al. 2015). The viral protein, gp120 activates macrophages, which subsequently release neurotoxic inflammatory mediators which include TNFα and Interleukin-1β (Moss et al. 2015; Jones et al. 2005).

Constant immune activation results in continuous T-cell activation which induces the release and increase of intracellular nuclear factor Kappa β that is responsible for the enhancement of the transcription of the virus (Appay & Sauce 2008). Furthermore, pro-inflammatory cytokines also contribute to the collective action of IL-6, IL-1β and TNFα, which leads to T-cell activation (Appay and Sauce, 2008; Alison et al., 2011).

Chronic immune activation also occurs in the context of other chronic viral infections that HIV-positive individuals may develop. This includes the cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (Sacre et al. 2012), which reactivate often due to the depletion of CD4 T-cells and they may be a consequence of chronic immune activation and promote inflammation as well (Alison et al., 2011; Sacre *et al.*, 2012).

1.2.3.3 Pain associated with HIV-SN

In the previous 2 sections I have discussed the pathogenesis of virus-mediated toxicity which leads to HIV-SN and the immune repose to HIV. In this section I will discuss the pathogenesis of neuropathic pain in HIV-SN.

One of the most common symptoms or complaints associated with HIV-SN is pain (Cherry et al. 2012; Phillips et al. 2010; Schütz & Robinson-Papp 2013). Studies have shown that the primary viral protein HIV-1 gp120 can produce pain when administered peripherally or centrally (Milligan et al. 2000; Eron et al. 1996). Viral gene products other than gp120 may also contribute to the development of hypernociception. Archarjee (2010) reported increases in neuronal excitability when the viral accessory protein Vpr was applied to cultured rat and human DRG cells (Acharjee et al. 2010). However, the majority of studies have used gp120, perhaps finding its administration and availability more convenient. Below I will review the literature first regarding peripheral administration of gp120 and then central effects of gp120.

In rodent models of HIV-SN, nerve exposure to gp120 causes allodynia and thermal hypernociception (Herzberg & Sagen 2001; Oh et al. 2001; Zheng et al. 2011; Wallace et al. 2007). The mechanism of hypernociception induced by gp120 exposure produces an inflammatory response involving several chemokine receptors, cytokines and increased macrophage infiltration around the nerve, at the level of the DRG.

Small nociceptive neurons express receptors for various chemokines and these chemokine receptors act as cellular binding sites for HIV-1 gp120. Therefore, gp120 is capable of producing pain by its indirect action on glial cells, which results in the release of inflammatory cytokines (Milligan et al. 2000; Zheng et al. 2011). Release of the chemokine CCL2 and pro-inflammatory cytokine TNFα is caused by massive macrophage infiltration around the rat sciatic nerve and leads to hypernociception seen in the dorsal root ganglion (DRG) and axons(Herzberg & Sagen 2001; Oh et al. 2001; Zheng et al. 2011; Wallace et al. 2007) (Figure 1.3). Indeed, the entry of HIV into cells requires the sequential interaction of gp120 with CD4 and chemokine receptors (CXCR4 and CCR5 as co-receptors of gp120) on the surface of the cell (Hao 2013).Therefore, gp120 also binds to chemokine receptor CXCR4 located on axon terminals, which produces neuronal dysfunction (Hao 2013).

Oh and colleagues (2001) showed that cultured rat DRG neurons express a large variety of chemokine receptors which include CXCR4 and CCR5. They found that when gp120 was injected intradermally into the rat paw it produced allodynia. Also, the intense peripheral stimulation caused by chemokines and gp120, resulted in the release of substance P from neurons, which also activated bradykinin receptors and TRPV1 (Transient receptor potential

vanilloid 1)(Oh et al. 2001; Hao 2013) (Figure 1.3). Therefore, when gp120 binds to its chemokine receptors this may cause activation of nociceptive neurons which may contribute to hypernociception which is seen in animals exposed to gp120 and neuropathic pain in HIV-positive individuals (For review: Kamerman et al. 2012; Oh et al. 2001).

Chemokines play a significant role in the mechanisms of hypernociception, however, pro-inflammatory cytokines also significantly contribute to the development and maintenance of neuropathic pain. Recent studies have linked neuropathic pain to significant immune system activation and highlighted the significant role of cytokine signalling (Austin & Moalem-Taylor 2010). Üçeyler (2007) assessed blood mRNA and protein levels in patients with painful and painless peripheral sensory neuropathies and found higher levels of pro-inflammatory cytokines (IL-1β and TNFα) and lower levels of anti-inflammatory cytokines such as IL-10 in the group of patients with painful neuropathy (Üçeyler et al. 2007) (Table 1.2). In rodents, intrathecal injection of gp120 caused increases in TNFα and IL-1β protein release into lumbrosacral cerebrospinal fluid, which were site specific and time-dependent (Milligan et al. 2000). Interestingly, Milligan (2000) reported that IL-1β and TNFα antagonists each prevented gp120-induced pain changes. This latter finding further confirms the role of gp120 in the development and perpetuation of neuropathic pain.

Table 1.2 Summary of pro and anti-inflammatory cytokines implicated in the development of painful and non-painful HIV-SN.

Sensory Neuropathy			
Cytokines	**Painful HIV-SN**	**Non-painful HIV-SN**	**References**
IL-1β	Increased*†	-	Lees et al. 2013
TNFα	Increased*†	Increased†	Taskinen et al. 2000; Tadokera et al. 2011 ;Zheng et al. 2011; Herzberg & Sagen 2001
IL-10	Decreased*	Increased*†	Taskinen et al. 2000
IL-2	Increased*	-	Uceyler et al. 2007
IL-4	Decreased*	Increased*	Tyor et al. 1995
IL-6	Increased*	-	Tadokera et al. 2011
IFNγ	Increased*†	-	Tadokera et al. 2011; Taskinen et al. 2000

Human studies denoted by (*) and animal models denoted by (†)

HIV-1 gp120 has been implicated in the development and perpetuation of neuropathic pain. When gp120 was applied peripherally to rat sciatic nerves, there was an upregulation in the expression of TNFα mRNA in the spinal cord (Zheng et al. 2011). Epineural exposure of gp120 in rat sciatic nerves induced allodynia and hyperalgesia for a period of 20-30 days, which also corresponded with increased expression of TNFα in the nerve trunk and axonal swelling (Herzberg & Sagen 2001). The findings from these two studies suggest that exposure of the peripheral nerve to gp120 causes painful peripheral neuropathy which is preserved by long-lasting spinal cord nerve pathology.

Interferon-γ is another cytokine that has a role in HIV-SN pain (Acharjee et al. 2010). Archarjee and colleagues (2010) reported that immunosuppressed mice that expressed the viral protein Vpr (a cytotoxic HIV-1 accessory protein) in the peripheral nervous system, displayed behaviour that might be consistent with mechanical allodynia together with increased levels of interferon-γ mRNA copies in the DRG and sciatic nerve (Acharjee et al. 2010)(Figure 1.3).

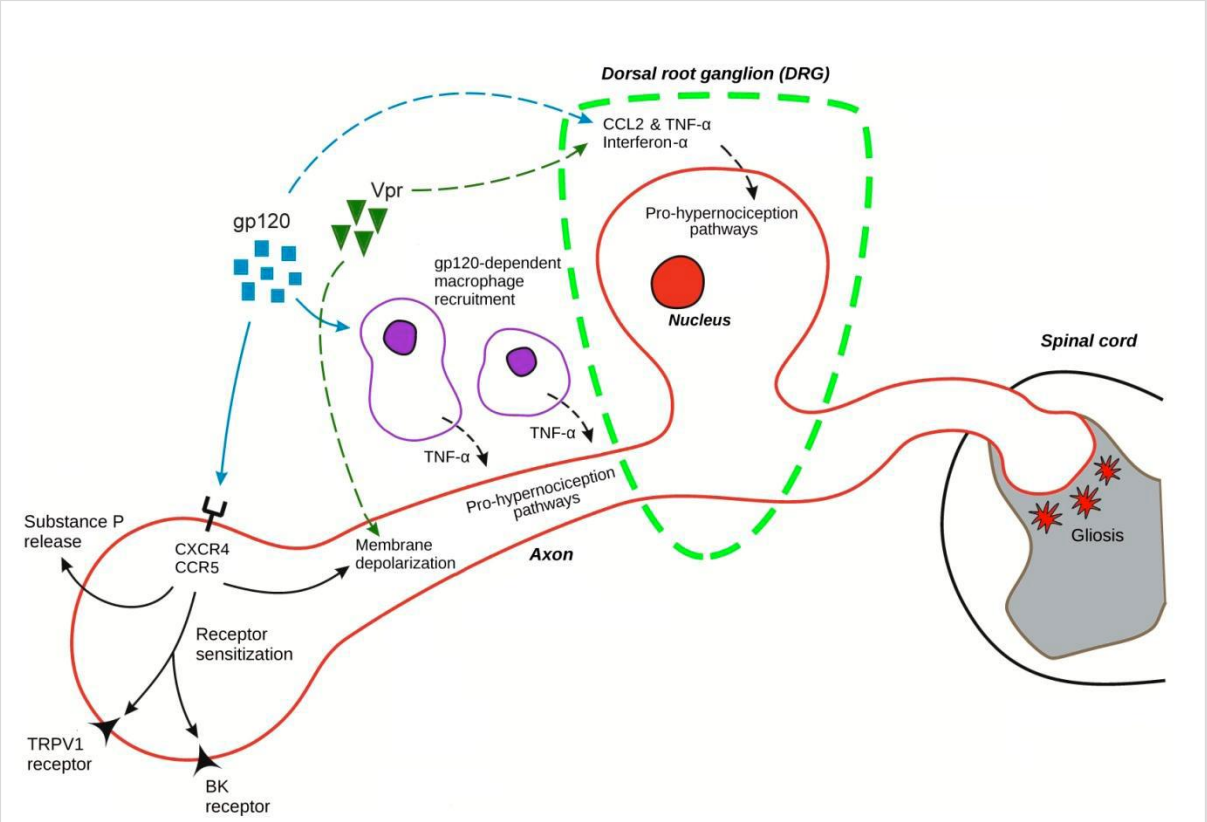


Figure 1.3. Mechanisms of hypernociception induced by HIV viral proteins in the dorsal horn of the spinal cord and peripheral nerves. Taken from (Kamerman et al. 2012).

Responses to gp120

Gp120 is the portion of HIV-1 that binds to and activates microglia and astrocytes and glial cells have been implicated in gp120-induced hyperalgesia and allodynia (Herzberg and Sagen, 2001; Wallace *et al.*, 2007). Wallace and colleagues (2007) also reported the occurrence of spinal gliosis in rodents after perineural exposure to gp120. The occurrence of spinal gliosis following gp120 administration is supported by Zheng (2011), who found an upregulation of GFAP and Iba1, which are activation markers of astrocytes and microglia, in the spinal cord, respectively. Substantial astrocyte and microglia activation, which corresponded with neuropathic pain behaviours, has also been reported by Herzberg & Sagen (2001), who performed their experiments on rats (Herzberg & Sagen 2001). The review by Mika and colleagues (2013) further associates glial activation and the development of neuropathic pain and describes the significant interaction between the nervous system and glial and immune cells (Mika et al. 2013). The findings of the abovementioned studies indicate that long-lasting spinal cord nerve pathology may contribute to the maintenance of neuropathic pain.

Hao (2013) also suggests that abnormal glia-neuron signalling, which is revealed in the pathogenesis of neurological complications in HIV-positive individuals, may be responsible for the development of neuropathic pain. Where, infected microglia release 'virotoxins' which largely constitute toxic viral proteins and inflammatory cellular toxins (Hao 2013). Whilst, astrocytes play a smaller role in the release of these virotoxins in comparison to the microglia, they can be activated via cellular toxins from the microglia (Hao 2013).

To summarise the above sections; viral coat proteins may mediate nerve fibre damage and hypernociception via direct and indirect mechanisms. Axonal pathology is mainly dominated by direct interaction between viral coat proteins and nerve fibres whereas indirect mechanisms that occur secondary to the virus-mediated activation of macrophage and glia infiltration into the DRG. Mitochondrial dysfunction is mainly implicated in antiretroviral induced peripheral sensory neuropathy, however the exact mechanism behind the mitochondrial dysfunction which leads to the nerve fibre damage remains unclear.

1.2.3.4 Pathogenesis of NRTI-induced peripheral neuropathy

Highly active antiretroviral therapy (HAART) typically contains three or more different drugs, typically including two nucleoside reverse transcriptase inhibitors (NRTIs) and either a protease inhibitor or non-nucleoside reverse transcriptase inhibitor (Table 1.3 and Table 1.4). NRTIs such as zalcitabine, didanosine and stavudine, which were previously used to treat HIV-infection, are strongly associated with the development of HIV-SN (Cherry et al. 2009; Ellis et al. 2010; Hao 2013). Due to their severe neurotoxicity, zalcitabine and didanosine have been phased out of first-line ARV drug regimens in developed and developing countries. In South Africa, the low-cost drug stavudine was phased out of first-line regimens in April 2010 (WHO 2009). Table 1.4 includes all ARV drugs currently approved for use in first and second line HAART regimens in South Africa (Stead 2017). These guidelines are consistent with recent WHO guidelines (World Health Organization 2017).

Growing evidence around the main pathological processes involved in NRTI-induced peripheral neuropathy implicate mitochondrial dysfunction (Gardener et al. 2014). However, the difficulty in identifying the exact causes of NRTI-induced neuropathy lies with the simultaneous viral-induced nerve damage, which is often referred to as the 'double hit hypothesis' (Keswani et al. 2006). Studies have suggested that an increase in neurotoxic inflammatory cytokines, caused by macrophage activation (via the viral coat protein gp120), may affect neurons by making them more sensitive and thus making them susceptible to the toxicity of NRTIs. This may be one reason behind non-symptomatic pre-existing peripheral

sensory neuropathy progressing to a symptomatic sensory neuropathy after initiation of ARV therapy.

Table 1.3 List of antiretroviral drugs included in first and second line HAART regimens. [adapted from South African National Department of Health 2015 Guidelines (Health 2015)].

NRTIs[a]	NNRTIs[b]	PI[c]	EI[d]
Zidovudine (Retrovir, AZT)	**Nevirapine** (Viramune, NVP)	**Saquinavir** (Invirase, SQV)	**Enfuvirtide** *(*Fuzeon, ENF, T-20)
Didanosine (Videx, Videx EC, ddI*)*	**Delavirdine** (Rescriptor, DLV)	**Indinavir** (Crixivan, IDV)	**Maraviroc** *(Selzentry or Celsentri, MVC)*
Stavudine (Zerit, d4T)	**Efavirenz** (Sustiva or Stocrin, EFV, also part of Atripla)	**Ritonavir** (Norvir, RTV)	
Lamivudine (Epivir, 3TC)	**Etravirine** (Intelence, ETR)	**Nelfinavir** (Viracept, NFV)	
Abacavir (Ziagen, ABC)	**Rilpivirine** (Edurant, RPV, also part of Complera or Epivlera).	**Amprenavir** (Agenerase, APV)	
Tenofovir a nucleotide analog (Viread, TDF)		**Lopinavir/ritonavir** (Kaletra or Aluvia, LPV/RTV)	
Combivir (combination of zidovudine and lamivudine)		**Atazanavir** (Reyataz, ATZ)	
Trizivir (combination of zidovudine, lamivudine and abacavir)		**Fosamprenavir** (Lexiva, Telzir, FPV)	
Emtricitabine (Emtriva, FTC)		**Tipranavir** (Aptivus, TPV)	
Truvada (combination of emtricitabine and tenofovir)		**Darunavir** (Prezista, DRV)	
Epzicom (combination of abacavir and lamivudine)			

[a] NRTIs=Nucleoside reverse transcriptase inhibitor
[b] NNRTIs=Non-Nucleoside reverse transcriptase inhibitor
[c] PI=Protease inhibitor
[d] EI-Entry inhibitor

Table 1.4 ARV drugs included in first and second line HAART regimens in South Africa. [adapted from South African National Department of Health 2015 Guidelines (Health 2015)].

	Type of regimen
First line (1a and 1b)	
All Adults All TB/HIV co-infection All HBV co-infection	1a: TDF + 3TC (or FTC) + EFV provide as fixed-dose combination (FDC)
If contraindication to EFV	1b: TDF + FTC (or 3TC) + NVP or LPV/r
If contraindication to TDF	1b: ABC+ 3TC + EFV (or NVP)
Second line (2)	
Failing on TDF-based first line	AZT + 3TC + LPV/r AZT + TDF + 3TC + LPV/r (if HBV co-infected)
Failing on a d4T or AZT-based first line regimen	TDF + 3TC (or FTC) + LPV/r
Dyslipidaemia (total cholesterol >6 mmol/L) or diarrhoea associated with LPV/r	Switch LPV/r to ATV/r
Anaemia and renal failure	Switch to ABC

Animal models of NRTI-induced neuropathy

In animal models of NRTI-induced neuropathy, zalcitabine is the drug that has most often been used and the mechanisms underlying neuropathy in these animals have been studied mainly in the context of the neuropathic pain-like behaviours in rodents (Joseph et al. 2004; Wallace et al. 2007; Zheng et al. 2011b; Blackbeard et al. 2012; Bhangoo et al. 2007)(Figure 1.4). Joseph (2004) created an animal model of NRTI-induced painful peripheral neuropathy using the neurotoxic NRTI's, stavudine, zalcitabine and didanosine. The administration of these three NRTI's produced dose-dependent mechanical hypersensitivity and allodynia. Furthermore, the hypersensitivity, induced by the three above-mentioned NRTI's, persisted even when various protein kinase inhibitors (which have shown anti-hyperalgesic effects in other models of toxic painful peripheral neuropathies) was administered (Joseph et al. 2004). The above-mentioned finding highlights the clinical importance of the elimination of stavudine, zalcitabine and didanosine in first-line ARV therapies in all countries. In support of the above-mentioned findings, Wallace and colleagues (2007) showed that zalcitabine, injected intraperitoneally (without gp120) three times a week for a period of three weeks, caused

mechanical but not thermal hypersensitivity which was also associated with anxiety-like behaviours (Wallace *et al.*, 2007). The hypersensitivity observed, increased with the perineural application of gp120, indicating a synergistic or additive effect consistent with the 'double knock' hypothesis. Interestingly, they also reported that when the microglial inhibitor minocyline was administered, hypersensitivity to mechanical stimuli was delayed and a reversal of anxiety-like behaviour was seen in those treated with zalcitabine and gp120 (Wallace *et al.*, 2007). Spinal gliosis was evident in the study by Wallace and colleagues (2007), and significantly increased when gp120 and zalcitabine were administered together. Indeed, glial cells found in the CNS are essential for homeostasis. Activated glial cells contribute to immune dysregulation and neuro-inflammation that contributes to the development of neuropathic pain (Lucin & Wyss-Coray 2009). Blackbeard and colleagues (2012) showed that spinal microgliosis was present in a model of zalcitabine exposure, either alone or in combination with perineural exposure of gp120. Using flow cytometry, they reported elevated levels of OX42, which is a marker of the presence of microglia (Blackbeard et al. 2012). In contrast, the model of zalcitabine exposure used by Zheng (2011a), showed no significant microglia hypertrophy or increase in markers of microglia (e.g. Iba1) in the spinal cord of animals who were treated with zalcitabine (Zheng et al. 2011a).

In another vital study involving zalcitabine exposure in rodents, which produced novel findings, Zheng and colleagues (2011b) investigated the role of the inflammatory cytokine, TNFα in a model of antiretroviral induced neuropathic pain (Zheng et al. 2011b). They found that a single high dose administration of zalcitabine, intraperitoneally, in non-HIV infected rats induced mechanical hypersensitivity within a period of three days, and which lasted for seven weeks. Also, zalcitabine exposure not only caused an over-expression of mRNA and TNFα in the spinal dorsal horn, but increased TNFα expression in the DRG (Zheng et al. 2011b). This was the first study, to report a link between administration of a single dose of zalcitabine, and subsequent increases in the expression of TNFα in the spinal dorsal horn and the DRG, highlighting the central role of TNFα in the development of neuropathic pain associated with antiretroviral treatment, independent of HIV-infection. To further corroborate the role of TNFα, when this cytokine was blocked by siRNA and recombinant TNFα soluble receptor, independently, mechanical allodynia was reduced (Zheng et al. 2011b).

TNFα has also been implicated in another model of NRTI-induced neuropathy, where inhibitors of mitochondrial transport chain complexes were administered and improved neuropathic pain-related behaviours in rats (Joseph 2006). In the model above, by Joseph and colleagues (2006), TNFα-induced hyperalgesia improved, when inhibitors of mitochondrial electron transport chain complexes were administered. Furthermore, inhibitors

of ATP-dependent mechanisms in the model improved the hyperalgesia induced by NRTI's but not by TNFα (Joseph 2006). The improvement of NRTI-induced hyperalgesia with inhibitors of ATP-dependent mechanisms reveals the importance of ATP-dependent mechanisms in NRTI-induced sensory neuropathy and highlights the pivotal role mitochondrial electron transport chains has in neuropathic and inflammatory pain.

Chemokines and NRTI-induced neuropathy

Chemokines may also play a role in NRTI-induced peripheral sensory neuropathy, however this has not directly been proven. Wallace and colleagues (2007) found no zalcitabine-induced alterations in the DRG of rats, however they reported that the chemokine CCL2 was significantly expressed in the DRG of the rats that were given perineural gp120, either alone, or in combination with zalcitabine (Wallace *et al.*, 2007). Furthermore, Bhangoo and colleagues (2007) investigated the role of chemokine signalling in NRTI treatment that included zalcitabine and reported that the expression of CXCR4 chemokine receptors in satellite glial cells in the DRG and Schwann cells in sciatic nerves was increased, following administration of a single dose of zalcitabine. In addition, the chemokine SDF-1/CXCL12 was also expressed in glial cells in the DRG and peripheral nerves. The findings of Bhangoo and colleagues (2007) emphasize that the mechanism of NRTI-induced pain hypersensitivity, may include the upregulation of CXCR4 signalling in the DRG (Figure 1.4).

In the study by Bhangoo (2007), increased CXCR4 mRNA expression was revealed by measuring CXCL12 induced calcium increases in the DRG neurons and glia (Bhangoo et al. 2007). Indeed, intracellular calcium has been linked to NRTI-induced pain. Joseph (2004) reported when calcium modulators (TMB-8 and Quin-2) were administered via spinal injection, improvement in the drug-induced hyperalgesia and allodynia were observed (Joseph et al. 2004). Therefore, implicating Ca in the development of hyperalgesia and allodynia seen in painful neuropathy and suggests a calcium dependent mechanism of NRTI-induced painful sensory neuropathy.

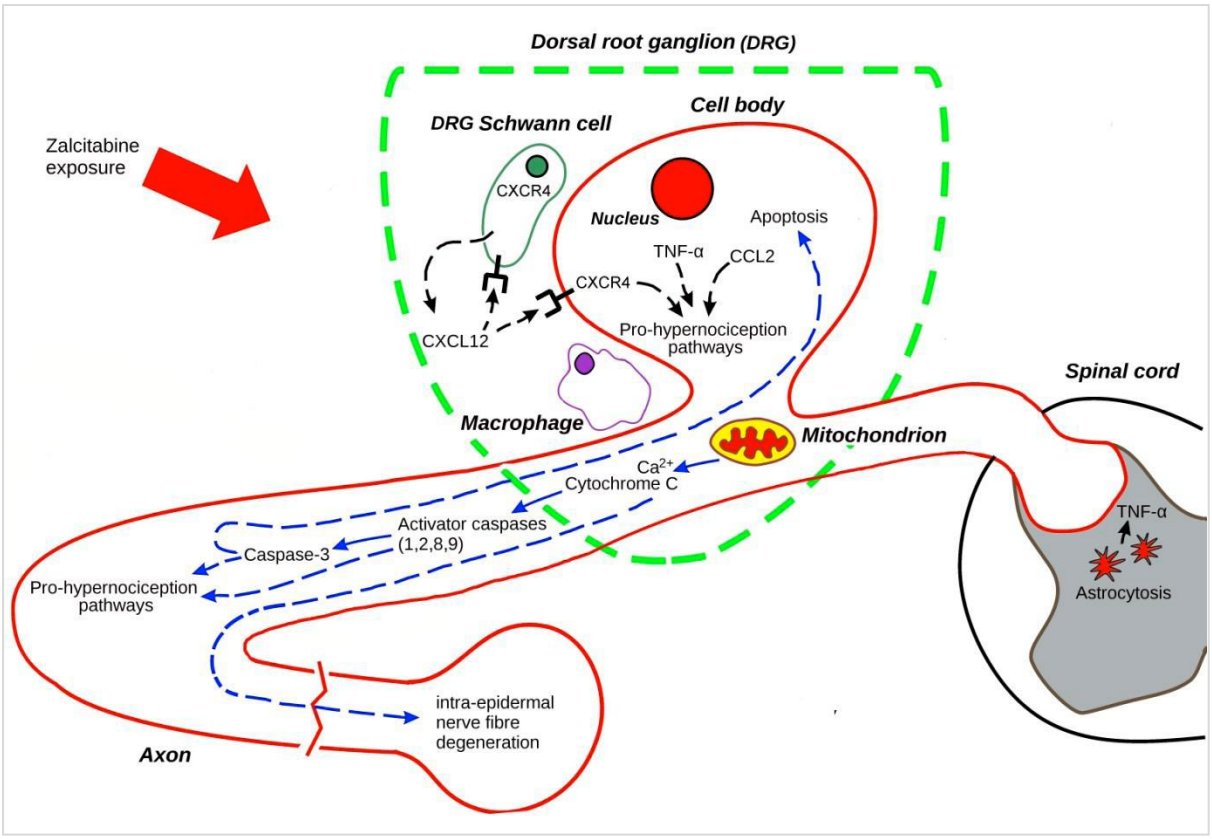


Figure 1.4. Mechanisms of NRTI-induced neuronal dysfunction in a model of zalcitabine exposure. Taken from: (Kamerman et al. 2012).

Mitochondrial dysfunction in NRTI-induced neuropathy

NRTI's block the replication of the HI virus by inhibiting viral reverse transcriptase. However, NRTI's also inhibit function of mitochondrial DNA polymerase gamma, which is the enzyme required for mitochondrial DNA maintenance and synthesis (Kakuda 2000). Mitochondrial DNA is also required for several oxidative phosphorylation complex 1 proteins (Hao 2013). Therefore, NRTI's may exert a large effect by inducing mitochondrial DNA depletion and causing oxidative phosphorylation deficits. Lehman and colleagues (2011) reported increased mitochondrial DNA mutation deletions and decreased mitochondrial protein levels, in sural nerve biopsies of HIV-positive individuals who were exposed to neurotoxic ARV therapy and had developed HIV-SN. Therefore, this data from HIV-positive individuals indeed confirms a model of mitochondrial dysfunction. In animal models, mitochondrial dysfunction induced by zalcitabine affects calcium homeostasis in cultured DRG neurons (Werth et al. 1994) and in a model of zalcitabine-associated painful sensory neuropathy (Joseph et al. 2004).

Other NRTI models of neuropathy

Zalcitabine-based animal models form the basis of investigation into mechanisms of NRTI-induced neuropathy due to the failure of didanosine and stavudine to create any compelling

morphological or electrophysiological evidence of peripheral nerve toxicities. A few studies have provided some findings, though minimal, using these drugs (Keswani et al. 2006; Zhu et al. 2007). Keswani and colleagues (2006) established a rodent model of HIV-SN by the oral administration of didanosine to transgenic mice that expressed gp120 (Keswani et al. 2006). They found a decrease in IENFD which is supported by Wallace (2007). Keswani and colleagues (2006) also reported that didanosine-treated transgenic mice showed a loss of unmyelinated axons but there was no evidence of axonal degeneration. Bhangoo (2007) also reported no evidence of demyelination, re-myelination or axonal degeneration in peripheral nerves (Bhangoo et al. 2007), however, this was observed in zalcitabine-treated rodents and not in a model including didanosine. Zhu and colleagues (2007) also failed to show any significant nerve fiber loss and any signs of axonal degeneration of IENF in cats that were administered oral didanosine daily for a period of six weeks (Zhu et al. 2007). Huang and colleagues (2013) developed a rodent model of d4t-induced peripheral sensory neuropathy, where intravenous injections of d4t (50mg/kg) were given to rats four days apart. Following the intravenous injection, the rats developed hind paw mechanical hypersensitivity and significant changes in both thigmotaxic and burrowing behaviours. They also reported possible injury to both peripheral and central terminals of the L5 dorsal root ganglion, evidenced by decreases in both IENFD in the hind paw and CGRP/IB4 immunoreactivity (Huang et al. 2013). After examining the sural nerve (seven days post injection), they suggested that down-regulated proteins associated with mitochondrial function may well be a possible mechanism involved in the vulnerability of distal axons to d4t neurotoxicity.

1.3 Factors associated with the development of HIV-SN:

Several risk factors may contribute to the development of HIV-SN. As a result, the HI virus and exposure to neurotoxic antiretroviral therapies are only one part of a larger group of risk factors which affect PLWHA. Table 1.5 lists demographic, anthropomorphic, clinical and genetic factors identified as risk factors for HIV-SN. In this section, a description of the various risk factors for HIV-SN in African and non-African based cohorts are given. Also, African and non-African cohorts are grouped separately to facilitate the discussion of risk factors which are common to these populations and to highlight the difference in prevalence and incidence rates between the different populations. Prevalence and incidence data on HIV-SN in African and non-African based cohorts will also be listed in the tables below.

Table 1.5 Factors commonly associated with HIV-SN.

Demographic and Anthropomorphic	AIDS/HIV-disease related	Clinical	Genetic
Age	Advanced HIV disease	Exposure to neurotoxic ARVs (stavudine, didanosine, zalcitabine)	Mitochondrial Haplotypes
Height		Comorbid disease (Diabetes mellitus and exposure to TB treatment	Immune-related genes
Sex			
Ethnicity			

Table 1.6 Prevalence and incidence of HIV-SN and risk factors for HIV-SN in stavudine and didanosine based African cohorts.

Study	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Associations with HIV-SN
(Wester et al. 2005)	NR	37%[a]	153	Botswana	d4t and ddi: n = 98	d4t and ddI use
(Forna et al. 2007)	Signs and symptoms	36%[a] FP(11-months)	1029	Uganda	All d4t	Age ≥35, TB Rx predicted severe SN
(Wadley et al. 2011b)	BPNS tool	57%[b]	395	South Africa African ancestry	d4t curr: n = 114 d4t ever: n= 395 ddI curr: n = 23 ddI ever: n =35	Increasing age, increasing height
(Sacktor et al. 2009)	Signs and symptoms	38%[a] FP(6-months)	102	NR	All d4t	None reported
(Maritz et al. 2010b)	BPNS tool	49%[b] 30%[b] (symptomatic)	598	South African African ancestry	d4t = 203, overall exposure to d4t: n = 269	Increased age, cART use, TB Rx, Increased waist to hip ratio
(Mehta et al. 2011)	Signs and symptoms	NR	199	NR	d4t: n = 125	Female sex

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.6 (continued)

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(van Griensven et al. 2010)	NR	8%[a]	2190	Rwanda African ancestry	All d4t	Increased age, advanced HIV, 40mg dose of d4t
(Isezuo et al. 2009)	NR	2.2%[b]	322	NR	n = 58 on ARV regimen incl. d4t	Stage IV HIV disease
(Beadles et al. 2009)	Symptoms (in medical records)	35%[b]	3341	NR	All d4t	Not reported
(Menezes et al. 2011)	Symptoms	17%[a]	9040	NR	All d4t	d4t use
(Shurie & Deribew 2010)	Signs and symptoms	25.5%[b]	318	NR	d4t ddc	Increasing age, cART use, d4t, 40mg dose of d4t, TB Rx which included isoniazid
(Watt et al. 2015)	BPNS and TNSr	21%[a] FP (24 weeks) 16%[b]	159	South Africa	not specified	Previous and current TB Rx

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.6 (continued)

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Kiwuwa-muyingo et al. 2014)	Symptoms on a case record form	11%[a] FP (4.9 years) 13%[b]	3316	Ugandan-Zimbabwean adults	d4t: n = 397 Isoniazid: n = 596	Current d4t use, current isoniazid use, current ddI use, increased age and increased pre-ART weight
(Luma et al. 2012)	BPNS	21%[b]	295	Cameroon	d4t: n = 47	Decreased CD4 count, alcohol intake, history of TB Rx
(Philomène et al. 2015)	BPNS	27.4%[b]	620	Gabon	d4t: n = 291	d4t use, stage of HIV disease, presence of diabetes mellitus
(Conradie et al. 2014)	Signs and symptoms	17%[a] FP (~1 year) 24.4%[b] (symptomatic)	246	South African	All d4t	Increased age, exposure to terizidone,d4t use
(Centner et al. 2014)	BPNS	56%[b]	116	South African	d4t: n =10 (60mg/day)	current and previous TB Rx, black race, slow acetylation
(Jamisse et al. 2007)	Symptoms	18%[a] FP (~10-months)	146	Mozambique	n = 76 (Nevirapine +lamivudine + zidovudine) n = 70 Nevirapine +lamivudine + stavudine)	NR

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.6 (continued)

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Hitchcock *et al.* 2008)	NR	18%[b]	354	SA	NR (possibility of d4t and AZT)	TB Rx, male sex, Increased viral load, decreased CD4 count
(Hawkins et al. 2007)	Symptoms	20.7%[a] FP (350 days ~11.6months)	1286	Kenya	Nevirapine +lamivudine+ stavudine): n = 797	Baseline AIDS symptoms and CD4 counts ≤ 100 cells/mm^3
(Centner et al. 2017)	BPNS & Reduced version of TNSr	18%[a] (18 per 100 person years)	184	SA	d4t n = 112 TDF: n = 51	≥ 40 years

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.7 Prevalence and incidence of HIV-SN and risk factors for HIV-SN in stavudine and didanosine based non-African cohorts.

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Affandi et al. 2008)	BPNS	34%[b]	96	Indonesia	d4t: n = 96	Increasing age, increasing height, carrier of TNF-1031*2
(Ances et al. 2009)	Signs only	55%[b]	130	NR	d4t: n = 76	Diabetes mellitus and increased triglycerides
(Cherry et al. 2006)	BPNS	Australia 55%[b] USA: 49%[b]	147	*Australia*: C-97%, AA-0%, H=0%, A-1% *USA:* C-13%, AA-86%, H-1%, A-0%	d4t ever: Australia n = 116 USA n = 105	d4t and ddi exposure
(Cherry et al. 2009)	BPNS	Melbourne:42% Kuala Lampur:19% Jakarta:34%	294	Caucasian:34% Malaysian:33% Indonesian:32%	d4t ever: none Melbourne: n = 11 Kuala Lampur: n = 138 Jakarta:n = 294	Increasing age and increasing height
(Evans et al. 2011)	BPNS	23%[b]	2140	Caucasians-44% African ancestry-32% Hispanic-21% Other-3%	d4t~ n = 513	Increasing age, increasing height, diabetes mellitus, exposure to d4t,ddi,ddc and protease inhibitors

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

C: Caucasian AA: African American H: Hispanic A: Asian

Table 1.7 (continued)

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Nakamoto et al. 2010)	Signs only	31%[a]	61	Caucasians: 51%	d4t~ n = 34	Increasing age and decreased nadir cd4 count
(Smyth et al. 2007)	BPNS	2001: 44%[b] 2006: 42%[b]	100	Australia	2001: n = 82 2006: n= 58	Increasing age and exposure to d4t, ddi and Indinavir
(Lichtenstein et al. 2005)	Symptoms only	13%[a]	2515	Causcasion:55%	n = 1332	Age> 40, diabetes mellitus, white race, CD4 count between 50-199 cells/mm^3, Viral load > 10 000 copies/mL, exposure to ddi, d4t, nevirapine, lamivudine, ritonavir, nelfinavir and sequanavir
(Robinson-Papp et al. 2009)	Signs only	50%[a]	104	Hispanic: 41% African ancestry:38% Caucasian: 21%	n = 52	Increasing age, increasing CD4 count, Hispanic race and intravenous drug use
(Watters et al. 2004)	Symptoms and signs	43%[b]	174	Hawaii	n = 121 with HIV-SN were exposed to NRTI's	≥ 50 years of age

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.7 (continued)

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Skopelitis et al. 2006)	Signs only	36%[b]	100	Caucasian: 98% African: 2%	n = 42	AIDS, increasing age, decreased nadir CD4 count, exposure to 2 neurotoxic ARVs
(Skopelitis et al. 2007)	Signs only	37%[b]	94	Caucasian: 95%	Single agent: n= 42 2 neurotoxic agents: n = 11	Increased nadir CD4 count, advanced HIV staging, previous exposure to NRTI combinations
(Wright et al. 2008)	Signs and symptoms	19.7%[b]	658	Asian Pacific region		Age > 35, previous d4t and ddi exposure, prior AIDS and neurocognitive impairment
(Anziska et al. 2012)	Signs only	36%[b]	973	USA		Older age, Hepatitis C seropositivity, African American race and diabetes mellitus. Hispanic race associated with reduced risk

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.8 Prevalence and incidence of HIV-SN and associations with HIV-SN in tenofovir-based African cohorts.

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Njuguna et al. 2013)	NR	0%: (TDF-based) 37.8%: (d4t-based) 12.1%: (AZT-based)	5095	SA	d4t: n=3438 AZT: n=209 TDF: n=948	NR
(Bygrave et al. 2011)	NR	TDF: 0% AZT: 0% d4t: 29%[b]	1124	Lesotho	TDF: 948 AZT: 255 d4t: 282	NR
(Tumusiime et al. 2014)	BPNS	59%[b]	507	Rwanda	TDF: 247 d4t: 53 d4t ever: 196	Older age, higher education level and an urban setting compared to a rural setting
(Tweya et al. 2014)	NR	TDF: 9%[b] d4t: 22%[b]	284 (all on TB+HIV treatment)	Malawi	TDF: 174 d4t: 110	NR
(Ndakala et al. 2016)	Symptoms only	29%[b]	320	Kenya	TDF: n = 58 d4t: n = 147	Older age with SN cases *Height could be a modulator

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.8 (continued)

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Watt et al. 2015)	BPNS TNsr	19%[a] (FP= 24 weeks)	133	SA	TDF: n = 56 d4t: n= 77	None
(Watt et al. 2014)	BPNS	27%[a] (FP= 24 weeks)	120	SA	TDF= 19 d4t= 92 AZT= 7	Increased CD4 counts, increased TNF/IL-4 ratios and increased IFN/IL-10 ratios

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

Table 1.9 Prevalence and incidence of HIV-SN and associations with HIV-SN in tenofovir-based non-African cohorts.

Study (Authors)	HIV-SN diagnostic tool (Criteria)	Prevalence/Incidence	N	Country/Ancestry	Drug exposure	Association with HIV-SN
(Shikuma et al. 2015)	BPNS Distal leg and proximal thigh IENFD assessment	10.6%[a] (FP=72 weeks)	150	Thailand	TDF: n=47 d4t/AZT: n=51 AZT: n=49	Drug toxicity (but not explicitly reported)
(Phanuphak et al. 2012)	BPNS	8.3%[a] (TDF-based) 9.8%[a] (d4t/AZT-based) 16.3%[a] (AZT-based) (FP= 72 weeks)	150	Thailand	TDF: n=49 d4t/AZT: n=51 AZT: n=50	Drug toxicity (but not explicitly reported)
(Sun et al. 2015)	NR	8.3%[a] (FP= 22 months)	3372	China	TDF: 474 d4t: 612 AZT: 2080	Drug toxicity, older age and CD4 count < 200cells/mm^3
(Phillips et al. 2014)	BPNS UENS TCSS	42%[b]	66	England	NR	Increased plasma triglycerides, increased depression, increased anxiety, increased catastrophizing and increased insomnia

[a]Incidence [b]Prevalence FP=Follow-up period NR=Not reported TB Rx=Tuberculosis treatment

1.3.1 Demographic and anthropomorphic risk factors

1.3.1.1 Age

Age is a well-documented and notable risk factor for the development of HIV-SN. In both African and non-African studies (Table 1.6, Table 1.7, Table 1.8), age is a consistent risk factor for HIV-SN (Forna et al. 2007; Wadley et al. 2011b; Maritz et al. 2010; van Griensven et al. 2010; Shurie & Deribew 2010; Kiwuwa-muyingo et al. 2014; Conradie et al. 2014; Affandi et al. 2008; Cherry et al. 2009; Evans et al. 2011; Nakamoto et al. 2010; Smyth et al. 2007; Lichtenstein et al. 2005; Robinson-Papp et al. 2009; Watters et al. 2004; Skopelitis et al. 2006, Wright et al. 2008; Anziska et al. 2011). Indeed, with increasing age, peripheral nerves become more fragile and weaker leading to decreased neurological function (Terman et al. 2010). If one considers that peripheral nerves are rather long, large and metabolically stressed cells, then increased vulnerability of these cells with aging is sure to induce damage (Inceoglu et al. 2015; Epel 2009). Improvements in cART regimens in both developed and developing countries, has seen the lifespan of PLWHA increase considerably. This increased lifespan of PLWHA together with an aging population that is well treated for HIV with cART may well ultimately lead to late-onset neuropathy and therefore an increased prevalence of both HIV-SN and symptomatic HIV-SN (Wadley *et al.*, 2011), which is independent of adaptable risk factors such as exposure to neurotoxic first line ARVs. Age remains a consistent risk factor even in the era of improved cART therapy which contains the less neurotoxic ARV drug tenofovir (Tumusiime et al. 2014; Ndakala et al. 2016; Sun et al. 2015). The recent study by Centner and colleagues (2017) demonstrates that age is still a consistent risk factor in the era of TDF-based cART. Their study (n=184) reported an incidence of neuropathy of 18% (follow-up period 24 months) in individuals on d4t-based (61%) and TDF-based (28%) cART. Age was the only risk factor identified in their cohort (Centner et al. 2017).

1.3.1.2 Height

Several studies have reported height as a risk factor for HIV-SN (Affandi *et al.*, 2008; Cherry *et al.*, 2009; Banerjee *et al.*, 2011; Evans *et al.*, 2011; Wadley *et al.*, 2011). Height has also been identified as a risk factor in diabetic sensory neuropathy (Sosenko *et al.*, 1986; Cheng *et al.*, 2006). Evans and colleagues (2011) describe that HIV-SN is a length-dependant neuropathy and therefore taller individuals are much more susceptible to the development of HIV-SN. Interestingly, Wadley and colleagues (2011) were the only African study to report increased height

as a risk factor for development of symptomatic HIV-SN (Wadley *et al.*, 2011). Another African study by Mehta and colleagues (2011) also assessed height in their cohort, however they did not find height as significant risk factor for HIV-SN. Unfortunately, several African studies did not assess height in their cohorts (Tumusiime et al. 2014; Bygrave et al. 2011; Tweya et al. 2014; Njuguna et al. 2013). Since height is a simple and non-invasive measurement to carry out, future studies should consider implementing this recording in their assessments of patients. Wadley and colleagues (2011) also suggest that prioritizing taller participants for less neurotoxic regimens could possibly decrease the prevalence of symptomatic HIV-SN in resource-limited settings.

Increased nerve length is associated with larger axon surface area, and thus any localised nerve injury to an axon results in impairment of the general conduction properties of the nerve (Cheng *et al.*, 2006). In addition, having a greater leg length may also be associated with a longer time for nervous tissue to completely regenerate after an injury which could result in increased prevalence of sensory neuropathy (Cheng *et al.*, 2006). The postulated theory of a longer leg length and SN is supported by Nyati and colleagues (2006), who reported that individuals of African ancestry may present with a longer leg length in comparison to trunk length as compared to Caucasians (Nyati et al. 2006). However, the findings of Nyati and colleagues (2006) are not supported by other studies specifically assessing height in individuals with HIV-SN (Affandi et al. 2008; Cherry et al. 2009) Two studies conducted on Asian populations i.e. Jakarta and Kuala Lampur, found height as a significant risk factor for HIV-SN (Affandi et al. 2008; Cherry et al. 2009). Furthermore, Mehta and colleagues (2011) conducted their study on a predominantly black African Kenyan population and found no association between height and HIV-SN (Mehta et al. 2011).

1.3.1.3 Sex

From the studies reviewed (Table 1.6), only two reported sex as a risk factor for HIV-SN (Mehta et al. 2011; Hitchcock, Meyer & Gwyther 2008). Furthermore, the results from these studies are inconsistent, where Mehta and colleagues (2011) reported female sex as a risk for HIV-SN and Hitchcock and colleagues (2008) reporting male sex as risk for HIV-SN. Men are generally taller than women, and thus the possibility of male participants being much taller and therefore at a higher risk of developing SN compared to female participants is one postulation (Cherry et al. 2009), however height was not measured and thus not considered in the Hitchcock (2008) cohort. In contrast, Mehta and colleagues (2011) reported that females were 9.6 times more like to develop SN compared to males. The association remained even after correction for height, age,

isoniazid exposure, type of ARV treatment regimen, WHO disease stage, BMI and CD4 count (Mehta et al. 2011). The female participants had higher BMI values and lower haemoglobin levels in comparison to the males. Higher BMI values (Herrera-rangel et al. 2014) and low haemoglobin levels (Steiner et al. 1988) have both been linked to risk of SN development.

1.3.1.4 Ethnicity and Genetics

Evidence for an association between race and HIV-SN is circumstantial. African ancestry was identified as a risk factor by a few studies (Mehta et al. 2011; Anziska et al. 2011; Centner et al. 2014). However, Caucasian (Lichtenstein et al. 2005) and Hispanic race (Robinson-Papp et al. 2009) have also been reported as risk factors for HIV-SN. Different genotypes may associate with HIV-SN (Cherry et al. 2008). Cherry and colleagues (2008) reported that carriage of the rs1799964*2 and rs9281523*2 single nucleotide polymorphisms (SNPs) increases risk of HIV-SN in in Caucasians. Increased risk of HIV-SN has also been reported with the carriage of the rs1799964*2 increases risk of HIV-SN in Malay individuals (Affandi et al. 2008). Furthermore, Chew and colleagues (2010) also reported that the TNFA haplotype group FVa6,7,8 increases risk of HIV-SN in Malay and Chinese individuals but not in Caucasians (Chew et al. 2011). In an African cohort, Wadley and colleagues (2015) reported that alleles of seven SNPs, spanning the TNF block, associated with reduced risk of HIV-SN. These alleles include: rs11796*A, rs3130059*G, rs2071594*C, NFKBIL1-62/rs2071592*A, rs2071591*A, LTA+252/rs909253*G and rs1041981*C (Wadley et al. 2015). Therefore, studies focusing on genetics within cohorts are providing novel information on ethnic differences in HIV-SN (Affandi *et al.*, 2008; Cherry *et al.*, 2008; Chew *et al.*, 2011; Hendry *et al.*, 2013, 2015, Wadley *et al.*, 2014, 2015). Genetic association studies on various ethnic groups may be highly beneficial to the treatment and management of symptomatic HIV-SN.

1.3.2 Clinical risk factors

1.3.2.1 Advanced HIV disease

Advanced HIV disease staging, usually identified by a lower CD4 T-cell count and higher plasma HIV-1 RNA load, is a significant risk factor for the development of HIV-SN (van Griensven et al. 2010; Isezuo et al. 2009; Philomène et al. 2015; Skopelitis et al. 2006; Wright et al. 2008; Luma et al. 2012; Hitchcock, Meyer & Gwyther 2008; Hawkins et al. 2007; Nakamoto et al. 2010; Lichtenstein et al. 2005). In the pre-cART era, higher plasma viral loads and lower CD4 counts

prior to the initiation of ARV therapy, increased the risk of HIV-SN (Childs et al. 1999; Tagliati et al. 1999). A diagnosis of AIDS also increased the risk of HIV-SN (Hawkins et al. 2007; Isezuo et al. 2009).

With the introduction of highly active antiretroviral therapy (HAART), most patients are able to achieve effective viral control and a significant boost in CD4 T-cells counts. As a result, these measures of disease severity no longer associate with HIV-SN (Wester et al. 2005; Evans et al. 2011; Cherry et al. 2009; Smyth et al. 2007). However, even in the era of HAART, several studies have found an association between nadir CD4 cell count and HIV-SN (Ellis et al. 2010; Robinson-Papp et al. 2009; Skopelitis et al. 2006). The findings of these studies correspond with the earlier trend (prior to HAART) of an association between decreased CD4 T-cell count and increased risk of HIV-SN development.

With the introduction of safer and non-neurotoxic cART regimens, the association with HIV-SN and CD4 counts persists. The study by Sun and colleagues (2015) found an association between decreased CD4 counts and increased risk of HIV-SN (Table 1.9), whilst Van der Watt and colleagues (2014) reported an increased risk of HIV-SN with increased CD4 counts (Van der Watt et al. 2014). The above-mentioned finding is not unique to Van der Watt and colleagues (2014) and has been reported by at least one other study (Skopelitis et al. 2007). One explanation for the development of neuropathy in the presence of increased CD4 counts may be that immune restoration brings with it increased risk of immune-mediated nerve damage if there is inappropriate activation of the recovering immune system (e.g. IRIS). The development of IRIS can occur in those patients with an existing opportunistic infection, within the first few weeks or months of the patient initiating ARV therapy, when the immune system is initially severely supressed and cannot initiate an immune response (Shelburne et al. 2005). After initiating ARV therapy, there is a pathological inflammatory response as the immune system responds to an active infection and attempts to restore itself. This massive inflammatory response can worsen pre-existing conditions (worsening signs and symptoms) as well as unmask previously unrecognized subclinical conditions (Shelburne et al. 2005; Said & Krarup 2013). A role for IRIS-like responses in HIV-SN is unclear, but decreased activity in the recovering immune system cannot be excluded as a possibility.

1.3.2.2 Exposure to neurotoxic ARVs

Stavudine has been associated with increased risk of HIV-SN in the majority of studies (Wester et al. 2005; Maritz et al. 2010a; van Griensven et al. 2010; Menezes et al. 2011; Kiwuwa-muyingo

et al. 2014; Philomène et al. 2015; Conradie et al. 2014; Cherry et al. 2006; Evans et al. 2011; Smyth et al. 2007; Lichtenstein et al. 2005; Skopelitis et al. 2006; Wright et al. 2008). The link between stavudine and risk of HIV-SN appears to be dose related (Gottlieb et al., 1995; Simpson and Tagliati, 1995; Skowron, 1995; van Griensven *et al.*, 2010). The study by Skowron and colleagues (1995) reported that a stavudine dose of 0.5mg/kg/day associated with an incidence of SN of 21%. Furthermore, when the dose of stavudine was increased to 12mg/kg/day, the incidence of SN increased drastically to 73%, with accompanying symptoms consisting of bilateral pain and numbness in the lower legs and feet. Stavudine doses of 20mg per day have also been associated with increased incidence of HIV-SN (Simpson & Tagliati 1995) between 15% and 17% (Gottlieb et al.,1995), and when the dose of stavudine was increased to 40mg daily in the two latter studies, the incidence of HIV-SN increased to 21% and 23%, respectively (Gottlieb et al.,1995; Simpson and Tagliati, 1995).

Didanosine exposure has also been associated with an increased risk of HIV-SN (Wester et al. 2005; Kiwuwa-muyingo et al. 2014; Cherry et al. 2006; Evans et al. 2011; Smyth et al. 2007; Lichtenstein et al. 2005; Skopelitis et al. 2006; Wright et al. 2008). However, didanosine is currently most often included in second line regimens. It is anticipated that with the introduction of less neurotoxic ARV regimens, NRTIs such as didanosine will eventually be completely phased out of ARV regimens.

Protease inhibitors (PIs) have been linked to HIV-SN (Evans et al. 2011; Smyth et al. 2007; Lichtenstein et al. 2005). Lichtenstein (2005) found that the PIs ritonavir, nelfinavir and saquinavir were linked to increased risk of HIV-SN. It has been suggested that PIs alone do not cause SN. Evans (2011) found that when PIs are added to neurotoxic cART regimens, the risk of HIV-SN increases. This is especially important in developing countries where second line therapies largely consist of pairs of PIs and neurotoxic NRTIs which could ultimately increase the risk of SN in these populations.

Unfortunately, HIV-SN persists despite the improved immunologic function and virologic control associated with using improved and less neurotoxic cART and decreased neurotoxic cART Despite the introduction of the supposedly less neurotoxic ARV tenofovir (TDF), studies have shown that HIV-SN continues to be a problem in the era of TDF (Shikuma et al. 2015; Phanuphak et al. 2012; Sun et al. 2015). Phanupak and colleagues (2012) reported an incidence of HIV-SN of 8.3% in Thai adult individuals with CD4 counts <350 cells/mm^3 on TDF-based cART. The cohorts of the recent studies by Shikuma (2015) and Sun (2015) were not universally exposed to TDF-based therapy but were also exposed to d4t and AZT. Therefore, it is difficult to ascertain which drug was associated with their reported incidences of HIV-SN, 10.6% and 8.3%,

respectively (Shikuma et al. 2015; Sun et al. 2015). Studies with cohorts only exposed to TDF-based therapies are lacking and there is a need for these studies to assess whether cART with TDF in first line therapy does indeed lower the risk of HIV-SN.

1.3.2.3 Comorbid disease

Comorbid conditions such as diabetes mellitus, which commonly occurs in the general population and is itself a risk factor for peripheral polyneuropathy, has been reported by several studies as a risk factor for HIV-SN (Philomène et al. 2015; Ances et al. 2009; Evans et al. 2011; Lichtenstein et al. 2005; Anziska et al. 2012; Banerjee et al. 2011). Evans and colleagues (2011) reported that HIV-SN was associated with both diabetes and hypertriglyceridemia in their cohort (Evans et al. 2011). They also found that together with age, diabetes was associated with a lesser chance of recovery of symptomatic HIV-SN and further reported that no other metabolic syndrome components were associated with HIV-SN. Zoest and colleagues (2016) recently reported a higher prevalence of hypertension in patients with HIV-1 infection and a higher prevalence of hypertension in those patients exposed to stavudine-based regimens (van Zoest et al. 2016). The findings reported by Zoest and colleagues (2016) has significant implications for Southern Africa, where the prevalence of HIV remains high and the majority of the population (over 6.2 million) have high blood pressure (SA Heart Foundation, 2016). Since stavudine has been phased out of first line, but not second line regimens in South Africa and novel ARV drugs have been introduced, further investigations into the relationship between metabolic syndrome and HIV-infection are warranted as long-term metabolic complications will certainly become more common in our population.

The association between increased HIV-SN risk and hypertriglyceridemia reported earlier by Evans and colleagues (2011) is corroborated by other studies (Ances et al. 2009; Phillips et al. 2014; Banerjee et al. 2011). Banerjee and colleagues (2011) reported that elevated triglycerides were independently associated with HIV-SN. In their cohort, a group of 145 individuals with triglyceride levels above 243mg/dL were 2.6 times more likely to present with sensory neuropathy as compared to those individuals with lower triglyceride levels (< 141mg/dL) (Banerjee et al. 2011). One possible reason for this might be that elevated triglyceride levels may modify the way in which mitochondria metabolise energy and thus could have an effect on nerve function. However, this remains a postulation and certainly further investigation into this process is required.

The association between HIV-SN and Hepatitis C coinfection are varied. Anziska and colleagues (2011) are the only study to report an association between Hepatitis C coinfection and risk for HIV-SN in their cohort of principally female individuals (Anziska et al. 2012). The majority of studies assessing risk factors for HIV-SN do not report an association between Hepatitis C seropositivity and risk for HIV-SN (Cherry et al. 2010; Banerjee et al. 2011; Anziska et al. 2012). Cherry and colleagues (2010) specifically investigated the above-mentioned association in their cohort of 873 individuals, which consisted of individuals from three different ethnicities (African, Asian and Caucasian) and six different research sites (Baltimore, Jakarta, Johannesburg, Kuala Lumpur, Melbourne and Sydney). They found that Hepatitis C seropositivity did not associate with HIV-SN at any research site and furthermore, found that Hepatitis C seropositivity was associated with reduced HIV-SN risk at the Melbourne and Baltimore sites (Cherry et al. 2010).

With the first introduction of isoniazid (INH) in the treatment of Tuberculosis (TB), there was a notable increase in the occurrence of peripheral sensory neuropathy in adults that were given the drug ± 60 years ago (Mandel 1959). Indeed, INH is neurotoxic. Peripheral sensory neuropathy associated with INH use can be attributed to a deficiency of biologically active pyridoxine (Vitamin B6). Pyridoxine together with INH, form a 'hydrazone' which is excreted in urine leading to a deficiency. Therefore, adequate supplementation of pyridoxine when INH is prescribed is a necessity (Mandel 1959). Currently, INH is used in TB treatment and prophylaxis in PLWHA and has been linked to the development of HIV-SN in numerous studies (Breen et al., 2000; Forna *et al.*, 2007; Hitchcock, Meyer and Gwyther, 2008; Maritz *et al.*, 2010b; Shurie and Deribew, 2010; Luma *et al.*, 2012; Centner *et al.*, 2014; Conradie *et al.*, 2014; Van Der Watt *et al.*, 2015). Breen and colleagues (2000) reported a significant increase in the risk of HIV-SN among patients who were receiving INH together with the neurotoxic NRTI stavudine. A total of 55% (12/22) patients developed HIV-SN within a median time of 5-months of starting the INH + stavudine therapy (Breen et al., 2000). This indicates that stavudine may exacerbate HIV-SN in the presence of INH. Since stavudine is still used in first line therapy in resource limited settings and in second line therapy in certain African regions, the concomitant use of INH and stavudine in these settings should be avoided.

1.3.3 Genetic risk factors

Genetic association studies have identified genes affecting mitochondrial function and genes that are involved in the inflammatory response which alter the risk for HIV-SN in patients who are exposed to neurotoxic NRTIs (Kamerman et al., 2012; Ngassa *et al.*, 2017). Studies that have been conducted in vivo and in vitro have shown a relationship between development of sensory neuropathy and the effect neurotoxic NRTIs and HIV have on immune and mitochondrial functions (Kamerman et al., 2012). Therefore, these two above-mentioned processes (immune and mitochondrial function) have resulted in the candidate gene approach which is used to investigate genetic associations related to HIV-SN (Ngassa *et al.*, 2017).

Genetic association studies encompass two approaches in investigating genetic associations:

a) The candidate gene approach
b) Genome wide association studies (GWAS)

The candidate gene approach targets specific genes which may influence disease pathogenesis (where biological function is known/confirmed). Whereas, GWAS studies are essentially 'hypothesis-free' and assess polymorphisms across the entire genome (Ngassa *et al.*, 2017).

1.3.3.1 Mitochondrial genes

With regards to genetic variations in mitochondrial DNA (Sinxadi et al. 2013), polymorphisms in mitochondrial DNA have been associated with HIV-SN risk in patients on cART (Hulgan *et al.*, 2005; Canter *et al.*, 2011; Kamerman et al., 2012). However, further investigation into this finding revealed that these results may only be relevant to individuals of European descent, due to the specific alleles' haplogroup division (Ngassa *et al.*, 2017). Intriguingly, in African American individuals, the mitochondrial haplotype *L1c* associated with increased HIV-SN risk (Canter et al. 2010). However, further investigation in the Southern African population could not be carried out due to the complete lack of the *L1c* haplotype in Southern Africans (Ngassa *et al.*, 2017). This finding is promising and warrants further investigation perhaps in west or central Africans where the authors propose that the *L1c* haplotype may be more common.

1.3.3.2 CaMKK2

There are several potential genetic markers related to immune function which have been associated with HIV-SN (Wadley *et al.*, 2014, 2015; Goullee *et al.*, 2016; Ngassa *et al.*, 2017). Immune related genes which are related to an increased risk of HIV-SN include: *IL4* (Wadley et al. 2014), *CAMKK2* and three *CAMKK2* haplotypes (Goullee et al. 2016). Immune related genes

related to decreased risk of HIV-SN include: *LTA, Bat1, NFKBILI* and *ATP6V1G2* (Wadley et al. 2015)*, CAMKK2* haplotype and two *CAMKK2* haplotypes (Goullee et al. 2016). Nonetheless, further research into the African genome is required with a need for the expansion of the database of reference genomes of African populations to further improve the design of association studies (Ngassa *et al.*, 2017).

1.3.3.3 TNF block

Although there is limited information on the genetics of pain associated with HIV-SN, Hendry and colleagues (2013, 2015) have identified haplotypes and a polymorphism within the block of immune-related genes on chromosome six (the TNF block), and haplotypes within the KCNS1 gene that associate with painful HIV-SN (Hendry et al. 2013, 2015). Indeed, the importance of TNF is supported by evidence that polymorphisms in the TNF gene and its neighbours affect HIV-SN in Caucasians, Africans and Asians. Ngassa and colleagues (2017) suggest that the set-up of larger cohorts may prove highly beneficial to GWAS studies which may help identify other relevant genes involved in HIV-SN. Findings from genetic association studies are significant in the field of HIV-SN as they have the potential to identify involved genes and proteins, which can assist in identifying potential drug targets.

1.3.4 Psychosocial risk factors and HIV-SN

1.3.4.1 Health related quality of life in individuals with HIV-SN

Health-related quality of life refers to the biological and psychological effects of illness and is made up of state of health, daily functioning, general quality of life, everyday functioning and well-being (Keltner et al. 2012). Studies assessing the relationship between HRQOL and pain intensity in individuals with HIV-SN are few (Ellis et al. 2010; Keltner et al. 2012; Lucey et al. 2011; Phillips et al. 2014). Furthermore, these studies have all been conducted abroad in American and UK populations, with no studies being carried out in Africa or in a South African population. The lack of data from indigenous studies, makes it difficult to relate the findings of these studies to our South African population, which further emphasises the need for such studies in our population. Despite the limited number of studies performed on the relationship between pain intensity and HRQOL in this area, the studies which have assessed this relationship have reported congruent and significant results (Ellis et al. 2010; Keltner et al. 2012; Lucey et al. 2011; Phillips et al. 2014).

The large cohort study performed by Ellis and colleagues (2010), found that even in the era of successful treatment with cART, neuropathic pain remained common and contributed to the excess burden of unemployment and disability in their cohort. Important to note is that a significant number of their participants were previously exposed to neurotoxic ARV drugs. Furthermore, they found that severe neuropathic pain was associated with lower HRQOL scores and increased functional impairment which is supported by the recent study conducted by Phillip and colleagues (2014) who also found that painful HIV-SN was associated with a higher pain interference score. Indeed, individuals who present with painful HIV-SN have a poorer HRQOL, consequently having a detrimental impact on their day-to-day living. Crucially, Ellis and colleagues (2010) reported individuals with no pain had significantly higher scores for their HRQOL, pain function and overall physical and mental health summaries in comparison to those who reported experiencing pain which ranged from slight to severe intensity.

1.3.4.2 Depression associated with HIV-SN

Ellis (2010) found that individuals with HIV-SN who presented with a current, but not a past, major depressive disorder were more likely to experience and report pain (Ellis *et al.* 2010). Several studies have linked depression and HIV-SN (Ellis et al. 2010; Lucey et al. 2011; Keltner et al. 2012; Phillips et al. 2014). One such study by Keltner and colleagues (year) investigated HRQOL 'well-being" in individuals with HIV-SN and found that a greater severity of depression, as measured by the Becks Depression Inventory second edition (BDI-II), was more strongly associated with a worse well-being than pain intensity. In contrast, Lucey and colleagues (2011), found that pain catastrophizing rather than depressive symptoms was associated with a greater pain disability in their cohort. Depressive symptoms were associated with ARV side effects rather than pain disability.

The above-mentioned findings highlight the significant role of depression and mood in the experience of neuropathic pain. These data may explain why neuropathic pain associated with HIV-SN is often difficult to treat and possibly why it does not respond to conventional therapies such as antidepressants (amitripyline), anticonvulsants (carbamazepine), opioids (codiene) and topical agents (capsaicin) (Turk et al. 2010; Chetty et al. 2012; Keltner et al. 2012). There is evidence for the efficacy of capsaicin, in the form of the 0.075% capsaicin cream and the single high-dose capsaicin 8% patch, in post-herpetic neuralgia and some postsurgical neuropathies (Derry et al. 2009) , however there have been mixed results with the patch for HIV-SN. Also, to date there have been no direct trials that have compared the capsaicin 8% patch and the 0.075%

capsaicin cream. The This suggests that a broader approach which encompasses all psychosocial factors such as depression and mood, together with pain intensity may be beneficial for HRQOL outcomes.

1.3.4.3 Pain catastrophizing and anxiety associated with HIV-SN

Depression is one psychosocial factor associated with reduced HRQOL and greater pain intensity but there are other factors which also play a significant role in HRQoL. These other factors include pain catastrophizing and anxiety (Lucey et al. 2011; Turk et al. 2010). Pain catastrophizing is described as the tendency to magnify the threat value of a painful stimulus and to often feel helpless in the presence of pain. Individuals who catastrophize may describe a pain experience in a much worse or exaggerated term than the average person would. Studies focusing on other pain states such as chronic low back pain and fibromyalgia found that high levels of anxiety and pain catastrophizing contributes to the development of chronic pain reported in these conditions (Picavet et al., 2002; Gracely *et al.*, 2004). Graceley and colleagues (2004) found that psychosocial factors can play an important role in the development and persistence of pain and pain catastrophizing in chronic pain patients and can have a huge impact on the pain intensity, HRQOL and physiological distress in these patients.

One of the challenges in better understanding the multifaceted relationship between the above mentioned psychosocial factors and pain intensity and HRQOL is that these factors are often studied in isolation (Hays et al. 2000). Often studies include two or three of these factors but, very few have assessed all of these factors together in individuals with symptomatic and asymptomatic HIV-SN. Furthermore, the biopsychosocial model which brings in the suggestion that clinicians should not only assess the physical source of pain, by examination and various diagnostic tests, but also the patients mood, fears related to the pain, QOL and coping strategies is not always executed by every clinician treating individuals with pain. Therefore, we are faced with two problems here i) the lack of investigation into the interplay of these factors and ii) the lack of assessment of these factors in clinical practice. However, time constraints within health care facilities and lack of sufficient training could be possible reasons why clinicians find it difficult to execute this approach. Indeed, by assessing all these factors, and not just the obvious physical symptoms and sites of pain, one would gain a much better understanding of the context of the individuals pain and this would be extremely beneficial to the individuals treatment (Phillips et al. 2014; Turk et al. 2010).

1.4 Thesis aims

HIV-SN is a common and often painful condition related to HIV and its treatment with certain ARV medications. This debilitating condition adversely affects quality of life, and yet it is often under-recognised and therefore undertreated. The neurotoxic ARV stavudine is associated with the development of painful HIV-SN amongst other side effects and has consequently been eliminated from first-line ARV therapies worldwide. It is anticipated that the worldwide replacement of stavudine with tenofovir, will significantly reduce the incidence of HIV-SN. Several risk factors have been linked to the development of HIV-SN in individuals on cART. These risk factors include, age, height, drug exposure, TB treatment, CD4 counts and a genetic component. South African cohorts of PLWHA are unique in comparison to cohorts from resource-rich settings, as most individuals initiating treatment in SA are younger, consist mainly of females and individuals frequently present with a TB co-infection. The identification of clinical, disease-related and demographic risk factors associated with the development of HIV-SN in an African population will help clinicians in identifying 'at risk' patients and contribute to the refinement of management options for these patients to reduce the burden of this disease.

It is anticipated that the incidence of SN may be reduced on TDF-based cART, but this is not known. Therefore, the first part of this thesis investigates the incidence of symptomatic and asymptomatic HIV-SN in treatment naïve neuropathy-free PLWHA and identifies clinical and demographic risk factors associated with its development on TDF-based cART.

I address the following questions in Chapter 3:

1. What is the six-month incidence of HIV-SN (both symptomatic and asymptomatic) in treatment naïve, neuropathy-free South African PLWHA initiating TDF-based cART?
2. Which clinical, disease-related, demographic and anthropomorphic risk factors associate with the development of HIV-SN in South African PLWHA on TDF-based cART?

Several chemokines have been implicated in the development and maintenance of neuropathic pain, which is often associated with HIV-SN. These chemokines include: CCL2 (MCP-1), CX3CL1 (Fractalkine), CCL5 (RANTES) and CXCL12 (SDF-1). Both CCL2 and CX3CL1 have been implicated in the chemokine modulation of pain via neuronal-glial interactions. CCL5 has been associated with the pathway that controls hyperalgesia in a rodent neuropathic pain model and

SDF-1 (CXCL12) has also been implicated in a model of neuropathic pain. An upregulation of chemokine receptors (CX3CR1, CCR2 and CCR5) in $CD3^+$ and $CD14^+$ has also been linked to the presence of HIV-SN in PLWHA. To date, no studies involving these chemokines have been conducted in an African population with respect to their role in HIV-SN.

I address the following question in Chapter 4:

1. Do CCL5 (RANTES), CCL2 (MCP-1), CX3CL1 (Fractalkine) and CXCL12 (SDF-1) levels in plasma associate with the development of HIV-SN within six-months of TDF-based cART?

Studies focusing on nerve pathology in symptomatic and asymptomatic HIV-SN, show only minor differences in somatosensory nerve function between symptomatic and asymptomatic HIV-SN and very few differences in skin biopsy data. Evidence from other chronic pain states such as fibromyalgia and chronic low back pain indicate that high levels of depression, anxiety and pain catastrophizing may contribute to the development of the chronic pain associated with these conditions. Psychological factors such as depression, anxiety and pain catastrophizing factors can have a huge impact on quality of life and function. These psychological factors have all been individually investigated for their role in HIV-SN, and no studies, to date, have assessed these factors together in an African population with HIV-SN. Neuropathic pain associated with HIV-SN is typically chronic and significantly impacts quality of life of affected individuals. Certainly, proven pharmacological agents for painful HIV-SN are lacking. Therefore, it is important that other, potentially modifiable risk factors for painful HIV-SN and its adverse effects are identified.

I address the following questions in Chapter 5:

Do greater levels of depression, anxiety and pain catastrophizing associate with:

1. the presence of symptomatic or painful HIV-SN in PLWHA?
2. increased pain intensity in PLWHA with symptomatic HIV-SN?
3. reduced quality of life in PLWHA with symptomatic and asymptomatic HIV-SN?

4. Does increased pain intensity in PLWHA with symptomatic HIV-SN associate with reduced quality of life?

CHAPTER 2
METHODS

This thesis comprises 3 projects:

Project 1: The six-month incidence of HIV-associated sensory neuropathy in individuals on TDF-based cART.

Project 2: Plasma chemokine profiles in HIV-positive individuals with and without HIV-SN, on TDF-based cART.

Project 3: Psychological factors associated with painful and non-painful HIV-associated sensory neuropathy.

In my methods section I will outline the methods for each project separately, as each project comprises a separate cohort. Where a methodology was common across projects (e.g. neuropathy assessment with the ACTG BPNS tool), I only specify the method in full in the first instance of its use.

Section 2.1: The six-month incidence of HIV-associated sensory neuropathy in individuals on TDF-based cART.

2.1.1 Ethical approval

Ethical approval for the study was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa, clearance no: M121018 (Appendix 1). Written, informed consent was obtained from all participants. An interpreter fluent in English and six indigenous South African languages explained the study rationale and procedures in the participant's language of choice.

2.1.2 Clinical aspect

One hundred and fifty treatment naïve HIV-positive individuals (18 years and older) starting tenofovir-based cART for the first time were recruited from the Mid Lawley/South of Lenasia Community Health Hospital, Johannesburg, South Africa between June 2016 to December 2016. Participants were followed up for a period of 6-months. Follow-up of participants ran from August 2016 to April 2017.

Study inclusion criteria

- ≥ 18 years of age
- confirmed HIV diagnosis
- Initiation of Tenofovir-based CART for the first time
- Free of signs and symptoms of distal symmetrical polyneuropathy (see neurological assessment)

Study exclusion criteria

- < 18 years of age
- Confirmed signs and symptoms of distal symmetrical polyneuropathy (see neurological assessment)
- Presence of conditions which may affect the feet and lower limbs e.g. Kaposi's sarcoma, any previous exposure to CART regimens.

Eligible participants had the following demographic and anthropometric data recorded: sex, age, height, weight, ethnicity (Black, White, Coloured, Indian), date of HIV diagnosis, (years of formal education), other potential causes of neuropathy [diabetes, alcohol consumption (units of alcohol consumed), vitamin B_{12} deficiency, treatment for current tuberculosis infection and diabetes

mellitus]. I also recorded data on whether eligible female participants were pregnant or not, at the time of recruitment. Those participants who were pregnant at the time of being recruited to the study, initiated treatment within the chronic clinic and thereafter were transferred to the antenatal clinic to collect their ART medications but still presented to the chronic clinic every ± 2-months for study follow-up.

The following haematological data (where available) were recorded from participants medical records: initial CD4 T-cell count, current CD4 T-cell count, HIV viral load, Hepatitis B status and current syphilis infection (if recorded).

2.1.3 Blood samples

Two blood samples (4ml EDTA tubes) were drawn from each participant by nurses within the chronic clinic at the Lawley/Lenasia South CHC at every visit. After collection, the blood samples were immediately stored on ice at the research site. From the two samples, one sample was sent to the Chris-Hani Baragwanath Hospital, Soweto for the computation of CD4 T-cell count, HIV viral load, HbA1c levels and Vitamin B12. The second sample was taken to the University of the Witwatersrand, where it was centrifuged by Dr Gaelle Ngassa, and the plasma removed and aliquoted into 2ml tubes for storage (to be analysed later for chemokine levels-Study 3). The remainder of the blood sample after plasma removal was stored for epigenetic analysis to be performed by Dr Ngassa in an independent study. I personally reviewed all participants medical records to ascertain the type and doses of analgesic medications participants were prescribed, the type and doses of vitamins prescribed and the cART regimens they were initiated onto.

2.1.4 Procedures

2.1.4.1 Neurological assessment

Participants were assessed for the presence of peripheral sensory neuropathy (SN) using the AIDS Clinical Trials Group Neuropathy (ACTG) Screening tool, Brief Peripheral Neuropathy Screen (BPNS). The BPNS is a validated screening tool for HIV-SN (Cherry et al. 2005) and was validated using both IENFD and QST testing as reference standards (Cherry et al. 2005; Mehta et al. 2011). This tool has been previously used in the South African population (Maritz *et al.*, 2010b; Wadley *et al.*, 2011). The ACTG tool encompasses the assessment of two signs (vibration sense in great toe and ankle reflex testing). We added the assessment of pin-prick sensitivity in the feet, to the ACTG tool used in our study which constituted the third sign of HIV-SN. Pin prick sensitivity testing is routinely used by neurologists to assess the integrity of non-myelinated pain

fibres and their inputs (Jacobs 2006). It has been used in diabetic peripheral sensory neuropathy assessments (Yang et al. 2014; Nather et al. 2008) and also forms part of the QST batch of tests developed by the German Research Network on Neuropathic Pain (DFNS), which aims to characterise the somatosensory phenotypes of individuals with neuropathic pain (Rolke et al. 2006). Pin prick assessment is also utilized in the Leeds assessment of neuropathic symptoms and signs (LANSS) Pain Scale (Bennett 2001) and the DN4 questionnaire (Bouhassira et al. 2005).

A diagnosis of *symptomatic* HIV-associated sensory neuropathy (HIV-SN) was made based on the bilateral presence of at least one sign (decreased vibration sense in the great toe, absent ankle reflex or decreased pin-prick sensitivity) and one symptom (pain, paraesthesias or numbness) in the feet. A diagnosis of *asymptomatic* HIV-SN was made based on the bilateral presence of at least two signs (decreased vibration sense in the great toe, absent ankle reflex or decreased pin-prick sensitivity) in the feet. The requirements for a positive diagnosis of SN which we used here were consistent with those of several studies that have used the BPNS screening tool (Cherry et al. 2005; Ellis et al. 2010), and thus was used as the 'gold standard' epidemiological definition of HIV-SN throughout the study. We chose to require the presence of two signs for the classification of *asymptomatic* HIV-SN to limit the number of false-positives on screening.

Vibration sense was assessed using a 128 Hz tuning fork. In order to first familiarize the participants to the sensation of the vibrating 128Hz tuning fork, the tuning fork was struck against the palm of the investigators hand and then placed onto the metacarpophalangeal joint of the participants' middle finger. The participant was asked to describe what sensation they had experienced and once the investigator was certain that this sensation was indeed the vibration or "buzzing" of the tuning fork and that the participant could comfortably identify this sensation, the participant was then informed that the procedure would be repeated by placing the 128Hz tuning fork on the distal interphalangeal joint of the great toe. The 128 Hz tuning fork was struck against the palm of the investigator's hand and then placed onto the distal interphalangeal joint of the participant's great toe (Figure 2.1a); the participant was told to inform the investigator when they could no longer feel the vibration or "buzzing" of the tuning fork. A stopwatch was used to record the time it took from the time the tuning fork was struck against the investigator's palm until the participant stopped feeling the vibration of the tuning fork. Vibration sense was recorded in both feet, with a recording of 10 seconds or less deemed abnormal (Cherry et al. 2005).

Figure 2.1 (b) illustrates the ankle reflex testing of participants. Participants were asked to sit in a comfortable position in their chair and to relax their foot and ankle. Once the participant relaxed their foot, the investigator placed her hand underneath the participants foot to support the ball of the foot. Using the rubber reflex hammer, the investigator gently struck the Achilles tendon to elicit the response. A positive ankle reflex response is indicated by the plantar flexion of the foot. The reflex test was performed three times to confirm the response. If no response was elicited, the investigator then asked the participant if he/she could perform the 'Jendrassik manoeuvre'. In the Jendrassik manoeuvre, the participant cups their fingers on each hand and attempts to pull their hands apart with a lot of strength. If no response was elicited after the Jendrassik manoeuvre was performed, the reflex was marked as 'absent'.

Pin-prick sensitivity was assessed using a size 4 'single use' safety pin. To first familiarise the participant with the sharp and blunt ends of the safety pin, the 'sharp' and 'blunt' ends of the safety pin were alternately placed on the dorsal surface of the participant's hand (with the participant viewing the procedure) and participants were asked to identify the sensation experienced as either 'sharp' or 'blunt. Thereafter, once the participant was comfortable in identifying both sensations, the procedure was carried out with the 'sharp' and 'blunt' ends of the safety pin being alternately placed on the dorsal surface of the participants great toes, and participants were asked to identify the sensation experienced as either 'sharp' or 'blunt'. Identification of the 'sharp' stimulus as being 'blunt' or that it was not felt at all was deemed abnormal. If participants gave an incorrect answer, I then tested medial and lateral to the point and then progressed proximally in order to identify the extent/borders of nerve dysfunction.

When symptoms were present, the anatomical distribution of the symptom(s) was(were) recorded and participants were asked to rate the severity of the symptom(s) on an 11-point numerical pain rating scale (NRS), anchored at 0 (no symptom experienced) to 10 (worst pain imaginable). Pain was classified as moderate to severe with a score of four or greater on the scale.

I personally carried out all assessments and received full training on the use of the tool by the validator, Dr Catherine Cherry and my co-supervisor Dr Antonia Wadley who successfully used the tool in a South African population previously (Wadley *et al.*, 2011).



Figure 2.1(a) Measurement of vibration sense



Figure 2.1 (b) Measurement of ankle reflexes

2.1.4.2 Recruitment and study procedure outline

All antiretroviral naive individuals attending the Mid Lawley/Ennerdale South of Lenasia Community Health Hospital, who were initiating cART for the first time were approached to partake in the study [Figure 2.1(c)]. Study participants were recruited over an 8-month period, and each participant was followed up for 6 months, with four visits with the investigator and interpreter present. Following recruitment and consenting, meetings were scheduled at 0 months (the day of

treatment initiation), 2 months, 4 months and 6 months after starting cART [Figure 2.1 (d)]. Participants were contacted telephonically a few days prior to their scheduled return to the hospital to confirm their appointment day and to remind them to meet with the investigator.

2.1.5 Study procedures at each visit

2.1.5.1 Participant recruitment, consenting and pre-screening (Visit 0)

An interpreter fluent in 6 native South African languages was employed for the duration of the study. The interpreter assisted with participant recruitment, consent and explanation of study procedures in the participant's home language.

2.1.6 Issues faced with visit scheduling

Despite having specific times for patient visits on our protocol and allowing ± 2 weeks before or after scheduled visits for patients to come in for their follow up visits, we faced several issues at the research site which were out of our control and which disrupted the timing of participants returning for follow-up visits.

Frequent service delivery protests at research site

The Lawley/Mid-Ennerdale Lenasia South CHC is situated in close proximity to a local informal settlement where locals illegally setup living areas on government owned land. When these informal dwellers were unhappy with service delivery to the area (poor sanitation, no electricity) they proceeded to carry out violent protests in the area, blocking all entrances to the Lawley/Mid-Ennerdale Lenasia South CHC. For the safety of the staff, investigators and our patients we could not carry out our follow-up visits during these days/weeks and had to be rescheduled outside of the ideal follow-up window period. Often patients were threatened by these protesters not to attend the clinic and therefore even when patients were willing to come in for their scheduled assessments they were forced to miss their scheduled appointments. This resulted in us having wide and irregular visits dates and data for our patients. In order to remedy the situation and proceed with our assessment we, on two occasions, contacted the local police authorities who escorted the study investigators to the facility to conduct our follow-up assessments. However, due to the protest often becoming violent, we had to cancel assessments in consideration of the safety of our patients. These sporadic protests occurred at least 4-5 times during the study period and severely impacted data collection. Not only were we forced to forfeit follow-up visits data, several patients who could not find us at the facility during these protests were subsequently lost

to follow-up. This irregular visit pattern also impacted the blood draws for plasma samples collection for our chemokine assays.

2.1.6.1 Improper handling and storage of blood sample at outside facilities

After blood samples were drawn at the research site, these samples were sent to two large academic teaching hospitals for testing and storage (a standard operating procedure for low-level facilities). Once the samples were tested for the various bio-markers, the remaining samples were to be stored at these facilities for collection at a later stage for chemokine analysis (testing that is not routinely available). All study-related blood samples were coded and bagged separately at the research site prior to being sent to these facilities. Regrettably, these facilities did not store the samples after testing and discarded the majority of our study samples. Despite liaising with heads of departments for both these facilities on several occasions and the promise that our samples would be stored appropriately, none of our samples were appropriately stored at these facilities. As a result, we lost the majority of our repeated patient samples and therefore could not assess multiple samples from the same individuals and were forced to assay single samples from our patient cohort.

2.1.6.2 Training of staff at facility

Our research was carried out at a government facility (Hospital) as most research studies in South Africa are carried out. At the beginning of our research project we were introduced to several clinicians and nursing staff at the facility that would assist us with data collection, oversee our research procedures and mainly to assist us with patient recruitment to facilitate the process and cause less chaos at the site. Since we were recruiting ARV naïve individuals for our project, we were paired with the ARV nurse that handles all ARV initiations within the clinic. We subsequently trained the nurse with regards to our recruitment procedures and requirements. Thereafter, following patient ARV initiation, the nurse would briefly explain the study to the individual and should they agree to meet us, she would send them to our research room. However, since this was a government facility, the ARV nurses were rotated every 3-4 weeks and we were given a new nurse every month. Due to the high patient numbers at the site, some of these nurses were often busy with their own clinic responsibilities and therefore failed to direct patients to our room. When new nurses were rotated, they often found it difficult to manage the patient schedule and as a result we lost several follow-up patients. Despite our telephonic reminder to these patients, nurses would often send patients for other procedures and tests at the hospital resulting in a ‘missed’ follow up visits to the study. Furthermore, despite placing notes in the patients clinic file

that the patient was part of an ongoing longitudinal research study, ARV nurses frequently down-referred our study patients to other facilities for the collection of ARV treatment which resulted in us losing these patients to follow-up. Down-referred patients tried their best efforts to make their scheduled appointments at our research site, however, public transport costs to and from the facility and work commitments often made this process near impossible for patients who were committed to having their neuropathy assessments done.

The abovementioned issues are just some of the issues faced by researchers in South African government facilities. Often the protocol is well planned, however, the challenges we as researchers face at these facilities are huge and often not easy to overcome. Not only have these challenges impacted our data and caused huge and irregular sampling intervals but it has also impacted our patients who often feel dismayed when they make the trip to visit the hospital for their scheduled assessments and only to find that the staff are not available due to service delivery protests.

For future studies, we have discussed these issues with hospital staff, who have brought it to the attention of ward councillors in the area. We continue to work with staff to suggest possible solutions to better facilitate our research at these government facilities, which will be beneficial to both investigators and patients.

Visit 1 (0 months-Baseline)

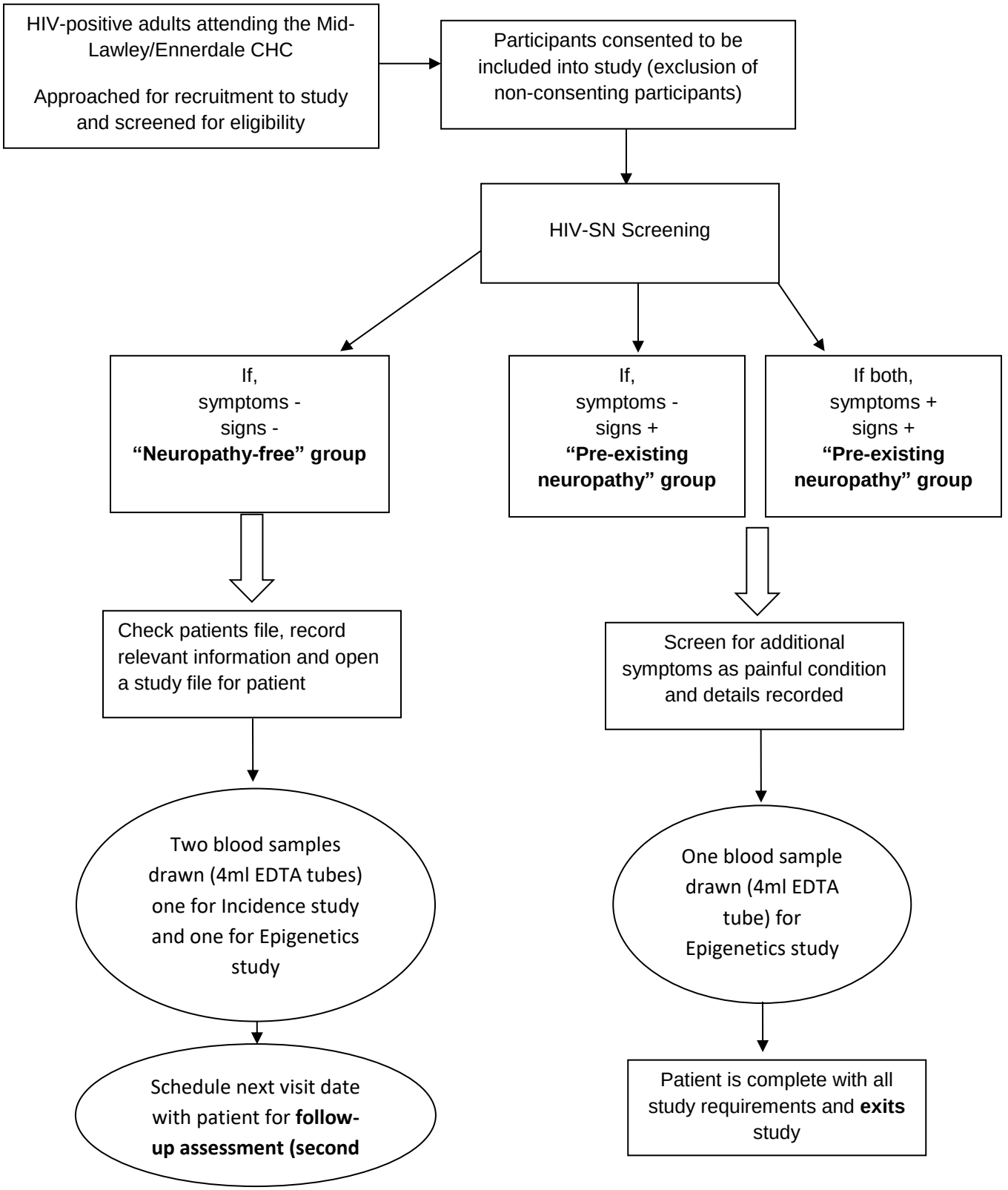


Figure 2.1(c) Flow diagram of recruitment and screening procedures at visit 1 (Baseline visit)

Follow up visits at 2, 4 and 6-months on treatment

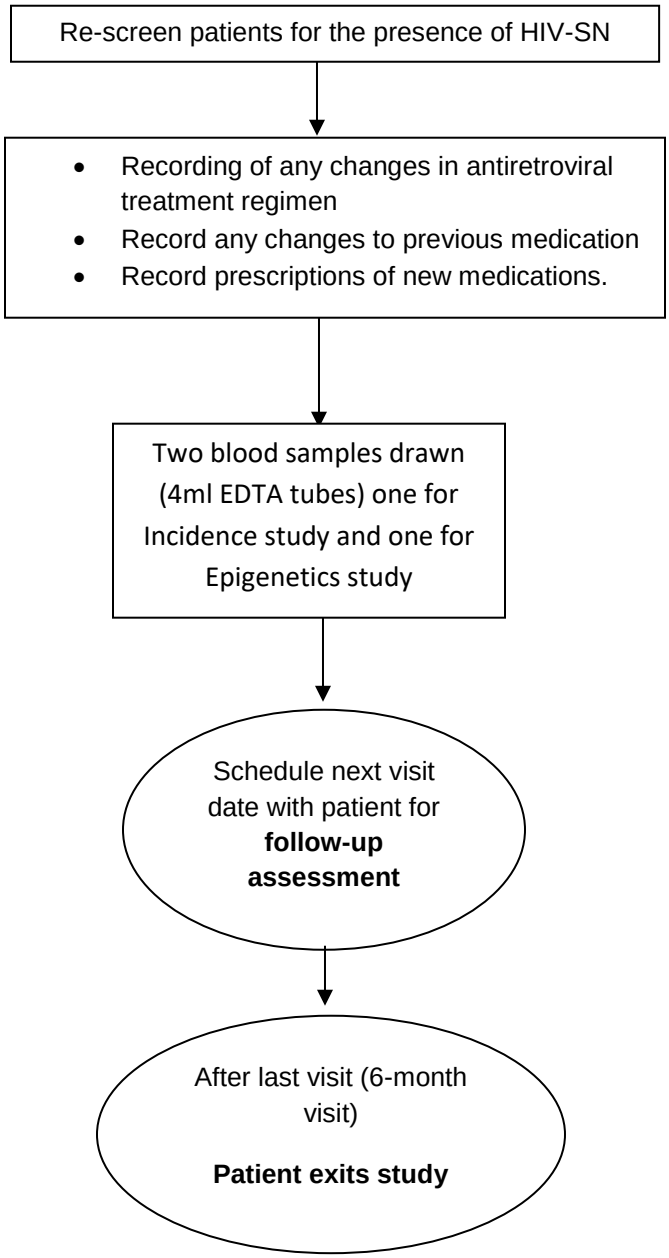


Figure 2.1 (d) Flow diagram of procedures at follow-up visits (at 2, 4, 6-months on treatment).

Section 2.2 Plasma chemokine profiles in HIV-positive individuals with and without HIV-SN, on TDF-based cART.

2.2.1 Ethical approval

All individuals in this cohort were participating in Project 2 (Incidence of HIV-SN) and an independent epigenetics study that was being conducted by the same laboratory at the same research site. Therefore, ethical approval for both the studies (incidence study and epigenetics study) was obtained in Project 2 from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa, clearance no: M121018 (Appendix 1). Written, informed consent was obtained from all participants. An interpreter fluent in English and six indigenous South African languages explained the study rationale and procedures in the participant's language of choice.

2.2.2 Aim

a) To measure CCL5 (RANTES), CCL2 (MCP-1), CX3CL1 (Fractalkine) and CXCL12 (SDF-1) levels in plasma samples of ARV naïve individuals initiating TDF-based cART
b) To determine whether CCL5 (RANTES), CCL2 (MCP-1), CX3CL1 (Fractalkine) and CXCL12 (SDF-1) levels in plasma are associated with the development of HIV-SN within the 6-month study period.

2.2.3 Samples

All plasma samples used for the chemokine analysis were extracted from whole blood samples. Blood samples were collected (in 4ml EDTA tubes) by nurses at the Lawley/Ennerdale-Lenasia South CHC. Within 5 minutes of collection, blood samples were stored on ice at the research site until they were transported to the University of the Witwatersrand on the same day of collection. At the University, blood samples were centrifuged, and plasma samples were aliquoted and stored at -80°C.

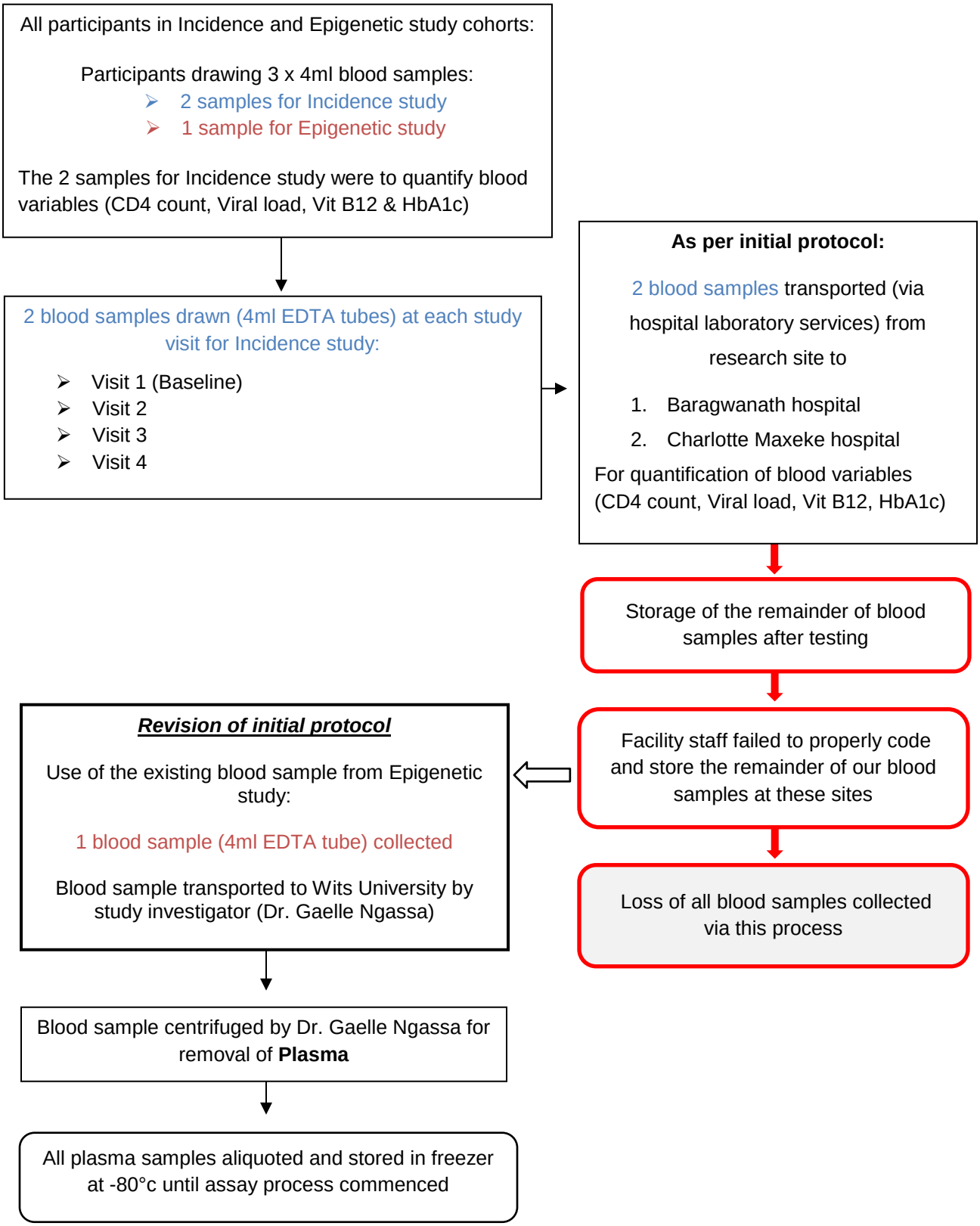


Figure 2.2(a) Flow diagram of blood collection process for chemokine assessment.

2.2.4 Human pre-mixed magnetic bead assays

Human pre-mixed magnetic bead Luminex assays, which are flexible bead-based multiplex assays, were selected for the analyses of the four selected chemokines. The Luminex assay system allows up to 50 target analytes (e.g. cytokines, chemokines etc.) to be simultaneously profiled.

To perform the assay, the sample is added to a mixture of colour-coded beads, which are pre-coated with analyte-specific capture antibodies. The antibodies then bind to the analytes of interest [Figure 2.2(b)]. Thereafter, biotinylated detection antibodies which are specific to the analytes of interest are added and form an 'antibody-antigen' sandwich. After this, phycoerythrin (PE)-conjugated streptavidin is added and binds to the biotinylated detection antibodies [Figure 2.2(c)]. For the magnetic beads aspect [Figure 2.2(d)], a magnet in the MAGPIX analyser captures and holds the magnetic beads in a monolayer, while two spectrally distinct light-emitting diodes (LEDs) brighten the beads. One LED is responsible for identifying the analyte that is being detected and the second LED is responsible for determining the magnitude of the PE-derived signal. We used the Bio-Rad Bio-Plex 200 system (Bio-Rad Laboratories, Hercules, California, USA) to read the plates.

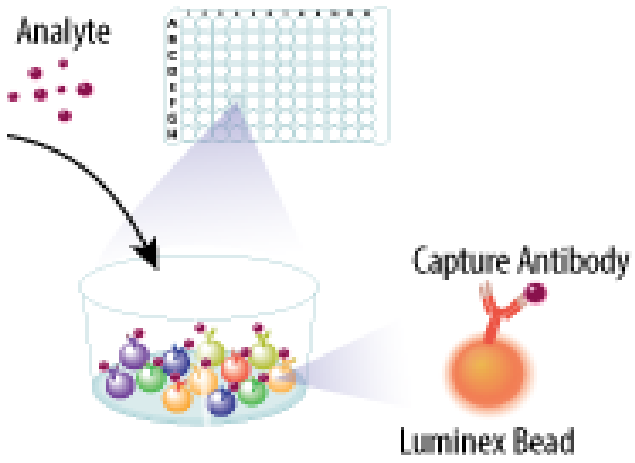


Figure 2.2(b). Pre-coated Luminex beads with analyte-specific capture antibodies binding to the analytes of interest.

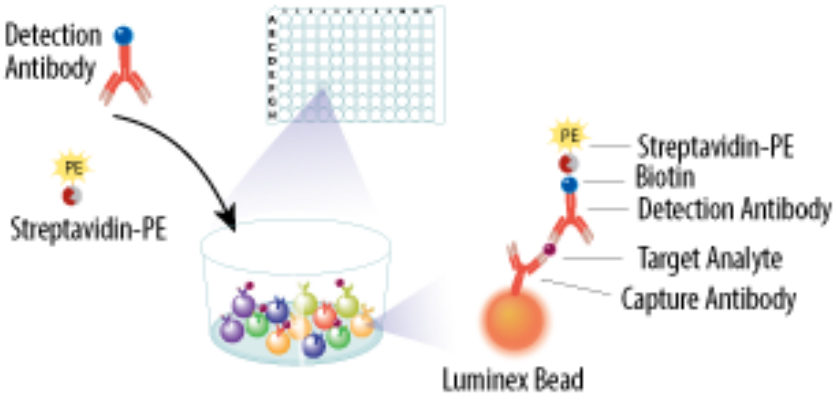


Figure 2.2(c). Step 2 of the Luminex assay, formation of the 'antibody-antigen' sandwich and addition of Phycoerythrin (PE)-conjugated streptavidin which binds to the biotinylated detection antibodies.

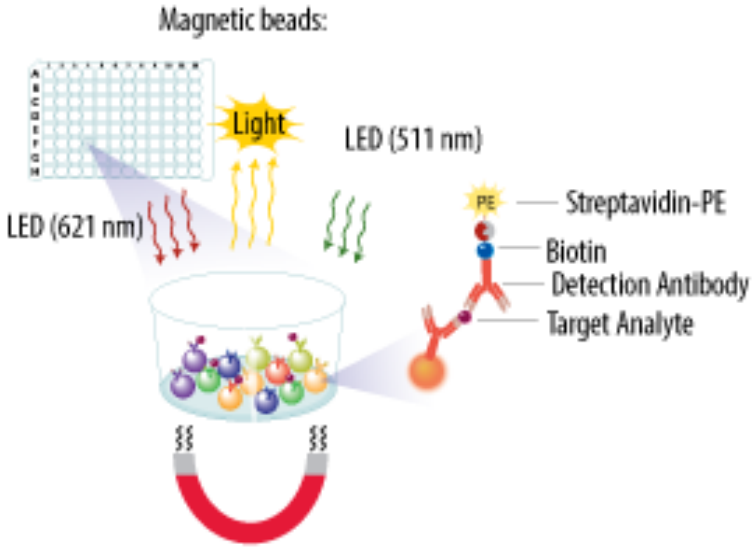


Figure 2.2(d). The magnet in the MAGPIX analyser capturing and holding the magnetic beads in a monolayer, while the two light-emitting diodes (LEDs- Red and Green) brighten the beads.

Due to the sensitivity and nature of the chemokines being analysed, two unique standard plates were designed to incorporate the four different chemokines. A total of four human pre-mixed magnetic bead kits were used for the analyses of the one hundred and nineteen samples:

1. Kit 1 consisted of 2 x Pre-mixed plates with analytes of CCL5, CCL2 and CX3CL1
2. Kit 2 consisted of 2 x Pre-mixed plates with the analyte of CXCL12

2.2.5 Preparation of materials for assays

All assays were performed at the University of Pretoria, Immunology Department, by Prinisha Pillay, under the supervision of the senior immunologist, Dr Helen Steel.

Reagent preparation

All reagents were brought to room temperature prior to use.

a) Wash buffer preparation

20mL of wash buffer concentrate was added to 480mL distilled water to prepare the 500mL of wash buffer required.

b) Standards

The standards in the kit differed according to the analytes that were chosen. Kit 1 contained two standard cocktails and Kit 2 consisted of one standard cocktail. We reconstituted the standard cocktails using the calibrator diluent (RD6-52) provided and used the certificate of analysis (provided in the kit) for reconstitution volumes. Prior to making the dilutions, we allowed the standard to sit for at least 15 minutes for all particles to dissolve appropriately. Upon reconstitution, each standard cocktail was a 10X concentrate.

1. From the table below, we added 200µL of calibrator diluent (RD6-52) into each of the test tubes that we labelled from 2-6
2. Tube 1 was standard 1 (labelled as tube 1)
3. We used standard 1 to produce a 3-fold dilution series until the 6th tube.
4. Standard 1 then served as the high standard and calibrator diluent RD6-52 served as the blank
5. Thus, we had a total of 6 standards that were added into the wells on the plates.

Table 2.2 (a) Preparation of the standard cocktails with the calibrator diluent (RD6-52).

Number of Unique Standard Cocktails Provided	Volume to Combine into a Single Tube	Volume of Calibrator Diluent Required	Total Volume of Standard 1
1	100 µL	900 µL	1000 µL
2	100 µL of each	800 µL	1000 µL
3	100 µL of each	700 µL	1000µL

c) *Diluted microparticle cocktail preparation:*

The microparticle cocktail vial was centrifuged for 30 seconds at 1000rps prior to opening to resuspend all the particles. While taking precaution not to invert the vial, the vial was gently vortexed to resuspend the microparticles. We diluted the microparticle cocktail using the diluent (RD2-1) provided.

We used all 96 wells on the plate and therefore mixed 500µL of the microparticle cocktail with 5mL of the diluent (RD2-1).

d) *Diluted Biotin antibody preparation:*

The Biotin antibody cocktail vial was centrifuged for 30 seconds at 1000 x g prior to opening. While taking precaution not to invert the vial, the vial was gently vortexed. We diluted the Biotin antibody cocktail using the diluent (RD2-1) provided.

As above, we used all 96 wells on the plate and therefore mixed 500µL of the Biotin antibody cocktail with 5mL of the diluent (RD2-1).

e) *Streptavidin-PE preparation:*

The Streptavidin-PE was protected from light during handling and storage at all times by keeping it in a drawer. We centrifuged the Streptavidin-PE vial for 30 seconds at 1000 x g prior to opening. The vial was then gently vortexed. We diluted the Streptavidin-PE Concentrate to a 1X concentration by adding 220µL of Streptavidin-PE Concentrate to 5.35 mL of Wash Buffer. This provided enough Streptavidin-PE to assay one 96-well microplate.

Control preparation:

The chemokines we were analysing were not available as high sensitivity analytes and therefore no specific controls were provided with the pre-mixed chemokine kits. We therefore used four different recombinant protein controls which were specific to the chemokines being analysed in order for us to measure the specificity and sensitivity of the kits. We used the following recombinant proteins:

1. Recombinant Human/Rhesus Macaque/Feline CXCL12/SDF-1 alpha (10µg) which was reconstituted at 100 µg/mL in distilled water.

2. Recombinant Human CCL2/MCP-1 Protein (10µg) which was reconstituted at 100 µg/mL in distilled water.

3. Recombinant Human CCL5/RANTES Protein (10µg) which was reconstituted at 100 µg/mL in distilled water.

4. Recombinant Human CX3CL1/Fractalkine (Full Length) Protein (25µg) which was reconstituted at 25 µg/mL in distilled water.

Plasma sample preparation:

All plasma samples were removed from the -80°c fridge 3 hours prior to running the assays. Once the plasma samples were fully thawed we proceeded with the dilution of the samples. For each plate that was prepared, we used a total of 60 x 2 mL tubes to dilute 60 samples (75uL of sample into 75uL of Calibrator Diluent RD6-52). This formed a 1 in 2 dilution of the plasma samples.

2.2.6 Assay procedure:

a) All reagents, standards, and samples were prepared as detailed in above sections.
b) The diluted microparticle cocktail was resuspended by gently vortexing it. We then added 50µL of the microparticle cocktail to each well of the microplate (96 wells).
c) Thereafter, we added 50µL of the standard samples into a total of 12 wells (6 wells in duplicate)
d) We left two wells open as ‘Blanks’
e) We added 50µL of the control samples to four wells (2 wells in duplicate)
f) We added 50µL of the 60 diluted plasma samples to the wells

g) The remaining wells were used for the duplication of samples (on average 10-15 samples were duplicated per plate depending on the number of wells available). In line with the recommendation of R & D systems (manufacturer of the chemokine kits), we did not duplicate all 60 samples on the plates, as the Bio-Rad Luminex system has a high specificity and sensitivity for the analytes.

h) After all standards, controls and samples were added to the plate, we securely covered the plate with a foil plate sealer and incubated for 2 hours at room temperature on a horizontal orbital microplate shaker set at 800 ± 50 rpm.

i) After the 2 hours were complete, we performed the wash procedure. The wash station was set up with a magnetic device that is designed to accommodate a microplate and washes the plate by applying the magnet to the bottom of the microplate. Each well was filled with 100µL of wash buffer and placed into the wash buffer station. The wash procedure was automatically performed three times.

j) After the wash procedure, we added 50µL of the diluted Biotin Antibody Cocktail to each well and securely covered the plate with a foil plate sealer and incubated for 1 hour at room temperature on a horizontal orbital microplate shaker set at 800 ± 50 rpm.

k) We then repeated the wash as in step (i)

l) Thereafter, we added 50µL of diluted Streptavidin-PE to each well and securely covered the plate with a foil plate sealer and incubated for 30 minutes at room temperature on a horizontal orbital microplate shaker set at 800 ± 50 rpm.

m) We then repeated the wash as in step (i)

n) Thereafter, we resuspended the microparticles by adding 100µL of Wash Buffer to each well and incubated for 2 minutes on the shaker set at 800 ± 50 rpm.

o) After this last step was performed, we then placed the plate into the Bio-Rad analyser for quantification.

2.2.7 Quantification of plasma concentrations from Bio-Plex 200

The Bio-Rad machine takes approximately 2-3 hours to analyse one plate. A high-speed digital manages data output and the Bio-Plex manager software presents the data as median florescence intensity (MFI) and as concentrations (pg/ml). The observed concentration of analyte bound to each bead is proportional to the MFI (Bio-Plex software, 200). Together with computing observed concentrations for each analyte, the Bio-Plex software also generates a ‘standard curve’ for each analyte that is assayed in the pre-mix assay. As we had a total of one hundred and nineteen samples to measure, we analysed four plates over a course of one week. Each assay took approximately 8 hours to complete from sample preparation to plate

analysis on the Bio-Rad Bio-Plex 200. All chemokine assays were performed at the same time relative to ART start i.e. Baseline, 2, 4 and 6-months on treatment.

2.2.8 Original plate design/layouts:

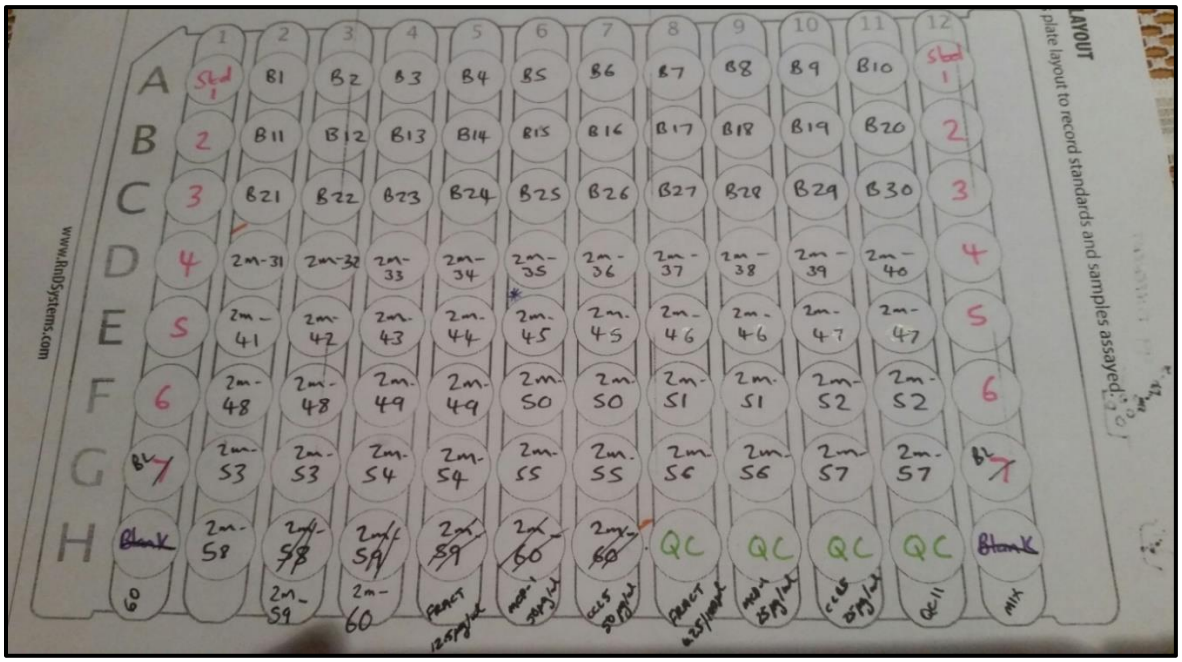


Figure 2.2(e). Plate layout for measurement of pre-mixed CCL2/CCL5/CX3CL1 at baseline and 2-month time-points. (B: Baseline sample; 2m: 2-month sample; QC: Quality control, BL: Blank; Std: Standard sample)

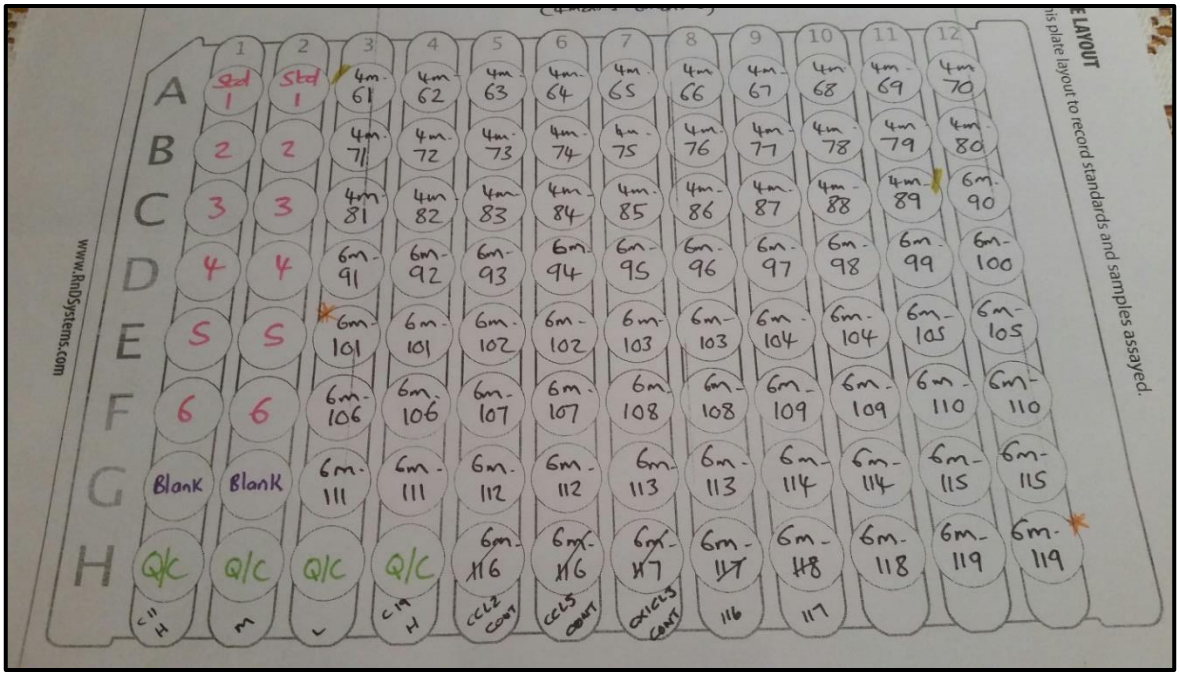


Figure 2.2(f). Plate layout for measurement of pre-mixed CCL2/CCL5/CX3CL1 at the 4-month and 6-month time-points. (4m: 4-month sample; 6m: 6-months sample; Q/C: Quality control, BL: Blank; Std: Standard sample)

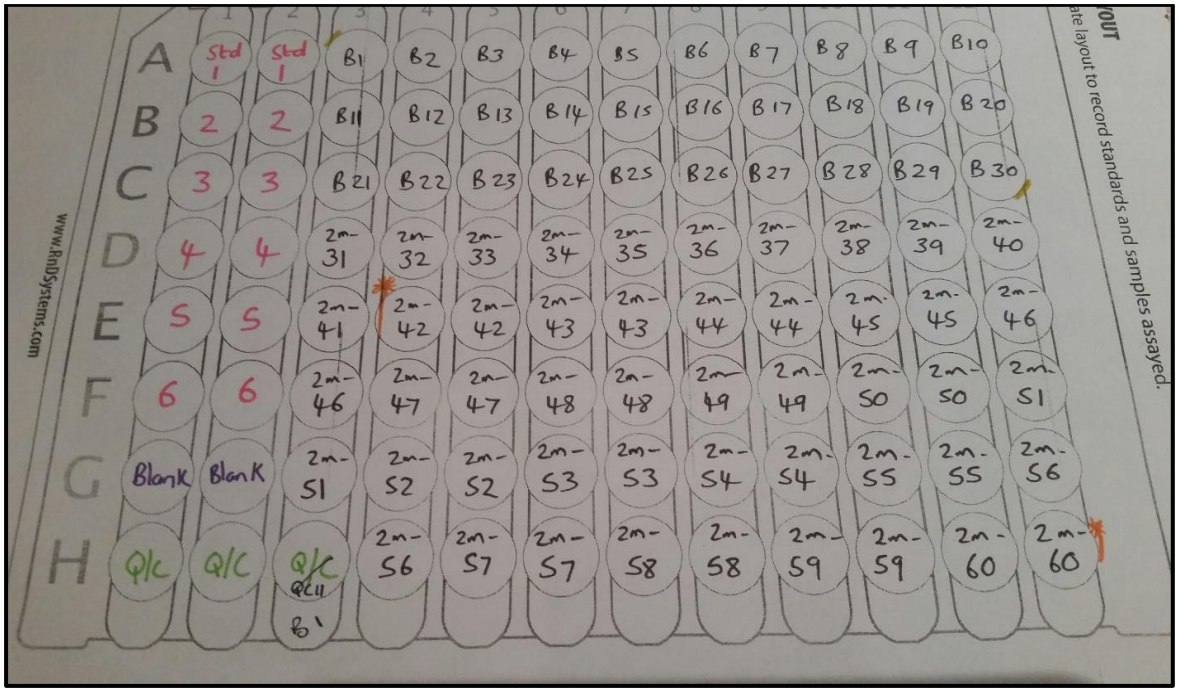


Figure 2.2(g). Plate layout for measurement of pre-mixed CXCL12 at the baseline and 2-month time-points. (B: Baseline sample; 2m: 2-month sample; Q/C: Quality control, BL: Blank; Std: Standard sample)

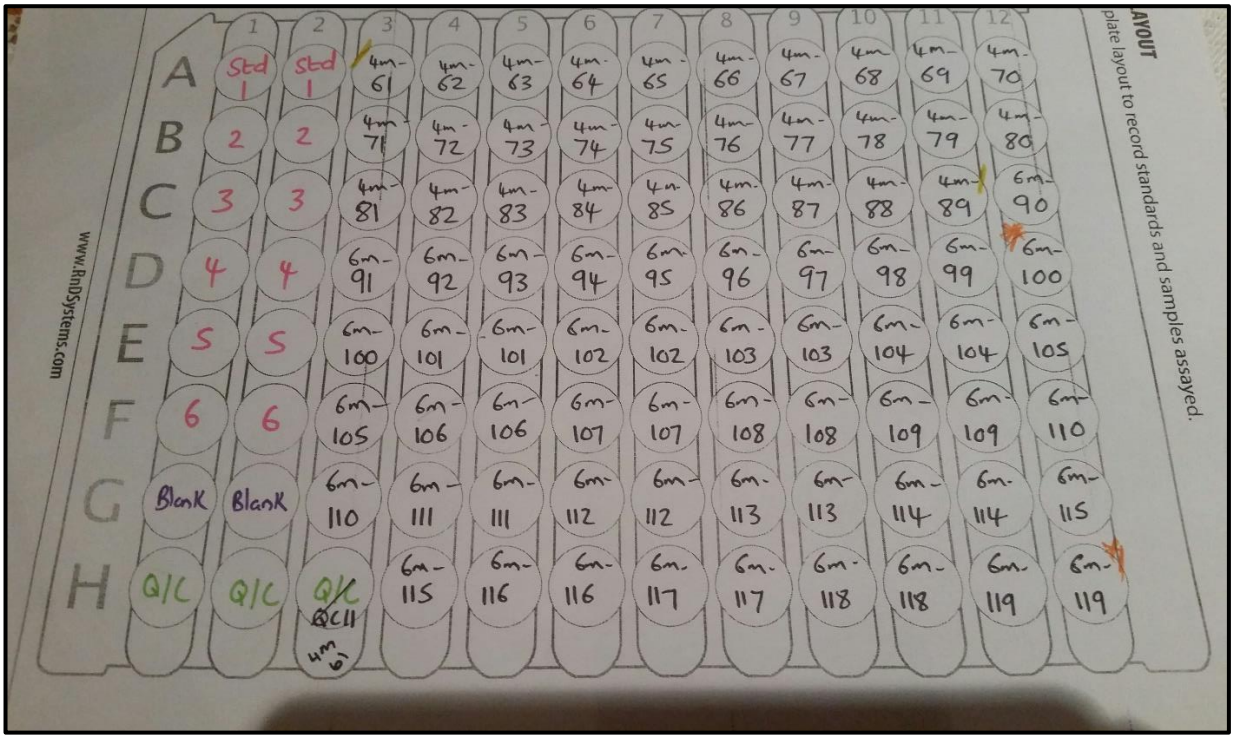


Figure 2.2(h). Plate layout for measurement of pre-mixed CXCL12 at the 4-month and 6-month time-points. (4m: 4-month sample; 6m: 6-months sample; Q/C: Quality control, BL: Blank; Std: Standard sample).

Section 2.3: Psychological factors associated with painful and non-painful HIV-associated sensory neuropathy.

2.3.1. Ethical approval

Ethical approval for the study was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa, clearance no: M121018 (Appendix 1). Written, informed consent was obtained from all participants. An interpreter fluent in English and six indigenous South African languages explained the study rationale and procedures in the participants' language of choice.

2.3.2 Clinical aspect

HIV-positive adults who were currently on combination antiretroviral therapy (cART) were recruited and screened for neuropathy at the Greenhouse Pharmacy, Chris Hani Baragwanath Hospital, Johannesburg, South Africa between May 2013 and January 2014.

Study inclusion criteria

- ≥ 18 years of age
- confirmed HIV diagnosis
- currently on antiretroviral treatment
- Positive diagnosis for SN using the ACTG BPNS (see neurological assessment)
- Mini-Mental State Examination (MMSE) score ⩾ 24 (Folstein *et al.* 1975)

Eligible participants had the following demographic and anthropometric data recorded: sex, age, height, weight, ethnicity [Black, White, Coloured (mixed race), Indian], date of HIV diagnosis, years of formal education, other potential causes of neuropathy [diabetes, alcohol consumption (units of alcohol consumed), vitamin B_{12} deficiency, treatment for current tuberculosis infection and diabetes mellitus]. The following haematological data were recorded from participants' medical records: initial CD4 T-cell count, current CD4 T-cell count, HIV viral load, Hepatitis B status and current syphilis infection (if present in the records). I personally reviewed all participants' medical records to ascertain the type and doses of analgesic medications and cART regimens they were being prescribed. It is important to note that the terms 'cART' and 'ART' are used interchangeably in the South African system. The current cART regimen refers to combination antiretroviral therapy i.e. a fix-dose combination (FDC) pill, containing either of the following combinations: 1. efavirenz + lamivudine (3TC) + tenofovir or 2. efavirenz + emtricitabine

(FTC)+ tenofovir. Also, the current first-line cART/ART regimen in South Africa does not include the drug stavudine.

2.3.3 Procedures

2.3.3.1 Neurological assessment/screening

Neurological assessment for HIV-SN was the same as described in section 2.1.4.1 (The six-month incidence of HIV-associated sensory neuropathy in individuals on TDF-based cART).

2.3.4 Study cohort characteristics

Three hundred and seventy-two black Southern African HIV-positive adults were screened at the Greenhouse Pharmacy. A total of one hundred and seventy-one participants were excluded for various reasons [see Figure 2.3 (a)], but mainly due to being HIV-SN free. The recruitment and exclusion of participants are shown in Figure 2.3 (a). Thus, in total, two hundred and one black African HIV-positive adults on cART were recruited to the study. Of these, one hundred and twenty-nine (129/201) patients presented with painful HIV-SN and seventy-two (72/201) patients presented with non-painful HIV-SN. To avoid repetition, demographic and clinical characteristics of the cohort are provided in Chapter 3 (results section), but briefly, the cohort was predominantly female and middle-aged. All the participants were currently on antiretroviral therapy and had well preserved CD4 T-cell counts. The painful HIV-SN group presented with a lower median current CD4 T-cell count, a shorter duration since HIV diagnosis and had a lower prevalence of recorded vitamin B_{12} deficiency.

2.3.5 Pre-study period (Pilot study)

Prior to commencing with the study, all study questionnaires were forward translated and back translated into isiZulu, Setswana and Sesotho (common indigenous languages spoken at the Chris-Hani Baragwanath Hospital in Johannesburg). The translation and validation processes were performed using the MAPI Institutes' guidelines for translating and validating questionnaires. The institute's ultimate goal, with regards to translation and validation of questionnaires is to produce a target language version of the questionnaire that is conceptually equivalent to the original questionnaire and allows data pooling and comparison across countries (MAPI 2004). The steps of the translation and validation process include: conceptual definition, forward translation, backward translation and lastly, pilot testing (validation). I have given a brief description below of what each of the translation processes involved:

2.3.5.1 Conceptual definition

This involved the clarification of the concepts that were investigated by each item of the questionnaires. The team of translators and investigators discussed the purpose of each of the items in the questionnaire in relation to what the questionnaire assessed.

2.3.5.2 Forward translation

The aim of the forward translation stage was to obtain a consensus target language version (MAPI, 2004). Figure 2.3(b) illustrates how the translation process was carried out. Two independent translations of the questionnaires in isiZulu, Setswana and Sesotho were produced.

2.3.5.3 Backward Translation

The aim of the backward translation process was to obtain a translation into English of the target language version, to assess and control its quality (MAPI, 2004). This involved three processes which are outlined below in Figure 2.3(c).

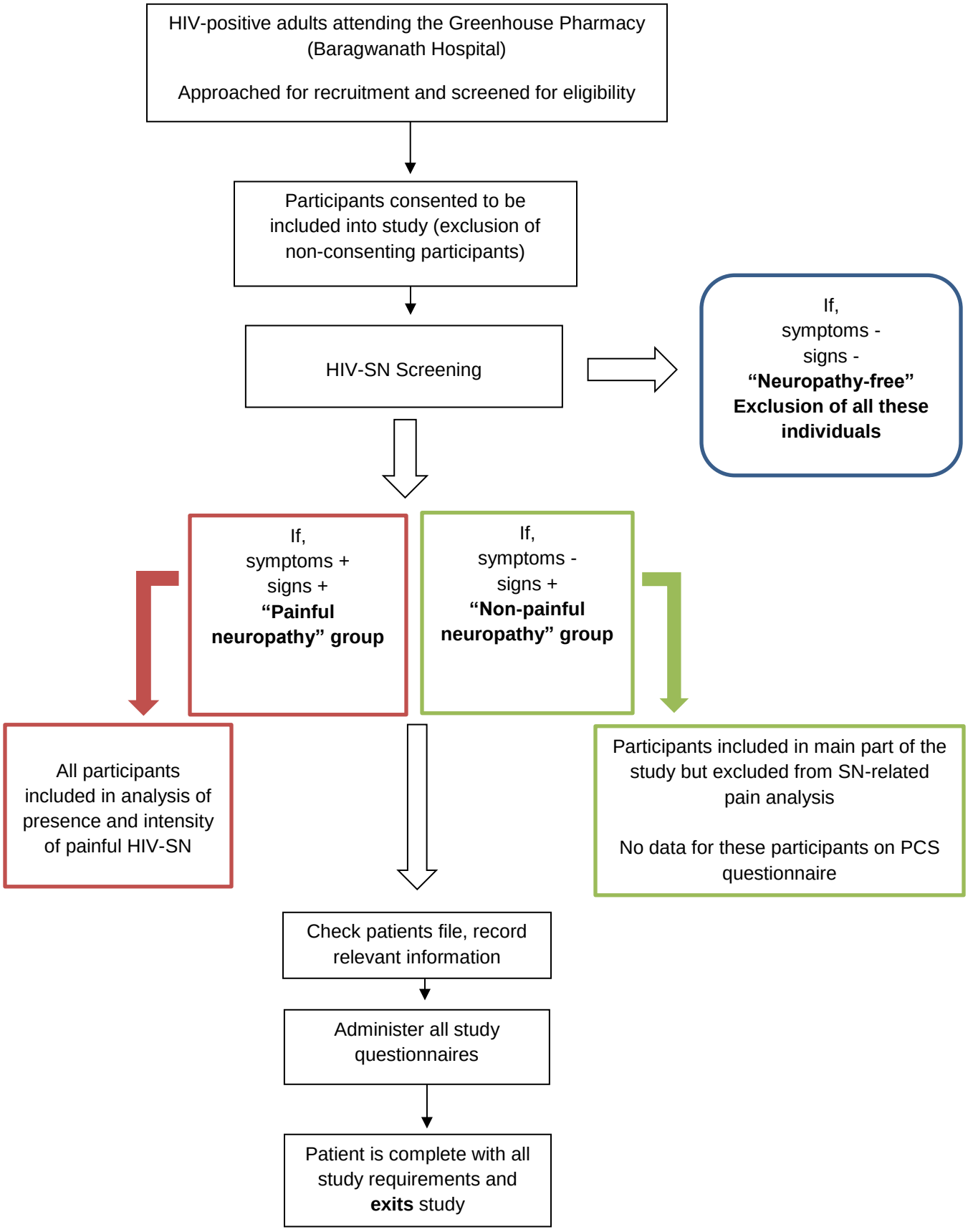


Figure 2.3 (a) Flow diagram of recruitment and screening procedures in study.

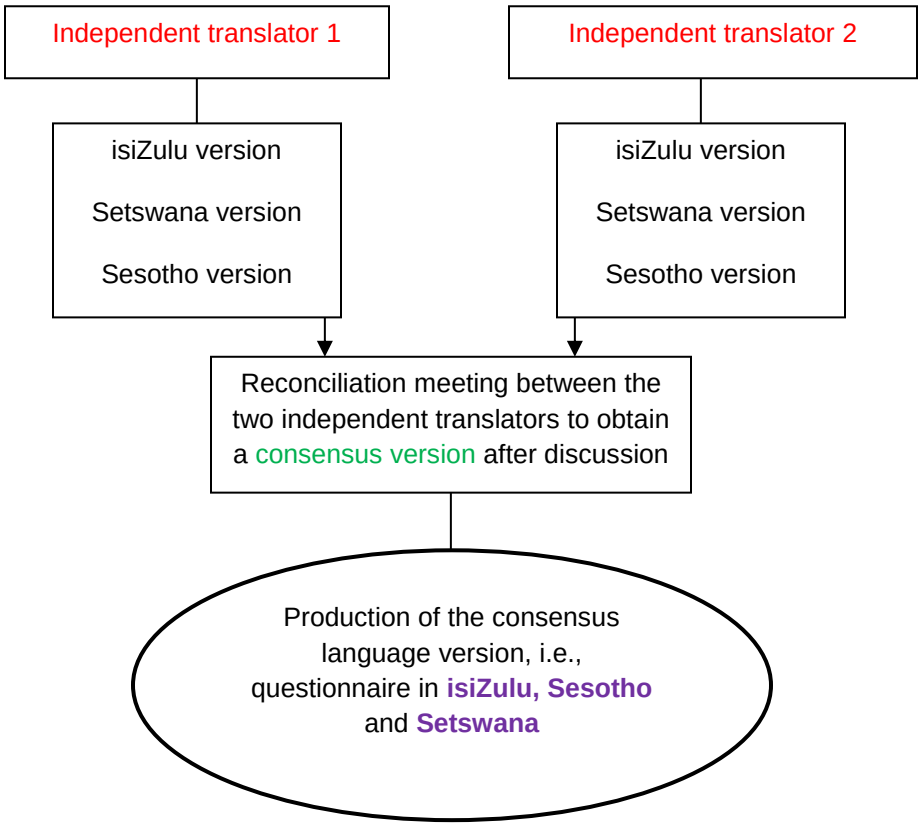


Figure 2.3 (b) Flow chart of the translation process of the questionnaire.

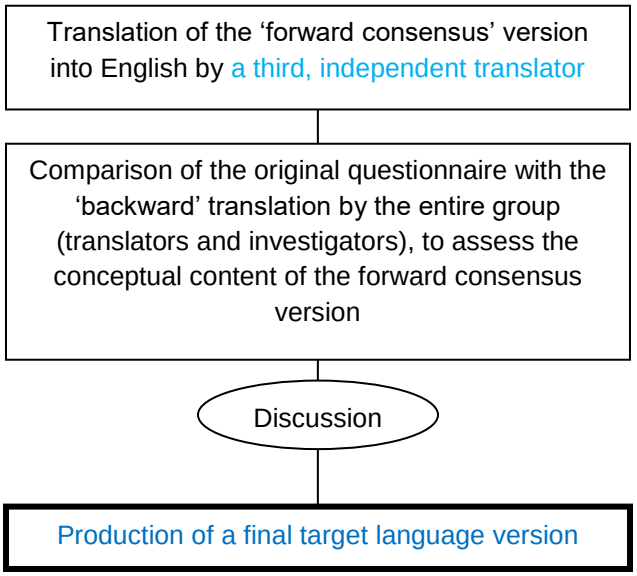


Figure 2.3(c) Flow chart illustration of the backward translation process.

2.3.5.4 Pilot testing of questionnaires

The aim of pilot testing was to acquire input from the individuals that would represent the future users of the questionnaire, i.e. the patients. The aim of cognitive debriefing was to assess the clarity, precision, appropriateness and cultural relevance of the translated questionnaire (target language version e.g. isiZulu version). This involved recruiting 50 participants to complete the questionnaires and thereby testing the interpretation of the translation. Thereafter, a final target language version of the questionnaire was produced.

2.3.6. Study Questionnaires

With the aid of an on-site study interpreter who was fluent in local South African languages, participants were asked to complete a series of questionnaires that assessed pain intensity, pain distribution, cognitive function, coping skills related to pain, pain related anxiety and depression, and health related quality of life. Below is a list of all the study questionnaires that were translated (aside from the EQ-5D) into three indigenous South African languages: IsiZulu, Setswana and Sesotho and validated in a sample of 50 individuals (protocol no. M110617) that were representative of the patient population which we used in the present study.

2.3.6.1 The Mini-Mental State Examination (MMSE)

The MMSE quantifies cognitive function and screens for cognitive loss (Folstein et al., 1975). It tests the participants' orientation, attention, calculation, recall, language and motor skills. The total score for the MMSE is out of 30 and a score of 24 or more is deemed normal. The MMSE has been used in similar populations in South Africa (Roos et al. 2010). The MMSE has been adapted for use in several populations including India, China and Bangladesh (Yu *et al.*, 1989; Kabir and Herlitz, 2000; Tiwari et al., 2009). The original MMSE has a bias with respect to education level and language and therefore has been adapted for use in the abovementioned populations with regards to local relevance. We used the original version of the MMSE and our own culturally adapted version of the MMSE (Appendix 7: SA-adapted MMSE). In our SA-adapted version of the MMSE we modified a total of eight questions in the questionnaire to make them more relevant to our South African participants. Modifications were made to the following questions: Place (orientation), Quick opinion, Presentation, Giving names (identifying objects), Repetition, section on reading, section on writing and section on drawing. We used the modifications that were made in the Hindi and Bangla versions of the MMSE to guide our alterations to the original MMSE.

2.3.6.2 Pain Catastrophizing scale (PCS)

The PCS assesses catastrophic thinking related to pain (Sullivan et al. 1995). It consists of 13 descriptions of thoughts and feelings related to pain. Participants were asked to indicate the level to which they experience these thoughts and feeling on a 5-point rating scale ranging from 0 (not at all) to 4 (always). The questionnaire consists of three subscales: rumination, magnification and helplessness. The total score for the questionnaire is calculated by adding the scores from each item answered and dividing this value by the total number of items answered (13).

2.3.6.3 EQ-5D-3L

The EQ-5D assesses health-related quality of life (Brooks 1996). It consists of two sections; the first section consists of descriptive questions and the second contains a visual analogue scale (VAS). The descriptive section is made up of five subscales: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Participants are asked to rate their own state of health on that particular day for each subscale. The scale ranges from 1 (no problems) to 3 (severe problems). In the second section, participants are asked to rate how good or bad their current state of health is by drawing a line on a VAS anchored at 0 (worst state of health imaginable and 100 (best state of health imaginable). The EQ-5D has been validated in a South African population by the EuroQol group who developed the questionnaire(The_EuroQol_Group 1990). The EQ-5D had previously been translated into isiZulu, Setswana and Sesotho by the EuroQol group and we requested and obtained permission from the EuroQol group to use their translated versions of the EQ-5D.

2.3.6.4 The Hopkins Symptoms Checklist-25 (HSCL-25)

The HSCL-25 assesses global psychological function (Derogatis et al. 1974). It consists of two subscales; anxiety and depression. The first scale consists of 10 questions which assess anxiety-related symptoms and the latter consists of 15 questions which assess depression-related symptoms. Participants are asked to rate how much the symptoms in the two subscales bothered or distressed them using a 4-point rating scale ranging from 1 (not at all) to 4 (extremely). Participants responses are summed and divided by the number of answered items (in each of the two sections) to generate a score for each of the sections. For the anxiety section, the sum of the responses is divided by 10 and for the depression section the total of the responses is divided by 15. A value >1.75 in each of the sections is considered symptomatic. The HSCL-25 has been used successfully in a South African HIV-positive population (Kagee & Martin 2010).

2.3.6.5 The Wisconsin Brief Pain Questionnaire (WBPQ)-adapted version

The WBPQ assesses pain intensity and pain interference (Daut et al.,1983). Using the WBPQ, participants rate the severity of their pain and the degree to which their pain interferes with common aspects of feelings and function. We used an adapted version of the WBPQ that included only the pain interference subscale, a pain body chart diagram and a NRS rating of participants 'other' unrelated SN pain. Participants were asked if they were currently experiencing any pain, if they answered yes, they were asked to mark their pain locations on the body chart diagram. Thereafter, with the aid of the onsite translator, they completed the pain interference subscale. The brief pain interference scale assesses seven items: general activity, mood, walking ability, normal work, relationships with people, sleep and enjoyment of life. Participants were asked to rate the extent to which their pain had interfered with each of these activities using a five-point rating system anchored at 0 (no interference) and 5 (interferes completely). The final score for the interference scale is a mean of the items answered (7). The WBPQ has been validated in a similar community of ambulatory HIV-positive South Africans that were recruited here (Mphahlele et al., 2008).

Chapter 3

The six-month incidence of HIV-associated sensory neuropathy in individuals on TDF-based cART.

3.1 Introduction:

As outlined in Chapter 1, HIV-associated sensory neuropathy is a common and frequently painful neurological complication associated with HIV-infection and its treatment (McArthur et al., 2005; Smyth *et al.*, 2007; Ellis *et al.*, 2010; Wadley *et al.*, 2011; Schütz and Robinson-Papp, 2013). HIV-associated distal sensory polyneuropathy (HIV-DSP) is primarily associated with the HIV-infection, while antiretroviral toxic neuropathy (ATN) is associated with the use of neurotoxic NRTIs that include stavudine, didanosine and zalcitabine (Rizzuto *et al.*, 1995; Smyth *et al.*, 2007; Kamerman et al., 2012). Indeed, Sub-Saharan Africa carries the largest burden of HIV and accounts for more than 70% of the global burden of infection (Kharsany & Karim 2016). ATN is the most common toxicity associated with stavudine and didanosine use in Sub-Saharan Africa (Forna et al. 2007; Ndakala et al. 2016; Sacktor et al. 2009; Wadley et al. 2011a; Maritz et al. 2010b; Shurie & Deribew 2010; Tumusiime et al. 2014).

Clinical, disease-related and demographic risk factors for ATN have been investigated and include age, height, drug exposure (NRTIs stavudine and didanosine), treatment for TB infection, CD4 T-cell counts, viral load, genetic factors and comorbid condition such as diabetes mellitus (Ngassa Mbenda et al. 2017; Hendry et al. 2013; Smyth et al. 2007; Ellis et al. 2010). With the worldwide elimination of stavudine in all first-line cART regimens, it is expected that the incidence of neurological conditions such as HIV-SN will decrease. However, several studies have reported a higher than expected prevalence of HIV-SN despite the use of TDF-based cART (Ellis et al. 2010; Cherry et al. 2010). Furthermore, no African-based studies have assessed the incidence of HIV-SN and factors related to its development in cohorts solely exposed to TDF-based cART (Njuguna et al. 2013; Tweya et al. 2014; Ndakala et al. 2016; Tumusiime et al. 2014; Bygrave et al. 2011; Van Der Watt et al. 2015; Watt et al. 2014). These previously mentioned cohorts have all assessed incidence of HIV-SN in cohorts with varied exposure to stavudine and TDF-based therapies. Therefore, we aimed to investigate the six-month incidence of HIV-SN in ARV naive South African PLWHA initiating TDF-based cART and to determine what clinical, disease-related and demographic risk factors associate with HIV-SN development in this population.

3.2 Methods:

The complete methodology for patient recruitment, neurological assessment, questionnaires and procedures is as described in Chapter 2 (Methods: section 2.1).

3.3 Results

From May 2016 through to December 2016, 151 confirmed HIV-positive individuals were invited to participate in the present study carried out at the Chronic Clinic within the Lawley/Lenasia South Community Health Hospital. Recruitment and exclusion of participants is summarised in Figure 3.1. A total of 31 participants were excluded from the study cohort. The remaining one hundred and twenty individuals participated in the study and were followed up for the duration of the study.

All study participants identified themselves as of Black African ancestry; 104 (87%) were South African, three (2%) were Zimbabwean, four (3%) were from Malawi, six (5%) were from Mozambique, two (2%) were from Lesotho and one (1%) was from the DRC Congo. All participants had a confirmed HIV infection, were 18-years or older, were ARV naïve and initiated TDF-based cART therapy on the day of recruitment.

All participants were free of peripheral neuropathy on initial assessment. Of the one hundred and twenty individuals who were free of neuropathy on initial assessment and enrolled into the study, 83 (69%) patients exited the study at 6-months. Patient flow and incident neuropathy for the 6-month duration of the study is summarised in Figure 3.2.

Demographic and clinical characteristics

Baseline demographic and clinical characteristics of the study cohort are shown in Table 1. Over two thirds of the cohort were categorized under WHO stage III and IV (92/120, 77%), indicating that the majority of our patients were immunocompromised with a baseline mean CD4 count of 228 cells/µL. No participants were exposed to stavudine based cART therapy or any other cART regimen that did not contain tenofovir. Non-HIV-related risk factors such as diabetes mellitus and vitamin B12 deficiencies were not common in our cohort.

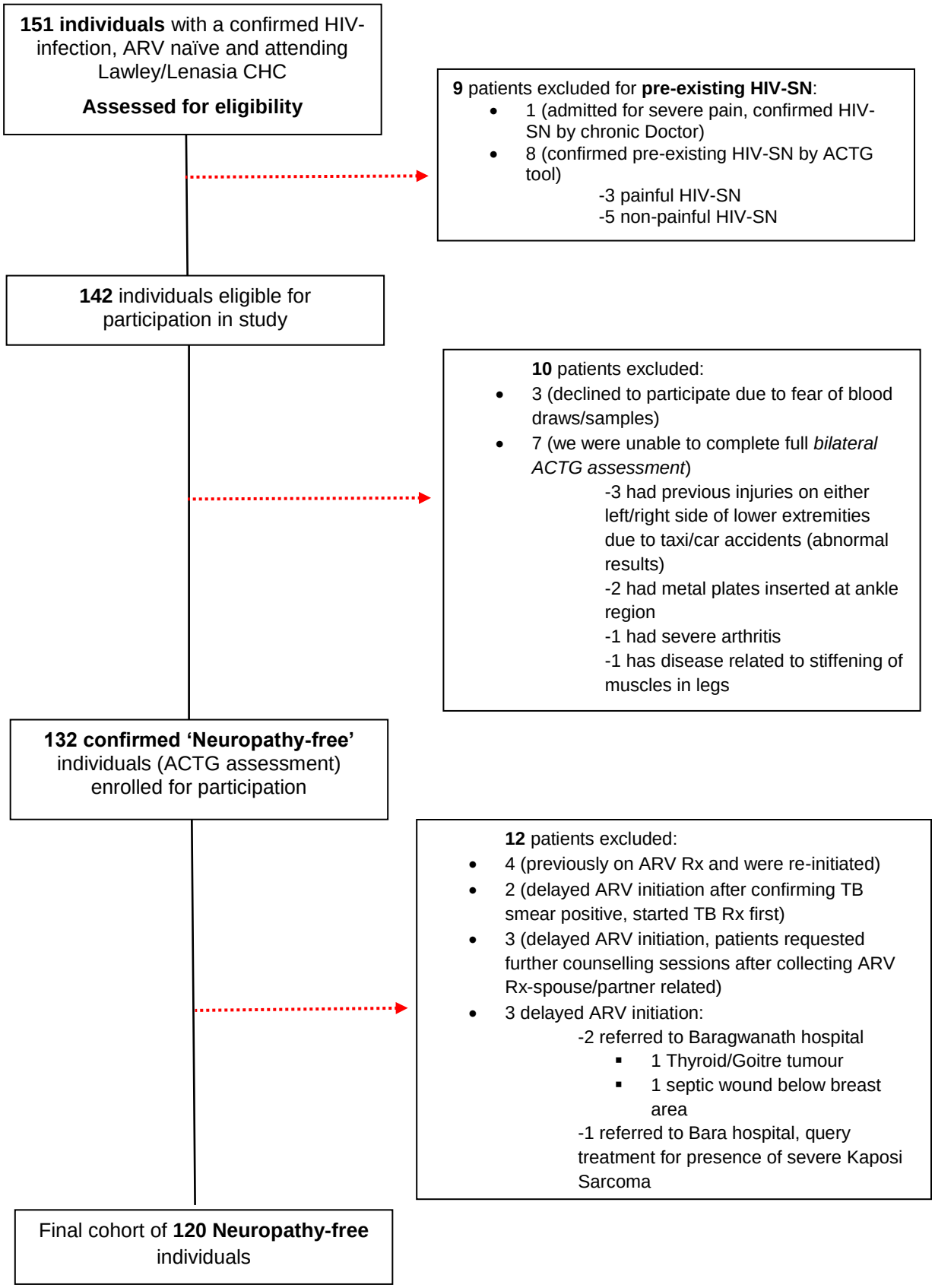


Figure 3.1 Flow diagram summarising patient recruitment and exclusion.

Table 3.1 Demographic and clinical characteristics of the study population at baseline (n=120).

Characteristic	Entire cohort (n=120)	Cohort completing 6-months of cART (n=83)
Female Sex, n (%)	66 (55)	46 (55)
Age (years)	39 (12)	38 (9)
Height (m)	1.63 (0.08)	1.63 (0.09)
Weight (kg)	65.0 (15)	66.0 (16)
Current CD4 count (cells/µL)	228 (1-1347)	234 (1-867)
log10 Viral load (copies/mL)	3.19 (1.69-6.5)	3.02 (1.69-6.5)
Current TB infection, n (%)	20 (17)	16 (19)
Standard units of alcohol consumed	15.0 (3-90)	9.6 (3.3-29.7)
Vitamin B_{12} deficiency, n (%)	1 (1)	-
Initial Vitamin B_{12} level (pmol/L)	316.5 (140-693)	308 (140-693)
Hepatitis B infection, n (%)	4 (3)	3 (4)
Current syphilis infection, n (%)	3 (3)	3 (4)
Type II diabetes, n (%)	1 (1)	1 (1)
Initial HbA1c percentage	5.4 (4.0-7.0)	5.4 (4.0 - 7.0)
WHO staging, n (%) *I* *II* *III* *IV*	 13 (11) 15 (12.5) 37 (31) 55 (46)	 5 14 26 38
Population group, n (%) *isiZulu* *isiXhosa* *Sesotho* *Setswana* *Other*	 45 (38) 20 (17) 26 (22) 7 (6) 22 (18)[a]	 34 (41) 12 (14) 16 (19) 5 (6) 16 (19)[b]
Treatment regimen, n (%): *TDF-based* *Non TDF-based*	 100 (100) 0 (0)	 100 (100) 0 (0)

[a]Other, n (%): Tsonga, 2 (2); Venda, 2 (2); Pedi, 3 (3); Afrikaans, 3 (3), Portuguese 7 (6), Chewa 4 (3), Shona 1 (1).
[b]Other, n (%): Tsonga 5, (6); Venda 3 (4); Pedi 3 (4); Portuguese, 2 (2); Chewa, 3 (4)
Dichotomous variables are presented as number (%), normally distributed continuous variables are presented as mean (standard deviation) and non-normally distributed continuous variables are presented as median (range).

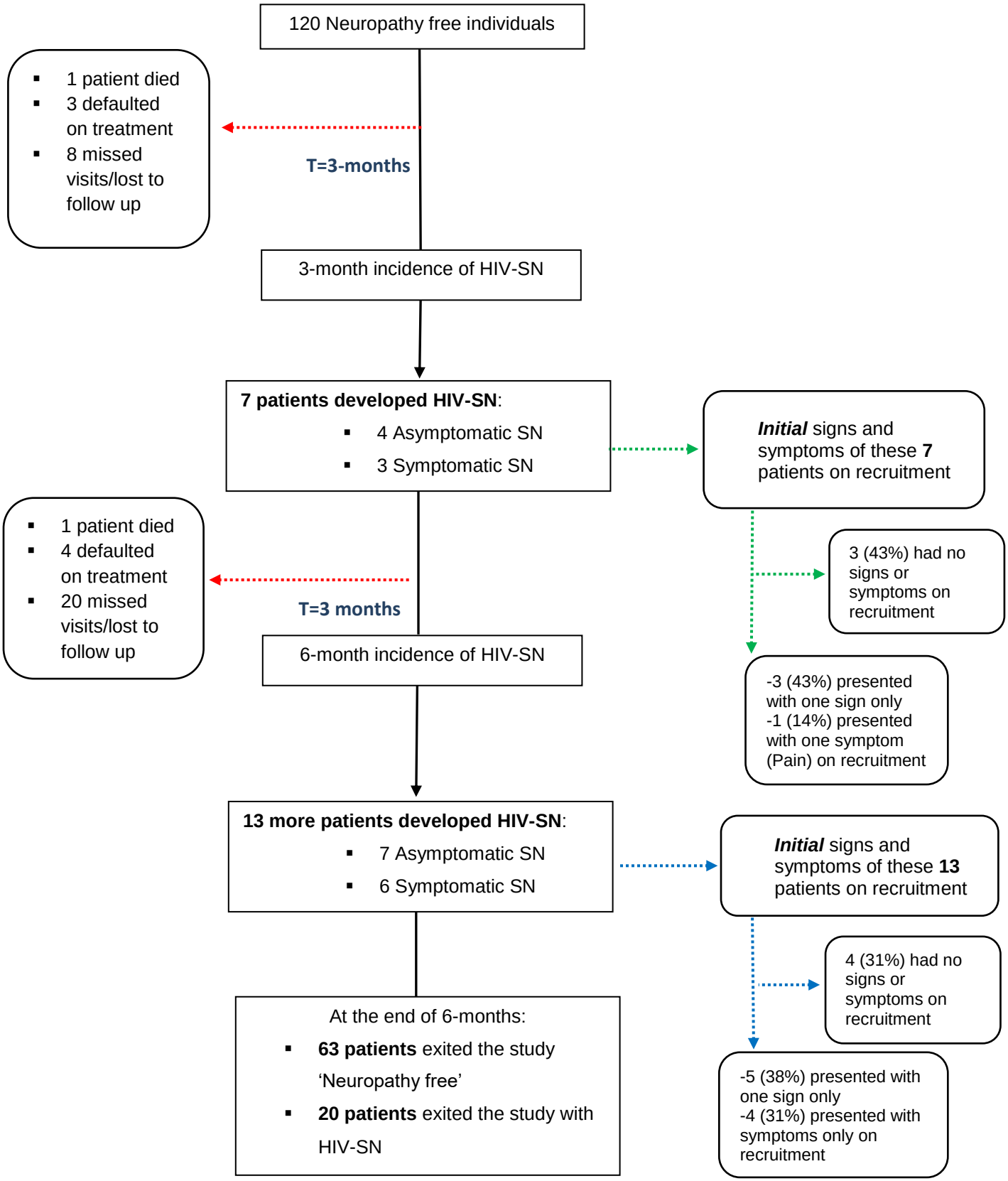


Figure 3.2. Flow diagram describing the development of HIV-SN in patients who initiated TDF-based cART.

Symptom prevalence at baseline

Of the 120 patients enrolled at baseline, 20% (24/120) presented with pain, 14% (17/120) had paraesthesias and only 5% (6/120) presented with numbness, however, despite the presence of these symptoms they were all 'neuropathy-free'. All symptoms were located primarily in the foot region for 85% (40/47) of these patients with only 15% (7/47) of patients experiencing the extension of these symptoms up to the ankle and knee regions. The median pain intensity was 5 (IQR 2-10) out of 10, with patients experiencing pain of a 'moderate' intensity. The median symptom intensities for paraesthesias and numbness, were 2 (IQR 1-10) and 4 (IQR 2-5), respectively.

Patient follow-up

Figure 3.3 illustrates the number of clinic visits for the entire cohort (n=83). Approximately 80% of the cohort had at least 3 clinic visits. Although participant follow-up was planned for 0, 2, 4 and 6-months on therapy, thereby allowing for a minimum of four visits for each participant, there was variability in the visit schedules of participants. Figure 3.4 illustrates the number of clinic visits, stratified according to the development of peripheral sensory neuropathy for the whole cohort (n=83).

The median number of clinic visits for the entire cohort (n=83) was [Median (IQR): 3 (3-3)]. For those individuals that remained 'neuropathy-free', the median number of clinic visits was 3 (IQR 3-3) and for those that developed neuropathy, the median was 3 (IQR 3-3.25). There was no significant difference in the number of clinic visits between those individuals that remained 'neuropathy-free' and those that developed neuropathy (p=0.67; Fishers exact test). There was also no significant difference in the number of days between the first and last clinic visit between those individuals that remained 'neuropathy-free' and those that developed neuropathy (p=0.51; Fishers exact test).

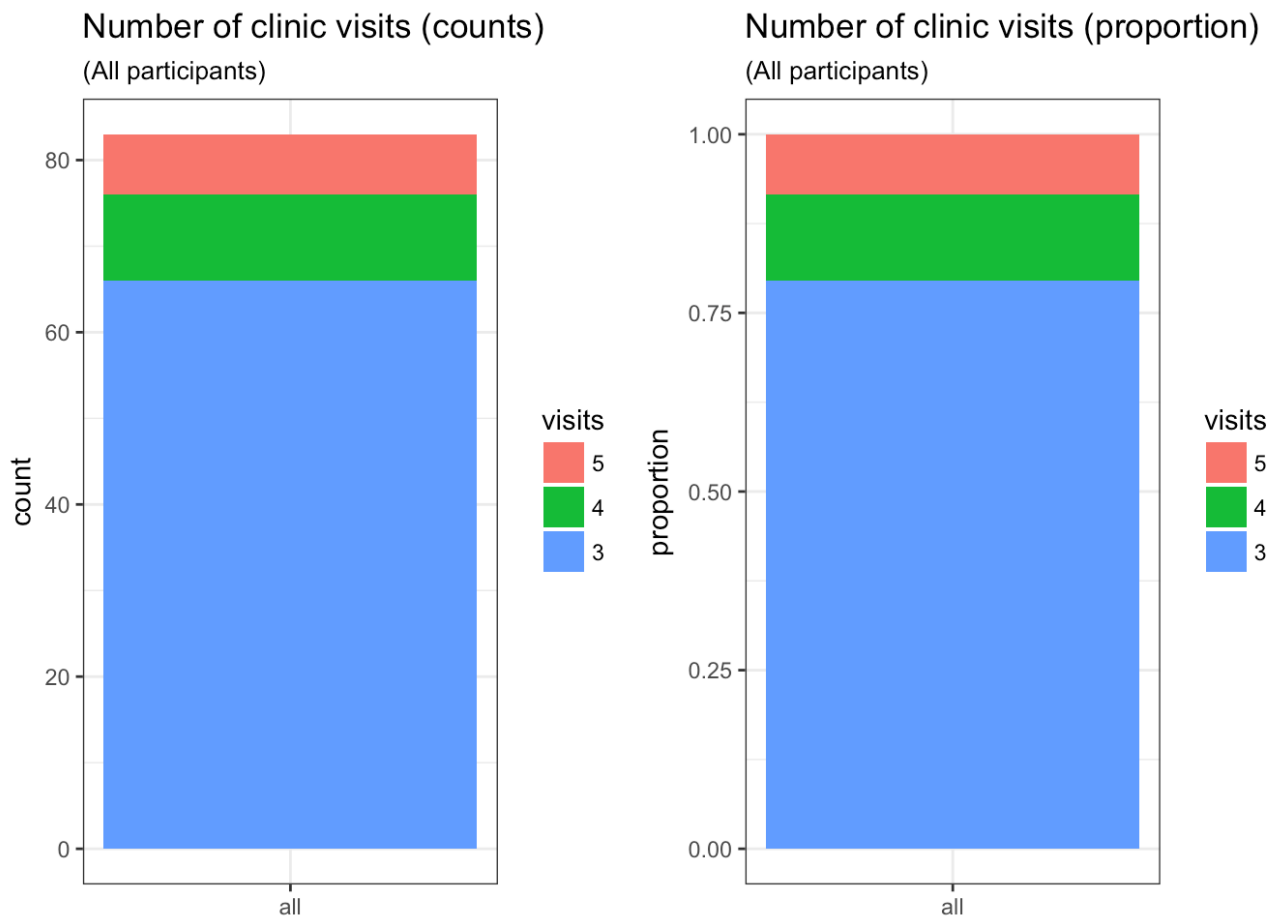


Figure 3.3 Bar graph illustrating number of clinic visits for the entire cohort (n=83).

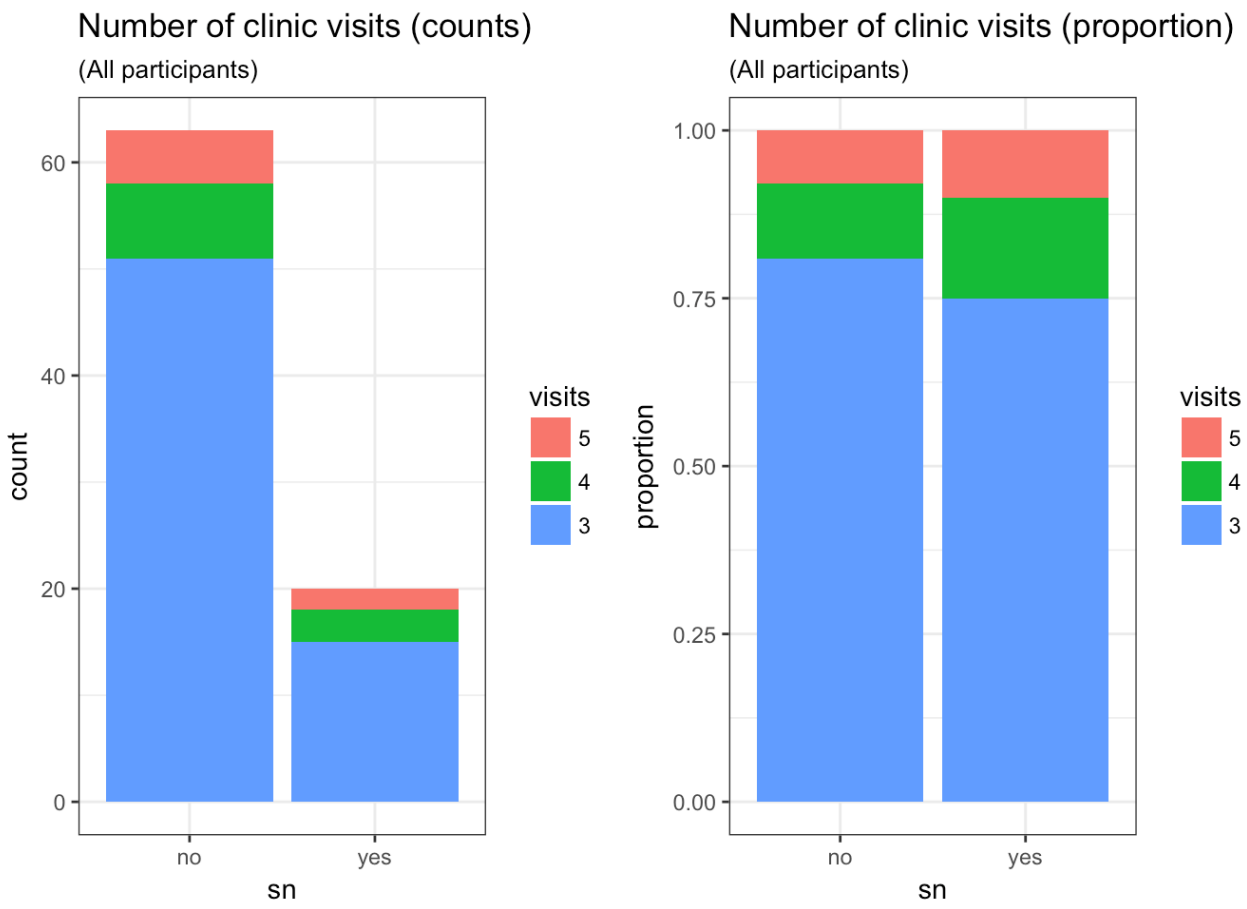


Figure 3.4 Bar graph illustrating number of clinic visits, stratified according to the development of peripheral sensory neuropathy for the whole cohort (n=83).

Development of neuropathy

Of the 120 confirmed 'neuropathy-free' patients who were enrolled at baseline, 10% (12/120) were lost to follow-up by the 3-month visit and a further 21% (25/120) were lost to follow-up by the 6-month period (Figure 3.2). Therefore, a total of 31% (37/120) of patients were lost to follow-up. Of the 69% (83/120) of patients who completed the study (completed at least three visits or more) and exited the study at 6-months, 24% (20/83) developed HIV-SN and 76% (63/83) remained 'neuropathy-free'.

The cumulative 6-month incidence (defined as a measurement of a specific disease during a period of time i.e. an entire lifetime) of HIV-SN was 200 cases per 1000 patients (95% CI: 120-290). The cumulative incidence over the first 3-months of follow-up was 80 cases per 1000 patients (95%: 20-140). The cumulative incidence over the second 3-months of follow-up was 130 cases per 1000 patients (95%: 50-200). The incidence rate (defined as the number of new cases

per population at risk in a given time period) of SN was 0.47 (95% CI: 0.3-0.67) per person-year. Nine (11%) individuals developed painful HIV-SN and eleven (13%) individuals developed non-painful HIV-SN. The median pain intensity of the 15% (3/20) patients who developed painful HIV-SN in the first 3-month period, was 4 (IQR 3-5) indicating a 'moderate' level of pain. The median pain intensity of the 30% (6/20) patients who developed painful HIV-SN in the second 3-month period, was 5 (IQR 4-10).

Kaplan-Meier survival curves

Figure 3.5 shows the Kaplan-Meier survival curve with the number and percentage of patients at risk for developing neuropathy across the study period. Of the 20 individuals that developed neuropathy, 10% (8/83) of patients developed neuropathy within the first 50 days of cART; one patient developed neuropathy as early as ± two weeks. Within the next 100 days of cART, 14% (12/83) individuals developed neuropathy. The last event of neuropathy development occurred at ~140 days (± 5-months of therapy).

The presence of pain was a significant risk factor for the development of neuropathy (p=0.0015). Furthermore, the presence of pain resulted in a quicker onset of the peripheral sensory neuropathy as shown in Figure 3.6 (orange curve), with the first painful neuropathy occurring at ± two weeks. The development of non-painful peripheral sensory neuropathy had a slightly delayed onset as depicted by the blue curve in Figure 3.6, with the first non-painful neuropathy occurring at ± day 25.

Univariate factors at baseline that did not asscociate with HIV-SN

Univariate factors at baseline that were not significantly associated with the development of neuropathy include: age [Mean (SD)] SN vs. No SN: 41.8 (8.5) vs. 37.7 (9.1)]; (p=0.07), CD4 count [Median (IQR)] SN vs No SN: 200 (72-308) vs. 235 (127-317)]; (p=0.5), log Viral load [Median (IQR)] SN vs. No SN: 3.0 (2.22 - 4.73) vs. 3.1 (2.25 - 4.22)]; (p=0.53), units of alcohol consumed [Median (IQR)] SN vs. No SN: 7.5 (5.75 – 9.25) vs. 9.0 (4.5 – 15.0)]; (p=0.6), alcohol consumption (p= 0.33; Chi-squared), Sex (p=0.12; Fishers exact test), and Rifafour and Pyridoxine prophylaxis (p=0.66; Chi-squared test).

Important to note, Rifafour refers to the standard first-line TB treatment in South Africa. This medication consists of four drugs, which include: Ethambutol, Isoniazid, Pyrazinamide and Rifampicin. Participants take Rifafour for two months and if this treatment is successful they are then changed to another medication called Rifinah, which contains Isoniazid and Rifampicin for

the next four months. Participants in our cohort who were on TB treatment all received Rifafour as their standard first-line TB treatment.

Univariate analysis of factors associated with the development of neuropathy at 3-months and 6-months of therapy

Risk factors associated with HIV-SN at 3-months and 6-months

At three-months of TDF-based therapy, increasing height (p=0.01) was the only factor significantly associated with the development of HIV-SN on univariate analyses (Table 3.2). At six-months of TDF-based therapy; increased viral load (p=0.003), increasing height (p=0.0006), increased weight (p=0.04) and a current TB infection (p=0.002) were all significantly associated with the development of HIV-SN on univariate analysis (table 3.3). Although current TB infection was significantly associated with the development of neuropathy at 6-months of therapy, receiving Rifafour and Pyridoxine prophylaxis treatment did not significantly associate with development of neuropathy at 6-months on univariate analysis (p=0.07).

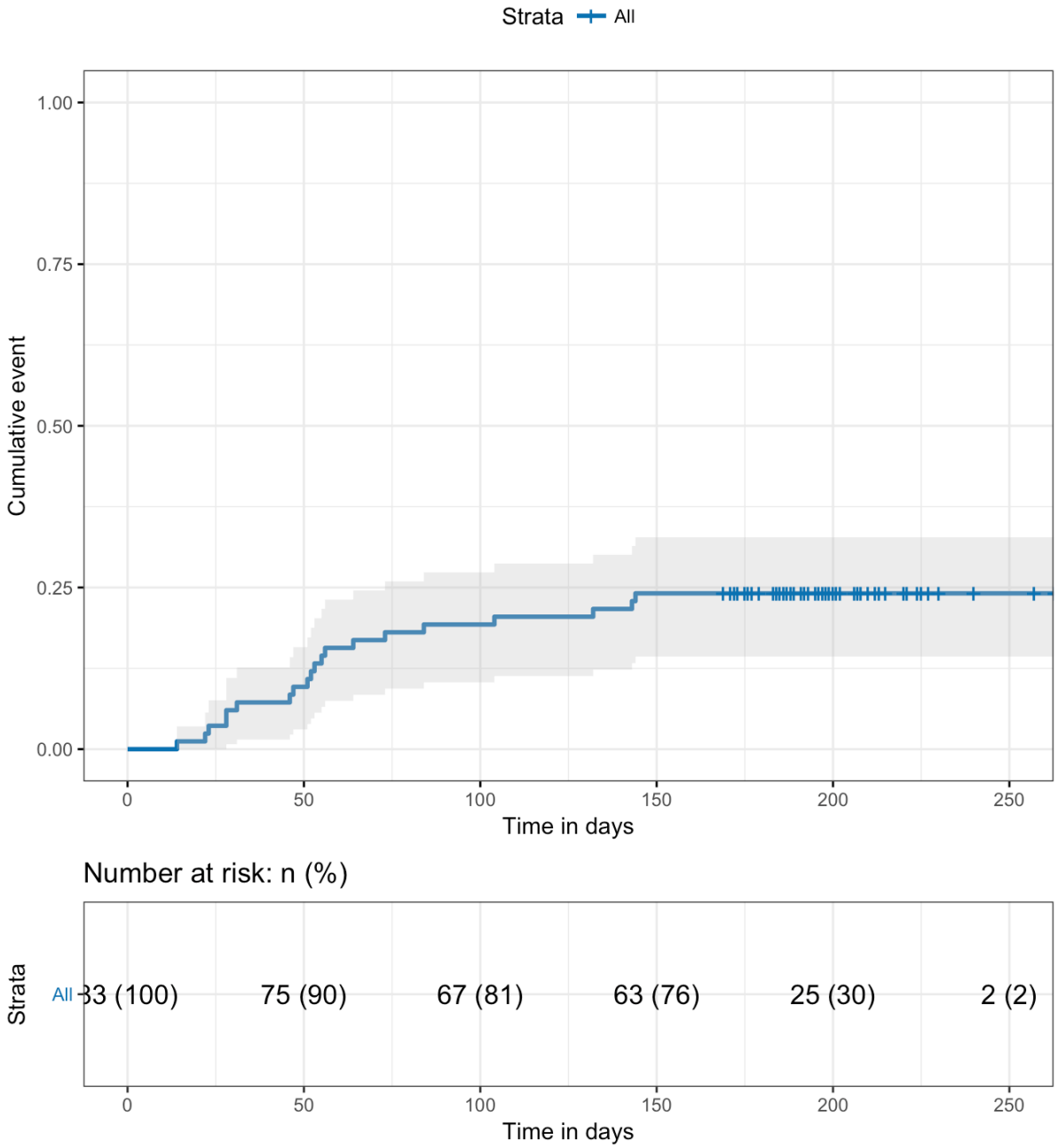


Figure 3.5 Kaplan-Meier survival curve illustrating the number and percentage of patients at risk for developing neuropathy across the study period. The y-axis represents the cumulative event (i.e. development of neuropathy) and the x-axis represents time in days.

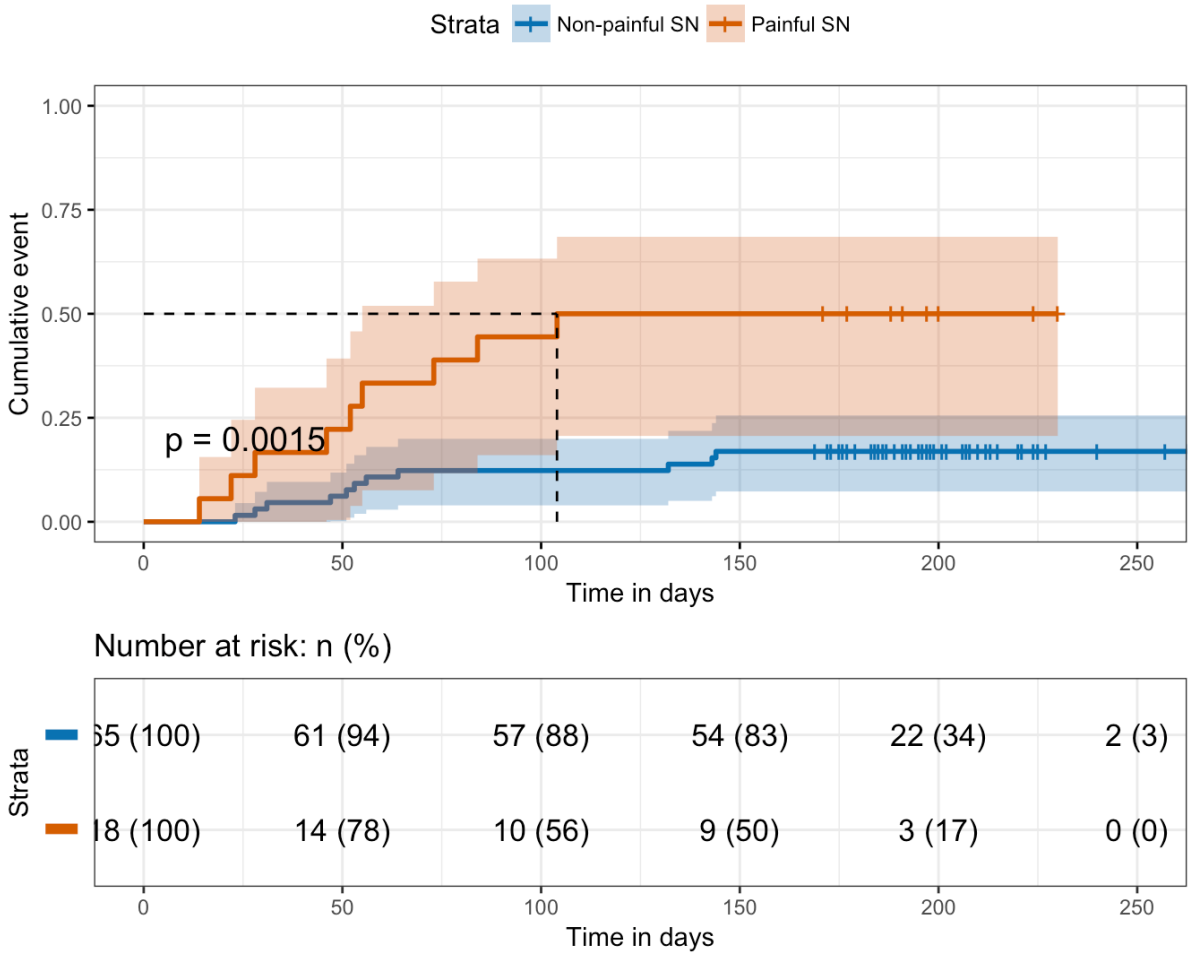


Figure 3.6 Kaplan-Meier survival curve illustrating the number and percentage of patients at risk for developing a painful vs. non-painful neuropathy across the study period and that the presence of pain at baseline predicts the development of painful versus non-painful neuropathy. The y-axis represents the cumulative event (i.e. development of neuropathy) and the x-axis represents time in days.

Table 3.2 Characteristics of HIV-positive individuals who initiated TDF-based cART and either developed a peripheral sensory neuropathy or remained neuropathy-free at **3-months** of follow-up (n=83).

Characteristic	No HIV-SN (n=76)	HIV-SN (n=7)	p-value (SN vs. No SN)
Age (years)[a]	39.2 (1.0)	35.29 (2.4)	p=0.28[c]
Female gender, n (%)	44 (58)	2 (29)	p=0.23[d]
CD4 count (cells/μL)[b]	271 (12-771)[n=73]	190 (19-519)[n=7]	p=0.72[e]
HIV RNA (Log_{10} copies/mL)[b]	2.47 (1.30-6.50)[n=65]	2.48 (1.96-3.71)[n=4]	p=0.88[e]
Height(cm)[a]	1.62 (0.009)	1.70 (0.03)	p=0.01[c]
Weight (kg)[a]	66.07 (1.84)	76.57 (5.17)	p=0.07[c]
Current TB infection, n (%)	13 (17)	3 (43)	p=0.12[d]
Pyridoxine treatment, n (%)	36 (47)	3 (43)	p > 0.1[d]
Rifafour treatment, n (%)	36 (47)	3 (43)	p > 0.1[d]
Standard units of alcohol consumed[b]	9.6 (3.3-29.7)[n=16]	nil	-
Vitamin B_{12} deficiency, n (%)	339 (146-786)[n=70]	283 (194-592)[n=7]	p=0.71[e]
HbA1c percentage, n (%)	5.4 (3.8-7.2)[n=73]	5.6 (5.4-6)[n=6]	p=0.11[e]

[a]Data shown as mean (SD) for parametric data [d]Fishers exact test
[b]Data shown as median (IQR) for non-parametric data [e]Mann-Whitney two sample statistic (non-parametric data)
[c]Unpaired t-test (parametric data)

Table 3.3 Characteristics of HIV-positive individuals who initiated TDF-based cART and either developed a peripheral sensory neuropathy or remained neuropathy-free at **6-months** of follow-up (n=83).

Characteristic	No HIV-SN (n=63)	HIV-SN (n=20)	p-value (SN vs. No SN)
Age (years)[a]	38.3 (1.2)	41.9 (1.9)	p=0.12[c]
Female gender, n (%)	38 (60)	8 (40)	p=0.12[d]
CD4 count (cells/μL)[b]	299.5 (8-832)[n=60]	280.5(19-575)[n=20]	p=0.36[e]
HIV RNA (Log_{10} copies/mL)[b]	1.87 (1.30-5.46)[n=43]	2.40(1.69-6.03)[n=16]	p=0.003[e]
Height(cm)[a]	1.61 (0.01)	1.69 (0.01)	p=0.0006[c]
Weight (kg)[a]	64.28 (1.2)	72.76 (4.1)	p=0.04[c]
Current TB infection, n (%)	7 (11)	9 (45)	p=0.002[d]
Pyridoxine treatment, n (%)	28 (44)	14 (70)	p=0.07[d]
Rifafour treatment, n (%)	28 (44)	14 (70)	P=0.07[d]
Standard units of alcohol consumed[b]	9.6 (3.3-297)[n=14]	7.7(4.05-11.25)[n=2]	p=0.71
Vitamin B_{12} deficiency, n (%)	355.5 (174-780)[n=60]	274 (161-592)[n=19]	p=0.07[e]
HbA1c percentage, n (%)	5.3 (4.1-6.5)	5.6 (3.8-7.2)[n=19]	p=0.07[e]

[a]Data shown as mean (SD) for parametric data [d]Fishers exact test
[b]Data shown as median (IQR) for non-parametric data [e]Mann-Whitney two sample statistic (non-parametric data)
[c]Unpaired t-test (parametric data)

Univariate analysis of baseline factors that associated with the development of neuropathy

The only univariate factors at baseline that associated with the development of neuropathy over the study period were current TB infection (p=0.002; Chi-squared test), increased body mass (p=0.02; Fishers exact test) and increasing height (p< 0.001; Fishers exact test). Of the 20 individuals that developed neuropathy, 45% (9/20) had a current TB-infection. Of the 63 individuals that remained 'neuropathy-free', 17% (11/63) presented with a current TB-infection (Figure 3.7).

Height was not correlated with body weight; however, body mass was influenced by sex. The mean for those individuals with neuropathy was [Mean (SD): 73.0 (18.0)] compared to those who remained 'neuropathy-free' with a mean of 63.9 (14.7) [Figure 3.8]. Due to sex differences height was analysed separately for males and females in the cohort [Figure 3.9]. For males with neuropathy the mean height was [1.72 (0.04); n=12], for those males who remained 'neuropathy-free' the mean was [1.69 (0.04); n=25], for females with neuropathy the mean height was [1.64

(0.09); n=8] and for those females who remained ‘neuropathy-free’ the mean was [1.57 (0.06); n=38]. There was a significant difference in mean height amongst the female participants who developed neuropathy and those that were ‘neuropathy-free’ (p=0.004; Fishers exact test), however there was no significant difference in mean height amongst the male participants who developed neuropathy and those that remained ‘neuropathy-free’ (p=0.07; Fishers exact test).

Despite the significant association of baseline TB-infection with neuropathy development (p=0.002; Fishers exact), baseline treatment for TB-infection with Rifafour and Pyridoxine was not significantly associated with the development of neuropathy (p=0.66; Chi-squared test) [Figure 3.10].

Diabetes (classified as HbA1c percentage > 7%) and Vitamin B_{12} deficiency (classified as < 141pmol/L) were not common in our cohort, as classified at baseline (only one individual had diabetes and one individuals had a Vitamin B_{12} deficiency) and therefore were not assessed under univariate predictors of neuropathy.

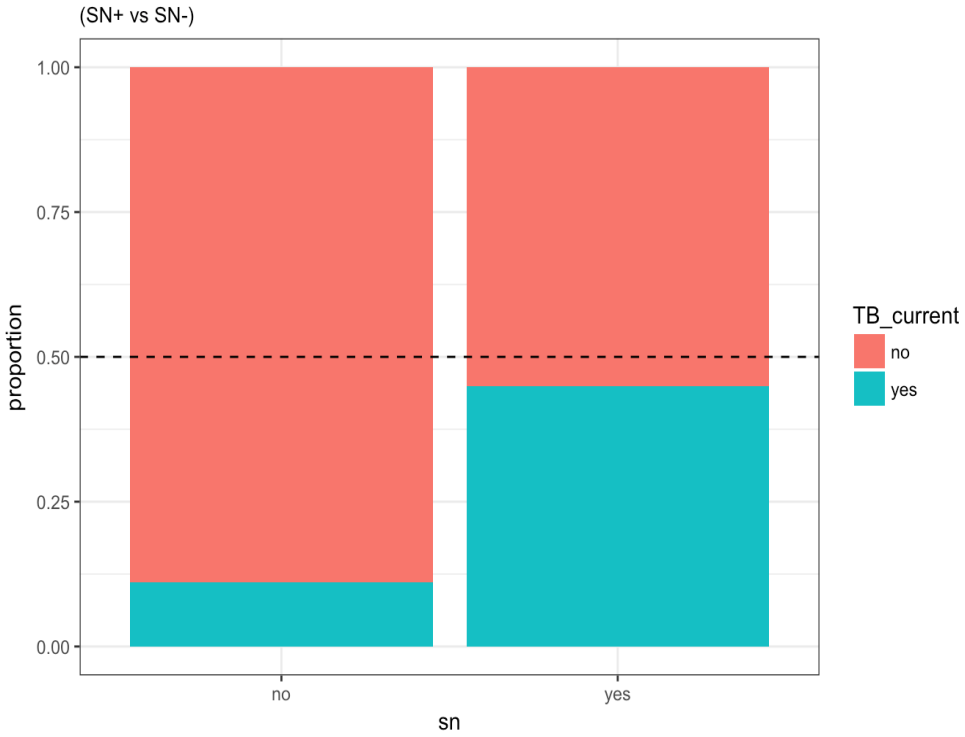


Figure 3.7 Bar graph illustrating proportion of patients at baseline with and without a current TB infection (y-axis), stratified according to the development of neuropathy (x-axis); (p=0.002; Chi-squared test).

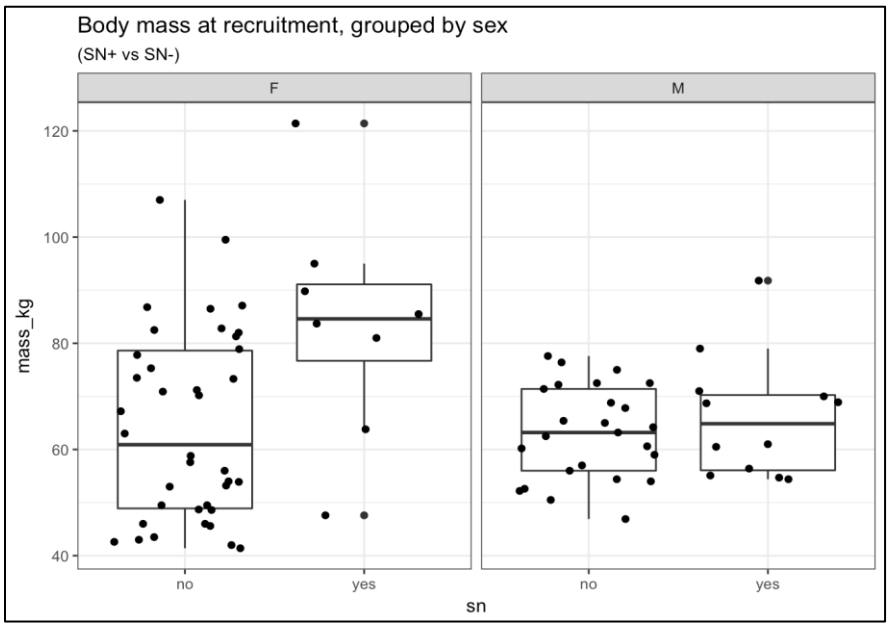


Figure 3.8 Box and whiskers plot illustrating baseline mass in kilograms (y-axis) of those participants who developed neuropathy and those that remained 'neuropathy-free' (x-axis), stratified according to male and female sex; (p=0.02; Fishers exact).

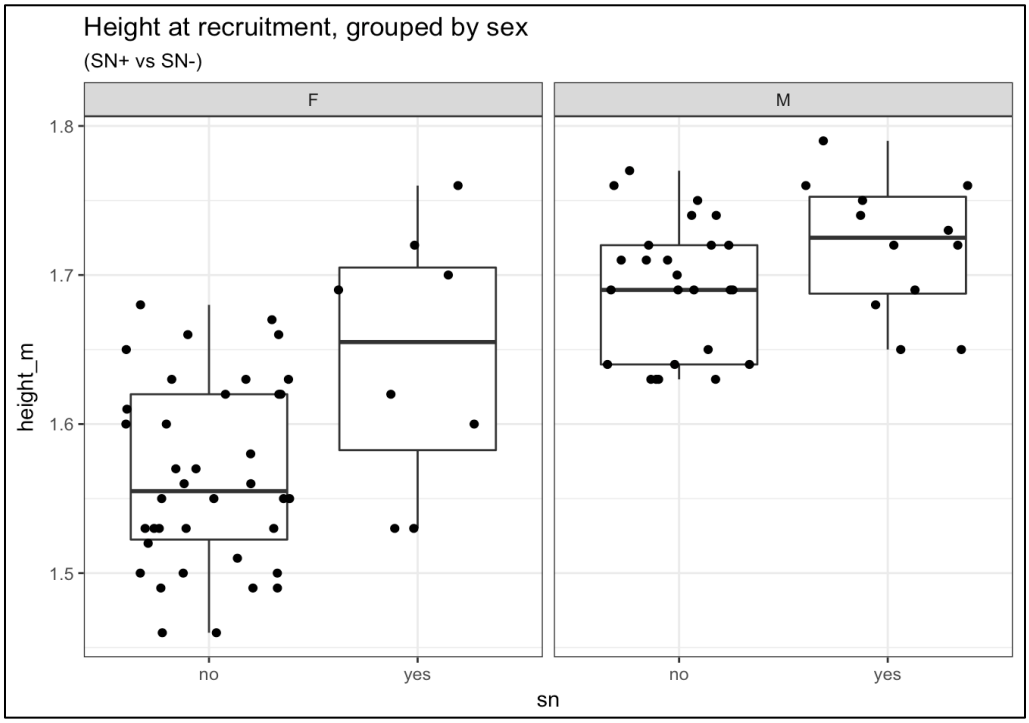


Figure 3.9 Box and whiskers plot illustrating baseline height in metres (y-axis) of those participants who developed neuropathy and those that remained 'neuropathy-free' (x-axis), stratified according to male and female sex; (p< 0.001; Fishers exact).

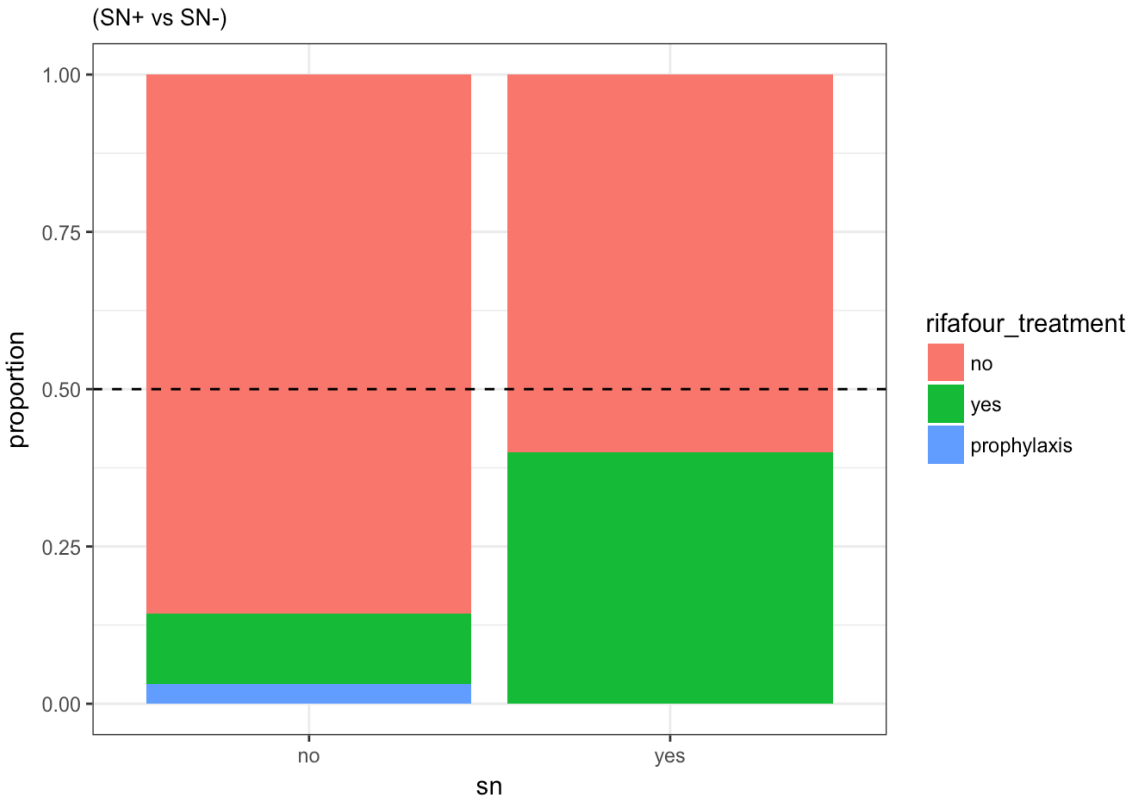


Figure 3.10 Bar graph illustrating proportion of patients at baseline (y-axis), who either received or did not receive Rifafour + Pyridoxine treatment, stratified according to the development of neuropathy (x-axis); (p=0.66; Chi-squared test).

Multivariate analysis

Variables yielding p-values< 0.1 on univariate analysis, and those previously shown to be associated with peripheral sensory neuropathy, were included in multivariate regression analyses. We performed logistic regression analysis as we only used data from visit 1 (baseline visit) to predict the development of neuropathy.

The logistic regression model did not include the following factors that have previously been associated with SN: Diabetes (as only one person had diabetes at baseline), Vitamin B_{12} deficiency (as only one person had a deficiency at baseline), Mass (as it is likely to be co-linear with height) and alcohol consumption (this data is encoded in units of alcohol consumed per week).

We included clinical, demographic, and physical factors in the modelling process. The full model included: height, sex, current TB-infection, age, Rifafour/Pyridoxine treatment, CD4 T-cell count, height (males) and viral load. Model selection was based on step-wise regression, using Akaike information criterion (AIC) as the basis for comparing models.

Independent predictors of peripheral sensory neuropathy included increasing height OR=12.8, 95% CI: 2.29 to 72.0, p=0.003) and having a current TB infection (OR=4.8, 95% CI: 1.03 to 22.4, p=0.04). The likelihood ratio for the model was (LR chi ^{2}= 22.82, d.f =4, p=0.0001) and the model had a predictive value of (R^2=0.38 and Kendall's tau A = 0.24).

3.4 Discussion:

We investigated the six-month incidence of HIV-SN in a cohort of 100% South African PLWHA of Black African ancestry who were universally exposed to TDF-based cART. We report a six-month cumulative incidence of HIV-SN of 200 cases per 1000 patients (95% CI: 120-290). The incidence rate was 0.47 (95% CI: 0.3-0.67) per person year. The number of individuals developing painful vs. non-painful SN in our cohort was relatively similar, as 11% (9/83) developed painful-SN and 13% (11/83) developed non-painful SN. The presence of pain was a significant risk factor for the development of HIV-SN (p < 0.001) and the onset of painful SN was more rapid as compared to non-painful SN. Increasing height (p=0.003) and having a current TB infection (p=0.04) were the only independent predictors of HIV-SN in our cohort.

Our study reports a cumulative incidence of HIV-SN of 200 cases per 1000 patients (95% CI: 120-290), in a cohort of Black African ancestry that was universally exposed to TDF-based cART. Our findings are novel and provide the first cumulative incidence data for individuals solely exposed to TDF-based cART. Our findings also highlight that both asymptomatic and symptomatic HIV-SN is still a problem in the post-stavudine era. Our findings are supported by previous studies who also reported the continued prevalence of HIV-SN in individuals exposed to non-d4t based cART (Ellis et al. 2010; Cherry et al. 2008).

The presence of a current TB infection or receiving treatment for a current TB infection was an independent predictor of HIV-SN in our cohort (p=0.04). This association between HIV-SN and current TB infection/receiving treatment for a current TB infection is supported by several studies (Forna *et al.*, 2007; Hitchcock et al., 2008; Maritz *et al.*, 2010a; Shurie and Deribew, 2010; Luma *et al.*, 2012; Centner *et al.*, 2014; Conradie *et al.*, 2014; Watt *et al.*, 2015). Indeed, isoniazid (INH) which forms part of the South African TB treatment regimen is neurotoxic (Mandel, 1959; Sanfeliu

et al., 1999; Steichen et al., 2006). Thus, the supplementation of pyridoxine (Vitamin B6) is included in the South African TB treatment guidelines (TB-Guidelines 2017). All participants who were receiving TB treatment in our cohort, also received pyridoxine supplementation, starting at 25mg/daily and increasing up to 100mg/daily. However, even with the supplementation of pyridoxine, participants in our cohort who initiated cART and were currently receiving TB treatment still developed HIV-SN. Pyridoxine supplementation is intended for the prevention of isoniazid-induced SN, however, pyridoxine supplementation has also been linked to the development of SN (Sanfeliu et al.,1999; Ghavanini and Kimpinski, 2014). Ghavanini and colleagues (2014) emphasize that while adequate pyridoxine supplementation may prevent isoniazid-induced SN, if given in an excessive amount >50mg/daily, this may cause SN. Over 50% of participants in our cohort were receiving pyridoxine between doses of 50-100mg/daily for the duration of the study. This amount of pyridoxine together with isoniazid-based TB treatment may well have contributed to the development of HIV-SN in our cohort. Increases in the dosage of pyridoxine supplementation should be carefully monitored by clinicians and certainly a reduction in these dosages is necessary in patients presenting with signs and/or symptoms suggestive of SN.

As part of the 2014 TB Guidelines and to acknowledge the growing epidemic of TB in South Africa, South Africa adopted a six-month isoniazid preventative therapy (IPT) programme for children and adults who are diagnosed as TB negative but have been in close contact with a confirmed TB positive case (Wood 2018; TB-Guidelines 2017). All participants in our cohort who were initiating TDF-based cART and were diagnosed as TB negative were all initiated onto IPT as part of the national TB guidelines. A systematic review by Wood and colleagues (2014) found that there is sufficient evidence to support the use of IPT in the high TB burden setting of South Africa (Wood & Bekker 2014). However, despite the reported effectiveness of IPT in preventing TB infections, the concern around the toxicity of isoniazid-based therapy in these individuals remains. Therefore, whilst IPT may prevent incident cases of TB, it may increase rates of SN. Initially IPT was given for 6-months, however this has been extended to a period of 12-months (TB-Guidelines 2017). We suggest that individuals who are receiving both cART and IPT be monitored regularly for the signs and symptoms associated with HIV-SN. Certainly, painful SN may be detected more easily in the clinic setting due to the associated symptoms (pain, burning, numbness and paraesthesias), however, asymptomatic SN, which is more common in the era of TDF-based cART, is not as easily detected due to the ‘slient’ nerve damage where there is no symptoms and therefore may be overlooked. Screening for asymptomatic SN in these individuals is equally important, as asymptomatic SN may transition into symptomatic SN over time (Malvar et al. 2015). Indeed, while reducing the rates of TB infection with IPT may be beneficial to TB

outcomes, the increased risk of SN must be addressed in these individuals. The association between IPT and risk of SN requires further investigation. No study has been carried out to evaluate the risk of IPT in the South African population which has a high burden of TB and HIV co-infection. Our findings highlight that PLWHA who are currently on cART and receiving isoniazid-based TB treatment are at risk for the development of HIV-SN.

Furthermore, it would have been highly beneficial to the study if we had additional information on whether patients TB diagnosis in our cohort, was pulmonary or extrapulmonary. However, we relied solely on patient's files within the chronic clinic to identify whether they were diagnosed with TB or not and this did not contiain the necessary information. Furthermore, nursing staff at the chronic clinic who were responsible for opening and filling out all files for ART naïve participants, who were subsequently recruited to our study, did not fill out a comprehensive TB diagnosis section where one would be able to ascertain the type of TB. Secondly, participants who were diagnosed with TB attended a separate "TB clinic" within the hospital for treatment and check-ups and therefore had a separate file at this clinic. We unfortunately did not have access to this file as it was not part of the chronic clinic records and these files remained within the TB clinic. We were only able to gain further information on TB treatment and the course of treatment from what was recorded in the chronic clinic file and from the medications that participants had brought with them after visiting the TB clinic. This lack of information in participants chronic files remains one of the disadvantages of conducting research in a public health facility.

We also found that increased height was independent predictor of HIV-SN in our cohort (p=0.003). Increasing height is a well stablished risk factor for polyneuropathies and has been linked to the development of HIV-SN in individuals exposed to stavudine (Affandi *et al.*, 2008; Cherry *et al.*, 2009; Banerjee *et al.*, 2011; Evans *et al.*, 2011; Wadley *et al.*, 2011) and diabetic sensory polyneuropathy (Sosenko *et al.*, 1986; Robinson *et al.*, 1992; Adler *et al.*, 1997; Cheng *et al.*, 2006). Since HIV-SN is a length dependent neuropathy, it is not surprising that taller individuals would be more susceptible to the development of SN (Evans et al. 2011). To date, Wadley and colleagues (2011) remain the only other African study, in addition to our study, to report height as a significant factor associated with the development of HIV-SN. Much like age, increased height remains a relative risk factor within cohorts for the development of SN, irrespective of the type of ARV exposure. Certainly, there is no critical cut-off point for height in terms of SN risk., as height is a continuous variable, where every mm/cm extra height increases risk of SN. Therefore, 'cut-off' values would be artificial. In this case, it would be beneficial to regularly screen all individuals for the signs and symptoms associated with SN. The early detection of this condition, and if symptomatic, will permit clinicians to treat this condition with suitable analgesic medications.

We did not find associations between established disease and demographic-related factors such as age, CD4 counts and viral loads with the development of SN in our cohort. The lack of association between age and development of HIV-SN may be explained by the fact that individuals in our cohort were much younger: the average age of our participants was 39 years (SD=12). Several studies have reported increased risk of HIV-SN in older PLWHA aged > 40 years (Cherry et al. 2008; Lichtenstein et al. 2005; Smyth et al. 2007). Furthermore, the lack of association between CD4 counts and viral loads with the development of HIV-SN may be the result of effective cART in our cohort. Lee and colleagues (2015) report that nerve injury, assessed by decreased vibratory loss in their cohort, was greater at pre-ARV therapy CD4 counts < 100 cells/mm^3 (Lee et al. 2015). This finding implies that nerve damage is more probable at very low CD4 counts. Certainly, the association between SN and CD4 counts is equivocal, with some studies reporting higher and others lower CD4 counts associated with SN (Richardson et al. 2009; Aouizerat et al. 2010; Ellis et al. 2010). Indeed, variations in the effect of CD4 counts may arise because the effects of a given count observed during immune recovery (early ART) may be different from the same count recorded in early HIV disease (i.e. never low) or treatment failure (i.e. falling counts).

There are several limitations to our work. One of these limitations remains the use a single screening tool to identify cases of SN (the BPNS ACTG). We relied solely on the use of the ACTG tool to ascertain all SN cases. The use of an additional screening tool in our setting would be beneficial, however the simple, rapid and non-invasive design of the ACTG was an advantage in our setting of a demanding and chaotic chronic clinic situated within the local hospital. Also, the ACTG tool was designed specifically for use by non-clinicians (Cherry et al. 2005), which was suitable in our research setting. Indeed, there is no accepted ‘gold-standard’ for the assessment of HIV-SN and the best option would be to encompass the assessment of both signs and symptoms of this condition which the ACTG tool achieves. A recent study investigating the utility of screening tools administered by non-clinicians and QST administered by trained individuals in the identification of SN, reported that the ACTG tool performed equally well when compared to QST findings (Gautron et al. 2014). Furthermore, for each participant assessed, the ACTG screening tool was repeated on the participant by two individuals (who had both received formal training on the ACTG tool). Therefore, we are confident of all SN cases identified. Certainly, in settings where QST equipment is available for use, we encourage the use of this tool due to its high specificity and sensitivity. However, in limited resource settings and especially in the situation of non-clinicians, the ACTG BPNS tool is a practical and reliable tool.

Another limitation was the sample size of our cohort. We aimed to recruit a sample size of n=150 individuals, although we did not achieve this number of partcipants, our cohort still consists of the largest number of ARV naïve individuals initiating TDF-based cART. Our sample size was a direct result of the down referral of ARV naïve individuals to larger tertiary-based hospitals when they presented to the local clinic (where our recruitment was based) with existing opportunistic infections such as cryptococcal meningitis, pneumonia and Kaposi's' sarcoma. These opportunistic infections are better managed in these large tertiary hospitals, due to the availability of specialist care. Therefore, participants were subsequently initiated onto ARV therapy at these tertiary hospitals and did not return to the local clinics, thereby reducing the amount of ARV naïve individuals available at site for study recruitment. Furthermore, we aimed to retain as many study participants as possible across the six-month period of our study. However, the research site was plagued by several community protests (as described in Chapter 2). Therefore, a number of our study participants were lost to follow-up during these periods of protest action where it was deemed unsafe by local policing authorities, for the study investigators, clinic staff and patients to attend the clinic. We aim to reproduce this work in a larger study cohort, at a larger tertiary facility. We may be able to achieve more controlled conditions for our future work, by approaching a research facility that has already established a 'research network' within a tertiary hospital. This 'research network' often includes reputable and skilled healthcare workers which can facilitate both study recruitment and participant retention within the study. We are also less likely to be affected by sporadic protest action at these larger tertiary hospitals. Because of administrative delays associated with seeking permission to conduct research at South African tertiary hospitals (often periods greater than six-months to approve a research study) and also the lack of 'research space' within these hospitals to conduct the study, we were unable to carry out the current work out at a tertiary hospital and therefore opted for a local clinic setting.

Lastly, our study was carried out in a resource-limited setting, as the clinic was located within 2 km of two large informal settlements. As a result of the, the majority of patients who visited the clinic and hence were recruited to our study were drawn from this population. Certainly, conditions in these informal settings are not pleasant with no access to proper sanitation i.e. toilet facilties and no electricity. We are indeed aware that the personal constraints faced by participnats in our cohort living in these informal settlemnts and 'tin-shacks' may well have contributed to the large loss of follow-up of participants in our cohort.

3.5 Conclusion:

Our findings highlight that HIV-SN (both symptomatic and asymptomatic) persists in the era of TDF-based cART. We report that the incidence of HIV-SN on TDF-based cART is indeed lower than that observed on d4t-based therapies and tended to be more asymptomatic. The development of HIV-SN was significantly associated with increasing height and TB treatment. Whilst, determining an 'at-risk' height is not possible, it is possible to identify who is receiving TB treatment or not and monitor these individuals more carefully for the signs and symptoms suggestive of SN. The development of HIV-SN in our cohort, despite no previous exposure to neurotoxic d4t-based therapy suggests a possible role of inflammatory mediators such as cytokines and chemokines and warrants further investigation into these mechanisms.

Chapter 4

Plasma chemokine profiles in HIV-positive individuals with and without HIV-SN, on TDF-based cART- A pilot study

4.1 Introduction

Chemokines are small chemotactic proteins which form part of the cytokine family (Wang et al., 2017). They are recognised as secondary pro-inflammatory mediators that are stimulated by primary pro-inflammatory mediators such as TNFα or IL-1 (Graves & Jiang 1995; Okuno et al. 2002). During chronic HIV-infection, there is sustained activation of the immune system leading to the elevation of numerous cytokines and chemokines which is often called the 'cytokine-storm' (Wang et al. 2017). This process contributes to HIV disease progression. Several chemokines have been implicated in the development and maintenance of neuropathic pain, which is often associated with HIV-SN. These include, CCL2/CCR2, CX3CL1 (Fractalkine)/CX3CR1, CCL5/CCR5 and CXCL12/CXCR4 (Abbadie et al. 2009; Liou, Lee & Day 2013; Yong-Jing 2010; Biber & Boddeke 2014).

Both CCL2 and CX3CL1 have been implicated in the chemokine modulation of pain via neuronal-glial interactions (Yong-Jing 2010; Abbadie et al. 2009). A recent study also reported that CCL2 in the injured dorsal root ganglion (DRG) can act as autocrine or paracrine signal and stimulate first or second line order neurons in the pain cascade (Biber & Boddeke 2014). Liou and colleagues (2013) reported the association of CCL5 with the pathway that controls hyperalgesia at the level of a peripheral injury site in a murine chronic neuropathic pain model (Liou et al., 2013). Furthermore, administration of CCL5 into the periaqueductal grey (area of the brain involved in pain modulation) caused significant hyperalgesia in rodents (Benamar at al., 2008). The chemokine SDF-1 (CXCL12) and its receptor CXCR4 have also been implicated in a model of in neuropathic pain, where elevated levels of CXCL12/CXCR4 were reported during chronic compression of the DRG in rodents (Yu et al. 2017). Therefore, we chose to investigate the possible role of the above-mentioned chemokines in the development of HIV-SN in our cohort. Understanding the mechanisms involved in chemokine-mediated nerve damage and associtated neuropathic pain, will largely assist with the development of adequate prophylactic and therapeutic medications for this condition.

4.2 Methods

The complete methodology for blood sample collection and procedures is as described in Chapter 2 (Methods: section 2.2).

4.3 Results

4.3.1 Chemokine selection

Based on reports in the literature, we focused on four chemokines, CCL5 (RANTES), CCL2 (MCP-1), CX3CL1 (Fractalkine) and CXCL12 (SDF-1) (Abbadie et al. 2009; Oh et al. 2001; Zheng et al. 2011; Mountford et al. 2017). We measured the levels of these chemokines at four time-points (0, 2, 4, and 6 months) in individuals starting TDF-based therapy and assessed whether they were associated with the development of HIV-SN.

4.3.2 Sample collection

All plasma samples used for the chemokine assays were collected from two study cohorts that were based at the same site (Lawley/Mid-Ennerdale CHC). The first cohort consisted of individuals that were screened and recruited for participation in the project 2 (See Chapter 2- Incidence of HIV-SN on TDF-based cART) and the second cohort consisted of individuals that were screened and recruited for participation in a study involving the analysis of epigenetics in individuals free of neuropathy and individuals that developed painful HIV-SN within six-months of initiating TDF-based therapy. The only difference between the two study cohorts were the objectives, all other aspects of the studies (study inclusion criteria, HIV-SN assessments, blood draws, visit schedules etc.) were the same. Also, although each cohort had a different study investigator (study investigator for cohort one: Prinisha Pillay and study investigator for cohort two: Dr. Gaelle Ngassa), both investigators worked together at the study site. A flow diagram illustrating the sampling collection and retention process is shown in Figure 4.3(a).

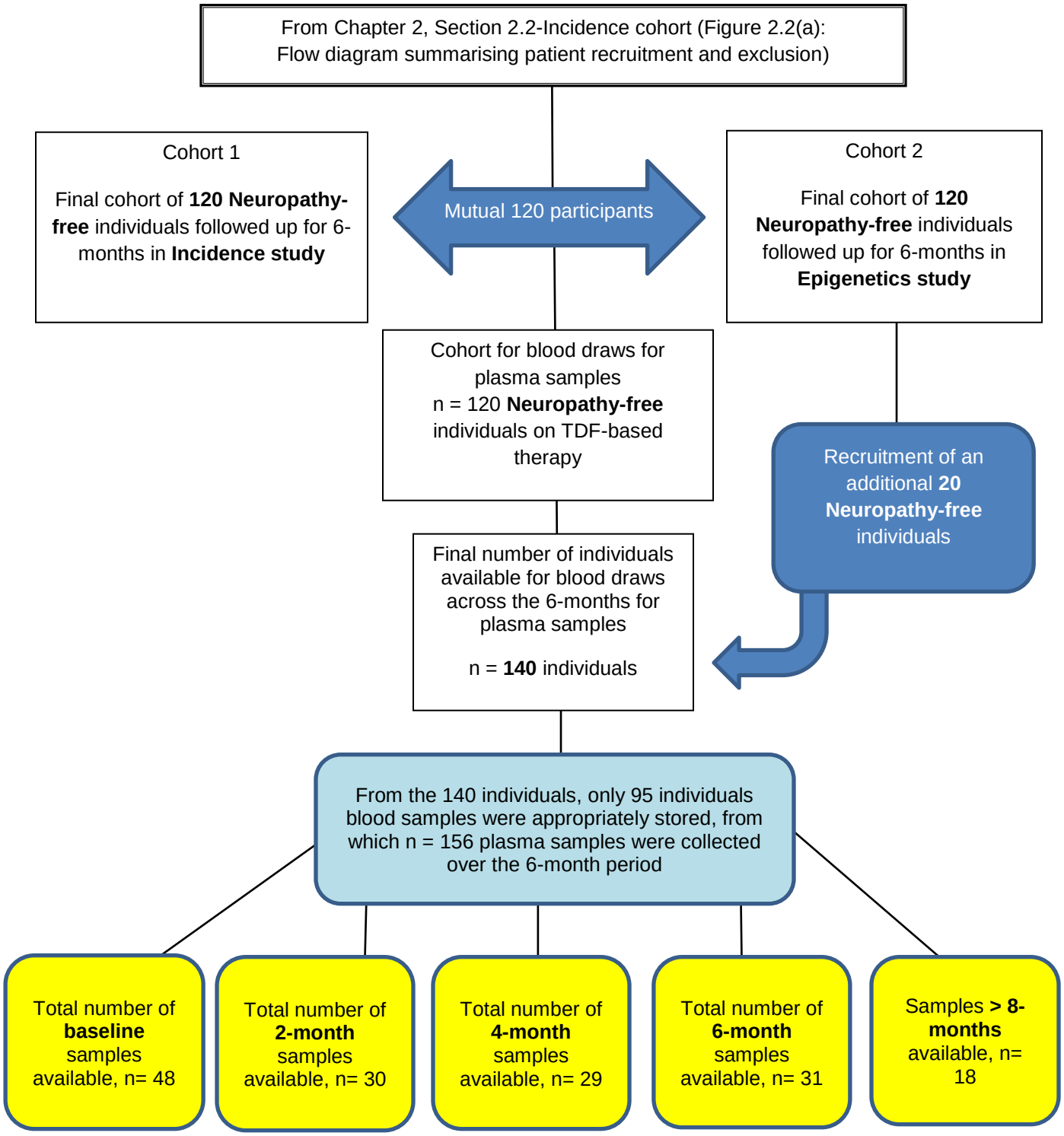


Figure 4.3(a). Flow diagram summarising collection of all plasma samples across the 6-months.

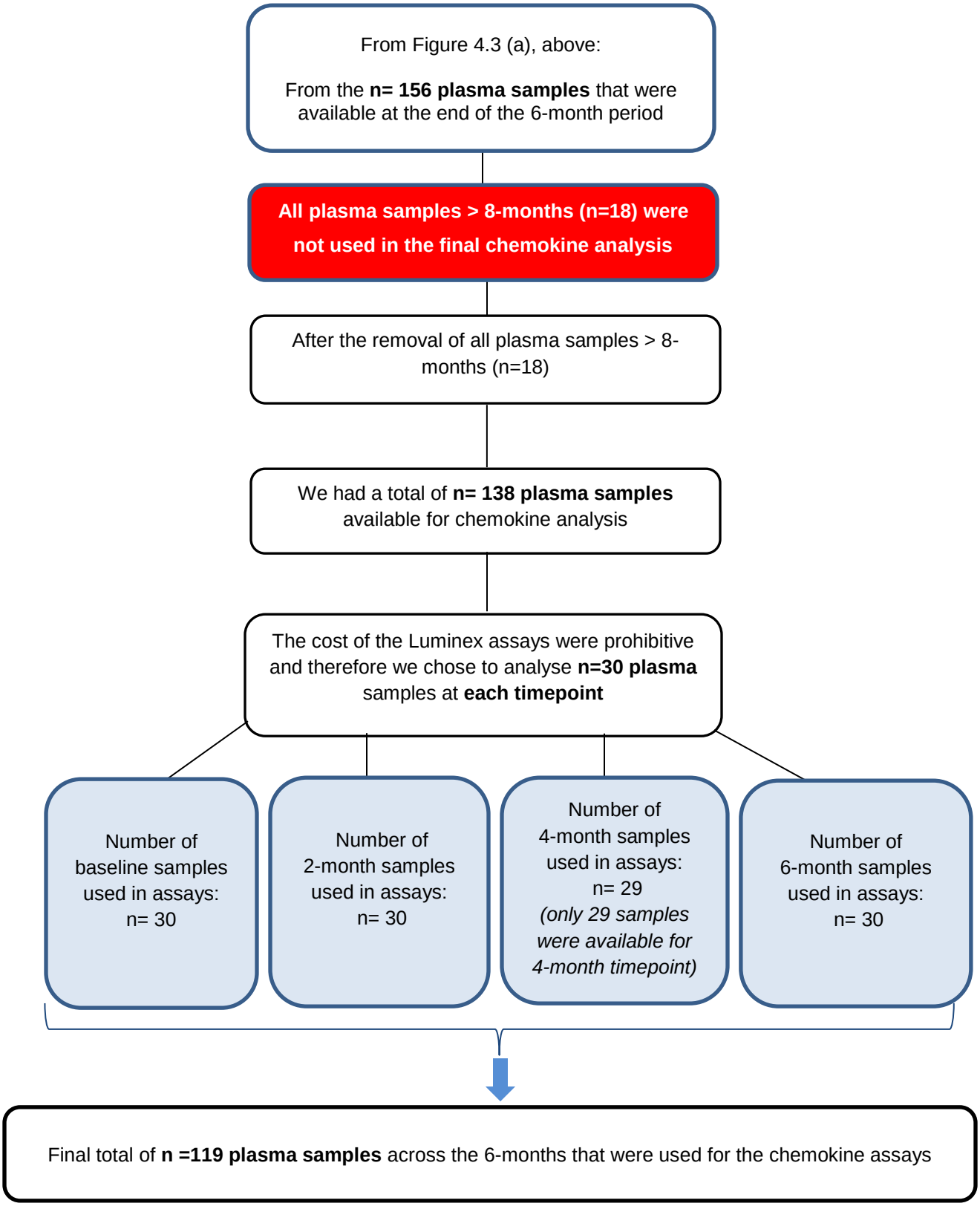


Figure 4.3(b). Flow diagram summarising collection and exclusion of plasma samples across the 6-months.

We aimed to perform chemokine assays on the full cohort of individuals participating in the study (n=140) across the 6-months, at all four timepoints. Therefore, blood samples were collected at each time-point (from baseline to the 6-months visit) and sent for testing and storage at two large academic teaching hospitals in Johannesburg. Regrettably, we faced several issues at these facilities where staff did not store and code study related-blood samples correctly resulting in the loss of the bulk of our blood samples [as described in Figure 2.2 (a)]. Also, because of protests routinely disrupting access to the hospital throughout the study, the sampling intervals could not be standardised (Chapter 2). For this reason, we had to revise our protocol based on the blood samples we had, and only look at trends across broadly defined time periods: [(baseline visit); (visit day > 25 days & visit day < 75 days); (visit day > 75 days & visit day < 150 days) and (Visit day > 150 days), and did not assess within-participant changes over time, but between-participant changes across time periods.

All plasma samples that were used for the chemokine assays were collected as whole blood samples (in EDTA tubes) by nurses at the Lenasia/Lawley CHC at four specific time-points:

a) Baseline (30 samples)
b) 2-months on ARV treatment (30 samples)
c) 4-months on ARV treatment (29 samples)
d) 6-months on ARV treatment (30 samples)

Because of the gross failure of the laboratory services which we used at both the Chris-Hani Baragwanath hospital and the Charlotte Maxeke Johannesburg hospital, we were forced to revise our study protocol regarding blood collection and storage for plasma samples [as described in Chapter 2, Figure 2.2 (a)]. With this revision of the blood storage protocol, instead of sending the blood samples to the laboratory service at the above-mentioned hospitals for storage, we chose to keep the blood samples on ice at the research site and transport them directly (via study the investigator) to the University of the Witwatersrand for the removal of plasma. Therefore, after collection, the whole blood samples were kept on ice at the research site and centrifuged on the same day of collection (by Dr. Ngassa) at the University of the Witwatersrand for the removal of plasma. After centrifuging, all plasma samples were aliquoted into duplicate 2ml collection tubes and stored at -80°c. Samples were aliquoted in duplicate to provide a back-up sample for all assays in the event of any issues during transporting and/or diluting of samples.

4.3.3 Samples available for chemokine assays

A total of n=156 plasma samples were available for the chemokine assays [Figure 4.3(a)]. We excluded n=18 plasma samples (these samples were drawn from participants who were on treatment for > 8-months and thus did not fall within the intended study period) [Figure 4.3(b)].

Therefore, a total of n=138 plasma samples were available for the chemokine analysis. The cost of the Luminex pre-mixed magnetic bead assays were prohibitive, therefore we randomly selected 30 samples to analyse at each time-point for the chemokine assays.

Randomly selecting 30 samples at each time-point allowed us to fit all standards, controls, blanks and samples on each plate as well as duplicate ± 15 samples. Thirty samples were available for each time-point (Baseline, 2-months and 6-months) except for the 4-month time-point, where only 29 samples were available. Therefore, a total of n=119 plasma samples were available for the chemokine assay procedure.

Of the 140 individuals that were available for blood draws across the 6-month study period, only 68% (95/140) individuals blood samples were appropriately stored after collection and could be further processed for plasma removal. From the 95 individuals, n=18 samples were excluded (as mentioned above, individuals on treatment > 8-months). Therefore, the n=119 plasma samples were collected from a total of 77 individuals. This was because of several individuals providing blood samples at one or more of the specified time-points, plasma samples were available for these individuals at more than one of the time-points e.g. at baseline, 4-months and 6-months for the same individual. While other individuals only had a single sample collected at one of the time-points (e.g. at baseline or at 4-months) and therefore plasma samples for these individuals were limited to the specific time-point of collection. Baseline demographic and clinic characteristics of the study cohort is shown in Table 3.1(a). No participants were exposed to stavudine based cART therapy or any other cART regimen that did not contain tenofovir.

Table 4.1(a) Demographic and clinical characteristics of cohort at baseline (n=77).

Characteristic	Entire cohort (n=77)
Female sex, n (%)	57 (74)
Age (years)	38 (12)
Height (m)	1.52 (0.05)
Weight (kg)	69.0 (12)
Current CD4 count (cells/µL)	236 (8-904)
log10 Viral load (copies/mL)	3.02 (1.21-5.5)
Current TB infection, n (%)	11 (14)
Standard units of alcohol consumed	12.0 (5.3-70)
Vitamin B_{12} deficiency, n (%)	-
Initial Vitamin B_{12} level (pmol/L)	278 (148-497)
Hepatitis B infection, n (%)	2 (2)
Current syphilis infection, n (%)	-
Type II diabetes, n (%)	1 (1)
Initial HbA1c percentage	5.4 (4.0-7.0)
Treatment regimen, n (%): *TDF-based* *Non TDF-based*	 100 (100) 0 (0)

Dichotomous variables are presented as number (%), normally distributed continuous variables are presented as mean (standard deviation) and non-normally distributed continuous variables are presented as median (range).

4.3.4 Development of neuropathy

Of the 140 confirmed 'neuropathy-free' patients who were enrolled at baseline for participation, 69% (85/140) patients completed the study (completed at least three visits or more) and exited the study at 6-months. Of these 85 participants (cohort one: n=83 + cohort two: n=2), a total of 24% (20/85) developed HIV-SN. From the 20 individuals that developed HIV-SN, 11% (9/85) developed painful HIV-SN and 13% (11/85) developed non-painful HIV-SN. Eighteen out of the 20 individuals that developed HIV-SN during the 6-month study period provided blood samples from which plasma samples were extracted for the chemokine assays.

4.3.5 Exclusion of CCL5 (RANTES)

Based on the observed concentration values from the assay for the chemokine (CCL5) RANTES, we opted to exclude CCL5 from the chemokine analysis as most values were far outside of the upper limit of the standard curve, indicating that the analyte had not be diluted enough for the assay. The dilution factor used was recommended by the manufacturer of the assay kits, however, for future work a much larger dilution factor for (CCL5) RANTES is required. Furthemore, we did not run a check on dilutions mainly because we were assaying the selected chemokines for the first time, with no solid evidence to guide us on the 'optimal' dilution number, except for the dilution number recommended by the manufacturer of the assay kits . We therefore had to proceed with the chosen dilution. Furthermore, repeating the dilution process would have been ideal to ascertain the 'optimal' dilution, however the cost of the assays was prohibitive and so we had to proceed with the current dilution.

4.3.6 Time intervals for analysis

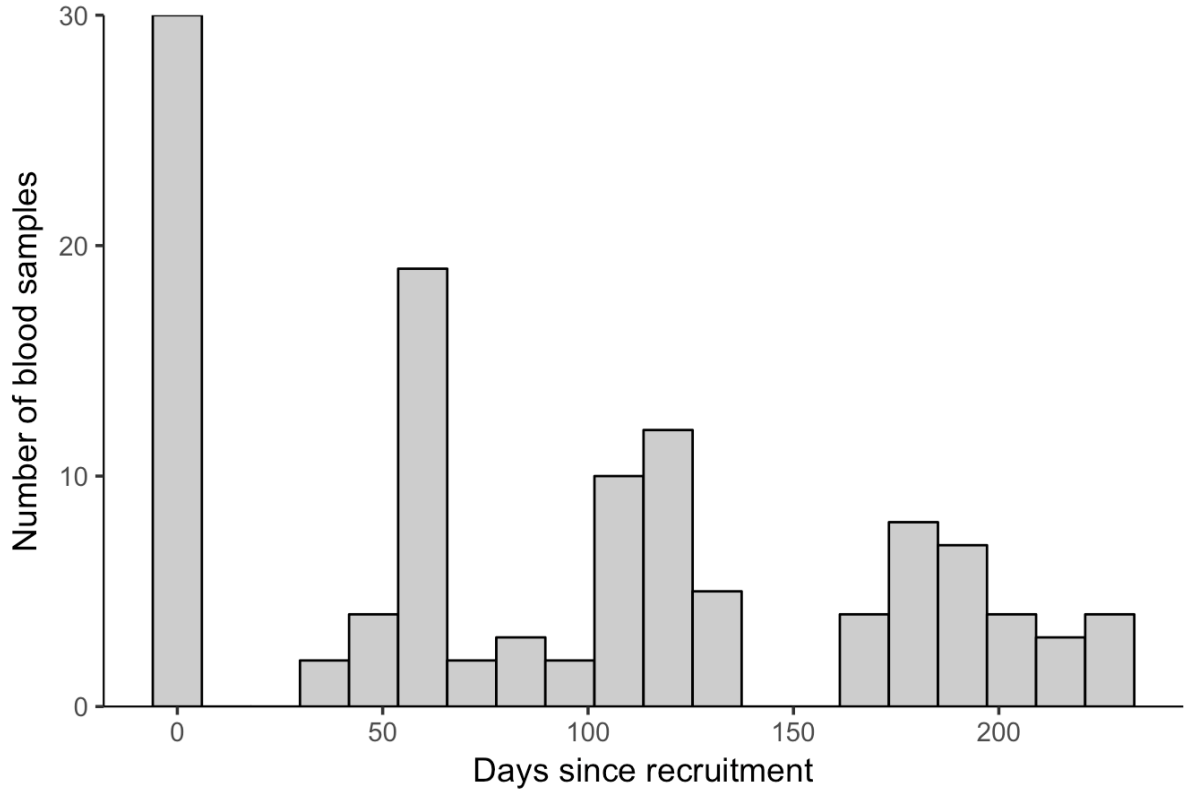


Figure 4.3 (c) Histogram of sample count over time.

As the samples were initially largely categorised into four time-points (based on date of collection), we created four broad time intervals for the final analysis. The four-time intervals included:

a) Period 1 (Baseline: Visit day = 0)
b) Period 2 (Visit day > 25 days & Visit day < 75 days)
c) Period 3 (Visit day > 75 days & Visit day < 150 days)
d) Period 4 (Visit day > 150 days)

4.3.7 Removal of 'multiple' measurements

Since some individuals provided multiple samples and others provided a single sample, we faced an issue of confounding intra- and inter-individual variation if we chose to group all the participants data together. Therefore, to avoid this issue, we chose to randomly select a single measurement from those individuals who provided multiple samples. The sampled measurements were standardised across the chemokines by performing the initial sample selection on the CXCL12 (SDF-1) data, and then selecting the matching samples codes in the other chemokine datasets.

4.3.8 Analysis of CXCL12 (SDF-1), CX3CL1 (Fractalkine) and CCL2 (MCP-1)

Permutation tests were used to test for significance as the data for all three chemokines did not conform to normality. The tests were performed using the *ezPerm* function in the *ez* package for R v3.4.2 (R Core Team 2014). The tests assumed a between-factor design with time and neuropathy status as main effects. P-values were calculated based on 10 000 permutations.

4.3.8.1 CXCL12 (SDF-1)

The concentration of CXCL12 varied significantly over time (main effect of time: $p = 0.015$), with concentrations being greatest over the first two time periods [Figure 3.3(b)]. However, there was no association between the levels of CXCL12 (SDF-1) and the development of HIV-SN (main effect of SN: $p=0.21$). There was no interaction between the two main effects (SN vs time interaction: $p = 0.56$).

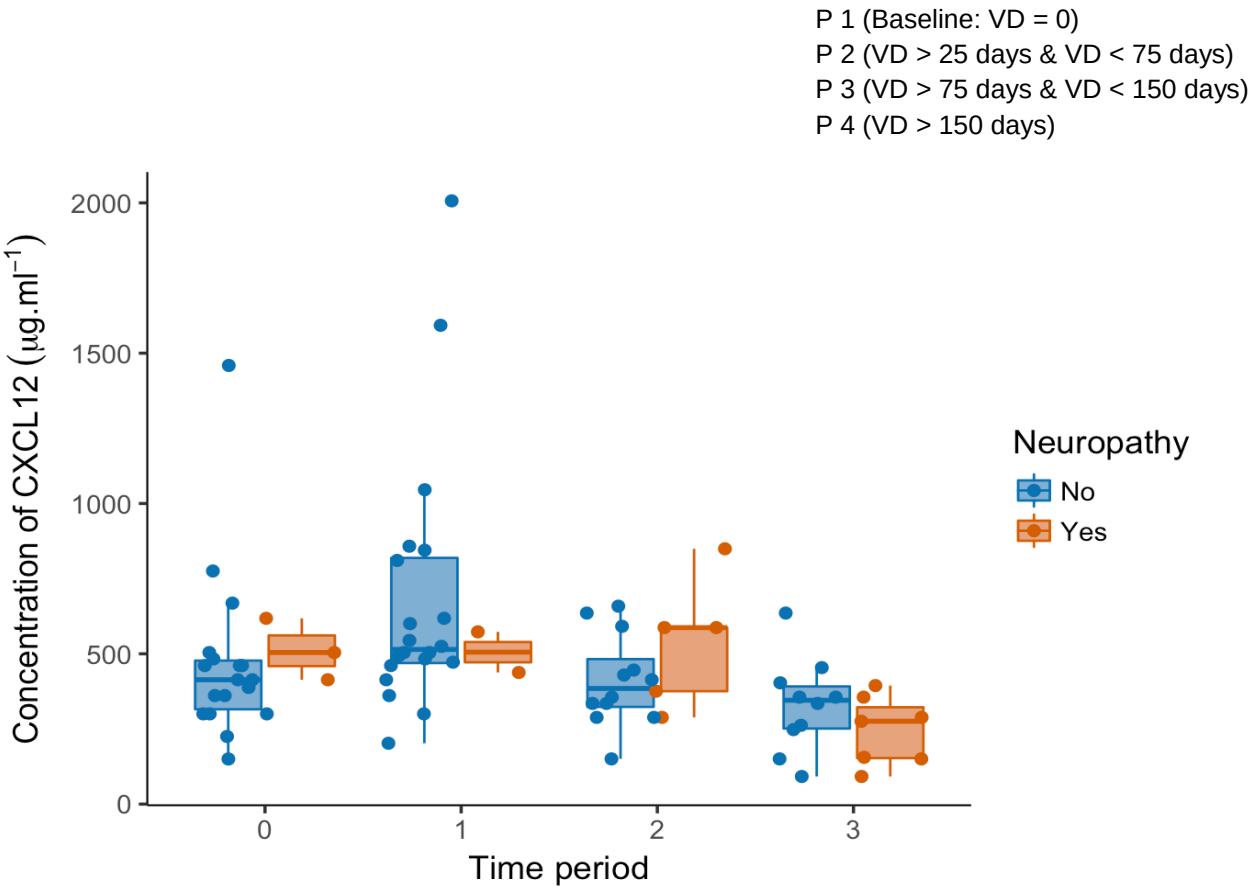


Figure 4.3(d). Plot illustrating the plasma concentration of CXCL12 (SDF-1) (y-axis) in those individuals developing neuropathy and those remaining neuropathy-free across the four-time periods (x-axis). P=Period; VD=Visit day

4.3.8.2 CX3CL1 (Fractalkine)

The concentration of CX3CL1 varied significantly over time (main effect of time: p=0.022), with concentrations being greatest over the first two time periods [Figure 3.3(c)]. However, there was no association between the levels of CX3CL1 (Fractalkine) and the development of HIV-SN (main effect of SN: p=0.90). There was no interaction between the two main effects (SN vs. time interaction: p=0.43).

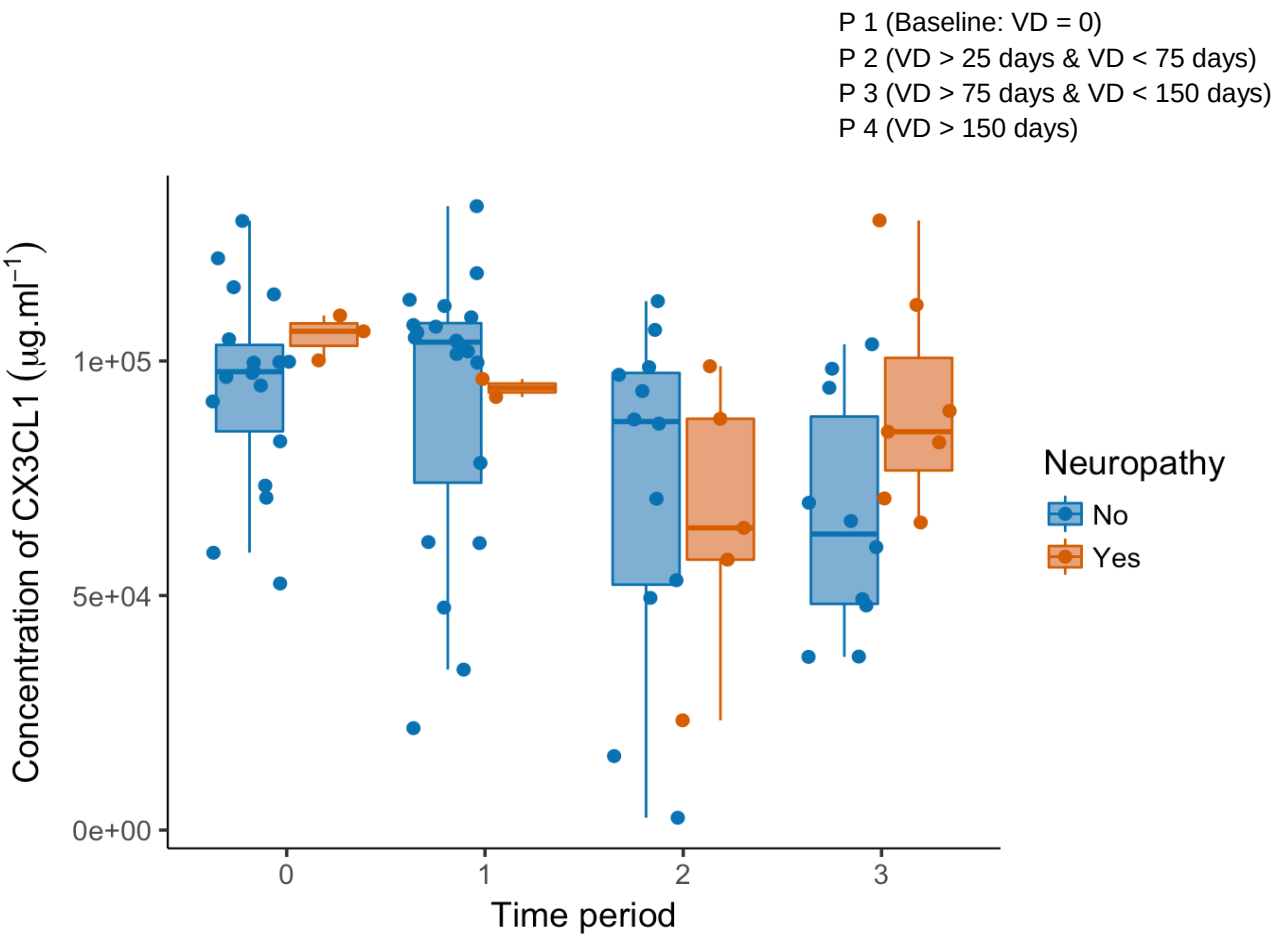


Figure 4.3 (e). Plot illustrating the plasma concentration of CX3CL1 (Fractalkine) (y-axis) in those individuals developing neuropathy and those remaining neuropathy-free across the four-time periods (x-axis). P=Period; VD=Visit day

4.3.8.3 CCL2 (MCP-1)

The concentration of CCL2 remained relatively constant across the time periods [Figure 3.3(d)]. There was no association between the levels of CCL2 (MCP-1) and the development of HIV-SN (main effect of SN: p=0.81). Also, there was no interaction between the two main effects (SN vs time interaction: p = 0.57).

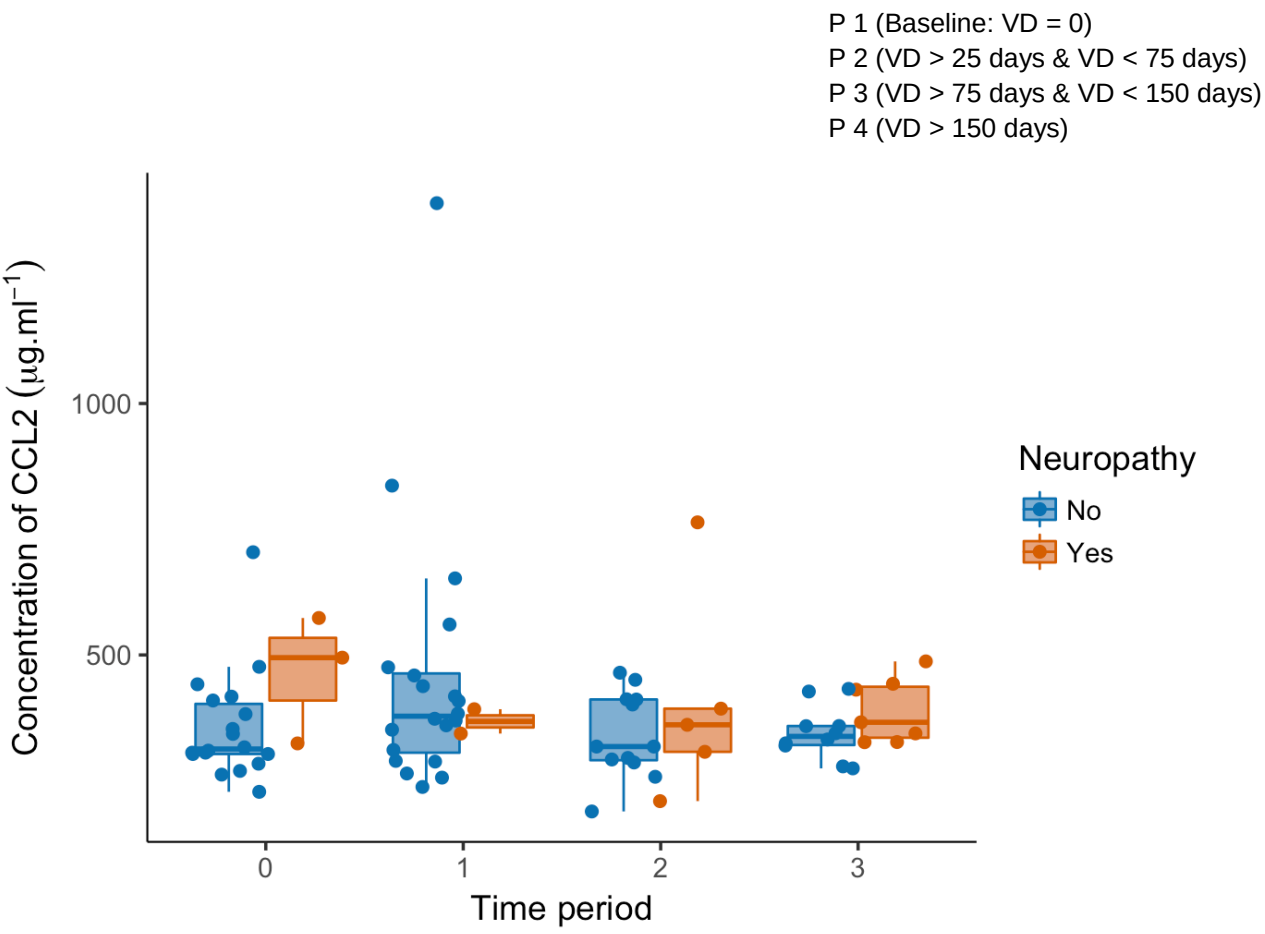


Figure 4.3(f). Plot illustrating the plasma concentration of CCL2 (MCP-1) (y-axis) in those individuals developing neuropathy and those remaining neuropathy-free across the four-time periods (x-axis). P=Period; VD=Visit day

4.4 Discussion:

We investigated the role of CCL2 (MCP-1), CX3CL1 (Fractalkine) and CXCL12 (SDF-1) in the development of HIV-SN in our cohort of South African PLWHA on TDF-based cART for a period of six-months. We did not find any significant associations between the concentrations of the three chemokines across the six-month study period and the development of HIV-SN in our cohort. The concentration of CXCL12 and CX3CL1 varied significantly over time (main effect of time: p=0.015

and p=0.022), respectively. While the concentration of CCL2 remained relatively constant across all the time periods assessed. It is unclear why we did not find any associations between concentrations of the various chemokines and development of SN. Perhaps the lack of association with plasma chemokines observed here could be because chemokines levels in plasma are simply are not critical and the expression of chemokine receptors in affected tissues may well be a more significant area to investigate. Another possibility for the lack of association observed between plasma chemokines and SN, may be the exposure of our cohort to TDF-based cART which may have induced a different mechanism involved in the development of HIV-SN as compared to the rodent ddc-models employed by Bhangoo and colleagues (2007) and Zheng and colleagues (2014). Certainly, it is difficult to compare our results to previous studies as our cohort was solely exposed to TDF-based cART and most studies investigating the role of chemokines in HIV-SN have not been conducted on TDF exposed cohorts (Bhangoo *et al.*, 2007; Abbadie *et al.*, 2009; Wang *et al.,* 2017). Indeed, the recent study by Mountford and colleagues (2017) investigated the expression of chemokine receptors on cells surrounding cutaneous nerves in PLWHA and reported a significant association between the presence of HIV-SN and an upregulation of chemokine receptors (CX3CR1, CCR2 and CCR5) in $CD3^+$ and $CD14^+$ cells in PLWHA (Mountford et al. 2017). The discrepancy in findings observed between our cohort and that of Mountford and colleagues (2017) could be the different populations that samples were drawn from or the fact that Mountford and colleagues (2017) used a completely different assay. Our cohort comprised 100% individuals of African Ancestry which was not the case of Mountford and colleagues (2017) that assessed samples from Indonesian/Asian participants. While polymorphisms in mitochondrial DNA have been associated with HIV-SN risk in individuals on cART (Canter et al. 2010; Hulgan et al. 2005; Kamerman et al. 2012; Kamerman, Wadley & Cherry 2012a), the polymorphisms associated with SN risk were only present in Europeans and not African populations (Mbenda *et al.*, 2017). Indeed, these genetic associations may not suggest different mechanisms but do possibly reflect different regulation of the same mechanisms which could be involved here. Furthermore, the lack of association observed between plasma chemokines and HIV-SN might also be explained by the fact that levels of chemokines in plasma are high in all PLWHA and are simply not limiting to the pathogenesis of HIV-SN. This could also be one of the reasons why we did not find any significant associations.

Coupled with the abnormal immune activation after HIV infection, there is a massive range of chemokines and cytokines which are elevated, which generates what is termed the ‘cytokine storm’. The upregulation of these chemokines and cytokines contribute to HIV disease

progression and mortality (Wang et al. 2017). In the South African setting, where we have the problem of a high HIV and TB co-infection rate (refer to Discussion section of thesis, page 148), the effect on chemokines and cytokines may be quite interesting especially with regards to the TB IRIS syndrome. Interestingly, Mihret and colleagues (2014) found no differences in the chemokine and cytokine profiles of HIV positive patients with tuberculosis before and after TB treatment. They assessed plasma levels of IFN-γ, IL-4, MCP-3, MIP1 and IP-10. They also reported that TB treatment significantly improves Th1 cytokines and chemokine levels in HIV negative patients, but this was not the case in HIV positive individuals (Mihret *et al.* 2014). The findings suggest that anti-TB treatment does not restore the immune response in HIV positive individuals i.e. there is prolonged impairment of the immune response to TB in HIV positive patients. These findings are supported by Kassa and colleagues (2016) who also reported that HIV co-infection decreased levels of IFN-γ, IL-17, IP-10 and IL-2 in patients on TB treatment. Furthermore, the initiation of highly active antiretroviral therapy (HAART) in these individuals did not improve levels of IFN-γ, IL-17, IP-10 and IL-2 (Kassa *et al.* 2016). These findings indicate that even in the face of successful TB treatment, the immune response in PLWHA may remain impaired. These are valuable findings and suggest that further investigation of chemokines and cytokines in individuals who are on concurrent TB and ARV treatments may be highly beneficial with regards to monitoring responses to treatment in these individuals.

We faced several issues with our blood sample collection for the chemokine aspect of this study. Due to wide data collection intervals, we could not ascertain the exact times of SN onset in our cohort and collect our blood samples at the specific time of SN onset. Indeed, Van de Watt and colleagues (2014) described a specific cytokine ‘burst’ which was evident at 2-4 weeks in previously ARV naïve individuals that had commenced ARV therapy. Our initial aim was to collect blood samples at each scheduled visit (Baseline, visit 2 [2 months later], visit 3 [4 months later] and visit 4 [at 6 months]) with the intention of assessing the variation in chemokine concentrations in these individuals across the six-month study period. However, due to the gross mismanagement and improper storage of the bulk of our blood samples by staff at government facilities associated with blood testing at our research site, we were unable to carry out these planned investigations. We were therefore, forced to amend our chemokine protocol and revise our samples available for chemokine assessment.

4.5 Conclusion:

The role of chemokines in the nerve damage associated with HIV-SN is becoming increasingly popular, with the promise of novel therapies to treat the neuropathic pain associated with HIV-SN. Unfortunately, we are unable to add to this knowledge as we found negligible results and the methodological issues preclude us from being confident about the lack of associations observed. Our aim is to repeat part of this work, under more controlled conditions, where we are able to optimise visit timings and proper sample storage to assess whether there is indeed a different pattern of chemokine expression related to TDF-based cART in the South African population.

Chapter 5

Painful and non-painful HIV-associated peripheral sensory neuropathy in HIV-positive individuals on TDF-based combination antiretroviral therapy (cART) – A comparison of psychological factors.

Data presented in this chapter have been published:

PILLAY P, WADLEY AL, CHERRY CL, KARSTAEDT AS, KAMERMAN PR

Psychological factors associated with painful vs. non-painful HIV-associated sensory neuropathy

Journal of AIDS and Behaviour, 2017

And presented at a conference:

PAIN SA Congress, 22-24 May 2015, Misty Hill Conference Centre, Muldersdrift, South Africa

5.1 Introduction

HIV-associated sensory neuropathy (HIV-SN) is a frequent complication of both HIV infection and its treatment, affecting up to 60% of ambulatory PLWHA (Kamerman et al., 2012). Unfortunately, the decrease in incidence and prevalence of the neuropathy that was anticipated with widespread introduction of less neurotoxic antiretroviral drug treatments appears to have been minuscule in Sub-Saharan Africa (Mehta *et al.*, 2011; Arenas-Pinto *et al.*, 2015; Kamerman PR, 2016), in comparison to the reductions reported by recent US cohorts, which are however, equivocal (Ellis et al. 2010; Evans et al. 2011; Lee et al. 2015). Indeed, there are reasons to believe there may be differences between the populations, which include, genetic (Hendry et al. 2015, 2013; Wadley et al. 2014) and behavioural (Wadley *et al*., 2016) differences.

Pain is a common feature of HIV-SN and causes significant physical, psychological, and social dysfunction (e.g., reduced physical independence, increased risk of depression, and increased risk of being unemployed) (Ellis et al. 2010). But, pain is not a universal symptom of the neuropathy, with 20 - 40% of individuals with HIV-SN being asymptomatic or having symptoms other than pain (Ellis *et al.*, 2010; Cherry et al., 2012; Phillips *et al.*, 2014; Arenas-Pinto *et al.*, 2015). Moreover, in individuals who have a painful neuropathy there is significant heterogeneity in the intensity of pain experienced, with patients reporting intensities anywhere between 'slight pain' and 'worst pain ever experienced' (Smyth *et al.*, 2007; Ellis *et al.*, 2010; Maritz *et al.*, 2010a; Wadley *et al.*, 2011). The presence of pain in HIV-SN, and the intensity of that pain have been positively associated with disease-related factors such as greater nadir CD4 T-cell count (Ellis et al. 2010), past exposure to neurotoxic antiretroviral drugs(Ellis et al. 2010), and increased viral load (Simpson et al. 2002). However, neither the presence of pain nor the variability in pain intensity is entirely explained by the extent of the neuropathology, as assessed by intraepidermal nerve fibre density (Simpson et al. 2002; Polydefkis et al. 2002; Zhou et al. 2007) and somatosensory nerve function (Martina et al. 2003; Phillips et al. 2014).

There is, however, growing evidence that psychological factors, including anxiety, depression and pain catastrophizing, are associated with the presence and intensity of pain, clinical outcomes, and quality of life in individuals with chronic pains such as chronic low back pain and arthritis (McWilliams *et al*., 2004; Wertli *et al.*, 2014). Consistent with these findings from other causes of pain, a small study by Lucey and colleagues (Lucey et al. 2011) reported that the intensity of the pain reported by patients with HIV-SN was positively associated with pain catastrophizing. And, in the large, multi-centre CHARTER cohort (https://www.charternntc.org), prevalent and incident painful HIV-SN has been associated with worsening depressive symptoms (Malvar et al. 2015;

Ellis et al. 2010). The presence and intensity of painful HIV-SN was also positively correlated with worse quality of life (Phillips et al. 2014; Keltner et al. 2012), but it appears that the severity of depressive symptoms may be a greater determinant of lower quality of life than is pain intensity (Keltner et al. 2012).

Neuropathic pain is typically chronic and PLWHA with access to medical care have a near-normal life expectancy (Van Sighem et al. 2010; Mills et al. 2011). Therefore, the impact of painful neuropathy on quality of life and long-term well-being of the affected individual, their support network and the healthcare system is substantial. Proven pharmacological agents for the treatment of painful HIV-SN are lacking (Clifford et al. 2012; Phillips et al. 2010). It is therefore particularly important that other, potentially modifiable risk factors for the pain and its adverse effects are identified. Consequently, we recruited a cohort of black HIV-positive South Africans with either painful or non-painful HIV-SN to investigate whether i) psychological factors associate with having painful HIV-SN (primary objective 1), and ii) whether pain and psychological factors affect quality of life in this population (primary objective 2). As a secondary objective, we also completed a sub-group analysis of the painful HIV-SN group only to determine whether psychological factors predict the intensity of their neuropathic pain (secondary objective 1).

5.2 Methods:

The complete methodology for patient recruitment, neurological assessment, questionnaires and procedures is as described in Chapter 2 (Methods, section 2.3). All patients currently on TDF-based combination antiretroviral treatment and who presented with a confirmed painful or non-painful distal symmetrical polyneuropathy were included in this analysis. The majority of participants spoke isiZulu as their first language [44%, (87/197)], followed by isiXhosa [18%, (36/197)]. Recruitment and screening of the sample of 197 participants is shown in Figure 3.1.

I reviewed all participant's medical records and clinic notes. Missing or incomplete information was supplemented by accessing the clinics computer records for patients (updated according to file number and day of visit). With the aid of the on-site study interpreter who was fluent in local South African languages, participants completed all study questionnaires in the language version of their choice (English, isiZulu, Sesotho and Setswana).

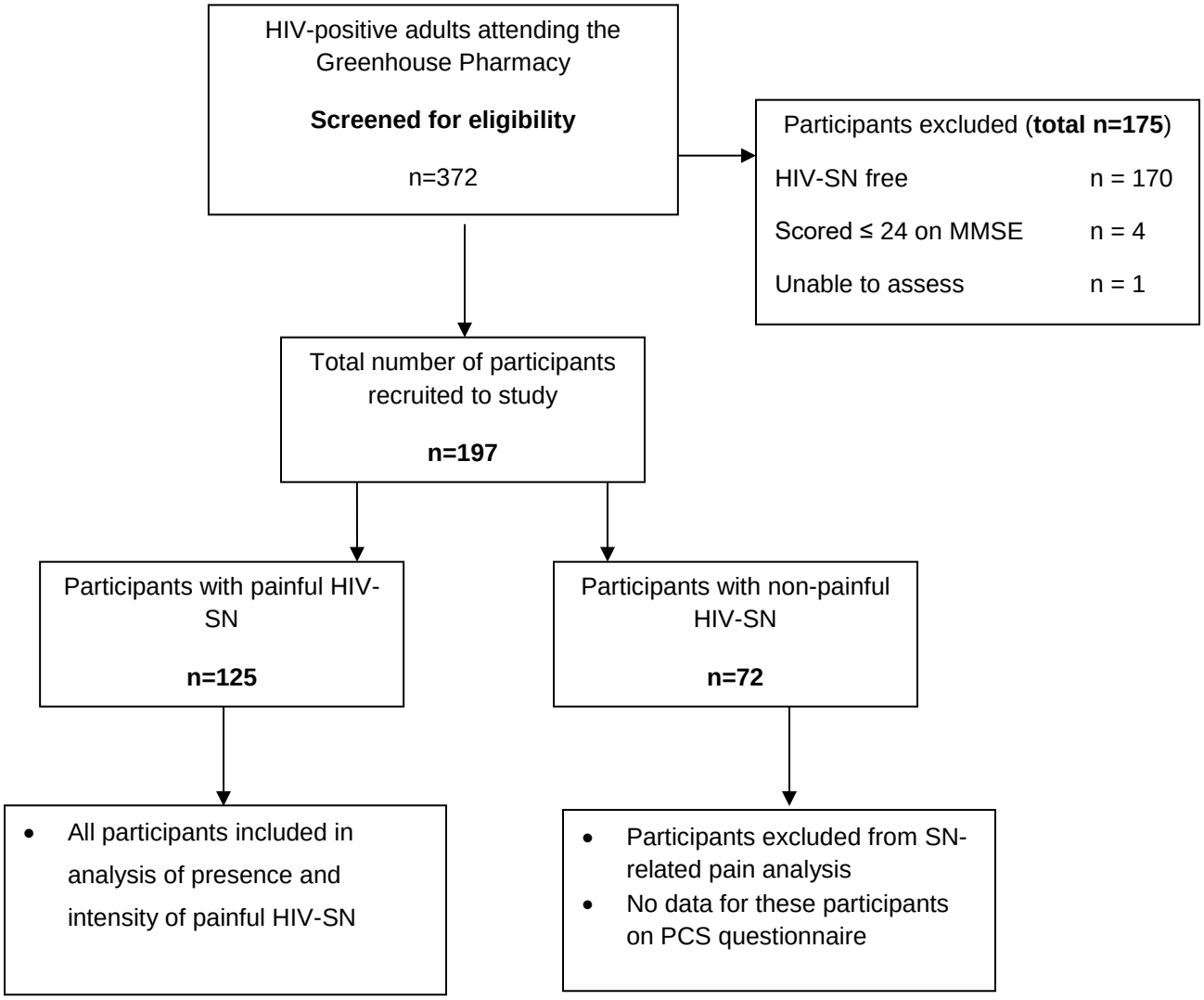


Figure 5.1 Recruitment and exclusion of study participants

5.3 Results

One hundred and twenty-five (125/197) patients with painful HIV-SN and seventy-two (72/197) patients with non-painful HIV-SN were recruited. Of the 197 patients, 53% (105/197) completed the English version of the questionnaire set, 25% (49/197) completed the isiZulu version, 11% (22/197) completed the isiXhosa version, 7% (14/197) completed the Sesotho version and 4% (7/197) completed the Setswana version.

Factors that associated with having painful HIV-SN (primary objective 1)

Univariate analyses

Physical and clinical characteristics of the predominantly female, middle-aged cohort are shown in Table 3.1. All the participants were currently on antiretroviral therapy and had well preserved CD4 T-cell counts. The painful HIV-SN group had, on average, lower median current CD4 T-cell count, a shorter time since HIV diagnosis at their assessment for this study, and a lower prevalence of vitamin B_{12} deficiency compared with the pain free HIV-SN group. Among participants with painful HIV-SN, 85% (106/125) reported their pain as “moderate” to “severe” in intensity [11-point Numerical Pain Rating Scale score (NRS): 4 to 10], with a median pain intensity of 7 (IQR 1-10) out of 10. All participants with painful HIV-SN presented with at least one additional pain site (pains outside the distribution of HIV-SN) compared with only a quarter of patients in the non-painful HIV-SN group. Moreover, patients in the painful HIV-SN group were more likely than the non-painful HIV-SN group to report the pain at these other sites as “moderate” to “severe”. Participants in the painful HIV-SN group were more likely to report experiencing numbness and/or paraesthesias in the distribution of their neuropathy than were individuals in the non-painful HIV-SN group but the severity of these other symptoms, when present, did not differ between the two groups.

Psychological and demographic factors (sex, education, and depressive and anxiety-like symptoms) for the painful and non-painful HIV-SN groups are presented in Table 3.2. There were no significant differences between the two groups in the proportion of females (~75%) or years of education completed (~6 years). A similarly high proportion of patients in the painful HIV-SN (88%, 110/125) and non-painful HIV-SN (93%, 67/72) groups reported a clinically relevant burden of depressive and anxiety-like symptoms (>1.75 on each of the HSCL-25 sub-scales). Consistent with this finding, ~60% of participants in both groups reported “moderate” or “severe” depression or anxiety on the EQ-5D-3L (Table 3.3).

Table 5.1 Participant physical and clinical characteristics

Characteristic	Entire cohort (n=197)	Painful SN (n=125)	Non-painful SN (n=72)	p-value
Age in years	43.2 (12.1)	42.4 (11.3)	44.6 (13.4)	0.23
Initial CD4 T-cell count	142 (94-142)	137.5 (92-231)$^{(n=114)}$	149 (96-221.5)$^{(n=64)}$	0.59
Current CD4 T-cell count	424 (256-674)	405 (227-674)$^{(n=125)}$	463.5 (352-651)$^{(n=72)}$	0.03
Time since HIV diagnosis	120 (72-162)	108 (60-144)$^{(n=104)}$	144 (120-186)$^{(n=55)}$	<0.0001
Height in cm	163.3 (8.8)	162.4 (8.5)$^{(n=115)}$	164.8 (9.2)$^{(n=67)}$	0.09
Weight in kg	71.7 (19.8)	73.5 (22.0)	68.6 (14.5)	0.13
Current TB infection	26 (13)	14 (11)	12 (17)	0.28
Standard units of alcohol consumed	13.5 (2-42)	12.2 (2-29.7)$^{(n=32)}$	13.5 (6-42)$^{(n=25)}$	0.29
Vitamin B_{12} deficiency	25 (13)	11 (9)	14 (19)	0.04
Hepatitis B infection	15 (7)	9 (7)	6 (8)	0.78
Type II diabetes	10 (5)	4 (3)	6 (8)	0.17
Treatment regimen				0.78
Non D4T-based	166 (84)	106 (85)	60 (83)	
D4T-based	24 (12)	14 (11)	10 (14)	
Other	7 (3)	5 (4)	2 (3)	
Number of patients with other pain sites	143 (73)	125 (100)	18 (25)	<0.0001
Number of other pain sites	3 (1-9)	3 (1-9)	2 (1-4)	0.02

Table 5.1 (continued)

Intensity of other pains				0.02
Mild	23 (11)	19 (15)	4 (6)	
Moderate	63 (32)	51 (41)	12 (17)	
Severe	57 (29)	55 (44)	2 (3)	
Prevalence of symptoms	116 (59)	106 (85)	10 (14)	<0.001
Pins and needles	115 (58)	102 (82)	13 (18)	<0.001
Numbness				
Intensity of symptoms (0-10 NRS score)				
Pins and needles	5 (1-10)$^{(n=106)}$	5 (1-10)$^{(n=98)}$	4.5 (2-10)$^{(n=8)}$	0.13
Numbness	6 (1-10)($^{n=102}$)	6 (1-10)$^{(n=90)}$	5 (2-10)$^{(n=12)}$	0.43

Parametric data shown as mean (standard deviation) and compared with painful SN status using an unpaired t test. Non-parametric data are shown as median (Interquartile range) and compared with painful SN status using a Mann-Whitney two-sample statistic. Dichotomous variables are shown as number (%) and compared with painful SN status using the Fishers exact test. Categorical data are also shown as number (%) and compared with painful SN status using the Chi_2 test. Numerical pain rating scale scores are shown as median (Interquartile range).

Table 5.2 Demographic and psychological characteristics

Characteristic	**Entire cohort (n=197)**	**Painful SN (n=125)**	**Non-painful SN (n=72)**	**p-value**
Female sex	149 (76)	94 (75)	55 (76)	1.00
Formal education in years	6 (0-10)	6 (0-10)	7 (0-10)	0.13
HSCL-25 (average sub-scale score)				
Anxiety symptoms	2.60 (2.1-3.4)	2.60 (2.1-3.4)	2.60 (1.0-4.0)	0.97
Depressive symptoms	2.53 (2.0-3.1)	2.53 (2.0-3.1)	2.53 (1.0-3.8)	0.70
PCS[b]	-	31.2 (14)	-	-

Non-parametric data are shown as median (Interquartile range) and compared with painful SN status using a Mann-Whitney two-sample statistic. Parametric data shown as mean (standard deviation). Dichotomous variables are shown as number (%) and compared with painful SN status using the Fishers exact test.

Table 5.3. Summary of quality of life outcomes from the EQ-5D questionnaire for painful and non-painful SN.

Characteristic	Entire cohort (n=197)	Painful SN (n=125)	Non-painful SN (n=72)	p-value
EQ-5D-3L				
Mobility (MO):				0.002
no problems	165 (84)	96 (77)	69 (96)	
moderate problems	27 (14)	24 (19)	3 (4)	
severe problems	5 (2)	5 (4)	0	
Self-Care (SC):				0.22
no problems	183 (93)	114 (92)	69 (96)	
moderate problems	14 (7)	11 (8)	3 (4)	
severe problems	0	0	0	
Usual Activities (UA):				0.03
no problems	170 (86)	102 (82)	68 (94)	
moderate problems	26 (13)	22 (18)	4 (6)	
severe problems	1 (1)	1 (1)	0	
Pain/Discomfort (PD):				<0.0001
no problems	89 (45)	31 (25)	58 (81)	
moderate problems	74 (38)	62 (50)	12 (17)	
severe problems	34 (17)	32 (25)	2 (3)	
Anxiety/Depression (AD):				0.65
no problems	79 (40)	51 (41)	28 (39)	
moderate problems	80 (41)	48 (38)	32 (44)	
severe problems	38 (19)	26 (21)	12 (17)	
EQ-5D-3L (VAS)	80 (50-100)	65 (40-80)	100 (50-100)	<0.0001

Non-parametric data are shown as median (Interquartile range) and compared with painful SN status using a Mann-Whitney two-sample statistic. Categorical data are shown as number (%) and compared with painful SN status using the Chi_2 test.

Multivariate analyses

Presence of additional pains outside the distribution of HIV-SN [Odds Ratio (OR)=3.47, 95% Confidence Interval (CI): $6.7x10^{-29}$ to 1.78, p=0.63)], time since HIV diagnosis (OR=2.02, 95% CI: 0.08 to 0.49, p<0.001) and body mass [OR=1.89, 95% CI: 0.76 to 4.64, p=0.16)] were retained in the final logistic regression model of predictors for having painful HIV-SN (Table 3.4). The model had good predictive value (R^2=0.76, Somers D = 0.87, area under the receiver operating characteristic curve = 0.93). However, only time since HIV diagnosis was independently associated with painful HIV-SN. A longer time since HIV diagnosis associated with a reduced likelihood of having pain.

Table 5.4 Final logistic regression model of predictors for having painful versus non-painful HIV-SN

Formula: SN pain status ~ other pain sites + time since HIV diagnosis+ mass *Complete sets of observations: 151 (96 with pain, 55 without pain)*				
	Coefficient	**Standard error**	**Wald Z**	**p-value**
Intercept	*-10.66*	*26.73*	*-0.40*	*0.69*
Other pain sites (yes)	*12.57*	*26.68*	*0.47*	*0.63*
Time since HIV diagnosis	*-0.017*	*0.004*	*-3.52*	*<0.001*
Mass	*0.033*	*0.023*	*1.39*	*0.16*

Factors associated with quality of life (primary objective 2)

Univariate analyses

Data from the EQ-5D-3L are reported in Table 3.3. When examining scores from the five subscales of the questionnaire, there was no difference between the painful and non-painful groups for the depression/anxiety subscale, but there were differences for the three remaining EQ-5D-3L items, with a greater proportion of participants with painful HIV-SN reporting having "moderate" or "severe" problems with mobility (p=0.002), "moderate" problems with their usual activities (p=0.03), and, as anticipated, "moderate" or "severe" pain and discomfort (p<0.0001). Consistent with these three EQ-5D-3L items, results from the EQ-5D-3L VAS differed between the two groups; painful HIV-SN participants had a lower median VAS score for their perceived state of health (65 IQR: 0-100 vs. 100 IQR: 50-100). We also measured pain catastrophizing in the patients with painful HIV-SN. The mean PCS score for the painful HIV-SN group was 31.2 with a standard deviation of 13.8, where a score >30 indicates a clinically relevant level of catastrophizing (Sullivan, Bishop & Pivik 1995).

In the full cohort, Hopkins depression mean score (r=-0.26, p<0.002), Hopkins anxiety mean score (r=-0.17, p=0.01) and intensity of pains outside the distribution of HIV-SN (r=-0.54, p<0.001) all correlated with quality of life VAS scores. Other factors considered, including age (r= 0.02, p=0.78), level of education (r=-0.12, p=0.07) and presence of pains outside the distribution of HIV-SN (r=-0.23, p=0.21) did not correlate with quality of life VAS scores on univariate analysis.

Within the painful HIV-SN group, Hopkins depression mean score (*r*=-0.34, p<0.001), Hopkins anxiety mean score (*r*=-0.25, p=0.004), PCS mean score (r=-0.29, p=0.001), presence of pains outside the distribution of HIV-SN (*r*=-0.23, p=0.008) and intensity of other pains (*r*=-0.41, p<0.001) all significantly correlated with quality of life VAS scores. Other predictors including age (r= 0.06, p=0.47) and level of education (r= 0.03, p=0.72) did not correlate with quality of life VAS scores on univariate analysis.

Multivariate analyses

Using general additive model regression analysis, we identified that having painful HIV-SN (mu coefficient = -0.91, t-value = -4.80, p < 0.001) and greater HSCL-25 depression scale scores (mu coefficient = -0.33, t-value = -3.14, p = 0.002) predicted lower EQ-5D-3L quality of life VAS score (Table 5.5). And in the painful HIV-SN cohort, greater HSCL-25 depression subscale scores were associated with lower EQ-5D-3L VAS score (mu coefficient = -0.35, t-value = -2.90, p = 0.004) (Table 5.6).

Table 5.5 Final general additive model of predictors of quality of life across the full cohort (painful and non-painful HIV-SN)

	Coefficients			
	Estimate	**Standard error**	**t-value**	**P-value**
Mean (μ) subscale				
Intercept	2.02	0.34	5.95	< 0.001
Painful SN (yes)	-0.91	0.19	-4.80	< 0.001
HSCL-25 depression score	-0.33	0.11	-3.14	0.002
Variance (σ) subscale				
Intercept	-0.32	0.08	-3.93	< 0.001

Table 5.6. Final general additive model of predictors of quality of life in patients with painful HIV-SN only

	Coefficients		t-value	P-value
	Estimate	Standard error		
Mean (μ) subscale				
Intercept	1.16	0.34	3.37	< 0.001
HSCL-25 depression score	-0.35	0.12	-2.90	0.004
Variance (σ) subscale				
Intercept	-0.23	0.09	-2.55	0.01

Factors that associated with increased pain intensity (secondary analysis 1)

Univariate analyses

On univariate analysis for associations with pain intensity, Hopkins depression mean score (r=0.21, p=0.01), presence of pains outside the distribution of HIV-SN (r=0.18, p=0.04) and intensity of pains outside the distribution of HIV-SN (r=0.22, p=0.01) all correlated with intensity of HIV-SN related pain. Other psychological factors, including: sex, education, PCS score, EQ-5D VAS score and Hopkins mean anxiety score did not correlate with pain intensity in this cohort (all p>0.05).

Multivariate analyses

Independent predictors of increasing pain intensity included increasing levels of depression (OR=1.95, 95% CI: 1.14 to 3.33, p=0.01), additional pains outside the distribution of HIV-SN (OR=1.40, 95% CI: 1.00 to 1.97, p=0.04) and being treated for TB infection (OR=0.33, 95% CI: 0.12 to 0.94, p=0.03) (Table 5.7). However, the model had poor predictive value (R^2=0.09, Somer's D = 0.25, area under the ROC curve=0.63).

Table 5.7 Multivarite supplementray results of the final ordinal logistic regression model of predictors of pain intensity in patients who presented with painful HIV-SN.

Formula: pain intensity ~ HSCL-25 depression + Additional pain sites + TB treatment *Complete sets of observations: 125*				
	Coefficient	**Standard error**	**Wald Z**	**P-value**
y≥2	2.3187	0.9708	2.39	0.0169
y≥3	0.6385	0.7365	0.87	0.386
y≥4	-0.1217	0.7097	-0.17	0.8638
y≥5	-0.4328	0.708	-0.61	0.541
y≥6	-1.4878	0.7187	-2.07	0.0384
y≥7	-1.7777	0.7257	-2.45	0.0143
y≥8	-1.995	0.7332	-2.72	0.0065
y≥9	-2.3548	0.7454	-3.16	0.0016
y≥10	-2.4618	0.7484	-3.29	0.001
HSCL-25 depression	0.5919	0.2420	0.0144	0.0144
Additional pain sites (yes)	0.1711	0.0867	0.0485	0.0485
TB treatment (yes)	-1.0802	0.5207	-2.07	0.038

1.2 Discussion

We recruited individuals with painful or non-painful HIV-SN to determine: 1) if psychological factors associate with having painful HIV-SN, 2) if pain and psychological factors affect quality of life, and 3) if psychological factors predict the intensity of their pain. We report that in this black, African cohort of PLWHA that: 1) depression, anxiety and pain catastrophizing did not associate with the presence of painful HIV-SN, 2) increased depressive symptoms and presence (but not intensity) of pain independently associated with reduced quality of life, and 3) greater depressive symptoms independently associated with increased pain intensity in patients with painful HIV-SN.

Factors associated with having a painful rather than non-painful HIV-SN

We found that a shorter time since HIV diagnosis was the only independent association with having painful HIV-SN in this cohort. This finding could be a consequence of the natural history of painful HIV-SN (i.e., progression from painful to non-painful), but this suggestion is speculative as the natural history of the neuropathy has not been properly described and the available evidence indicates that the symptoms are progressive (Hung et al. 2008). Also, although current CD4 T-cell count was lower in the painful HIV-SN group on univariate analysis, it was not retained as a predictor in the final multivariate model. Thus, we do not believe that the time since HIV diagnosis is merely an indirect measure of more recent CD4 T-cell count recovery and viral elimination. Further investigations into this finding are required.

Having additional pains outside the distribution of HIV-SN was retained in the final model of associations with painful HIV-SN, however this was not an independent risk factor (p=0.63). As our study was cross-sectional, we cannot comment on the order in which additional pains outside the distribution of HIV-SN and painful SN occurred. However, the data do suggest that those with painful HIV-SN may be predisposed to having pain of any sort. Indeed, it may be that these individuals are generally more sensitive/susceptible to pain and other symptoms, including numbness and paraesthesias. The presence of multiple pain sites could also be indicative of central sensitisation of the central nervous system (Latremoliere & Woolf 2009). Nevertheless, our data provide clear evidence of a high burden of pain among individuals with HIV-SN. Eighty-five percent (85%) of those in the painful HIV-SN group reported that the pain of their neuropathy was moderate to severe in intensity. Furthermore, all 125 individuals in the painful HIV-SN group had at least one additional pain site, and these sites were on average also rated as moderate to severe in intensity. Thus, painful HIV-SN was just one part of a larger pain burden in this group.

Reports of associations between CD4 T-cell count and pain are mixed. Like some cohorts we found a lower current CD4 T-cell count associated with having a painful SN (Aouizerat et al. 2010; Richardson et al. 2009), but Ellis and colleagues reported a significant association between higher nadir CD4 count [Median (IQR): 150 (50-255)] and presence of painful SN (Ellis et al. 2010). In our case, as we previously reported (Pillay et al. 2015) (Appendix 6), it is possible that patients in this clinic setting perceive painful sensory neuropathy to be a complication of cART. Fear of drug-associated harm may lead to reduced adherence with cART and consequently impaired HIV control. With few published studies specifically addressing associations with pain in HIV-SN, the role of CD4 count remains unclear.

Depression and anxiety did not associate with having a painful SN in our cohort, unlike previous studies(Uebelacker & Bailey 2015; Phillips et al. 2014; Ellis et al. 2010). Indeed, we found a similarly high burden of depressive and anxiety-like symptoms in those with HIV-SN, regardless of whether pain was present. The use of different tools to screen for depression by us (HSCL-25) and other investigators (Becks Depression Inventory (Ellis et al. 2010; Keltner et al. 2012; Lucey et al. 2011); and Centre for Epidemiologic Studies Depression Scale [CES-D] (Uebelacker & Bailey 2015) prevents direct comparisons of the scores reported across studies. Overall, however, we found 93% of the non-painful and 88% of the painful HIV-SN group had clinically relevant levels of depressive symptoms, compared with13% of non-painful and 20% of the painful HIV-SN group in the study by Ellis and colleagues (Ellis et al. 2010). The high level of depressive symptoms in our cohort is not remarkable given the high levels of depression reported in the population from which our study cohort was drawn (Freeman *et al.*, 2008; Thom, 2008; Jonsson *et al.*, 2011). Certainly, only by conducting our research in a unique setting have we been able to demonstrate that high levels of background depression in the community may erode the often reported link between depression and chronic pain (Dworkin and Gitlin, 1991; Blackburn-Munro and Blackburn-Munro, 2001; Holmes et al., 2013).

Effect of pain and psychological factors on quality of life

As in other studies, patients with painful HIV-SN had a lower overall rating of quality of life than SN patients without pain (Ellis et al. 2010; Keltner et al. 2012; Phillips et al. 2014). Analysis of subscale data from the EQ-5D-3L also identified that patients with painful HIV-SN were more likely to have problems with their mobility and usual activities, as expected.

In multivariate analyses we found depressive symptoms and presence of pain associated with reduced quality of life. These data fit with reports from other investigators, where coincident pain and depression have associated with reduced quality of life in HIV-positive patients (Mwesiga et al. 2015; Miaskowski et al. 2011). Of note, we found that the presence of pain, but not its intensity associated with reduced quality of life. Indeed, this is not the first time that factors other than the intensity of pain have associated more strongly with quality of life. In US patients with HIV-SN, depression affected quality of life more than pain intensity (Keltner et al. 2012). Neuropathic pain has associated with reduced quality of life in individuals with HIV-SN (Lucey et al. 2011; Phillips et al. 2014; Keltner et al. 2012; Ellis et al. 2010). Severe neuropathic pain was associated with reduced quality of life and pain function scores as well as higher pain interference scores (Ellis et al. 2010; Phillips et al. 2014). However, Ellis and colleagues also found that mild neuropathic pain

associated with a profoundly reduced quality of life (Ellis et al. 2010). Similarly, high levels of depressive symptoms and the high pain burden (neuropathic pain and the presence of additional pains) observed in our cohort may have made patients more sensitive to any pain, regardless of its intensity. This may explain why we did not find any association between pain intensity and quality of life but rather an association between the presence of pain and reduced quality of life. Indeed, socioeconomic and sociocultural factors may also have a huge effect on a patient's quality of life (Refer to Discussion section of thesis, section 6.4 Psychological factors associated with painful and non-painful HIV-SN, last paragraph).

Effect of psychological factors on pain intensity

Although, depressive symptoms did not associate with having a painful SN, they did associate with pain intensity. These data fit with other reports that higher depression scores associate with increased pain intensity in US HIV- positive patients (Richardson et al. 2009; Miaskowski et al. 2011).

Anxiety level did not associate with pain intensity here. Anxiety has not been assessed in relation to intensity of pain in HIV-SN previously, but greater anxiety has been associated with greater pain intensity in arthritis, migraine and back pain in US cohorts (McWilliams et al., 2004).

Having additional pains outside the distribution of HIV-SN was independently associated with greater pain intensity in this cohort. This may be suggestive of a spatial summation effect or may suggest that depression and anxiety (also associated with pain intensity) worsen with an increased load of chronic conditions. Indeed, the observed association between depressive symptom burden and pain burden intimates possible dysfunction in brainstem serotonergic and noradrenergic pathways and/or diffuse noxious inhibitory control system (Ossipov et al., 2014).

Catastrophizing has associated with pain intensity in other chronic pain conditions (Quartana et al., 2009) including intensity of HIV-SN pain (Lucey et al. 2011; Phillips et al. 2014) but did not associate with pain intensity here. Levels of pain catastrophizing were very high in this cohort: the mean (SD) PCS score was 31.2 (13.8) vs. 19.6 (13.7) in Lucey's U.S cohort (Lucey et al. 2011) and a score >30 indicates a clinically relevant level of catastrophizing (Sullivan et al., 1995). Thus, similar to our findings for depression, universally high prevalence of pain catastrophizing in this population confounds the detection of an association between catastrophizing and pain.

The limitations of the study include that it was a cross-sectional based study. Therefore, although this provided a good representation of the participants attending the clinic daily, we had to rely on current medical information provided in the patients file to assess risk factors for painful and non-painful HIV-SN rather than collect this data prospectively. This also influenced the amount of data available for analysis of each risk factor. However, we did access the clinics main PC to obtain any additional information missing from the patient's own clinic file. Furthermore, due to the cross-sectional nature of the study we were unable to follow patients prospectively to investigate possible development of painful HIV-SN in the later months following the initial screening where they may have presented with a non-painful neuropathy. However, the onsite translator advised all patients to make the senior clinician or myself (for the duration of the study, when we were present at site) aware should pain develop in the distribution of the neuropathy.

5.5 Conclusion:

In conclusion, in this cohort of black African PLWHA attending a public healthcare facility, I found a significantly high pain burden in patients with SN, demonstrated by painful SN being only one of multiple pains experienced by these patients. In this first assessment of psychological factors in a non-US or European cohort with HIV-SN, I also found high levels of depression, and depression associated with increased pain intensity and reduced quality of life. Painful HIV-SN is difficult to treat pharmacologically (Finnerup et al. 2015; Phillips et al. 2010) and recent commentary suggests that reducing pain intensity may not be a realistic therapeutic goal for chronic pain conditions (Ballantyne & Sullivan 2015) but improving quality of life and function may be more valuable. Therefore, placing a greater emphasis on assessing and treating depressive symptoms in patients with SN could be highly advantageous. Where successful treatment of depressive symptoms in these patients may result in a reduction in pain intensities reported and therefore lead to a reduction of copious amounts of analgesic medication and this warrants further investigation in African populations.

CHAPTER 6

Discussion and Conclusion

HIV-SN is a common neurological complication of HIV infection and its treatment with certain classes of ARV drugs (Luciano et al., 2003; Cherry *et al.*, 2012; Kamerman PR, 2016). The prevalence of HIV infection is high in sub-Saharan Africa and is expected to stay high for the foreseeable future. This high prevalence of HIV persists despite reduced infection rates because of improved roll-out of ARV therapies, which improve survival (Avert statistics, 2016). Longer survival means that there is potential for chronic complications of HIV infection and its treatment to accumulate in the population, and in my research, I have focused on HIV-SN, a chronic comorbid condition that has the potential to significantly affect quality of life. In the first part of my thesis, I investigated the incidence of HIV-SN in individuals on updated cART, which consists of the single fixed dose combination pill that contains the ARV tenofovir. I also investigated what clinical factors may be associated with the development of HIV-SN in this population. This work is the first to assess the incidence of HIV-SN in a cohort of individuals that were universally exposed to TDF-based cART, and clinical risk factors associated with the development of HIV-SN in the South African population. I also investigated the role of chemokines in the development of HIV-SN on this regimen. In the second part of my thesis, I explored the role of psychological factors including depression, anxiety and catastrophizing in individuals who developed a painful or non-painful HIV-SN. This second study is also the first to assess the relationship between psychological factors and painful and non-painful HIV-SN in the South African population.

6.1 Development of HIV-SN

I recruited 120 ARV naïve individuals who were initiating TDF-based cART. A total of 83 individuals completed the study, and the cumulative incidence of neuropathy at the end of six-months was 200 cases per 1000 patients (95% CI: 120-290). The incidence rate was 0.47 (95% CI: 0.3-0.67) per person year. Eleven (13%) individuals developed non-painful HIV-SN and nine (11%) developed painful HIV-SN. Our finding of a 6-month cumulative incidence of 200 cases per 1000 patients who had never been exposed to any form of cART certainly contradicts the belief that updated cART therapies, which do not include drugs associated with neurotoxicity, will almost eliminate new cases of HIV-SN (Rosen et al. 2008; Gallant et al. 2004, 2006). Instead, our findings indicate that HIV-SN is certainly not a thing of the past, and that the number of new cases is still high, even if substantially lower than what we observed in a similar cohort initiating d4t-based cART (Pillay 2011) . In the previously mentioned cohort, 44 individuals initiated d4t-based therapy, and 41% (18/44) of these individuals developed SN within 6-months (Pillay, 2011). Our findings in the era of TDF-based cART highlight two key facts:

1. Incidence of HIV-SN is lower on TDF-based cART, but still higher than what was anticipated on this updated drug regimen
2. HIV-SN in the TDF-based era tends to be slightly more asymptomatic, as compared to d4t-based therapy where symptomatic SN was more common [~25%(Pillay 2011)]

Eleven (13%) individuals developed asymptomatic SN in our cohort. The development of asymptomatic SN had a slightly later onset in comparison to symptomatic SN, which was more rapid (most cases occurred within first 3-months of cART). Our finding of the rapid development of symptomatic SN is supported by Arenas-Pinto and colleagues (2008) who also reported that the development of incident neuropathic symptoms peaks within 12 weeks of individuals commencing cART (Arenas-Pinto et al. 2008). In the above-mentioned study, patients received zalcitabine, didanosine and zidovudine. None of the patients in this study cohort received stavudine. Furthermore, even though our findings indicate that the development of asymptomatic HIV-SN in the era of TDF-based cART is common, data on whether asymptomatic HIV-SN will transition into a symptomatic SN is limited. Indeed, only one study, by Malvar and colleagues (2015) provides evidence that asymptomatic SN may well transition into painful SN over time, within a 12 to 24 month period on cART (Malvar et al. 2015). The lack of data in the field of HIV-SN regarding the transition from asymptomatic to symptomatic SN is surprising and certainly warrants further investigation. It is also important to address the key factors which may be contributing to the abovementioned transition. This is because at face value, cases of asymptomatic SN may not be a huge concern with limited patient complaints and many patients not even being aware of the nerve damage. However, should an asymptomatic SN transition into a painful SN, where there is an increased presence of pain, burning and paraesthesias (Maritz *et al.*, 2010; Wadley *et al.*, 2011; Cherry et al., 2012; Pillay *et al.*, 2015), consequently, there may be a significant impact on these patients quality of life and function. Furthermore, data from our previous work indicate that PLWHA who present with SN prior to the initiation of cART often report numbness as common symptom (Pillay 2011). Whether the initiation of TDF-based cART worsens or improves this symptom remains to be seen. Certainly, we found that asymptomatic SN is common on TDF-based cART. If TDF-based cART does indeed worsen pre-existing numbness, this could lead to the development of further conditions such as ulcerations in the lower limbs (Singer et al., 2017), as often seen in diabetic polyneuropathy. As noted later in this discussion, chronic venous insufficiency, a condition which affects the venous system of the lower extremities, has been observed in both the diabetic population and PLWHA (Pieper *et al.*,2006) and therefore the development of ulcerations in these patients could be common (Refer to section on height, second paragraph, Page 144).

Our cohort and findings are unique, as individuals in our cohort were solely exposed to TDF-based cART. We are confident that all individuals were ARV naïve at the time of recruitment and subsequently ARV initiation, as we were provided with current and previous clinic-based records, for all our study participants. Several previous studies have reported an association between HIV-SN and treatment with TDF-based cART in their cohorts (Tumusiime et al. 2014; Tweya et al. 2014; Ndakala et al. 2016; Watt et al. 2014, 2015; Centner et al. 2017). However, findings from these studies need to be interpreted with caution as none of these cohorts were universally exposed to TDF-based cART, but rather consisted of individuals previously exposed to d4t and who were currently on TDF-based cART. It is also important to note that several of these studies had a greater proportion of their cohorts exposed to d4t rather than TDF (Table 7.1) (Ndakala et al. 2016; Watt et al. 2014, 2015; Sun et al. 2015; Shikuma et al. 2015; Phanuphak et al. 2012). Therefore, in the case of these studies we are unable to ascertain what the probable causative agent for the development of HIV-SN in these cohorts was. As such, our study is the first in a global context to report on the incidence of HIV-SN in a cohort solely exposed to TDF-based cART.

The most recent work in the field of HIV-SN was conducted by Centner and colleagues (2017) who investigated the incidence of symptomatic and asymptomatic HIV-SN in South African individuals on cART. Their incidence rates were: symptomatic HIV-SN = 18% and asymptomatic HIV-SN = 62% (Follow-up Period = 24-months). However, a significant proportion of their cohort were currently on a d4t-based regimen (61%) and a much smaller proportion were currently on a TDF-based regimen (28%). The remainder of their cohort was on a zidovudine-based regimen (11%) (Centner et al. 2017). Despite reporting that d4t use did not predict onset of symptomatic SN, the authors failed to report whether their symptomatic and asymptomatic HIV-SN incidence rates were related to the use of a specific drug regimen i.e. exclusive to d4t, TDF or zidovudine-based therapy. This lack of information makes one assume that the incidence rates reported may well be associated with all three regimens and makes it difficult to associate these findings to a specific drug.

Table 6.1 Prevalence and incidence of HIV-SN and associations with HIV-SN in mixed tenofovir and d4t-based cohorts.

Authors	Prevalence/ Incidence	ARV exposure	Tool used	Associations reported
(Ndakala et al. 2016)	29%[b]	TDF: 18.1% **d4t: 45.7%**	Symptoms only	> age
(Watt et al. 2014)	27%[a]	TDF: 15.8% **d4t: 76.6%**	[c]BPNS	>CD4 counts, >TNF/IL-4 ratios, >IFN/IL-10 ratios
(Watt et al. 2015)	19%[a]	TDF: 42% **d4t: 58%**	[c]BPNS [d]TNSr	None
(Shikuma et al. 2015)	10.6%[a]	TDF: 31.3% **d4t/AZT: 34%**	[c]BPNS [e]ENFD assessment	Drug toxicity
(Phanuphak et al. 2012)	8.3%[a] (TDF) 9.8%[a] (d4t/AZT)	TDF: 32.6% **d4t/AZT: 34%**	[c]BPNS	Drug toxicity
(Sun et al. 2015)	8.3%[a]	TDF: 14% **d4t: 18.1%**	Not reported	Drug toxicity, CD4 count < 200cells/mm^3, > age

[a]Incidence [b]Prevalence [c]BPNS: Brief Peripheral Neuropathy Screening Tool
[d]TNSr: Total Neuropathy Score-reduced [e]ENFD: Epidermal nerve fiber density

In contrast to our finding of a cumulative incidence of 200 cases per 1000 patients, two other studies reported a 0% incidence of HIV-SN in their participants on TDF-based cART (Njuguna et al. 2013; Bygrave et al. 2011). As above, these cohorts were not universally exposed to TDF, but rather d4t, AZT and TDF-based cART, with the majority being exposed to d4t (d4t: 67% vs. TDF: 18.6%). In the case of the participants that were specifically on TDF-based cART they reported a 0% incidence rate for HIV-SN. Bygrave and colleagues (2011) reported a follow-up period of 12-months and Njuguna and colleagues (2013) carried out a retrospective analysis of records collected over ten years, with a minimum follow-up period of 12-months for all eligible records. However, both these studies failed to report the method of assessing SN in their cohorts. This lack of information makes it difficult for us to compare our results to these studies. Their tool used to test for SN may well have included symptoms alone, thereby grossly underestimating the actual incidence of SN in the study. Indeed, we found that 11 (13%) individuals developed asymptomatic

SN in our cohort. The assessment of both signs and symptoms is especially important in the context of non-painful or asymptomatic HIV-SN, where, until the SN becomes symptomatic, individuals themselves are unaware of SN development as the underlying nerve damage is 'silent'. Indeed, by not describing their tool efficiently, we are unable to appreciate their result of a 0% incidence rate of SN. That is, these are very low-quality data.

No evidence, to date, suggests that TDF is directly neurotoxic to sensory nerves and evidence which implicates TDF in neuropathy is weak. A single case study conducted in Kenya is the only study to date which implies that TDF is a neurotoxic agent (Owino et al. 2013). The study reported that a participant had developed pain, paraesthesias, numbness and tingling sensations in her upper limbs after initiating eight weeks of TDF-FTC treatment, administered alone (300mg daily) or together with emtricitabine (200 mg daily). They report that all symptoms had resolved once the participant stopped TDF-based therapy. However, the attending neurologist did not identify SN as the cause of the participants symptoms and suggested that a previous lesion in the spinal column may be the source. Since TDF is not directly neurotoxic, as is the case for d4t, there may well be other factors, e.g. inflammation associated with immune activation in HIV-infection, which may have played a role in the development of SN in our cohort.

Chronic inflammation associated with chronic immune activation has been investigated in individuals on ARV therapy (Klatt et al. 2013; Robbins et al. 2009; Hunt et al. 2003). One key factor is the translocation of microbial products from the gastrointestinal tract to the systemic circulation, which has been implicated as a major contributor to chronic immune activation (Marchetti, Tincati & Silvestri 2013). Evidence suggests that mucosal immune dysfunction and the loss of mucosal cells such as Th17 (T-helper 17 cells) may persist during cART, which can lead to increased microbial translocation and therefore persistent inflammation in individuals on cART (Pandiyan et al. 2016; Mudd & Brenchley 2016). Whether this inflammatory process may have contributed to increased inflammation and the resultant SN in our cohort is speculative, but we suggest that there may be a role for inflammation in the development of SN observed in our cohort.

Van de Watt and colleagues (2014) reported in their cohort of symptom-free individuals, that 27% of individuals developed neuropathic symptoms within 12 weeks of starting cART. The onset of symptomatic SN in our cohort was also rapid and occurred within the same timeframe as reported by Van de Watt and colleagues (2014) i.e. within 3-months. Their study reported that the symptomatic group had elevated baseline levels of interleukin-1-receptor (IL-1R)-antagonist. After two weeks on cART, the symptomatic group also had higher levels of soluble interleukin-2 receptor-alpha and tumour necrosis factor (TNF) receptor-II. Significantly high ratios of pro-

inflammatory vs. anti-inflammatory cytokines in the group of symptomatic individuals was also reported with elevated levels of TNF-alpha/IL-4 and interferon-gamma/IL-10 (Van der Watt et al. 2014). The authors suggest a role for sub-clinical inflammation and IRIS in the group of individuals that developed symptoms. Indeed, we did not assess cytokine levels in our cohort and our chemokine data show no obvious differences between those individuals who developed SN and those who remained SN free. However, we speculate that perhaps the occurrence of immune restoration after cART initiation, through the immune reconstitution inflammatory syndrome (IRIS) may have increased the risk of immune-mediated nerve damage in our cohort (Shankar et al. 2007). Indeed, TB has been well identified as a significant risk factor for SN (Forna *et al.*, 2007; Hitchcock et al., 2008; Maritz *et al.*, 2010; Shurie and Deribew, 2010; Luma *et al.*, 2012; Centner *et al.*, 2014; Conradie *et al.*, 2014; Watt *et al.*, 2015). We suggest a possible role for TB-IRIS in our cohort, as 45% (9/20) of individuals who had a current TB infection and were receiving TB treatment, had developed SN. The occurrence of TB-IRIS has also been linked to patients in advanced stages of HIV (stages 3 and 4)(Braitstein et al. 2006; He et al. 2013). The majority of individuals in our cohort (77%) were at disease stages III and IV when they initiated cART. Whether a TB-IRIS component contributed to the nerve damage observed in our cohort is hypothetical, however, our findings certainly warrant further investigations into IRIS-like responses in HIV-SN which are currently lacking.

Furthermore, there is evidence of augmented inflammation related to first-line regimens containing TDF and EFV-based cART. Hileman and colleagues (2015) investigated the effects of antiretroviral therapy on inflammation and monocyte activation by measuring levels of sCD14, sTNFa, IL-6 and high sensitivity C-reactive protein (CRP). Their study compared two ARV regimens, the first regimen included the integrase inhibitor elvitegravir/cobicistat (EVGc) together with FTC+TDF and the second regimen included EFV+FTC+TDF (Hileman et al. 2015). They reported that the regimen which included the integrase inhibitor was associated with lower levels of monocyte activation and lower levels of systemic inflammation. These findings indicate that regimens containing EVGc+ FTC+ TDF rather than EFV+FTC+TDF may be beneficial in terms of lowering systemic inflammation in individuals on TDF-based cART therapy.

6.2 Risk factors associated with the development of HIV-SN in our cohort

Clinical risk factors associated with the development of HIV-SN in our cohort included increasing height ($p=0.003$) and a current TB infection ($p=0.04$). Our data fit well with previous findings where both height (Wadley et al. 2011b; Cherry et al. 2009; Evans et al. 2011; Banerjee et al. 2011) and

a current TB infection or receiving treatment for a current TB infection (Forna et al. 2007; Maritz et al. 2010a; Shurie & Deribew 2010; Watt et al. 2015; Luma et al. 2012; Conradie et al. 2014; Centner et al. 2014; Hitchcock, Meyer & Gwyther 2008) have been associated with the development of SN.

As discussed in Chapter 3, increasing height is a well stablished risk factor for polyneuropathies and has been linked to the development of HIV-SN (Affandi *et al.*, 2008; Cherry *et al.*, 2009; Banerjee *et al.*, 2011; Evans *et al.*, 2011; Wadley *et al.*, 2011) and diabetic sensory polyneuropathy (Sosenko *et al.*, 1986; Robinson *et al.*, 1992; Adler *et al.*, 1997; Cheng *et al.*, 2006). Height is a non-modifiable risk factor and therefore it is difficult to manage its risk within cohorts. To reiterate, there is no particular cut-off point for height which can be applied to mitigate risk. In a cohort of diabetic individuals, increased height was significantly associated with increases in lower-extremity amputations (Tseng 2006). The authors noted that the occurrence of ulcers in the lower extremities may be linked to increased amputations. A possible cause for increased ulcers, pain, swelling and oedema in the lower extremities of diabetic individuals may be linked to the occurrence of chronic venous insufficiency which is common in diabetes (Eberhardt & Raffetto 2005; Rüttermann et al. 2013). Pieper and colleagues (2006) reported that chronic venous insufficiency is also evident in PLWHA (Pieper et al., 2006). Therefore, increased risk of ulcers and pain due to chronic venous insufficiency may well occur in PLWHA. Data on the risk of ulceration, swelling and oedema in PLWHA with HIV-SN are lacking. This could be due to the fact that many do not assume ulceration to be a common occurrence in HIV-SN and therefore it may often be missed in the clinic setting. We note, anecdotally, that those individuals who developed painful SN in our cohort often presented with swelling of the lower limbs and/or ankles within the first month of cART. Benjamin and colleagues (2014) also noted swelling of the ankles in an individual who developed painful HIV-SN (Benjamin & Jelsma 2014). Together with regularly screening all individuals who are on cART for the signs and symptoms associated with SN, we also suggest adequately assessing these individuals for the occurrence of ulcerations, swelling and pain possibly related to chronic venous insufficiency. Indeed, the early detection of HIV-SN, dependant on whether it presents as symptomatic, will permit clinicians to treat this condition with suitable analgesic medications and at the same time treat co-occurring symptoms such as ulcerations, pain and swelling.

Current national TB treatment guidelines in South Africa consist of treatment with the following drugs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol) (National TB-Guidelines, 2017). These drugs are prescribed in combination, according to the participants weight, and can increase

to five pills a day in the intensive phase treatment (2-months) to 3 pills a day in the continuation phase (4-months). All participants in our cohort that were receiving treatment for a current TB infection and who had developed HIV-SN were receiving the appropriate TB treatment doses recommended under these guidelines. Indeed, isoniazid (INH) which forms part of the current TB treatment regimen is neurotoxic (Mandel 1959). Therefore, pyridoxine (Vitamin B6) is continuously supplemented with the use of INH to mitigate its neurotoxicity. All participants receiving TB treatment in our cohort were receiving pyridoxine supplementation, which started at 25mg daily and was titrated up to 100mg (with the intention of increasing to the maximum dose of 200mg daily) during the 6-month study period. Unfortunately, the recommended supplementation of pyridoxine in our cohort did not prevent the development of SN. Our finding that HIV-SN was associated with the concomitant use of TDF and Isoniazid-based TB treatment is disturbing. Since, the TB and HIV co-infection rate in South Africa currently lies above 60% (CDC-statistics 2017) and recent data from the Centers for Disease Control (CDC) and prevention indicate that South Africa is ranked 6th among 22 TB high burden countries with a HIV+TB incidence of 781/100,000 (TB/HIV reports, 2018). This underlines the fact that neither HIV nor TB infection are showing signs of decreasing in our population and highlights the detrimental synergistic effect of cART and isoniazid-based therapy which remains a huge burden in the South African healthcare system. Certainly, with TB treatment guidelines being revised over the years, the removal of isoniazid from current TB regimens may not be possible in the South African setting. The effectiveness of isoniazid-based therapy in treating TB-infections and the cost-effectiveness of Isoniazid-based therapy certainly outweighs its adverse effects and therefore we cannot envisage the elimination of isoniazid from TB treatment regimens in South Africa.

Several African-based studies have linked Isoniazid-based TB treatment and stavudine exposure to the development of HIV-SN (Forna *et al.*, 2007; Hitchcock et al., 2008; Maritz *et al.*, 2010; Shurie and Deribew, 2010; Luma *et al.*, 2012; Centner *et al.*, 2014; Conradie *et al.*, 2014; Watt *et al.*, 2015), however, our study is the first study to associate TDF-based cART and concomitant TB treatment with the development of HIV-SN. The high risk of SN development with TB treatment containing isoniazid seems to be limited to African cohorts (as noted above), with no non-African based cohorts reporting TB treatment as a significant risk factor for SN development. The lack of association probably reflects population-specific comorbidity of TB and HIV, with low rates of coinfection of the two in developed countries (e.g., USA =2.7/100,000 population, in 2014)(CDC USA 2014), and high rates of coinfection in sub-Saharan Africa (e.g., RSA = 781/100,000 population, in 2017). Indeed, the link often observed between HIV-SN and TB may well be a feature of resource-poor settings, which includes many of the African countries and possibly why

we do not see such associations (between HIV-SN and TB) in resource-rich countries such as the US.

As described earlier, Africa and South Africa are overwhelmed by these two epidemics (HIV and TB), and we certainly require a more integrated and collaborative framework with regard to safer and appropriate treatment options to combat these conditions which will ultimately benefit patients at the level of their local clinics. Current South African TB guidelines only identify the ARV d4t as a possible offender in SN development with concurrent TB treatment (TB-Guidelines 2017). A starting point would be to revise current national TB guidelines to highlight that initiating TB treatment together with cART may increase the risk of SN and also to recommend, within these guidelines, appropriate treatment strategies for the debilitating symptoms associated with SN.

6.3 Role of chemokines in the development of HIV-SN

I investigated the role of three chemokines that were previously shown to have a link in the development of HIV-SN (Abbadie et al. 2009; Oh et al. 2001; Milligan et al. 2000; Mountford et al. 2017). These chemokines were CCL2 (MCP-1), CX3CL1 (Fractalkine) and CXCL12 (SDF-1). We did not find any significant associations between the concentrations of the three chemokines across the 6-month study period and the development of HIV-SN in our cohort. A recent study by Mountford and colleagues (2017) investigating the expression of chemokine receptors on cells surrounding cutaneous nerves in PLWHA reported a significant association between the presence of HIV-SN and an upregulation of chemokine receptors (CX3CR1, CCR2 and CCR5) in $CD3^+$ and $CD14^+$ cells in PLWHA (Mountford et al. 2017).

The lack of association observed in our cohort may be due to several reasons. We may have missed the optimal timing of blood sample collection for many of our participants with HIV-SN. Indeed, Van de Watt and colleagues (2014) described a specific cytokine ‘burst’ which was evident at 2-4 weeks in previously ARV naïve individuals that had commenced ARV therapy. Their study also reported a significant peak of these cytokine levels at 2 weeks of therapy and a return to baseline levels by the 12th week (Watt et al. 2014). Due to our irregular visit schedules we missed the opportunity to collect our samples at these time-points.

A possible role for chemokines in the maintenance of neuropathic pain associated with HIV-SN is becoming an increasing popular line of thinking. However, as a first line of study we wanted to assess the role of chemokines in the development of HIV-SN. Understanding the exact role of these chemokines is key and could directly benefit and inform treatment strategies for debilitating

neuropathic pain. Therefore, our aim is to repeat part of this work, including in people with painful SN, under more controlled conditions. We may be able to achieve this by conducting this work as part of a tightly controlled clinical trial where we may be able to optimise visit timings and proper sample storage.

Limitations to this piece of laboarty work include relying plasma samples to quantify chemokine concentrations limited our analysis. This was due to the issues we faced with collecting and storing the plasma samples. Perhaps the use of tissue biopsies, where RNA extraction could be performed via qPCR, could have benefited the study protocol. However, tissue biopsies are not without its own issues, which include feasibility, especially in the setting where we were conducting our research and secondly the healing time for patients after a biospy. Wound healing in PLWHA is very slow and adding to this already complex condition in these patients may not be ideal.

6.4 Psychological factors associated with painful and non-painful HIV-SN

As outlined in Chapter 3, we recruited individuals with painful (63%) or non-painful HIV-SN (37%) to determine: 1) if psychological factors associate with having painful HIV-SN, 2) if pain and psychological factors affect quality of life, and 3) if psychological factors predict the intensity of their pain. We found that 1) depression, anxiety and pain catastrophizing did not associate with the presence of painful HIV-SN in our cohort, 2) increased depressive symptoms and presence (but not intensity) of pain independently associated with reduced quality of life, and 3) greater depressive symptoms independently associated with increased pain intensity in individuals with painful HIV-SN.

The psychological factors which we investigated in our cohort included, depression, anxiety and pain catastrophizing. Although these factors have all been individually investigated for their role in HIV-SN (Blackburn-Munro and Blackburn-Munro, 2001; Thom, 2008; Lucey *et al.*, 2011; Keltner *et al.*, 2012; Phillips *et al.*, 2014), very few studies have studied these factors together in the HIV-SN population. Certainly, the relationship between the above-mentioned factors is a multifaceted one. Our study is unique as it is the first, in a global context, to compare depression, anxiety and pain catastrophizing between those with symptomatic and asymptomatic HIV-SN. Phillips and colleagues (2014), a UK-based study, investigated these factors in healthy individuals, SN-free individuals and individuals with SN. However, their group of non-painful SN individuals was very small and they did not provide further details on this group within their study (Phillips et al. 2014). Therefore, our study remains the first globally to assess the impact of psychological factors on

painful and non-painful HIV-SN in South African PLWHA. Certainly, sociocultural context affects the experience of pain. A high burden of unemployment and financial difficulties relegated pain to a lower priority in South African PLWHA (Wadley et al., 2016). This finding highlights the uniqueness of South African PLWHA who experience pain and affirms why it was essential to conduct the current study in a South African population.

We found that depression, anxiety and pain catastrophising did not associate with the presence of painful-SN in our cohort. However, several earlier studies have reported an association (Uebelacker & Bailey 2015; Phillips et al. 2014; Ellis et al. 2010). Indeed, our participants displayed high levels of depression and anxiety, which was evident for both our symptomatic and asymptomatic groups. The setting where our study was conducted may also have influenced the lack of association between depressive symptoms, anxiety and pain catastrophizing with painful-SN. Participants were recruited the Chris-Hani Baragwanath hospital located in the township of Soweto, located just out of Johannesburg. While, the socioeconomic status of this township has grown considerably over the years, it remains a heavily populated region (population density of Soweto in 2017 = 6400 persons per square kilometre) where unemployment and low income is a problem (Gilbert & Soskolne 2003). Our participants did not report high education levels (median years of schooling for entire cohort = 6 years) and the majority (83%) of our cohort were currently unemployed at time of recruitment. Participants often relied on study remuneration to aid with transport and additional household costs. A recent study carried out in South Africa reported that low income and socioeconomic status was associated with increased depression (Mungai, 2016). This study findings are supported by that of Bhatia and colleagues (2014) who also reported that socioeconomic factors such as unemployment and low income may significantly contribute to increased levels of depression in PLWHA (Bhatia & Munjal 2014). Given that the majority of our cohort was unemployed, this factor may have played a role in the equally high levels of depression reported across both groups and the lack of association observed between depressive symptoms, anxiety and pain catastrophizing with painful-SN.

It is important to address these high levels of background depression in our cohort and identify the factors that may be driving these high levels of depressive symptoms. One factor which may be associated with these high levels of depression observed is the stigma associated with HIV status. Although, we did not measure stigma in our cohort we propose that it may explain the depressive symptoms noted across both study groups (painful SN and non-painful SN). HIV-related stigma and lack of social support can negatively affect PLWHA and contribute to the development of mental health issues such as depression (Li et al. 2010). From subjective patient reports recorded in patient clinic files by ARV counsellors, 54% of individuals in our cohort did not

disclose their 'HIV status' to their current partners/spouses or other immediate family members for fear of being excluded or ridiculed. Furthermore, 32% of individuals in our cohort expressed to the ARV initiating nurse that they had faced some form of discrimination after disclosing their HIV status to family members and friends. This may well be a form of 'enacted stigma' where there is social exclusion (Earnshaw & Chaudoir 2009). We postulate that the burden of HIV-related stigma was high in our cohort based on these patient reports. Wadley and colleagues (2017) recently conducted a preliminary analysis of the association between perceived stigma and HIV-related pain. They report that 88% (44/50) participants experienced some form of HIV-stigma and that depressive symptoms correlated with the total stigma score (Wadley, Pincus & Evangeli 2017). However, they could not confirm whether depression mediated this relationship. We speculate that HIV-related stigma may have contributed to high levels of depression observed in our cohort, however, this certainly needs to be investigated further in our population.

The type of ARV regimen that both individuals with painful SN and non-painful SN were currently taking may also explain the high levels of depression noted. The non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz has been linked to increased levels of neuropsychiatric symptoms which include, depression, anxiety, stress and paranoia (Apostolova et al. 2015; Mothapo et al. 2015). First-line ARV therapy in South Africa includes the NNRTI efavirenz and 100% of our cohort were exposed to this drug. In support of this notion, recent work conducted on rodents by Cavalcante and colleagues (2017) report that sub-chronic treatment with efavirenz induced both anxiogenic-like and depressive-like behaviours which were dose related (Cavalcante et al. 2017). Efavirenz has also been linked to suicidal ideation and suicide attempts (Mollan et al. 2014). A two-fold increased hazard of suicidality in participants on efavirenz containing regimens vs. those on regimens without efavirenz, was reported by Mollan and colleagues 2014. Suicide risk was also observed in our cohort, with 4% (7/197) participants attempting suicide (as evidenced by clinic notes from the psychiatric room at our research site). Unfortunately, two out of these seven individuals in our cohort who attempted suicide passed away. We cannot be 100% certain that efavirenz contributed to the psychiatric condition of suicide and increased depressive symptoms in our cohort as we did not directly measure this, however the evidence suggests that efavirenz may well contribute to increased neuropsychiatric adverse effects and certainly warrants further investigation into this drug which forms the backbone of first-line ARV therapy in Sub-Saharan countries.

Increased depressive symptoms and the presence, but not intensity, of pain associated with reduced quality of life in our cohort. This finding emphasises the fact that any pain, irrespective of its intensity, can significantly impact an individual's QoL and should be treated sufficiently and

promptly to reduce the suffering experienced by these individuals. Out data fit with previous reports from USA and Uganda based studies, of the dual effect of pain and depression on QoL in PLWHA (Mwesiga et al. 2015; Miaskowski et al. 2011). The comorbidity of pain and depression and their effect on QoL in our cohort was also noted through patient reports suggestive of depressive symptoms. These include, missed scheduled visits due to their pain, 'sleeping all day' and 'not eating anything the entire day'. There is an urgent need to address the high levels of depression and pain and their association with reduced QoL in this South African population. Recent work from Redman and colleagues (2018) suggest that poor sleep quality in South African PLWHA correlates with pain and depression (Redman *et al.,* 2018). Unfortunately, we did not include any measure of sleep quality in our set of study of questionnaires. Since, poor sleep associates with pain and depression it would be worthy to assess this factor in panful and non-painful SN. Verma and colleagues (2004) also identify the interrelationship between poor sleep, chronic pain and depression, where each factor has a significant impact on the other (Nicholson & Verma 2004). As an example, depression can be the cause of poor sleep quality and can also be the result of poor sleep quality. Furthermore, as our data indicate, increased depressive symptoms and the presence of pain associate with reduced quality of life. Therefore, by evaluating sleep quality in these individuals we may be able to identify a treatable factor that could lead to improvement in QoL for these individuals. Certainly, in this triad of pain, depression and sleep quality an improvement in one of these factors can lead to improvements in the other two factors (Teh *et al.*, 2010; Gaynes *et al.*, 2015; Huang *et al.*, 2017).

We also found that although depressive symptoms did not associate with having a painful SN, they associated with increased pain intensity in people with pain. These data fit with other reports that higher depression scores associate with increased pain intensity in PLWHA in the US population (Richardson et al. 2009; Miaskowski et al. 2011). Bhatia and colleagues (2014) supports this association and also reported that increasing levels of HIV disease severity (progression from stage I to stage IV) was associated with increased levels of depression in PLWHA. We are unable to comment on the staging of disease for our entire cohort as data from the clinic files at this facility were limited. Current guidelines for the management of neuropathic pain in South Africa include the tricyclic antidepressant amitriptyline (Chetty et al. 2012). Unfortunately, from our recent data we report that despite the use of amitriptyline, either alone or in combination therapy, the majority of patients with painful HIV-SN still described their pain as 'moderate to severe' in intensity (Pillay et al. 2015). This is not altogether surprising, as amitriptyline was no more effective then placebo in reducing neuropathic pain in the randomised, double blind, crossover trial for analgesia in painful HIV-SN (Dinat et al. 2015). A recent application to the 21st meeting of the WHO expert committee on selection and use of essential

medicines by Kamerman and colleagues (2018), represented a small glimmer of hope for the treatment of neuropathic pain associated with HIV-SN. Their group, which consisted of members from the International Association for the Study of Pain (IASP), Neuropathic Pain Specialist Interest Group (NeuPSIG) and the International Association of Hospice and Palliative Care (IAHPC), applied for the inclusion of gabapentin as an analgesic agent for the management of neuropathic pain in adults (central and peripheral) on the WHO model list of essential medicines (Kamerman et al. 2018). Regrettably, this application was denied based on concerns regarding the abuse potential of gabapentin (Smith *et al.*, 2012; Smith et al., 2016). The rejection of this application is unfortunate, as the inclusion of gabapentin under the essential medicines list, would certainly have provided renewed hope for individuals affected by painful SN, since gabapentin was effective in reducing neuropathic pain associated with diabetic neuropathy (Backonja & Glanzman 2003). Indeed, the rate of depression and its association with pain intensity in PLWHA is very high but as described earlier, attempting to treat either one of these factors (i.e. either pain intensity or depressive symptoms) may result in a significant improvement in the other. Concomitantly treating pain and depression in these individuals would be ideal, but in limited settings, focusing on treatment of at least one of these factors could be beneficial to quality of life.

6.5 Limitations of current work

We faced a significant number of issues with regards to data collection, which severely impacted our patient visit schedules and consequently affected the way we assessed our data (e.g. chemokine sample collection and storage). For full description, refer to last paragraph on Page (59 – 61).

6.6 Future recommendations

6.6.1 Incidence of TDF-based cART

Our findings here highlight that the incidence of HIV-SN on TDF-based cART is indeed lower than that seen on d4t-based therapies, but still higher than what was anticipated for this drug. Our findings also highlight the development of SN in individuals who were not previously exposed to neurotoxic d4t-based therapies. This certainly warrants further investigation to identify possible mechanisms that are driving the development of SN where there is no exposure to neurotoxic ARVs. We propose that inflammatory mediators such as cytokines and chemokines may have had role in the development of HIV-SN in our cohort and therefore suggest that further studies be carried out to identify the mechanisms of these inflammatory biomarkers in HIV-SN.

6.6.2 Importance of regularly screening individuals for sign and symptoms and referrals

Indeed, our data confirms that HIV-SN is not a thing of the past and that its development on TDF-based cART tends to be more asymptomatic. Perhaps employing the approach used for diabetic SN in the clinic setting may work equally well for individuals with PLWHA who develop SN. Diabetic individuals have their feet regularly screened in the clinic setting as they are identified as 'at risk' patients for SN due to prolonged exposure to high blood sugar and various other metabolic factors (Papanas & Ziegler 2015). Certainly, our study highlights that individuals that are concurrently exposed to isoniazid-based therapy and cART are also 'at risk' for the development of SN and should be regularly screened for signs and symptoms suggestive of SN. Carefully monitoring these individuals and prioritizing them for visits to occupational therapists, physiotherapists and podiatrists within the clinic can assist in several ways. Podiatrists can inform these individuals about appropriate foot care to prevent infection. Diabetics are often advised not to wear tight shoes (which could restrict blood flow in the foot) or open-toed shoes which could lead to severe injury in case the toe is stubbed. It will be beneficial to convey the same information to individuals with HIV-SN who are often overlooked in the clinic setting. It is perhaps due to the lack of knowledge that HIV-SN is an often painful and debilitating neurological condition, identical to diabetic SN, that this condition is not given the priority which it is due. Diabetic neuropathy has been associated with an increased risk of bone fractures in the lower limbs and hip (Gill et al. 1997) and falls due to balance impairment (Timar et al. 2016; D'Silva et al. 2016). Erlandson and colleagues (2012) reported SN as a significant risk factor for 'single' and 'recurrent' falls in their cohort of PLWHA. Their study reported that 43% of PLWHA with SN were more likely to experience single or recurrent falls (Erlandson et al. 2012). Although, diabetic SN and HIV-SN may differ in their etiology, the signs and symptoms of both these neurological conditions show similarities. Therefore, one can postulate that the same risk may apply for individuals with HIV-SN. It is possible that the main focus with regards to HIV-SN in the era of d4t was the occurrence of painful SN, with little focus on the co-occurrence of falls or related balance issues and therefore no studies have reported on this in their investigations of HIV-SN. Furthermore, if one agrees that PLWHA tend to be of a younger group [59% of new HIV infections worldwide were among young people aged 15-24 (UNAIDS 2017)], in comparison to diabetic individuals who tend to fall more in the middle-aged category, concerns around falls and related injuries in PLWHA with SN would be minimal. Therefore, this could be the reason behind the limited information on associated risk of falls in individuals who develop HIV-SN. Certainly, in severe cases of SN, where there may be several symptoms including pain, numbness and paraesthesias, individuals may find it difficult to perform mundane daily activities such as walking or even maintaining proper balance. Therefore,

routine visits to occupational therapists and physiotherapists in the clinic may assist these individuals in easing their discomfort and prevent severe injuries.

6.6.3 The use of Chronic Clubs in South Africa

Furthermore, the introduction of 'Chronic Clubs' in local clinics in South Africa is becoming increasingly popular. These chronic clubs include individuals with chronic conditions such as HIV, diabetes and hypertension who are attending the clinic for treatment. Individuals are invited to partake in these chronic clubs by nursing staff, on the promise that they will follow strict instruction with regards to treatment, diet and lifestyle and exercise regimens. Individuals who are members of these clubs get monthly visits to a clinician, dietician and nursing staff who are dedicated specifically on that day to these clubs (Tshabalala & Mkhondo 2014; Wilkinson 2013). The main purpose of these clubs is to promote a healthy lifestyle and proper treatment adherence. Given that HIV is recognised as chronic disease and individuals affected by this epidemic suffer immensely, we questioned why chronic clubs for PLWHA were not initiated at all local clinics in Johannesburg? Currently, chronic clubs are well established in Khayelitsha (an informal township in Cape Town, South Africa) and in Soweto, a heavily populated region in Johannesburg which is home to the largest tertiary care hospital in South Africa, the Chris-Hani Baragwanath Hospital (Tshabalala & Mkhondo 2014; Campion 2015). The implementation of these chronic clubs for PLWHA at local clinics could benefit ARV treatment adherence, monitoring of adverse events related to ARV therapy and the rapid treatment of co-infection with TB (Luque-Fernandez et al. 2013; Bemelmans et al. 2014). Within these chronic clubs for PLWHA, we suggest expanding the network of health care providers to perhaps include a clinician and a health care worker with experience in treating PLWHA, a dietician, an occupational therapist and a physiotherapist. This option may assist those individuals with painful nerve damage to receive appropriate physical therapy and analgesic treatment to treat the severe pain and numbness that is often associated with symptomatic SN (Pillay et al. 2015). Where possible, if resources permit, we also propose the inclusion of a podiatrist that could routinely monitor patients feet for signs and symptoms suggestive of neuropathy.

6.6.4 Educating and training Health care workers treating individuals with HIV-SN

The South African TAC (treatment action campaign) (TAC 2010), emphasised in 2010 (when the large-scale roll-out of TDF commenced) that TDF was a safer and more cost effective drug than d4t and, also if available as single pill in the form of fixed dose combinations, would be easier for patients to adhere to and more cost effective for the public health sector. Despite the observed decrease in the incidence of HIV-SN with TDF-based cART reported by our study, HIV-SN has

not drastically reduced as expected. Contrary to the belief of the TAC, individuals who develop HIV-SN and specifically, painful HIV-SN are less likely to adhere to ARV medications (Surratt et al. 2015) and this is likely to increase the rates of ARV non-compliance leading to viral load rebounds, immune system complications, and disease progression. Certainly, despite large scale educational programmes being rolled out on HIV/AIDS and its treatment across South Africa (Motsoaledi A, 2015), my impression anecdotally, is that knowledge on HIV-SN seems to be poor in the local clinic setting. Therefore, HIV-SN is still not a well-recognised or well understood condition related to HIV and its treatment. During our data collection at the various clinics and hospital sites, myself and my interpreter (Florence Mtsweni) used the opportunity to train and educate nursing staff on the signs and symptoms of this painful and debilitating condition. Indeed, peripheral sensory neuropathy is listed under 'specific conditions' related to HIV and its treatment in the recent treatment guidelines for HIV/AIDS, however the actual understanding and recognition of this condition, by nursing staff at our research site (from our investigation) is poor. Our findings highlight that there is a drop in the incidence of HIV-SN from d4t-based regimens in comparison to TDF-based cART, however, this condition is far from being eliminated. Thus, perhaps the focus could shift to training programmes coordinated by Health Institutes such as the Anova Health Institute, a non-governmental organisation (located in Parktown, Johannesburg, South Africa) that provide nursing staff to local clinics for the initiation of ARV therapy. These nursing staff are in the ideal position to be trained on the signs and symptoms of SN and their services could also be included in the 'chronic club' concept described above.

6.6.5 Using a simple tool to screen for HIV-SN

The ACTG BPNS tool is a simple screening tool designed specifically for use by non-clinicians and can easily be incorporated into local clinic settings. We exposed staff to this tool and emphasised the importance of the early detection of HIV-SN in these settings. From our own experience, clinic staff were keen to implement this tool and were eager to learn about this condition. This suggests that there is an excellent opportunity to implement this tool in local clinics. Implementing this tool will not only help with the early detection of this condition but will also reduce the amount of referrals to already inundated clinicians, as nursing staff could easily identify and treat this condition.

6.6.6 Treating depression – a starting point

Our findings also highlight the significant burden of depression in the South African population. Unfortunately, mental health facilities within provincial/local clinics in South Africa are limited. These facilities are most often located within tertiary government hospitals and require referral

from provincial/local clinics. However, the issue stems from local clinics not having enough resources (counselling staff) to identify these individuals and 'down-refer' to tertiary government hospitals for proper treatment. As an example, at our research site which is a primary care facility, only one counsellor is available within the chronic clinic setting. This counsellor is expected to provide services to all patients attending the chronic clinic including PLWHA, who often require more time for counselling sessions with counsellors to cover adherence, adverse effects, depressive symptoms etc. Therefore, these counsellors are often expected to see ± 35 to 40 patients per day (Masango-Makgobela et al., 2013; Igumbor *et al.*, 2016). The South African Depression and Anxiety Group (SADAG) has introduced mobile treatment stations which employ wellness coaches, psychologists and nursing staff that have received mental health training at non-governmental intuitions. These mobile treatment stations travel around resource-limited areas within provinces, to provide mental health services to individuals who cannot access them. This initiative is aimed at improving the quality of mental healthcare and making these resources freely available in areas where patients have limited access to primary and tertiary health care facilities. Furthermore, this mobile mental health care initiative is also aimed at reducing the pressure faced within primary health care clinics where psychologists and psychiatric staff are inundated with huge patient volumes. Although this initiative is fairly new, and requires adequate funding to sustain its function, it may well hold the key to addressing depressive and anxiety symptoms in resource-limited settings. We also suggest further investigations into the triad of chronic pain, sleep and depression as it may well be a significant factor playing a role in the development and persistence of pain observed in our cohort.

6.6.7 The introduction of the ARV Dolutegravir in South Africa

Dolutegravir (DTG) is an intergrase inhibitor which is prescribed in combination with other ARV drugs. As it is an intergrase inhibitor, it blocks the the HIV enzyme intergrase, which prevents HIV from multiplying. Dolutegravir has been introduced in resource-rich countries such as the US, however, it's introduction in middle-income countries such as South Africa has been delayed with clinical trials still being conducted (Van de Watt *et al.* 2017). This is indeed surprising as Van de Watt and colleagues (2017), describes this drug as being cost effective and having minimal side effects in comparison to the current first-line cART regimen being prescribed in South Africa. Certainly, the cost of providing ARV therapy consumes the majority of the budget for most HIV programmes in South Africa. Therefore, this drug would be ideal in the South African setting. Essentially, DTG would replace the ARV efavirenz, which is susceptible to resistance and has several side-effects including increasing risk of depressive symptoms in individuals on therapy (refer to second paragraph, page 149). Van de Watt and colleagues (2017) note that DTG is not

only a more cost-effective drug with minimal side effects, but also that it presents with a higher resistance barrier which ultimately decresases the need to transition to other lesser tolerated second-line treatments. The roll-out of DTG has also been delayed due to the need to convert the drug to a fix dose combination (FDC) pill, which is what current first-line therapies in South Africa consist of (WHO, 2009). This delay is unfortunate but critical, as patients would likely not prefer to switch back to the previous type of 'three-pills a day' ARV regimen which was phased out after FDC was introduced in South Africa (WHO, 2009). Although, the introduction of DTG in South Africa is promising, Brook and colleagues (2018) report that the use of DTG may not be ideal in the high TB + HIV setting of South Africa (Brook et al, 2018). Their study reported serious toxicities where DTG was combined with isoniazid and rifapentine. Individuals reported a flu-like syndrome and levels of interferon-y, CXCL10, C-reactive protein and cytokines were temporarily associated with these symptoms. As noted in section 6.2, first paragraph (page 145) South Africa has a massive TB + HIV co-infection rate, and isoniazid forms the backbone of current first-line TB treatment in South Africa. Although, the abovementioned study had a small sample size, it certainly raises concerns and warrants further investigation into the drug interactions of DTG before it is added into first-line therapies in South Africa. Perhaps further clinical trials may provide solid evidence for DTG, but it remains a drug of the 'future' for South Africa.

6.7 Conclusion:

In conclusion, my work highlights an important clinical condition related to HIV and its treatment in South Africa. On the positive side, we found that the incidence of HIV-SN on TDF-based cART was lower than that observed on d4t-based therapies and we also found that HIV-SN in the era of TDF-based cART tends to be more asymptomatic. These benefits may be tempered by other factors, however. With the expected longer survival rates of PLWHA, due to the increased availability of cART, we foresee an increasing prevalence of this neurological condition in the South African population. Furthermore, our high TB/HIV co-infection rates certainly make South African PLWHA more susceptible to the development of HIV-SN. Therefore, those individuals who present with these comorbid conditions need to be monitored carefully and screened regularly for the signs and symptoms suggestive of SN, starting at the level of primary clinics. Within primary care clinics, a greater emphasis needs to be placed on the signs and symptoms of SN to ensure that this condition is adequately managed. Educational programmes centred on HIV-SN and incorporating the simple screening tool for HIV-SN in the primary clinic setting could be beneficial to both patients and healthcare workers, in helping them to properly identify the signs and symptoms of SN. Another area that warrants attention is the high burden of depression

in the HIV-postivie South African population. PLWHA who present with comorbid pain and depression need to be treated within the framework of primary care clinics, and where this is not possible, rapid ‘down referral’ of these individuals to tertiary care needs to be implemented. The goal with regards to PLWHA who present with comorbid pain and depression must be improving quality of life and function in these individuals.

APPENDIX 1

Ethics clearance and Ethics amendments

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mrs Antonia Wadley

CLEARANCE CERTIFICATE

PROJECT	**M121018** HIV-Associated Sensory Neuropathy in the Era of Tenofovir-Based Highly Active Anti-Retroviral Therapy (HAART)
INVESTIGATORS	Mrs Antonia Wadley.
DEPARTMENT	School of Physiology
DATE CONSIDERED	26/10/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 22/02/2013 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel +27 (0)11-717-1252
Medical School Secretariat: Medical School Room 10M07, 10th Floor. Tel +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za. Fax +27 (0)11-717-1265

29 April 2014

Mrs Antonia Wadley
School of Physiology
University of the Witwatersrand
Sent by email to: anronia.wadley@wits.ac.za

Dear Mrs Wadley

Re: **Protocol Ref no: M121018**
Protocol Title: HIV-Associated Sensory Neuropathy in the Era of Tenofovir-Based Highly Active Anti-Retroviral Therapy (HAART)
Principal Investigator: Mrs Antonia Wadley
Protocol Amendment

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has approved the amendments on the above mentioned protocol as detailed in your letter dated 25 March 2014. However, the approval is subject to providing written permission to conduct the study from the CEO of Charlotte Maxeke Johannesburg Academic Hospital

Thank you for keeping us informed and updated,

Yours Sincerely,

.....

Ms Zanele Ndlovu
Administrative Officer
Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
29 APR 2014
RESEARCH OFFICE
JOHANNESBURG

APPENDIX 2

AIDS CLINICAL TRIALS GROUP BRIEF PERIPHERAL NEUROPATHY SCREEN

ACTG BPNS NEUROLOGICAL SCREENING TOOL

INSTRUCTIONS FOR RECORDING SUBJECTIVE ELICITED SYMPTOMS

Ask the subject to rate the severity of each symptom listed in question 1 on a scale of 01 (mild) to 10 (most severe) for right and left feet and legs Enter the score for each symptom in the column marked Presence/Severity. If a symptom has been present in the past, but not since the last visit, enter '00-Currently Absent' If the symptom has never been present, enter '11-Always Been Normal'

Always been normal	Currently absent	Mild ——— Severe									
11	00	01	02	03	04	05	06	07	08	09	10

1. SYMPTOMS R L

   a. Pain, aching, or burning in feet, legs* …… ☐ ☐

   b. "Pins and needles" in feet, legs …… ☐ ☐

   c. Numbness (lack of feeling) in feet, legs …… ☐ ☐

   * Option "a" refers to pain of any type, which is not described as "pins and needles" or "numb". If the patient chooses option "a" to describe their pain, please record what term(s) the patient used to describe the pain in the space below.

   _____

   _____

INSTRUCTIONS FOR GRADING SUBJECTIVE ELICITED SYMPTOMS

Use the single highest severity score from question 2 above to obtain a subjective sensory neuropathy score. If all severity scores are'00' or '11', the subjective sensory neuropathy score will equal '0'

Presence/Severity Score of:
01 – 03 = Grade of 1
04 – 06 = Grade of 2
07 – 10 = Grade of 3
11 or 00 = Grade of 0

R L

2. Subjective sensory neuropathy grade …… ☐ ☐

1. Location of symptoms

Use Score of:
0 = None
1 = feet (or hands) only
2 = extends to ankles (or wrists)
3 = extends above ankle (wrist) but not to knee (elbow)
4 = extends to knees (or elbows)
5 = extends above knees (or elbows)

R L

   a. Pain, aching, or burning in feet, legs …… ☐ ☐

   b. "Pins and needles" in feet, legs …… ☐ ☐

   c. Numbness (lack of feeling) in feet, legs …… ☐ ☐

INSTRUCTIONS FOR EVALUATING PERCEPTION OF VIBRATION

Strike the end of a 128 Hz tuning fork hard enough that the sides touch. Place the vibrating tuning fork on a bony prominence on the subject's wrist to be sure that they can recognise the vibration or "buzzing" quality of the tuning fork. Again, strike the ends of the tuning fork hard enough so that the sides touch. Immediately place the vibrating tuning fork gently but firmly on the top of the distal interphalangeal (DIP) joint of one great toe and begin counting the seconds. Instruct the subject to tell you when the "buzzing" stops. Repeat for the other great toe.

4. Vibration sense

a. Great toe DIP joint perception of vibration in seconds

R	L

b. Vibration perception score

R	L

Vibration Perception
0 = felt ≥10 seconds
1 = felt < 10 seconds

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, the examiner then strikes the Achilles tendon. The tendon reflex is felt by the examiner's hand as a plantar flexion of the foot, appearing after a slight delay from the time the Achilles tendon was struck. Use reinforcement, if necessary, by having subject clench fist.

Ankle reflexes
0 = absent
1 = Hypoactive
2 = Normal deep tendon reflexes
3 = Hyperactive (prominent spread)
4 = Clonus
8 = unable/did not assess

5. Ankle reflexes ……..

R	L

APPENDIX 3

PATIENT QUESTIONNAIRE

PARTICIPANT QUESTIONNAIRE

GENERAL INFORMATION

(CONTACT DETAILS)

Reference number: _____

Name: _____

Residential address: _____

_____

_____

Telephone number: _____ (home)

_____ (work)

_____ (cell)

This information shall be kept separate from the patients other information, in a locked cupboard

GENERAL INFORMATION

(DEMOGRAPHICS)

Age (years)**:** _____

Date of birth: _____

Sex: **Male** ☐ **Female** ☐

Population group: _____

Ethnicity: _____

Country of birth: _____

Mass: _____ (kg) **Height:** _____ (cm)

MEDICAL INFORMATION

1. Does the participant have a history of:
Please tick the appropriate box

	Yes	**No**	**Unknown/ not tested**	**Comments** (current/historical, treatment)
Alcoholism				
Tuberculosis infection				**Isoniazid therapy?** Yes ☐ No☐ **Pyridoxine prophylaxis?** Yes ☐ No☐
Diabetes mellitus				
Syphilis infection				
Hepatitis B Infection				
Hepatitis C infection				
Peripheral neuropathy (of any cause)				
Vitamin B_{12} deficiency				

2.1. Date first diagnosed with HIV: _____ (day/month/year)

2.2. CD4 T-cell count when first diagnosed with HIV: _____ (cells/mm^3)
Date: _____

2.3. Plasma HIV viral load when first diagnosed with HIV: _____ (copies/ml)
Date: _____

3. Nadir CD4 T-cell count: _____ (cells/mm^3) **Date**: _____

4.1. Current CD4 T-cell count: _____ (cells/mm^3) **Date**: _____

4.2. Current HIV viral load: _____ (copies/ml) **Date**: _____

5.1. Date antiretroviral treatment first started: _____ (day/month/year)

5.2. AIDS-defining illnesses when treatment started and dates:

_____

_____

_____

5.3. Disease staging (at time of starting ARV's)

_____

_____

5. 4. Baseline assessment

ARV name	Dose	Date started	CD4 count & viral load when started	Other medications being taken concurrently
			CD4: Viral Load:	

5. 5. Second assessment (2 months)

ARV name	Dose	Date started	CD4 count & viral load when started	Other medications being taken concurrently	Reason for change
			CD4: Viral Load:		
			CD4: Viral Load:		
			CD4: Viral Load:		
			CD4: Viral Load:		

5. 6. Third assessment (4 months)

ARV name	Dose	Date started	CD4 count & viral load when started	Other medications being taken concurrently	Reason for change
			CD4: Viral Load:		
			CD4: Viral Load:		
			CD4: Viral Load:		
			CD4: Viral Load:		

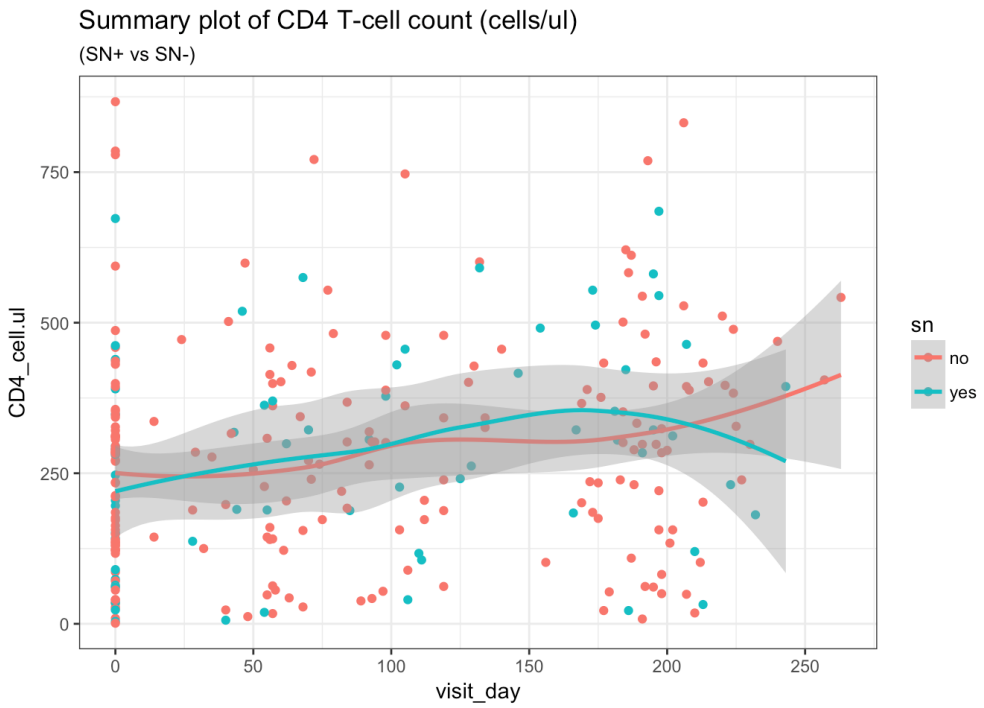
5. 7. Last assessment (6 months)

ARV name	Dose	Date started	CD4 count & viral load when started	Other medications being taken concurrently	Reason for change
			CD4: Viral Load:		
			CD4: Viral Load:		

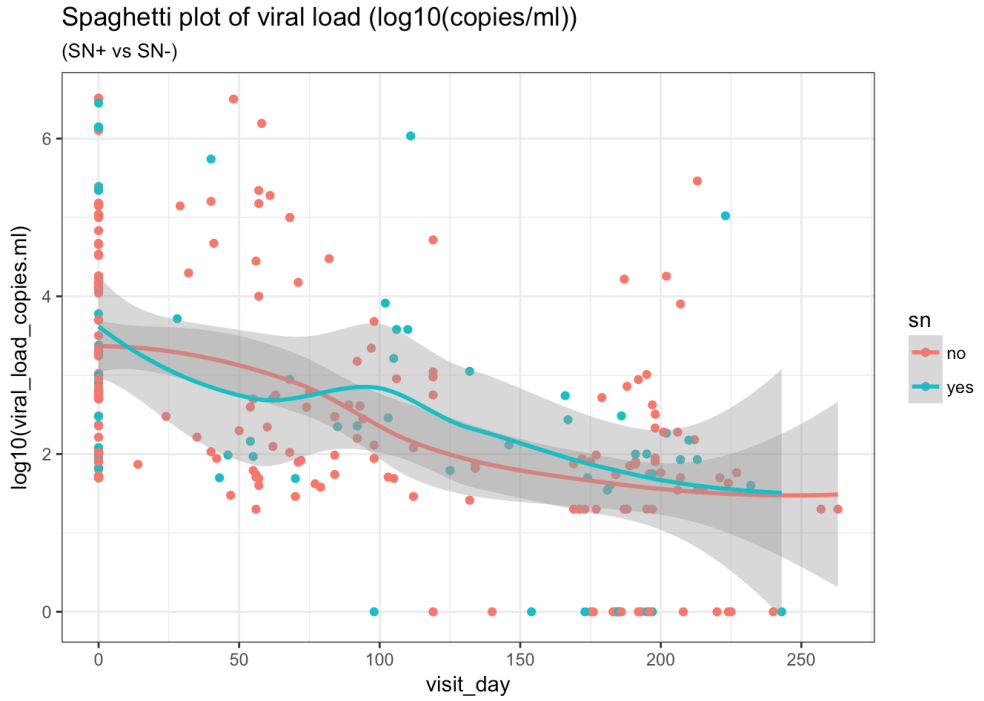
			CD4: Viral Load:		
			CD4: Viral Load:		

APPENDIX 4

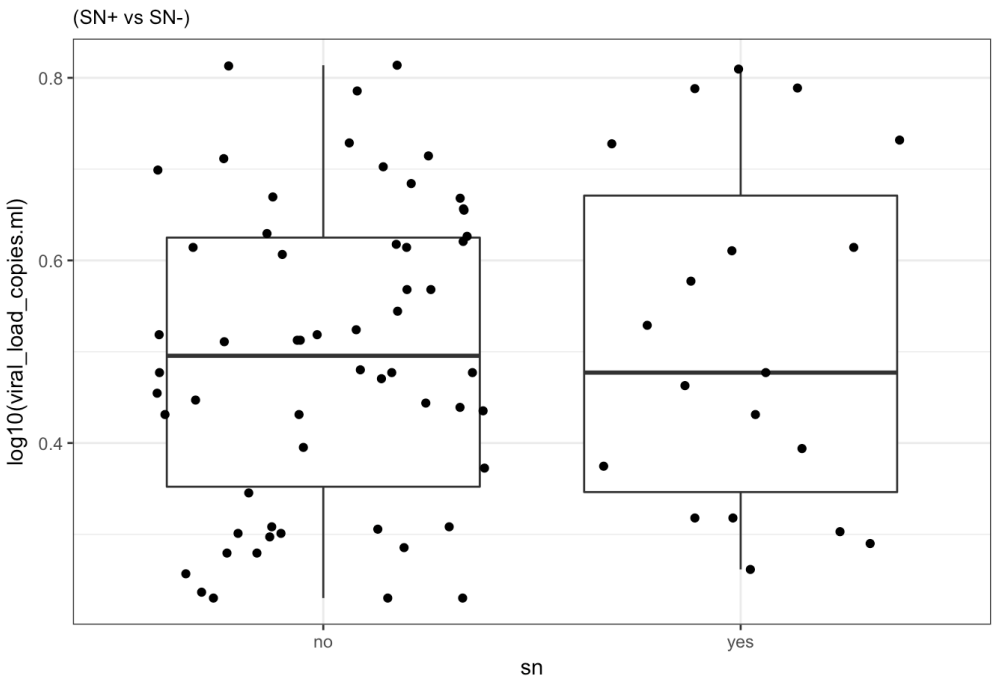
ADDITIONAL PLOTS AND GRAPHS FOR DESCRIPTIVE DATA IN INCIDENCE STUDY



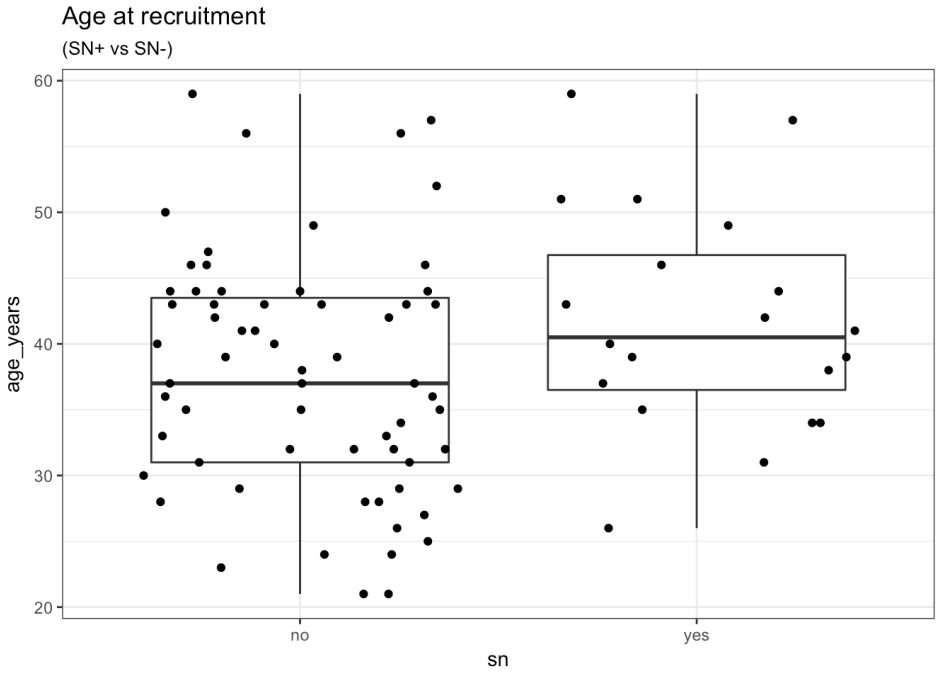
Summary plot of CD4 count for entire cohort, stratified according to neuropathy development (n=83)



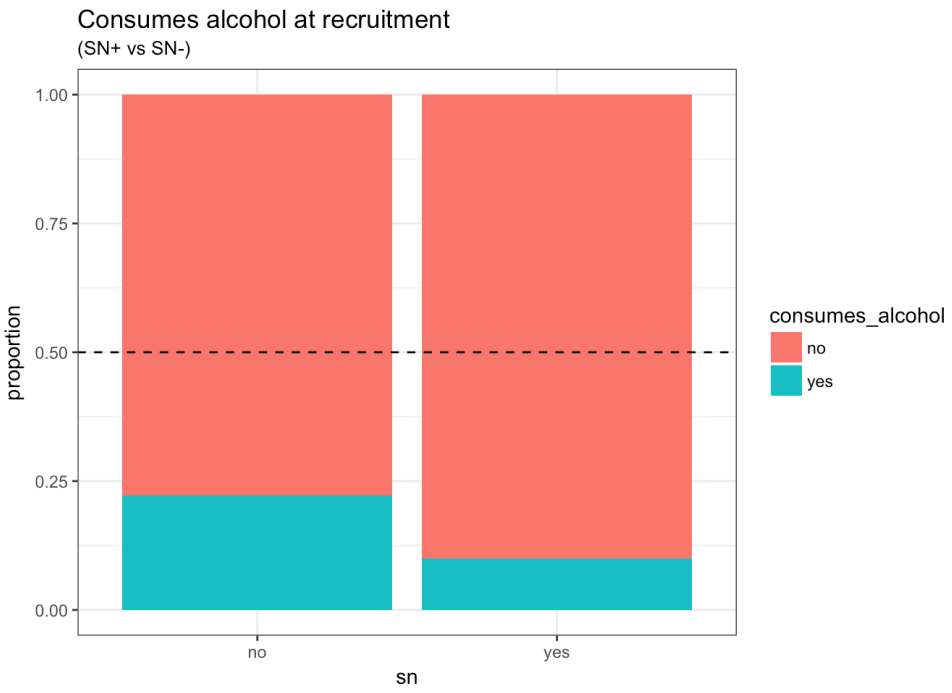
Summary plot of viral load for entire cohort, stratified according to neuropathy development (n=83).



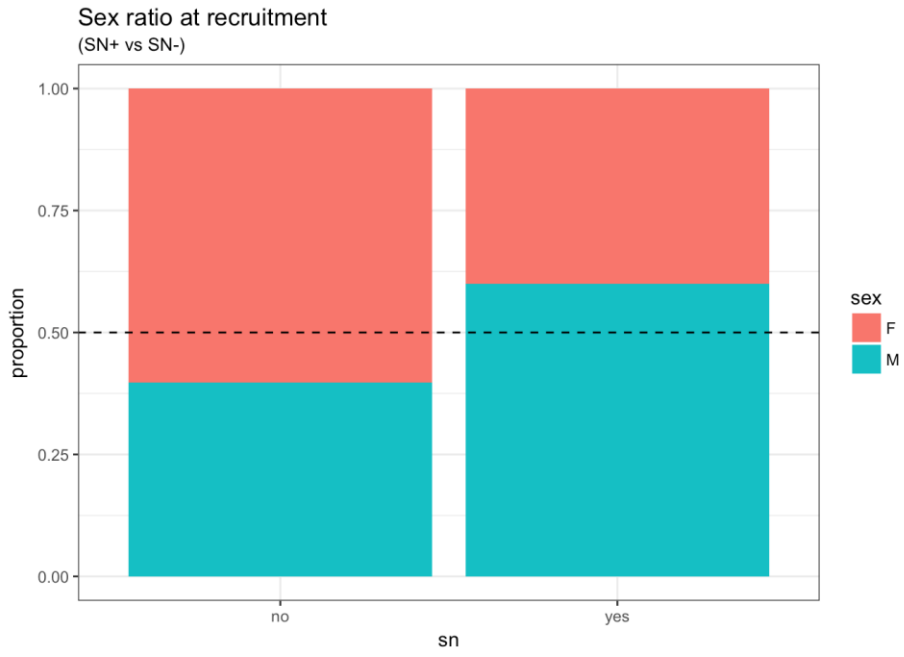
Box and whiskers plot of viral load for entire cohort, stratified according to neuropathy development (n=83)



Box and whiskers plot of age at baseline for entire cohort, stratified according to neuropathy development (n=83).



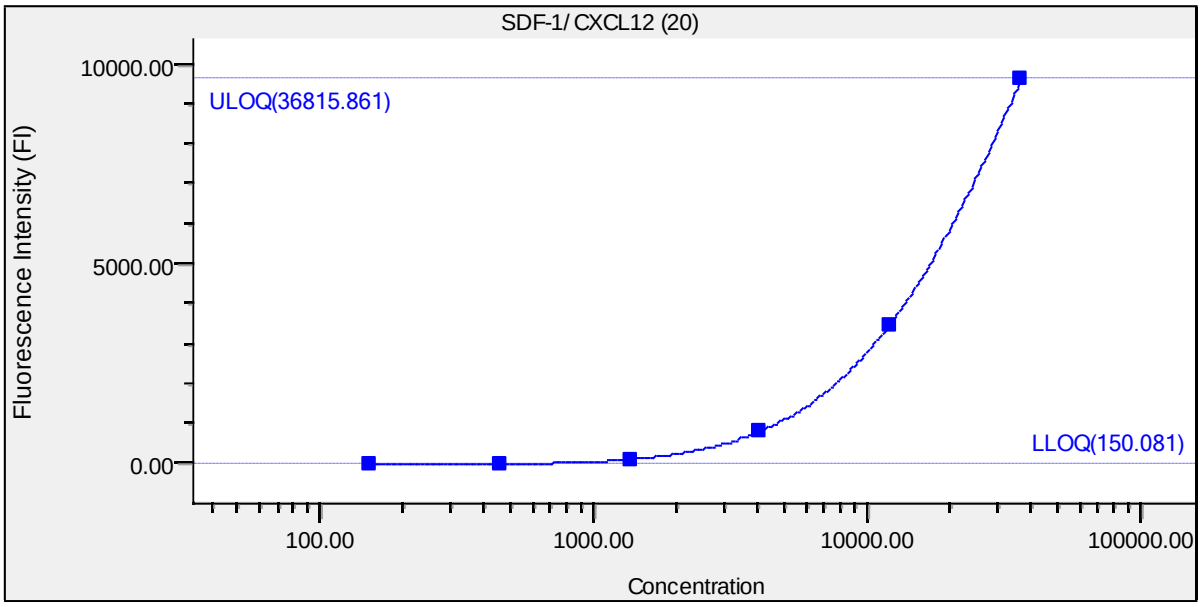
Bar graph of alcohol consumption at baseline for entire cohort, stratified according to neuropathy development (n=83).



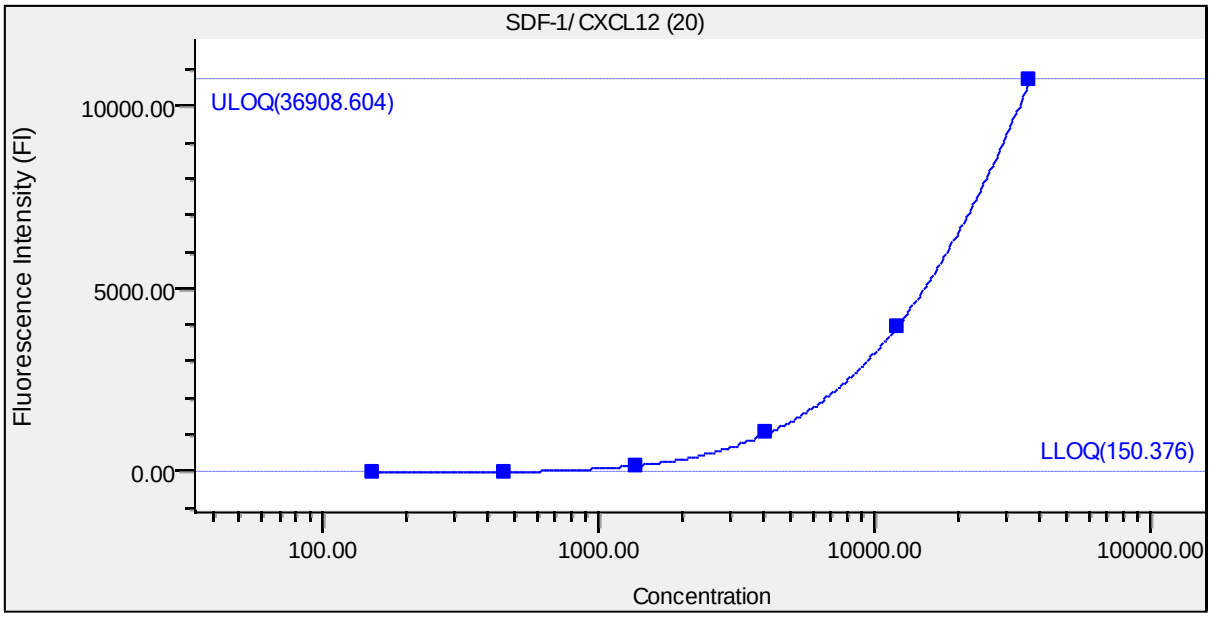
Bar graph of sex ration (male vs. female) at baseline for entire cohort, stratified according to neuropathy development (n=83).

APPENDIX 5

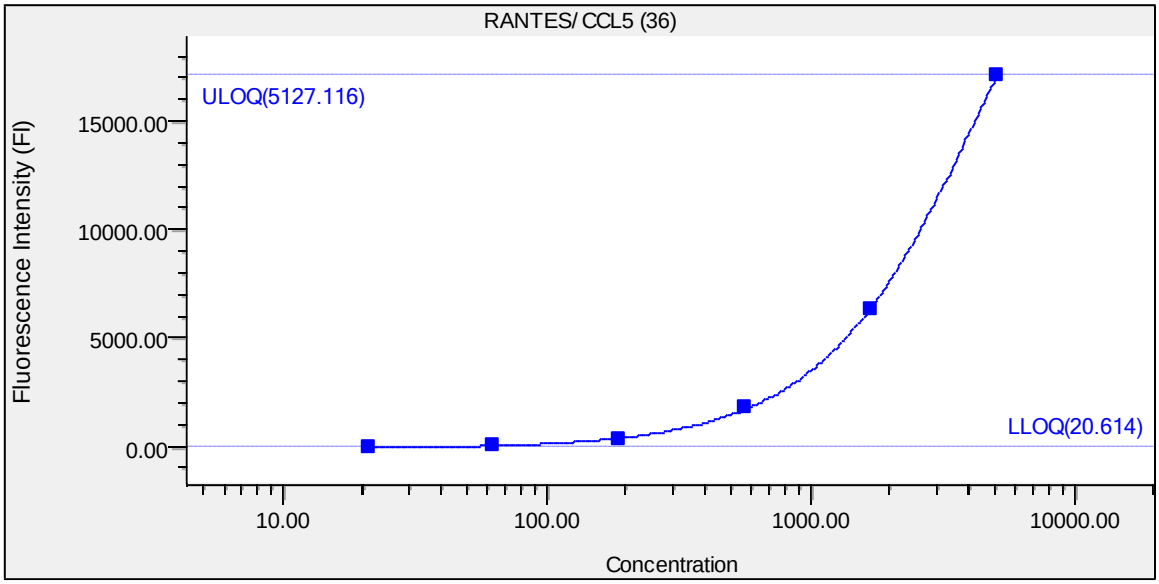
ADDITIONAL GRAPHS FOR CHEMOKINE ANALYSIS



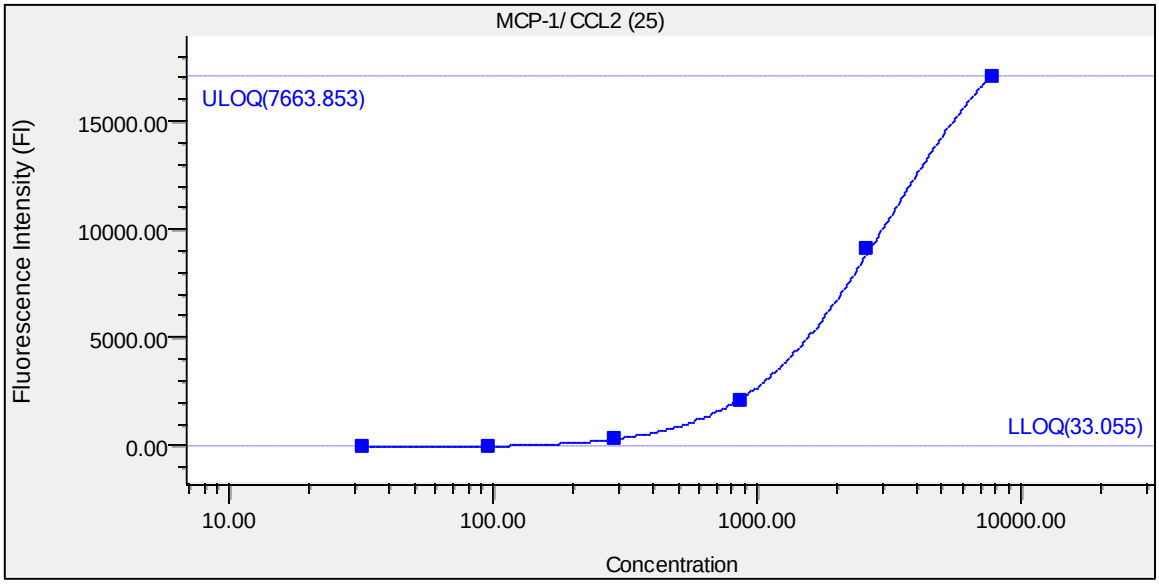
Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CXCL12 (SDF-1), Baseline and 2-month samples.



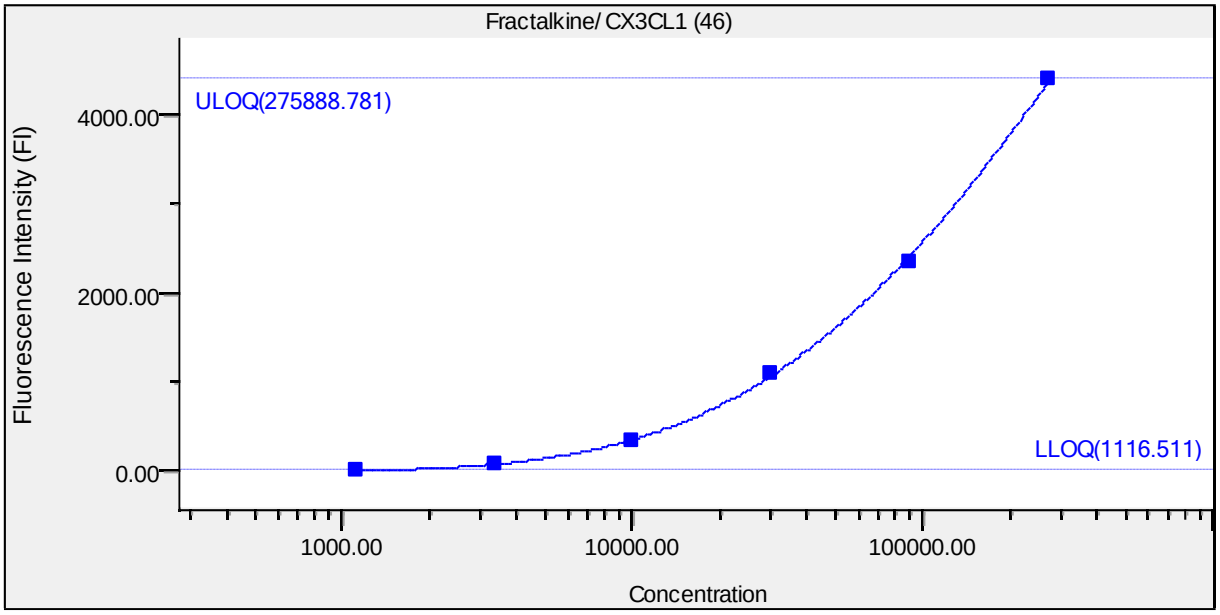
Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CXCL12 (SDF-1), 4-month and 6-month samples.



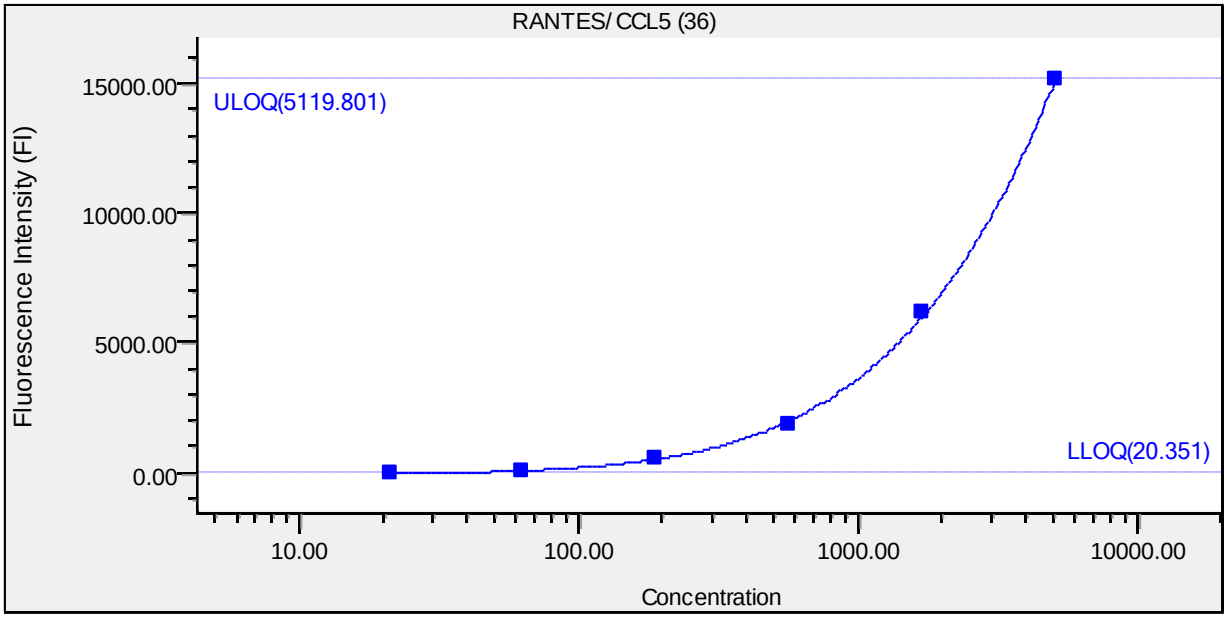
Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CCL5 (RANTES), Baseline and 2-month samples. (*Data excluded from analysis*)



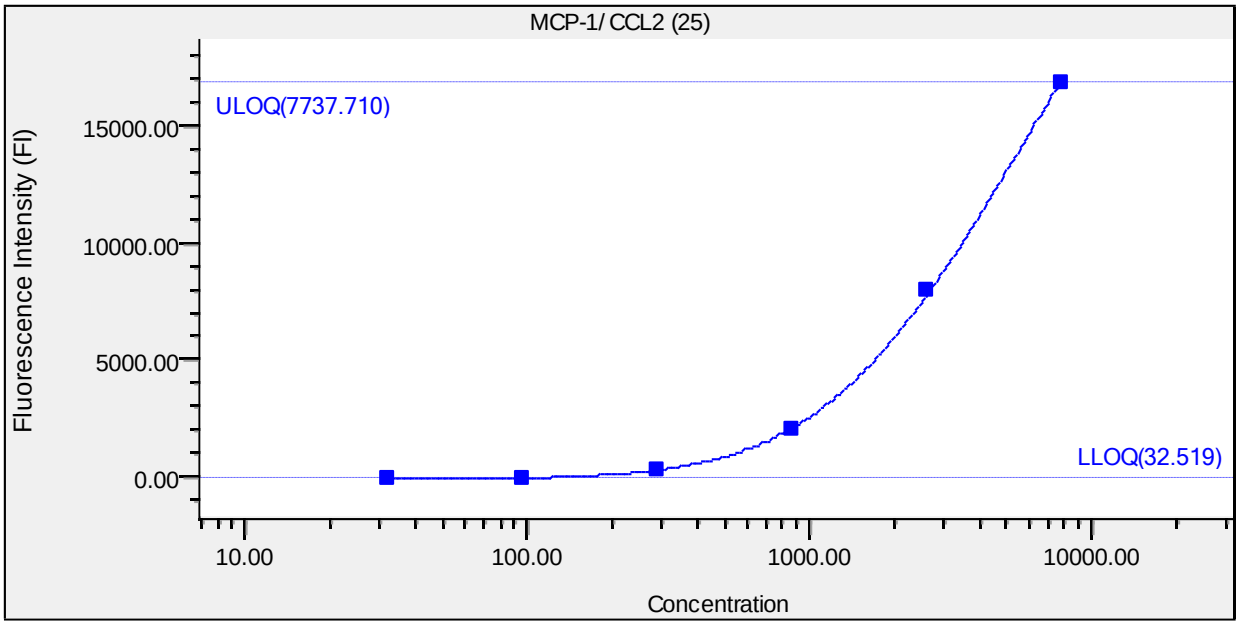
Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CCL2 (MCP-1), Baseline and 2-month samples.



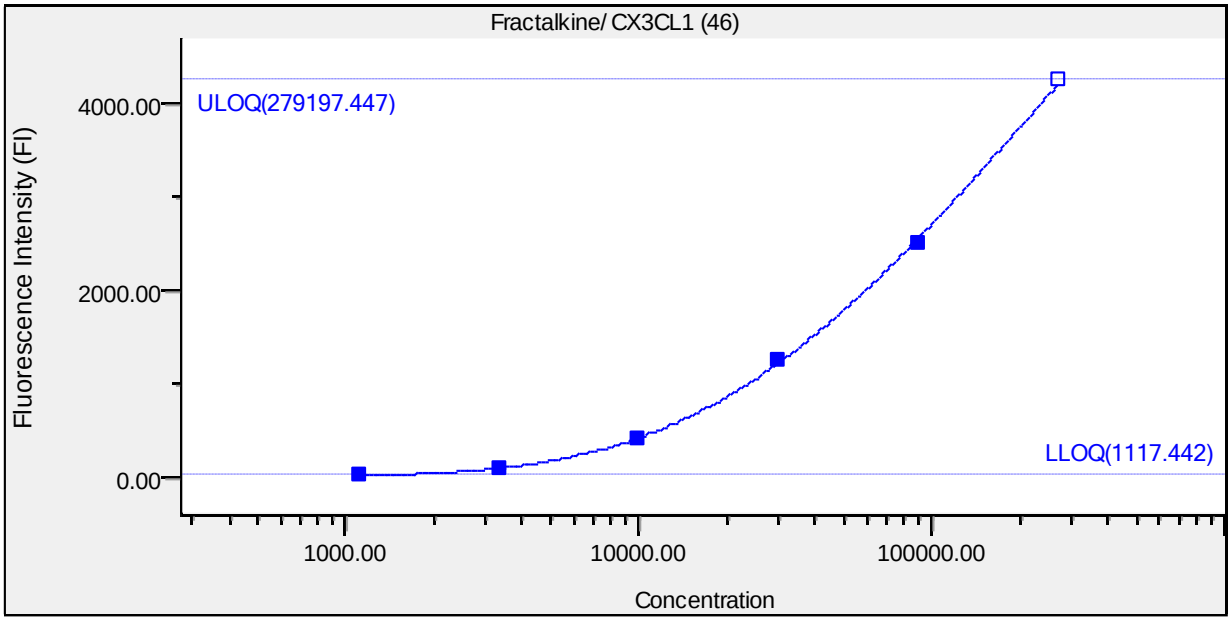
Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CX3CL1 (Fractalkine), Baseline and 2-month samples.



Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CCL5 (RANTES), 4-month and 6-month samples. (*Data excluded from analysis*)



Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CCL2 (MCP-1), 4-month and 6-month samples.



Standard curve generated by Bio-Rad Bio-Plex 200 for plasma concentration of CX3CL1 (Fractalkine), 4-month and 6-month samples.

APPENDIX 6

PILLAY P, WADLEY AL, CHERRY CL, KARSTAEDT AS, KAMERMAN PR

(2015) Pharmacological treatment of painful HIV-associated sensory neuropathy. *S Afr Med J.* 105 (9): 769-772.

Pharmacological treatment of painful HIV-associated sensory neuropathy

P Pillay,[1] MSc; **A L Wadley**,[1] PhD; **C L Cherry**,[1,2,3] MBBS, PhD; **A S Karstaedt**,[4] MB BCh; **P R Kamerman**,[1] PhD

[1] *Brain Function Research Group, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[2] *International Clinical Research Laboratory, Centre for Biomedical Research, Burnet Institute, Melbourne, Victoria, Australia*
[3] *Infectious Diseases Unit, Alfred Hospital and Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Victoria, Australia*
[4] *Department of Medicine, Chris Hani Baragwanath Hospital, Johannesburg, South Africa*

Corresponding author: *P Pillay (prinishapillay_13@yahoo.com)*

Background. HIV-associated sensory neuropathy (HIV-SN) is a common and frequently painful complication of HIV infection and its treatment. However, few data exist describing the frequency, type and dosage of pain medications patients are receiving in the clinic setting to manage the painful symptoms of HIV-SN.
Objective. To report on analgesic prescription for painful HIV-SN and factors influencing that prescription in adults on combination antiretroviral therapy.
Methods. Using validated case ascertainment criteria to identify patients with painful HIV-SN, we recruited 130 HIV-positive patients with painful HIV-SN at Chris Hani Baragwanath Hospital, Johannesburg, South Africa. Demographic and clinical data (including current analgesic use) were collected on direct questioning of the patients and review of the medical files.
Results. We found significant associations, of moderate effect size, between higher pain intensity and lower CD4 T-cell counts with prescription of analgesic therapy. Factors previously identified as predicting analgesic treatment in HIV-positive individuals (age, gender, level of education) were not associated with analgesic use here. Consistent with national guidelines, amitriptyline was the most commonly used agent, either alone or in combination therapy. Importantly, we also found that despite the relatively high analgesic treatment rate in this setting, the majority of patients described their current level of HIV-SN pain as moderate or severe.
Conclusion. Our findings highlight the urgent need for both better analgesic options for HIV-SN pain treatment and ongoing training and support of clinicians managing this common and debilitating condition.

S Afr Med J 2015;105(9):769-772. DOI:10.7196/SAMJnew.7908

HIV-associated sensory neuropathy (HIV-SN) is a common and frequently painful complication of HIV infection and its treatment, and is likely to remain prevalent for the foreseeable future.[1-3] Painful HIV-SN is associated with substantially reduced health-related quality of life,[2] but evidence-based analgesic options are lacking.[4] Several national and international agencies[5-10] have recommended the tricyclic antidepressant amitriptyline for HIV-SN pain, despite evidence that this is no better than placebo.[11,13]

Despite the high prevalence[1,2] and considerable impact of HIV-SN, and the lack of proven effective analgesics, few data exist describing the frequency, type and dosage of pain medication patients are receiving. We are aware of only one retrospective case review, with low-quality case ascertainment criteria, that attempted to describe the treatment of HIV-SN pain in a clinical setting.[14] This showed that only 7% of a cohort of Malawians who may have had painful HIV-SN were prescribed amitriptyline.

We used validated case ascertainment criteria to identify patients with painful HIV-SN, and provide the first report on analgesic prescription for HIV-SN among South African (SA) adults on combination antiretroviral therapy (cART).

Methods

Consecutive consenting HIV-positive adults on cART were screened for HIV-SN at the Greenhouse Pharmacy, which services patients attending the Ntabiseng Clinic at Chris Hani Baragwanath Hospital, Johannesburg, SA, between June 2012 and January 2014. Researchers attended all pharmacy days servicing ambulatory adults infected with HIV. All individuals on stable cART (any regimen) for longer than 6 months and who had HIV-SN (painful or non-painful) were included in the study. The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg. Informed consent was obtained from all participants.

Patients were screened for HIV-SN using the AIDS Clinical Trials Group (ACTG) Brief Neuropathy Screening Tool (BPNS).[15] HIV-SN was diagnosed on the basis of at least one bilateral sign (vibration sense <10 seconds using a 128 Hz tuning fork in the great toe or absent ankle reflexes) and at least one symptom (pain, paraesthesiae or numbness) in both feet. Symptom severity was rated on an 11-point numerical pain rating scale (NRS) ranging from 0 (not present) to 10 (most severe imaginable). Demographic and clinical data (including current prescribed and self-medicated analgesic use) were collected on direct questioning of the patient and review of the medical file.

Descriptive statistics are presented as means (standard deviation (SD)) for parametric data, medians (interquartile range (IQR)) for non-parametric data, and percentages for frequency data. Univariate analyses comparing patients receiving and not receiving analgesic therapy included Fisher's exact test (gender, years of formal education, number of pain sites, ART regimen, current tuberculosis (TB) infection, sensory symptoms other than pain), the Wilcoxon rank sum test (pain intensity, CD4 T-cell count, years of formal education), and Student's *t*-test (age). Variables with $p<0.1$ on univariate analysis were included in multivariate logistic regression analysis. For those patients who were receiving analgesic therapy, the Cochrane-Armitage test for

trend was used to assess whether there was an association between the dose of analgesic prescribed and pain intensity. Pain was categorised as mild if rated 1 - 3, moderate if rated 4 - 7, and severe if rated 8 - 10, as set out in the ACTG BPNS.[15]

Results

One hundred and thirty black SA patients with painful HIV-SN were recruited. Consistent with the population seen in clinics in Johannesburg,[16] the subjects were predominantly middle-aged women (72% female, mean (SD) age 45.7 (9.4) years) with well-preserved CD4 T-cell counts. Current HIV-SN pain was rated as severe by two-thirds of the subjects, with an overall median pain intensity of 7.5 (IQR 2 - 10) on the numerical rating scale. Details of the clinical and demographic features of the cohort are shown in Table 1.

Significant associations were observed between the use of analgesic medication and current pain severity (individuals with greater pain were more likely to be using analgesics: median difference in pain intensity rating on the 11-point NRS (95% CI of difference in medians) 4 (0 - 5)), and between the use of analgesic medication and the latest CD4 T-cell count (individuals with lower CD4 T-cell counts were more likely to be using analgesics: median difference in CD4 T-cell count (95% CI of difference in medians) −138 (−315 - 21) cells/μL). Multivariate logistic regression models incorporating combinations of pain intensity, CD4 T-cell count and number of pain sites ($p<0.1$ on univariate analysis) were not significantly better than a model only incorporating pain intensity as an independent variable (analysis of deviance $p>0.05$). CD4 T-cell count was not associated with pain intensity (Spearman's rho −0.08, $p=0.35$).

Pharmacological treatments prescribed to the patients are shown in Table 2. Overall, 64% of patients were using at least one analgesic medication, including three-quarters of those with severe pain, half of those with moderate pain and one-quarter of those who described their current pain as mild. Consistent with national guidelines, amitriptyline was the most commonly used agent, either alone or in combination therapy.

In patients receiving analgesic therapy, there was no association between drug dosage prescribed and pain intensity for amitriptyline ($\chi^2=1.88$, $p=0.18$), or for paracetamol and codeine ($\chi^2=0.01$, $p=0.94$).

During the patient interview, we also collected information on over-the-counter medications participants were taking. The majority of patients were only taking those medications provided at the pharmacy, as they could not afford to buy their own medications. Of the few patients who did so, none purchased pain medications.

Table 1. Demographic and clinical characteristics of the cohort and univariate associations with use of any analgesic medication for HIV-SN

	Entire cohort (*N*=130)	Participants prescribed analgesics (*N*=83)	Participants not prescribed analgesics (*N*=47)	*p*-value
Female gender, *n* (%)	93 (71.5)	57 (68.7)	36 (76.6)	0.42
Age (years), mean (SD)	45.7 (9.4)	45.4 (9.3)	46.2 (9.8)	0.63
CD4 T-cell count (cells/μL), median (IQR)	409 (34 - 1 606)	369 (34 - 1 092)	507 (63 - 1 606)	0.04
Formal education (years), median (IQR)	6 (0 - 8)	6 (0 - 8)	6 (0 - 8)	0.94
Current TB infection, *n* (%)	8 (6.2)	6 (7.2)	2 (4.3)	0.71
Treatment regimen, *n* (%)				0.26
TDF based	81 (62.3)	52 (62.7)	29 (61.7)	
AZT based	25 (19.2)	17 (20.5)	8 (17.0)	
D4T based	17 (13.1)	12 (14.5)	5 (10.6)	
Other	7 (5.4)	2 (2.4)	5 (10.6)	
Pain intensity, median (IQR)	7.5 (2 - 10)	10 (2 - 10)	6 (2 - 10)	0.01
Pain intensity rating, *n* (%)				<0.01*
Mild	12 (9.2)	3 (3.6)	9 (19.1)	
Moderate	34 (26.2)	18 (21.7)	16 (34.0)	
Severe	84 (64.6)	62 (74.7)	22 (46.8)	
Number of pain sites, median (IQR)	3 (1 - 9)	3 (1 - 9)	3 (1 - 7)	0.06
Prevalence of other symptoms, *n* (%)				
Paraesthesiae	114 (87.7)	79 (95.2)	35 (74.5)	0.001
Numbness	116 (89.2)	77 (92.8)	39 (83.0)	0.13

TDF = tenofovir; AZT = zidovudine; D4T = stavudine.
**Post-hoc* analysis identified a greater proportion of patients with severe pain in the group receiving analgesics.

Table 2. Pharmacological medications prescribed for patients with painful HIV-SN

Analgesic (mg)	*n* (%)	Median daily dose (mg) (min - max)*
Ami 25, monotherapy	23 (27.7)	25 (25 - 50)
Ami 25 + para 320 + cod 8	33 (39.8)	Ami 25 (25 - 75)/para 2 560 (1 920 - 2 560)/cod 64 (48 - 64)
Ami 25 + ibu 200	3 (3.6)	Ami 25 (25 - 25)/ibu 200 (200 - 200)
Ami 25 + para 320 + cod 8 + ibu 200	4 (4.8)	Ami 37.5 (25 - 75)/para 2 560 (2 560 - 2 560)/cod 64 (64 - 64)/ibu 200 (200 - 400)
Ami 25 + para 320 + cod 8 + carb 200	6 (7.2)	Ami 25 (25 - 50)/para 2 240 (1 920 - 2 560)/cod 56 (48 - 64)/carb 100 (100 - 200)
Para 320 + cod 8 only	14 (16.9)	Para 1 920 (1 920 - 2 560)/cod 48 (48 - 64)

Ami = amitriptyline; para = paracetamol; cod = codeine; ibu = ibuprofen; carb = carbamazepine.
*Dose frequency was prescribed according to recommended dosing strategies for individual drugs in all cases.

Discussion

We provide the first report of analgesic use for HIV-SN pain in which HIV-SN was defined using standard, objective criteria. Ninety per cent of patients in our sample had moderate or severe pain, despite almost two-thirds being on some form of analgesic therapy. In most cases the tricyclic antidepressant amitriptyline was included in the analgesic regimen, consistent with national guidelines.[6,7] We found significant associations, of moderate size, between pain intensity and analgesic therapy as well as between CD4 T-cell count and analgesic

therapy; subjects with more severe pain and those with lower CD4 T-cell counts were more likely to be receiving treatment. However, greater pain was not associated with analgesic dose used, and lower CD4 T-cell counts were not associated with pain intensity. Factors previously identified as predicting analgesic treatment in HIV-positive individuals (age, gender, level of education) were not associated with analgesic use in this study.[17]

Our finding that 36% of patients with HIV-SN pain were not receiving any analgesic therapy is worrying. Nevertheless, analgesic use in this cohort was high compared with the very low rates we and others have reported in similar ambulatory HIV-positive populations with pain of similar intensity, but of any origin.[18-21] Clinicians may be more aware of HIV-SN as a common cause of pain, and therefore be more likely to recognise and treat HIV-SN pain than other pain conditions. Although more than a third of patients with HIV-SN pain in this cohort were not receiving analgesics, we cannot exclude the possibility that they may previously have used agents such as amitriptyline and ceased these owing to poor tolerability or efficacy. Indeed, the high levels of pain reported by many patients using amitriptyline in this cohort highlight the need for more effective treatments for HIV-SN pain,[12] or greater awareness of optimal dosing of amitriptyline for the management of neuropathic pain.

Our finding that use of some form of analgesic agent increases with pain severity may indicate that physicians believe that regular analgesics are required only for HIV-SN pain of greater severity, despite evidence that health-related quality of life is reduced across all levels of pain in this condition.[2] However, it is also possible that patients do not report mild pain. In addition, our finding that analgesic use increases with lower initial CD4 T-cell count may indicate that patients perceive sensory neuropathy to be a complication of ART, and more of those patients with severe pain and severe paraesthesiae (median 8, IQR 2 - 10) may therefore not take their ART reliably, possibly thinking that they are being harmed by these drugs, and therefore have a poor CD4 T-cell count.

Amitriptyline was prescribed to most patients receiving analgesia in this cohort. While there is no evidence that amitriptyline is more effective than placebo for painful HIV-SN,[11-13] its proven efficacy in other types of neuropathic pain[22] prompted an expert panel to recommend it as a first-line option for HIV-SN pain in SA.[7] These patients were therefore receiving a recommended treatment, albeit at a relatively low dose (25 - 50 mg/d).[7] Amitriptyline was commonly used in combination with codeine and paracetamol. Neither of these drugs have proven efficacy in neuropathic pain,[22] but their use may reflect concurrent sources of pain (common in HIV) that may be responsive to standard opioid and non-opioid analgesics and non-steroidal anti-inflammatory agents.[23] Use only of agents with no evidence of efficacy for neuropathic pain by 16% of our cohort may reflect poor knowledge of neuropathic pain management on the part of clinicians, inadequate efficacy and/or tolerability of available neuropathic pain treatments, or treatment of concurrent nociceptive pains while the neuropathic pain went unrecognised. This finding highlights the need for both better analgesic options for HIV-SN pain treatment and ongoing training and support of clinicians managing this difficult condition.

Six patients (7%) were prescribed amitriptyline together with the anticonvulsant carbamazepine, largely owing to the preference of a single clinician. Carbamazepine decreases levels of amitriptyline and paracetamol by affecting hepatic/intestinal enzyme CYP3A4 metabolism.[24] There is also potential for increased sedation in patients taking codeine and amitriptyline concurrently, but at the doses of codeine prescribed, this interaction was probably not significant.[25]

Study limitations

Our study has a number of limitations. The modest sample size limits our ability to understand factors associated with use of less common analgesic choices. The cross-sectional nature of this work means that we are unable to comment on patients' previous level of HIV-SN pain or prior use of analgesics for this condition. We therefore cannot comment on the level of pain relief achieved by the treatments used. Patients also express preferences for analgesic therapy, with some not wanting certain analgesics because they fear side-effects, which may affect treatment choices. Moreover, we do not have data on comorbid diseases for which analgesics may have been prescribed, including depression (amitriptyline) and seizure disorders (carbamazepine), although we would have expected higher doses to have been used in these situations.[26,27]

Conclusion

We found that most patients with HIV-SN pain in a large SA HIV care clinic were receiving analgesics consistent with the recommendation in the national guidelines. However, about one in six patients were only receiving agents with no demonstrated efficacy for neuropathic pain. Furthermore, despite the observed high level of treatment coverage, the majority of patients described their current level of HIV-SN pain as moderate or severe, highlighting the urgent need for better therapies for this common and disabling condition.

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APPENDIX 7

QUESTIONNAIRES (ENGLISH)

QUESTIONNAIRES (ISIZULU TRANSLATION)

QUESTIONNAIRES (SESOTHO TRANSLATION)

QUESTIONNAIRES (SETSWANA TRANSLATION)

QUESTIONNAIRES (ENGLISH)

Pain Catastrophizing Scale (PCS)

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures or surgery.

We are interested in the types of thoughts and feeling that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

	Not at all	To a slight degree	To a moderate degree	To a great degree	All the time
I worry all the time about whether the pain will end	0	1	2	3	4
I feel I can't go on	0	1	2	3	4
It's terrible and I think it's never going to get any better	0	1	2	3	4
It's awful and I feel that it overwhelms me	0	1	2	3	4
I feel I can't stand it anymore	0	1	2	3	4
I become afraid that the pain will get worse	0	1	2	3	4
I keep thinking of other painful events	0	1	2	3	4
I anxiously want the pain to go away	0	1	2	3	4
I can't seem to keep it out of my mind	0	1	2	3	4
I keep thinking about how much it hurts	0	1	2	3	4
I keep thinking about how badly I want the pain to stop	0	1	2	3	4

There's nothing I can do to reduce the intensity of the pain	0	1	2	3	4
I wonder whether something serious may happen	0	1	2	3	4

Mini Mental State Examination (MMSE-SA) (South African adapted version)

Mini-Mental Status Examination

The Mini-Mental Status Examination offers a quick and simple way to quantify cognitive function and screen for cognitive loss. It tests the individual's orientation, attention, calculation, recall, language and motor skills.

Each section of the test involves a related series of questions or commands. The individual receives one point for each correct answer.

To give the examination, seat the individual in a quiet, well-lit room. Ask him/her to listen carefully and to answer each question as accurately as he/she can.

Don't time the test but score it right away. To score, add the number of correct responses. The individual can receive a maximum score of 30 points.

A score below 20 usually indicates cognitive impairment.

The Mini-Mental Status Examination

Name: _____ DOB: _____

Years of School;_____ Date of Exam: _____

Orientation to Time	Correct	Incorrect
What is today's date?	☐	☐
What is the month?	☐	☐
What is the year?	☐	☐
What is the day of the week today?	☐	☐
What season is it?	☐	☐

Total: ____

Orientation to Place		
What place is this?	☐	☐
Whose room is this?	☐	☐
What city are we in?	☐	☐

What country are we in? ☐ ☐
What province are we in? ☐ ☐

Total:____

Immediate Recall
Ask if you may test his/her memory. Then say "bed", "chair", "pot" clearly and slowly, about 1 second for each. After you have said all 3 words, ask him/her to repeat them – the first repetition determines the score (0-3):

Bed ☐ ☐
Chair ☐ ☐
Pot ☐ ☐

Total: ____

Attention
A) A man has R20.00 for taxi fare. Everyday he spends R3.00 on his taxi fare. After spending the first day's taxi fare, he will be left with R17.00. How much will be left after spending the next days taxi fare? And the next day's taxi fare... (Five times)

R14.00 ☐ ☐
R11.00 ☐ ☐
R8.00 ☐ ☐
R5.00 ☐ ☐
R2.00 ☐ ☐

Total:_____

B) Name the days of the week backwards e.g. (before Sunday comes Saturday, and before Saturday comes Friday and so on...)

Friday ☐ ☐
Thursday ☐ ☐
Wednesday ☐ ☐
Tuesday ☐ ☐
Monday ☐ ☐

Total:_____

Delayed Verbal Recall
Ask the individual to recall the 3 words you previously asked him/her to remember.

Bed ☐ ☐
Chair ☐ ☐
Pot ☐ ☐

Total: _____

Naming
Show the individual a cup and ask him/her what it is. Repeat for pen.

Cup	☐	☐
Pen	☐	☐

Repetition

Ask the individual to repeat the following:

"Neither this, nor that"	☐	☐

3-Stage Command

Give the individual a plain piece of paper and say, "Take the paper in your hand, fold it in half, and put it on the floor."

Takes	☐	☐
Folds	☐	☐
Puts	☐	☐

Reading

Examiner says"look at me and do exactly what I do" and then raises his/her right hand for 3 seconds. Score correctly only if the individual actually raises his/her right hand

☐ ☐

Sentence construction

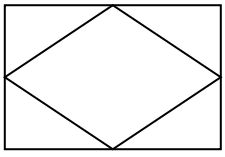
The individual is asked, 'If you did not know name, how would you find out my name?'

☐ ☐

Copying

Give the individuals a set of sticks and ask him/her to construct a figure with sticks following a laid out construction of a square with a diamond inside.

☐ ☐



Total Score:_____

Hopkins Symptoms Checklist 25 (HSCL-25)

Hopkins Symptom Checklist 25

_____DATE _____SERIAL NUMBER

Listed below are some symptoms or problems that people sometimes have. Please read each one carefully and decide how much the symptoms bothered or distressed you in the last week, including today. Place a check in the appropriate column.

Extremely	**Quite a bit**	**A little**	**Not at all**	**Part 1** **Anxiety symptoms**	
				suddenly scared for no reason	1
				feeling fearful	2
				faintness, dizziness or weakness	3
				nervousness or shakiness inside	4
				heart pounding or racing	5
				trembling	6
				feeling tense or keyed up	7
				headaches	8
				spells of terror or panic	9
				feeling restless, can't sit still	10
				Part 2 **Depression symptoms**	
				feeling low in energy, slowed down	11
				blaming yourself for things	12
				crying easily	13
				loss of sexual interest or pleasure	14
				poor appetite	15
				difficulty falling asleep, staying asleep	16
				feeling hopeless about the future	17
				feeling blue	18
				feeling lonely	19
				thoughts of ending your life	20
				feeling of being trapped or caught	21
				worrying too much about things	22
				feeling no interest in things	23
				feeling everything is an effort	24
				feelings of worthlessness	25

Wisconsin Brief Pain Questionnaire
(revised study version)

HIV Pain
Brief pain questionnaire

Patient reference no. _____

Date:_____

Dear patient,

Please read and answer the questions below. We would like you to tell us about any pain you might be experiencing and also indicate where you are experiencing this pain on the body diagram provided.

1. When you first received your diagnosis, was pain one of your symptoms? Please tick the correct box.

   ☐ Yes ☐ No

2. If your answer to the first question was Yes, mark one of the following boxes.

   ☐ Pain was a reason I went to the doctor

   ☐ Pain was present but not the reason why I visited the doctor

3. Have you ever had pain due to your present disease?

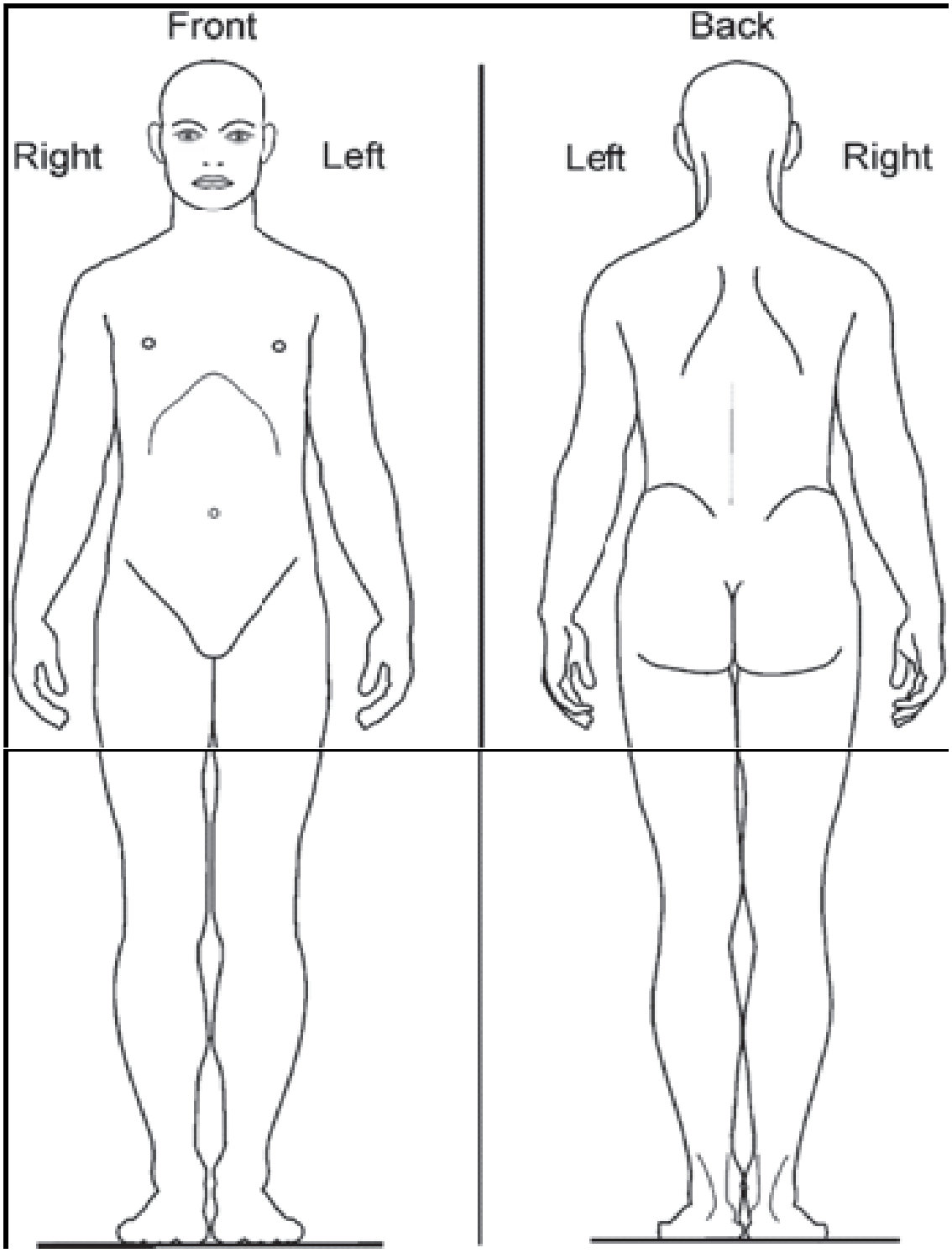
   ☐ Yes ☐ No

4. Throughout our lives, most of us have had pain from time to time (such as headaches, muscle aches and tooth aches). Have you had pain other than these everyday kinds of pain during the last month? Please tick the correct box.

   ☐ Yes ☐ No

   If you answered Yes, please continue to question 5 on the next page.

5. Show on this picture where your pain occurs by colouring in the painful area. Write the letter "S" if the pain is just on the surface or the letter "D" if the pain is much deeper inside your body, next to the place where you coloured in. Also show where the **main** pain is on the picture.



6. The “other” pain, besides your “main” pain which you coloured in on the picture is important and we would like you to please describe it using the scale below. Tick the correct box to describe the feeling of this pain:

☐ Pains a little bit (Mild)

☐ Pains quite a bit (Moderate)

☐ Pains quite a lot (severe)

7. What other words could you use to describe this "other" pain, which is not your main pain?

_____

_____

Additional comments:

_____

_____

ED-5D

Health Questionnaire
(English version for South Africa)

By placing a tick in one box in each group below, please indicate which statements best describe your own state of health TODAY.

Mobility

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

Self-Care

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

Usual Activities (*e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

Pain/Discomfort

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

Anxiety/Depression

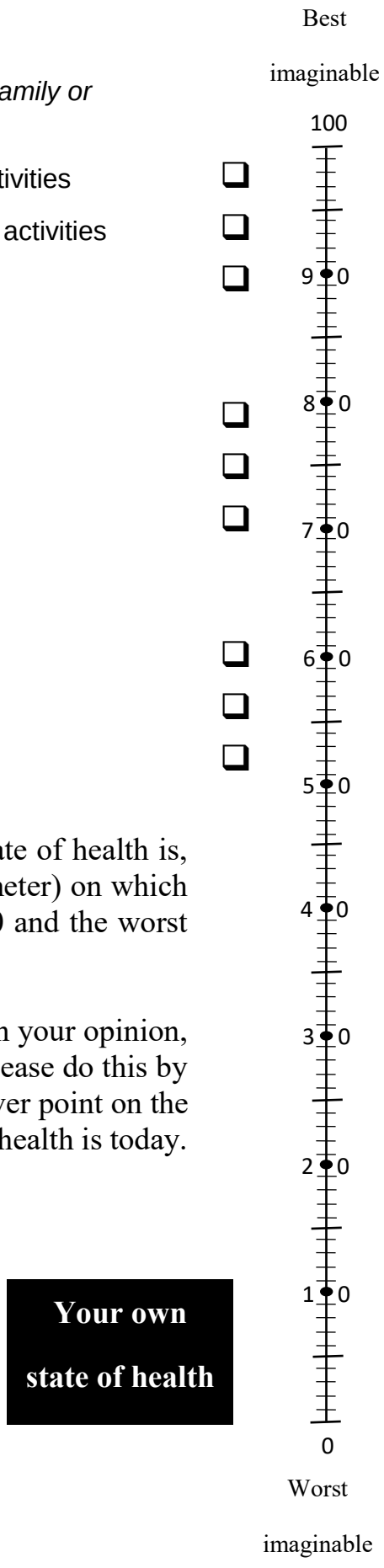
I am not anxious or depressed ❑

I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

To help people say how good or bad their state of health is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale, in your opinion, how good or bad your own health is today. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your state of health is today.



QUESTIONNAIRES (ISIZULU TRANSLATION)

Isikali Sehaba Lobuhlungu (Pain Catastrophizing Scale (PCS))

Wonke umuntu uzwa ubuhlungu ekuphileni kwakhe. Kungase kube yikhanda elinkenkethayo, ubuhlungu bezinyo, ubuhlungu bamalunga noma bemisipha. Kuhlale kunezimo ezibangela ubuhlungu ezinjengokugula, ukulimala, ukukhishwa kwezinyo noma ukuhlinzwa. Singathanda ukwazi ngemicabango nendlela ozizwa ngayo uma usezinhlungwini. Ethebuleni elingezansi, sinezincazelo ezingu-13 ezichaza imicabango nemizwa ehlukahlukene engase ihlotshaniswe nobuhlungu.

	Lutho neze	Kancane	Kumaphakathi	Kakhulu	Njalo
Ngihlale ngikhathazeka ngokuthi ubuhlungu buzophela nini.	0	1	2	3	4
Ngizizwa sengathi angikwazi ukuqhubeka kanje	0	1	2	3	4
Kuyethusa kakhulu futhi ngicabanga ukuthi ngeke kube ngcono.	0	1	2	3	4
Kuyethusa futhi ngizizwa ngingenawo amandla.	0	1	2	3	4
Nginomuzwa wokuthi angisakwazi ukukubekezelela.	0	1	2	3	4
Nginovalo lokuthi ubuhlungu buzoba bubi kakhulu.	0	1	2	3	4
Ngihlale ngicabanga ngobuhlungu noma izenzakalo ezizwisa ubuhlungu	0	1	2	3	4
Ngifisa sengathi ubuhlungu bungaphela ngokushesha.	0	1	2	3	4
Ngihlale ngicabanga ngabo.	0	1	2	3	4
Ngihlale ngicabanga indlela okubuhlungu ngayo.	0	1	2	3	4
Ngihlale ngicabanga indlela engifisa ngayo ukuthi ubuhlungu buphele.	0	1	2	3	4
Akukho engingakwenza ukunciphisa ubuhlungu obuhlabayo.	0	1	2	3	4
Angazi noma kungase kwenzeke okuthile okubi.	0	1	2	3	4

Ukuxilongwa Okufushane Kwesimo Sengqondo (MMSE) (uhlelo olwenzelwe iNingizimu Afrika)

Ukuxilongwa Okufushane Kwesimo Sengqondo

Ukuxilongwa okufushane kwengqondo kusiza ekuboniseni ngokushesha nangendlela elula ukuthi ucabanga kanjani, ukuthi uyakwazi yini ukukhumbula izinto, ukubala, nokuqonda ulimi.

Lokhu kuhlola kunemibuzo nemiyalelo futhi umuntu oxilongwayo uzothola iphoyinti ngombuzo ngamunye awuphendula ngokufanele. Mcele ukuba alalelisise futhi aphendule imibuzo ngendlela efanele.

Ngesikhathi exilongwa, umuntu oxilongwayo kufanele ahlale endaweni ethule enokukhanya okwanele. Mcele ukuba alalelisise futhi aphendule imibuzo ngokwethembeka.

Akukho sikhathi esinqunyiwe sokuphendula imibuzo kodwa nikeza amamaki ngaleso sikhathi uma ephendula. Amamaki aphezulu akufanele eqele ngaphezu kwamaphoyinti angu-30.

Amamaki angaphansi kuka-20 abonisa ukuthi ingqondo iphazamisekile.

Ukuxilongwa Okufushane Kwesimo Sengqondo

Igama: _____Usuku Lokuzalwa: _____

Iminyaka Esikoleni:_____Usuku Lokuxilongwa: _____

Ukuqaphela Isikahthi	Kulungile	Kuyiphutha
Umhla kabani namuhla?	☐	☐
Sikuyiphi inyanga manje?	☐	☐
Unyaka bani lo esikuwo?	☐	☐
Iluphi usuku lwesonto namuhla?	☐	☐
Iyiphi inkathi yonyaka namuhla?	☐	☐
		Ingqikithi: ____

Ukuqaphela Indawo		
Sikuphi lapha?	☐	☐
Elikabani leli gumbi esikulo?	☐	☐
Sikuliphi idolobha?	☐	☐
Sikuliphi izwe?	☐	☐
Sikusiphi isifundazwe?	☐	☐
		Ingqikithi:____

Ukukhumbula Ngokushesha
Buza ukuthi ungayihlola yini ingqondo yakhe. Manje yisho la magama: “umbhede”, “isihlalo”, “ibhodwe” ngokucophelela futhi unense, uthathe umzuzwana ngegama ngalinye. Ngemva kokusho womathathu la magama, cela le ndoda/owesifazane ukuba awaphinde. Igama lokuqala aliphinda kahle lizothola amamaki (0-3):

Umbhede ☐ ☐
Isihlalo ☐ ☐
Ibhodwe ☐ ☐

Ingqikithi: ____

Ukugxilisa Ingqondo
Indoda ethile ino-R20.00 wokugibela itekisi. Nsuku zonke isebenzisa/ikhokha u-R3.00 etekisini. Ngemva kokukhokha leli nani ngosuku, iyobe isisele ngo-R17.00. Iyobe isele ngamalini ngemva kokukhokhela itekisi ngakusasa? Ngosuku olulandela lolo (izikhathi ezinhlanu/izinsuku ezinhlanu)?

R14.00 ☐ ☐
R11.00 ☐ ☐
R8.00 ☐ ☐
R5.00 ☐ ☐
R2.00 ☐ ☐

Ingqikithi:_____

B) Yisho izinsuku zesonto uhlehle. Isibonelo: (Ngaphambi kweSonto kuba uMgqibelo, futhi ngaphambi koMgqibelo kuba uLwesihlanu njalonjalo …)

ULwesihlanu ☐ ☐
ULwesine ☐ ☐
ULwesithathu ☐ ☐
ULwesibili ☐ ☐
UMsombuluko ☐ ☐

Ingqikithi:_____

Ukukhumbula Amagama Kamuva
Cela le ndoda/owesifazane ukuba akhumbule futhi aphinde asho amagama amathathu ombuze wonke ekuqaleni.

Umbhede ☐ ☐
Isihlalo ☐ ☐
Ibhodwe ☐ ☐

Ingqikithi: _____

Amagama Ezinto
Bonisa lo muntu inkomishi bese umbuza ukuthi iyini. Phinda okufanayo nangepeni.

Inkomishi ☐ ☐
Ipeni ☐ ☐

Ukuphindaphinda
Cela lo muntu ukuba aphinde lo musho:

“Akukhona lokhu futhi akukhona lokhuya” ☐ ☐

Umyalo Onezigaba Ezingu-3
Nika lo muntu iphepha elingenalutho bese uthi, “Thatha leli phepha, ulisonge, bese ulibeka phansi”.

Thatha	☐	☐
Songa	☐	☐
Beka	☐	☐

Ukufunda
Umhloli uthi: “Ngibheke bese wenza lokho engikwenzayo” bese umhloli ephakamisa isandla sakhe sokudla imizuzwana emithathu. Mnike amamaki afanele uma ephakamisa isandla sakhe sokudla.

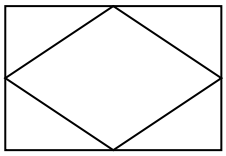
☐ ☐

Ukwakha Imisho
Lo muntu uyabuzwa: ‘Ukube ubungalazi igama lami, ubuzolazi kanjani?’

☐ ☐

Ukukopisha
Nika le ndoda/owesifazane izinti bese umcela ukuba akhe isithombe ngalezi zinti, isikwele esinephethini eyidayimane phakathi nendawo.

☐ ☐



Amamaki Esewonke:_____

Uhla Lokuhlola Izimpawu Ze-Hopkins 25 (HSCL-25)

Uhla Lokuhlola Izimpawu ze-Hopkins 25

_____USUKU _____I-SERIAL NUMBER

Ngezansi kubhalwe izibonelo zobunzima noma izinkinga abantu abavame ukuba nazo. Sicela ufunde ngayinye ngokucophelela bese ukhetha ukuthi yiziphi izibonelo ezikunike izinkinga noma ukukhathazeka kula masonto ambalwa adlule, kanye nanamuhla. Phawula uhla olufanele.

Kakhulu	**Maphakathi**	**Kancane**	**Lutho**	**Ingxenye 1** **Izimpawu zokukhathazeka**	
				unokwesaba ngaphandle kwesizathu.	1
				uzizwa wesaba	2
				isiyezi, awunamandla	3
				uyaqhaqhazela futhi unovalo	4
				inhliziyo yakho ishaya ngamandla kakhulu	5
				uyaqhaqhazela/uyathuthumela	6
				umzimba wakho uqinile	7
				izinhlungu zekhanda	8
				ukushaqeka nokungakhululeki	9
				awuhlaliseki, awunasinqe	10
				Ingxenye 2 **Izimpawu zokucindezeleka ngokomzwelo**	
				ukuzizwa ubuthaka nokuzidonsa	11
				uyazisola	12
				ukhala njalo	13
				awusenandaba nocansi nokuzijabulisa	14
				awusathandi ukudla njengakuqala	15
				unenkinga yokulala, uyaqwasha	16
				uphelelwe yithemba ngekusasa	17
				udangele	18
				unesizungu nesithukuthezi	19
				ucabanga ukuzibulala	20
				uzizwa uthwele kanzima	21
				ukhathazeka kakhulu ngezinto	22
				awunandaba nalutho	23
				kunzima ukuba wenze izinto	24
				uzizwa ungento yalutho	25

EQ-5D

Uhlelo Lwemibuzo Ngempilo

(Zulu version for South Africa)

Ngokufaka uphawu ebhokisini elilodwa kulelo nalelo qoqo elingezansi, sicela ukhombise ukuthi yisiphi isitatimende esichaza kahle kakhulu isimo sempilo yakho namhlanje.

Ukuhamba/ukunyakaza

Anginazinkinga ukuzihambahambela ❑

Nginezinkinga ezithile ukuzihambahambela ❑

Ngihlala ngisembhedeni ❑

Ukuzinakekela

Anginazinkinga ngokuzinakekela ❑

Nginezinkinga ezithile zokuzigeza noma ukuzigqokisa ❑

Angikwazi ukuzigeza noma ukuzigqokisa ❑

Imisebenzi ejwayelekile (*isibonelo: ukusebenza, ukutadisha, umsebenzi wasendlini, imisebenzi yomndeni noma eyokungcebeleka)*

Anginazinkinga ukwenza imisebenzi yami eyejwayelekile ❑

Nginezinkinga ezithile ukwenza imisebenzi yami eyejwayelekile ❑

Angikwazi ukwenza imisebenzi yami eyejwayelekile ❑

Izinhlungu/ukungaphatheki kahle

Anginazinhlungu noma ukungaphatheki kahle ❑

Nginezinhlungu noma ukungaphatheki kahle okulingene nje ❑

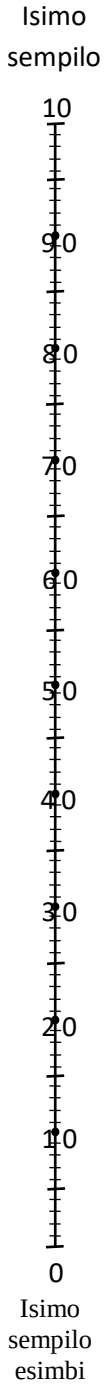
Nginobuhlungu obedlulele nokungaphatheki kahle ❑

Ixhala/ukudangala

Anginalo ixhala noma ukudangala ❑

Nginexhala noma ukudangala okulingene nje ❑

Nginexhala nokudangala ngokweqile ❑



Ukuze sisize abantu basho ukuthi isimo sempilo yabo sihle noma sibi kangakanani, sidwebe isikali (esifana netemometha) okuqoshwe kuso isimo esihle kakhulu ongase usicabange sabekwa ku 100 naleso esibi kakhulu saba ku- 0.

Singathanda ukuba ukhombise kulesi sikali ukuthi yinhle noma yimbi kangakanani impilo yakho namhlanje, ngokwakho ukubona. Siza wenze lokhu ngokudweba umugqa osuka ebhokisini ngezansi uye kunoma yiliphi izinga esikalini elikhombisa ukuthi sihle noma sibi kangakanani isimo sempilo yakho namhlanje.



Iphepha-mbuzo LaseWisconsin Lobuhlungu Elifushane (uhlelo locwaningo olubukeziwe)

Ubuhlungu be-HIV
Iphepha-mbuzo lobuhlungu elifushane

Ino. ye-reference yesiguli _____

Usuku:_____

Siguli esithandekayo,

Sicela ufunde futhi uphendule imibuzo engezansi. Singathanda ukuba usitshele nganoma ibuphi ubuhlungu okungenzeka uyabuzwa futhi ubonise ukuthi ubuzwa kuphi lobu buhlungu emfanekisweni womzimba ongezansi.

8. Uma uqala ukuthola ukuthi uphethwe yini, ingabe wawuzwa ubuhlungu bomzimba phakathi kwezinye izinto? Sicela uthikhe ibhokisi elifanele.

☐ Yebo ☐ Cha

9. Uma impendulo yakho yombuzo wokuqala ithe Yebo, sicela ukhethe elilodwa lamabhokisi alandelayo.

☐ Ubuhlungu yibo obangenza ngaya kudokotela

☐ Babukhona ubuhlungu kodwa akusona isizathu esangenza ngaya kudokotela

10. Ingabe wake wezwa ubuhlungu ngenxa yesifo onaso manje?

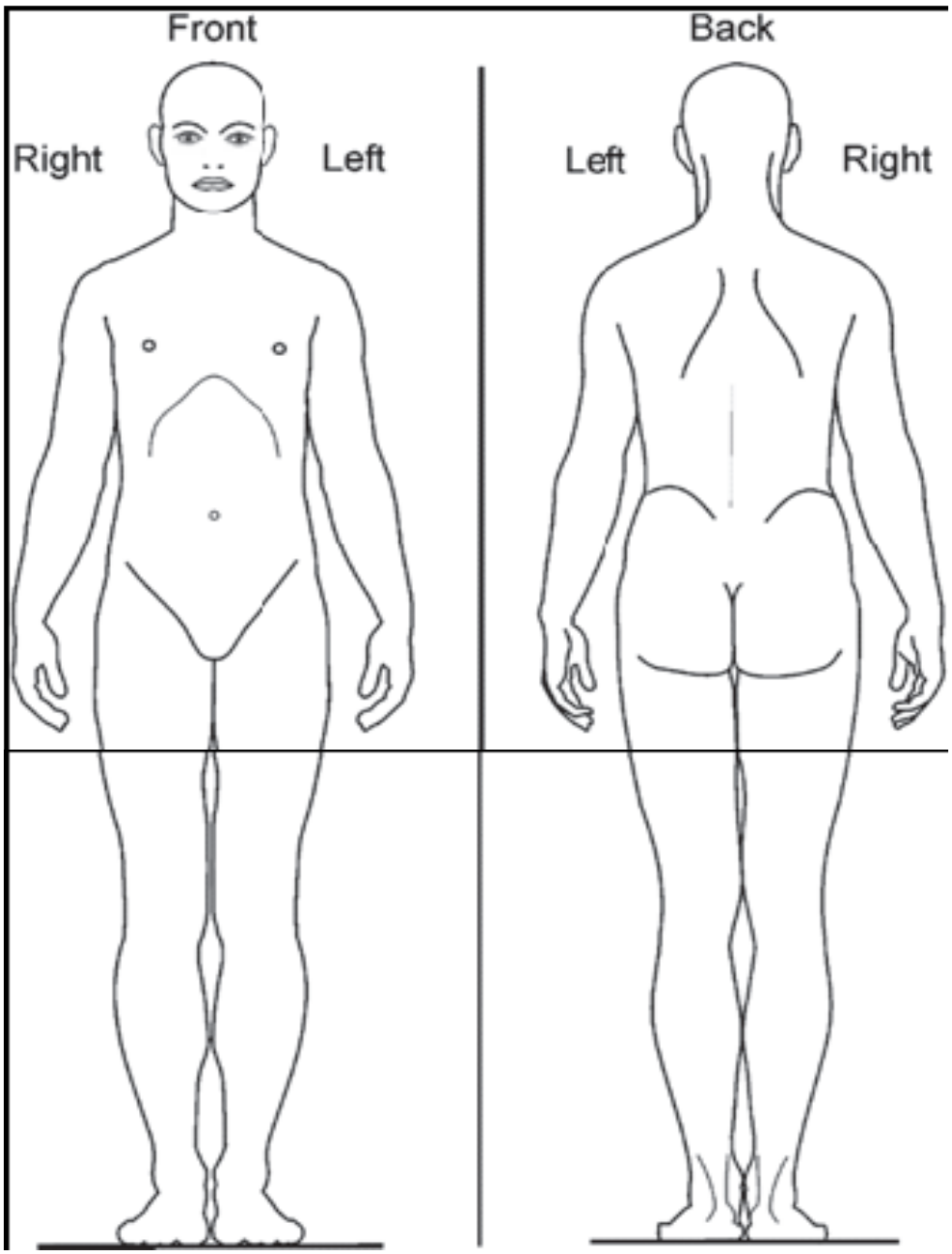
☐ Yebo ☐ Cha

11. Ekuphileni kwethu konke, iningi lethu lake lezwa ubuhlungu ngezikhathi ezithile (njengobuhlungu bekhanda, bemisipha nobamazinyo). Bukhona yini ubuhlungu obe nabo ngaphandle kwalobu buhlungu bansuku zonke kule nyanga edlule? Sicela uthikhe ibhokisi elifanele.

☐ Yebo ☐ Cha

Uma uphendule ngokuthi Yebo, sicela udlulele embuzweni 5 ekhasini elilandelayo.

12. Bonisa kulesi sithombe ukuthi ubuhlungu bakho bukuphi ngokufaka umbala endaweni ebuhlungu. Bhala uhlamvu “S” uma ubuhlungu buphezulu noma uhlamvu “D” uma ubuhlungu busekujuleni ngaphakathi emzimbeni, eduze nendawo oyibhale ngombala. Bonisa nokuthi ubuhlungu **obukhulu** bukuphi esithombeni.



13. “Obunye” ubuhlungu, ngaphandle kobuhlungu “obukhulu” obufake umbala esithombeni bubalulekile futhi singathanda ukuba ubuchaze usebenzisa isikali esingezansi. Thikha ibhokisi elifanele ukuze uchaze umuzwa walobu buhlungu:

K☐hlungwana (Buphansi)

K☐hlungwana impela (Bumaphakathi)

K☐hlungu kakhulu (Buyanjunjutha)

14. Yimaphi amanye amagama ongawasebenzisa ukuchaza lobu buhlungu “obunye”, okungebona ubuhlungu obukhulu obuzwayo?

_____

Okunye ukuphawula:

QUESTIONNAIRES (SESOTHO TRANSLATION)

Sekala se Lekanyang Bohloko ba Lehlaba [Pain Catastrophizing Scale (PCS)]

Motho e mong le e mong o ba le nako moo a bang le lehlaba ka nako e itseng bophelong ba hae. Lehlaba lena e ka nna ya ba ho opa ha hlooho, leino kapa lenonyeletso. Batho ba iphumana ba le maemong a ka etsang hore ba be le lehlaba jwaloka bolwetsi, ho tswa kotsi le ho etsa opareishene. Ka tlase ho na le mela e leshome le metso e meraro e hlalosang menahano le maikutlo a fapaneng a ka nnang a amana le lehlaba. Sekaleng se fanweng, ka kopo kgetha se hlalosang menahano le maikutlo a hao ha o utlwa lehlaba.

	Le hanyeny ane	Hanyeny ane	Mahareng	Haholo	Ka nako tsohle
Ke kgathatseha ka nako tsohle hore na lehlaba le tla fola.	0	1	2	3	4
Ke utlwa eka ha ke sa kgona ho tswelapele.	0	1	2	3	4
Le bohloko haholo mme ke nahana hore le ka se be betere.	0	1	2	3	4
Le bohloko haholo mme ke nahana hore le ntlhola ka matla.	0	1	2	3	4
Ke nahana hore ha ke sa kgona ho mamella lehlaba.	0	1	2	3	4
Ke ya tshoha hore lehlaba le tla mpefala le ho feta.	0	1	2	3	4
Ke ntse ke nahana ka dinako tse ding ha ke ne ke na le lehlaba.	0	1	2	3	4
Ha ke na mamello mme ke batla lehlaba le fole.	0	1	2	3	4
Ha ke kgone ho lebala ka lehlaba.	0	1	2	3	4
Ke ntse ke nahana hore le bohloko jwang.	0	1	2	3	4
Ke ntse ke nahana hore ke lakatsa haholo ha kae hore lehlaba le fole.	0	1	2	3	4
Ha ho na seo ke tla se etsa ho fokotsa lehlaba lena.	0	1	2	3	4
Ke ya ipotsa haeba ho na le ntho e itseng e mpe haholo e tla nketsahalla.	0	1	2	3	4

Tlhahlobo e Kgutshwanyana ya Boemo ba Kelello [Mini Mental Status Examination (MMSE)]

Tlhahlobo e Kgutshwanyana ya Boemo ba Kelello

Karolo le ha ele efe a teko ena e akaretsa dipotso tsa bohlokwa le ditaelo le ho hlahloba boemo ba kelello ba motho ka tsela e bonolo le ka potlako. Motho ka mong o tla fumana ntlha e le nngwe bakeng sa karabo ka nngwe e nepahetseng.

Ho etsa tlhahlobo ena, motho o lokela ho fumana sebaka se kgutsitseng mme a dule sebakeng seo hore a hlahlojwe; ya fanang ka tlhahlobo o lokela ho bolella motho ka mong hore a mamele ka hloko le ho araba potso ka nngwe ka nepo.

O se ke wa nahana ka nako ya teko empa kopanya dipalo tsa dikarabo tse nepahetseng. Motho ka mong o tla fumana dintlha tse ka fihlang ho mashome a mararo (30).

Dintlha tse ka tlase ho mashome a mabedi (20) di bontsha ho fokola ha kelello.

Thlahlobo e Kgutshwanyana ya Boemo ba Kelello

Lebitso: _____ Mohla wa Tswalo: _____

Dilemo tsa Thuto;_____ Mohla wa tlhahlobo: _____

Boemo ba Nako	E Nepahetse	E Fosahetse
Mohla wa kajeno ke eng?	☐	☐
Re kgweding efe?	☐	☐
Re selemong sefe?	☐	☐
Ke letsatsi lefe la beke kajeno?	☐	☐
Ke nako efe ya selemo?	☐	☐

Dimaraka: ____

Boemo ba Sebaka		
Ke ntlo ya mang ena?	☐	☐
Ke kamore ya mang ena?	☐	☐
Re toropong efe?	☐	☐
Re naheng efe?	☐	☐
Re porofinseng efe?	☐	☐

Dimaraka:____

Ho hopola hanghang

Mo kope haeba o ka hlahloba kellello ya hae. Mme o re “Bethe”, “Setulo”, “Pitsa” o sa potlake le ka lentswe le utlwahalang ka motsotswana o le mong bakeng sa lentswe ka leng. Kamora ho bitsa mantswe a mararo kaofela, mo kope hore a phete mantswe ana a mararo – o tla fumana dimaraka (0-3) ka ho araba lentswe la pele le phetilweng ka nepo.

Bethe	☐	☐
Setulo	☐	☐
Pitsa	☐	☐

Dimaraka: ____

Ela hloko

A) Monna o na le R20.00 ya ho patala tekisi. Letsatsi le leng le le leng o sebedisa R3.00 ho lefa tekisi. Kamora ho lefa ka letsatsi la pele o tla sallwa ke R17.00. O tla sala ka bokae ka mora ho lefa tekisi ka letsatsi le latelang? Le kamora letsatsi le hlahlamang leo… (ka makgetlo a mahlano/ka matsatsi a mahlano)

R14.00	☐	☐
R11.00	☐	☐
R8.00	☐	☐
R5.00	☐	☐
R2.00	☐	☐

Dimaraka:_____

B) Mpolelle matsatsi a beke o qala qetellong, ka mohl., (pele ho Sontaha ho tla Moqebelo, pele ho Moqebelo ho tla Labohlano, j.j.)

Labohlano	☐	☐
Labone	☐	☐
Laboraro	☐	☐
Labobedi	☐	☐
Mantaha	☐	☐

Dimaraka:_____

Ho Hopola Mantswe Kamora Nako
Kopa motho ho hopola mantswe a mararo ao o mmolelletseng ona pejana.

Bethe	☐	☐
Setulo	☐	☐
Pitsa	☐	☐

Dimaraka:_____

Ho bitsa ntho ka lebitso
Bontsha motho kopi e be wa mmotsa hore ke eng. Etsa se tshwanang le ka pene.

Kopi	☐	☐
Pene	☐	☐

Phetapheto
Kopa motho ho pheta se latelang:

E seng Sena, kapa Sane	☐	☐

Taelo ya Mokgahlelo - 3
E fa motho pampiri e hlwekileng mme o re “Nka pampiri e leng letsohong la hao, e mene, mme o e behe fatshe”

Ho nka	☐	☐
Ho mena	☐	☐
Ho beha	☐	☐

Ho bala
Mohlahlobi o re “ntjhebe mme o etse seo ke se etsang” e be mohlahlobi o phahamisa letsoho la hae le letona ka metsotswana e meraro. Fana ka dimaraka hafeela motho a phahamisa letsoho la hae le letona.

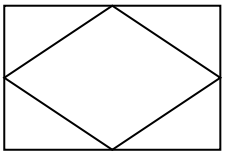
☐ ☐

Tsela ya ho etsa polelo ka mantsoe
Botsa motho “haeba o sa tsebe lebitso la ka, ke eng seo o lokelang ho se etsa hore o le tsebe?

☐ ☐

Ho torowa
Efa motho ka mong dithupana tse mmalwa mme o mo kope hore a etse setshwantsho seo a se boneng se nang le sebopeho sa taemane ka mahareng.

☐ ☐



Paloyohle ya Dimaraka**:**_____

Lenanenetefatso la bo 25 la Matshwao a ho Kula la Hopkins [Hopkins Symptoms Checklist 25 (HSCL-25)]

Lenanenetefatso la bo 25 la Matshwao a ho kula la Hopkins

_____MOHLA _____NOMORO YA SERIALE

Motho ka mong o lokela ho iketsetsa qeto hore o ba le matshwao ana a ho kula ka makgetlo a makae, beke e sa tswa feta ho kopanyelletsa le kajeno. Ka kopo kgetha karabo e nepahetseng ka ho kenya sefapano ho yona.

Hah olo	Haholwa nyana	Hanyane	Le hanyenyane	Karolo 1 **Matshwao a ho kula a ho kgathatseha haholo**	
				Ho tshoha ka ntle le lebaka	1
				Ho ikutlwa o tshohile	2
				Bofokodi	3
				Ho kgathatseha	4
				Pelo e otlang ka potlako	5
				Ho thothomela	6
				Ho tsitsipana	7
				Hlooho e opang	8
				Ho hloka botsitso le ho kgathatseha haholo	9
				Ho ikutlwa o sa lokoloha le kgutso	10
				Karolo 2 **Matshwao a ho kula a kgatello ya maikutlo**	
				Ho ikutlwa o fokola mme o sa kgone ho phakisa	11
				Ho ikutlwa o le molato ka dintho tse itseng	12
				Ho lla habonolo	13
				Ho lahlehelwa ke takatso ya tsa thobalano kapa boithabiso	14
				Ho lahlehelwa ke takatso ya dijo	15
				Ho ba le bothata ba ho tshwarwa ke boroko, ho se robale hantle.	16
				Ho lahlehelwa ke tshepo	17
				Ho ikutlwa o nyahame	18
				Ho ikutlwa o na le bodutu	19
				Ho nahana ho ipolaya	20
				Ho ikutlwa o na le morwalo	21
				Ho kgathatseha haholo ka dintho	22
				Ho lahlehelwa ke thahasello ya dintho	23
				Ho ikutlwa eka ntho e nngwe le e nngwe ke morwalo	24
				Ho ikutlwa o sa tshwanelehe mme o sa ratwe.	25

Letoto la Dipotso tsa Lehlaba la Nakwana la Wisconsin (kgatiso ya phuputso e ntjhafaditsweng)

Lehlaba la HIV
Letoto la dipotso tsa lehlaba la nakwana

Nomoro ya bopaki ya mokudi. _____

Mohla:_____

Mokudi ya ratehang,

Ka kopo bala le ho araba dipotso tse ka tlase. Re kopa hore o re bolelle ka lehlaba leha e le lefe leo o nang le lona mme hape o re bontshe moo lehlaba le leng teng dayakeramong ya mmele e fanweng.

15. Qalong ha o fumana hore o tshwerwe ke eng, na lehlaba e ne e le le leng la matshwao a bolwetse? Ka kopo tshwaya lebokose le nepahetseng.

    ☐ Ee ☐ Tjhe

16. Haeba karabo ya hao potsong ya pele e ne e le Ee, tshwaya le leng la mabokose a latelang.

    ☐ Lebaka le entseng hore ke ye ngakeng ke lehlaba

    ☐ Lehlaba le ne le le teng empa ha se lebaka le entseng hore ke ye ngakeng

17. Na o kile wa ba le lehlaba ka lebaka la bolwetse boo o nang le bona?

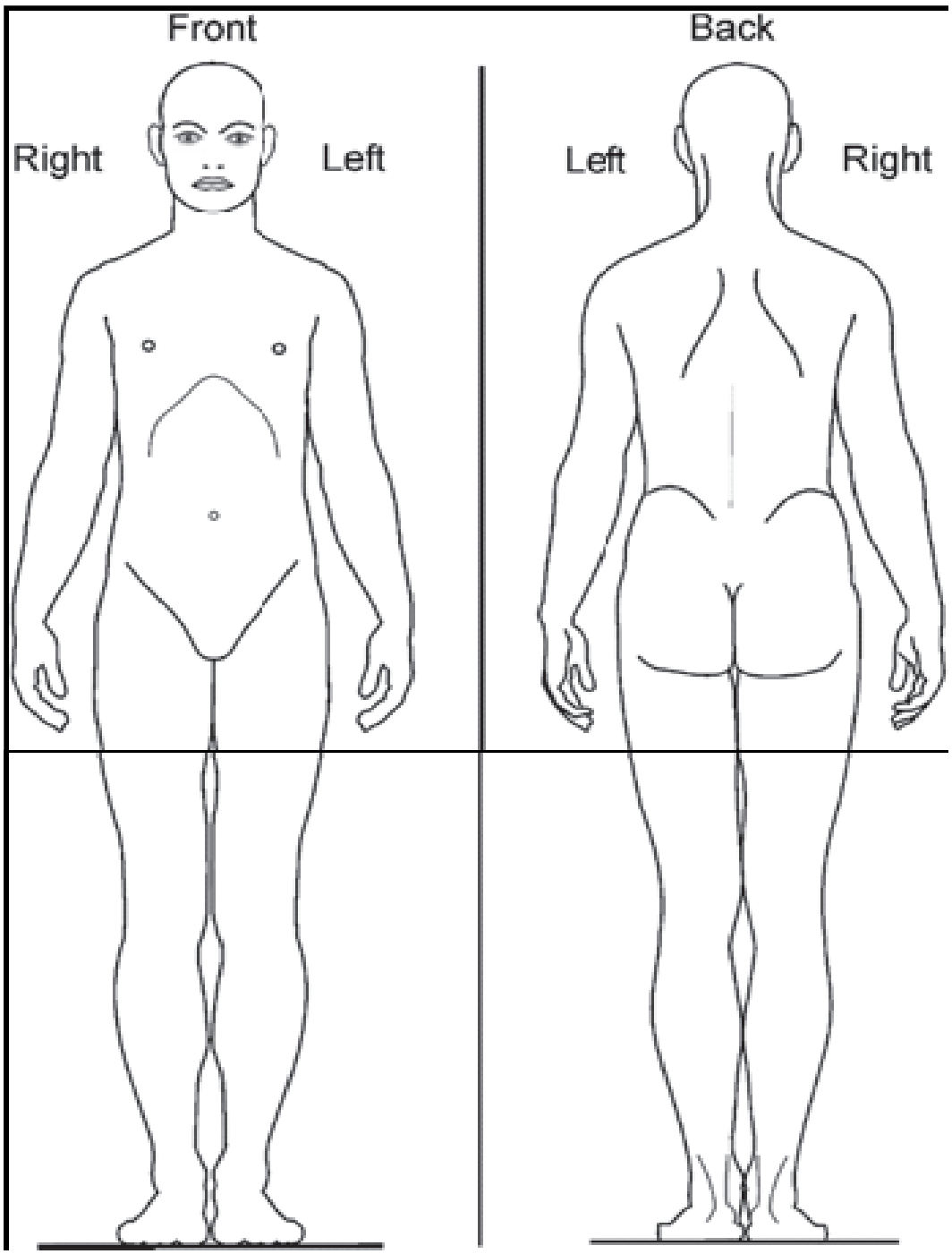
    ☐ Ee ☐ Tjhe

18. Bophelong ba rona, bongata ba rona re ba le lehlaba nako le nako (jwaloka ho opa ha hlooho, ho opa ha mosifa le ho opa ha leino). Na o kile wa ba le lehlaba le leng kantle le mefuta ya mahlaba ana a kamehla mahareng a kgwedi e sa tswa feta? Ka kopo tshwaya lebokose le nepahetseng.

    ☐ Ee ☐ Tjhe

    Haeba karabo ya hao ke Ee, ka kopo tswelapele ho potso 5 leqepheng le latelang.

19. Bontsha setshwantshong sena hore lehlaba le ho kae ka ho kenya mmala sebakeng se nang le lehlaba. Ngola tlhaku “N” haeba lehlaba le le ka Ntle ho mmele kapa tlhaku “H” ha lehlaba le le ka Hare ho mmele, ka pela sebaka seo o kentseng mmala ho sona. Hape bontsha hore lehlaba le bohloko **haholo** le ho kae setshwantshong.



20. Lehlaba “le leng”, ka ntle le lehlaba la hao le bohloko “haholo” leo o le kentseng mmala setshwantshong ke la bohlokwa mme re kopa hore o re hlalosetse ka lehlaba lena o sebedisa sekala se ka tlase. Tshwaya lebokose le nepahetseng ho hlalosa hore lehlaba lena le jwang:

- ☐ Lehlaba le seng bohloko haholo (Fokolang)
- ☐ Lehlaba le bohlokonyana (Itekanetseng)
- ☐ Lehlaba le bohloko haholo (Matla)

21. O ka sebedisa mantsoe afe a mang ho hlalosa lehlaba lena “le leng”, leo e seng lehlaba le bohloko haholo?

_____

_____

Dintlha tse eketsehileng:

_____

_____

EQ-5D

Lenane la Dipotso la tsa Bophelo bo botle
(Phetolelo ya Sesotho)

(Sesotho version for South Africa)
(Best available)

Ka ho tshwaya ka hara lebokoso le le leng sehlopheng se seng le se seng mona tlase, bontsha hle hore na ke dipolelo dife tse hlalosang ka botlalo boemo ba bophelo ba hao kajeno.

Ho tsamaya

Ha ke na bothata ba ho tsamaya ❑

Ke na le bothata bo itseng ba ho tsamaya ❑

Ke hlola betheng ❑

Ho itlhokomela

Ha ke na bothata ba ho itlhokomela ❑

Ke na le bothata bo itseng ka ho itlhatswa kapa ho itentsha/ikapesa ❑

Ha ke kgone ho itlhatswa kapa ho itentsha/ikapesa ❑

Mesebetsi ya setlwaheli *(mohlala: mosebetsi, boithuto, mosebetsi wa ka tlung, mesebetsi ya lelapa, kapa ya ho iketla)*

Ha ke na bothata ba ho etsa mesebetsi ya ka ya setlwaheli ❑

Ke na le bothata bo itseng ho etsa mesebetsi ya ka ya setlwaheli ❑

Ha ke kgone ho etsa mesebetsi ya ka ya setlwaheli ❑

Ho opelwa le ho se ikutlwe monate

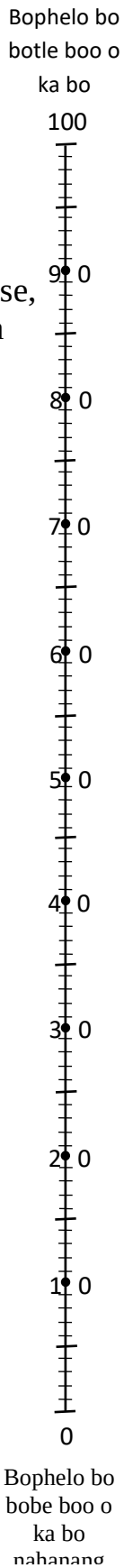
Ha ke opelwe kapa ho se ikutlwe monate ❑

Ha ke opelwe hakaalo kapa ho se ikutlwe monate ❑

Ke opelwa hampe kapa ha ke ikutlwe monate ho hang ❑

Ho tshwenyeha kapa ho wa ha maikutlo

Ha kea tshwenyeha kapa hona ho wa maikutlo ❑



Ha kea tshwenyeha hakaalo kapa ke wele maikutlo ❑

Ke tshwenyehile hampe kapa ke wele maikutlo haholo ❑

Ho thusa batho ho bolela hore na boemo ba bophelo bo botle kapa bo bobe, re takile sekala (se batlang se tshwana le themometa) seo ho sona boemo bo botle haholo boo o ka bo nahanang bo tshwailweng ka 100 boemo bo bobe ka ho fetisisa boo o ka bo nahanang bo tshwailweng ka 0.

Re ne re ka rata ha o ne o ka bontsha sekaleng sena ka moo bophelo ba hao bo leng botle kapa bo leng bobe ka teng kajeno, ho ya ka wena. Etsa hona hle ka ho seha mola ho tloha lebokosong hle ka tlase ho ya fihla kae kapa kae sekaleng ho bontsha ka moo boemo ba hao ba bophelo bo leng botle ka teng kapa bo leng bobe ka teng kajeno.

QUESTIONNAIRES (SETSWANA TRANSLATION)

Sekale sa Go lekanya Tsietsi ya Setlhabi (Pain Catastrophizing Scale (PCS))

Mongwe le mongwe ka tsela ngwe o nna le diemo tsa go nna le ditlhabi mo matshelong a bona. Diemo tse di ka nna sta akaretsa tlhogo e e opang, mesifa e e botlhoko le malokololo a a botlhoko. Batho gantsi ba iphitlhela ba le mo diemong tse di ka ba bakelang botlhoko tse di jaaka bolwetse, kgobalo, kalafo ya meno le karo.
Re na le kgatlhego mo mefuteng ya megopolo le maikutlo a o nang le ona fa o na le ditlhabi. Fa tlase go na le mela e e lesometharo e e kayang maikutlo a a ka amanngwang le setlhabi. O dirisa sekale, tsweetswee kaya palo e o nang le yona mo kakanyong ya gago fa o itemogela botlhoko.

	Le fa le ka gope	Go le gonnye	Fa gare	Thata	Dinako tsotlhe
Ke tshwenyega ka dinako tsotlhe ka ga gore setlhabi se nyelele	0	1	2	3	4
Ke ikutlwa e kete ga ke kake ka tswelela pele	0	1	2	3	4
Se maswe thata le gone ke akanya gore ga se ka ke sa tokafala	0	1	2	3	4
Se maswe thata le gone se a mphekeetsa	0	1	2	3	4
Ga ke kgone go itshokela botlhoko	0	1	2	3	4
Ke boifa gore setlhabi se tlaa gakalela pele	0	1	2	3	4
Ke nna ke akanya ka dinako tse dingwe tse ke neng ke na le setlhabi ka tsona	0	1	2	3	4
Ke fela pelo e bile ke batla gore setlhabi se fele	0	1	2	3	4
Ga ke kgone go se ntsha mo tlhaloganyong ya me	0	1	2	3	4
Ke nna ke akanya ka setlhabe se ke nang le sona	0	1	2	3	4
Ke nna ke akanya ka fa ke batlang gore setlhabi se fele	0	1	2	3	4
Ga ke kgone go dira sepe go fokotsa setlhabi se	0	1	2	3	4
Ke ipotsa gore a sengwe se se masisi se ka diragala	0	1	2	3	4

Tlhatlhobo-Potlana ya Maemo a Tlhaloganyo (MMSE) (Mofuta o o diretsweng

Aforikaborwa)

Tlhatlhobo-Potlana ya Maemo a Tlhaloganyo

Tlhatlhobo-Potlana ya Maemo a Tlhaloganyo e fa tsela e e bofefo le e e bonolo ya go lekanya kgolo ya tlhaloganyo le go tlhatlhoboa tatlhegelo ya tlhaloganyo. E sekaseseka katlhego, tlhagafalo, kgopolo le bokgoni jwa go buisa, puo, le go kgona go tshikhinyega dikarolo tsa mmele sentle.

Karolo nngwe le nngwe e kopanya dipotso le ditaelo tsa tsona. Motho o fiwa ntlha e le nngwe fa a file karabo e e nepagetseng.

Go fa teko, baya motho mo lefelong le le didimetseng, mo phaposing e e bonisitsweng sentle. Kopa motho go reetsa ka kelotlhoko le go araba potso nngwe le nngwe ka boikanyego jo a ka bo kgonang.

O se ka wa baya nako e tlhatlhobo eno e tlaa nnang ka yona. Le fa go le jalo, fa maduo morago ga karabo. Go fa maduo otlhe, tlhakanya dipotso tsotlhe tse di arabilweng ka nepagalo. Motho mongwe le mongwe a ka kgona go nna le maduo a a a fitlhang ka dintlha tse di masometharo (30).

Maduo a a ka fa tlase ga masomepedi (20) a supa bokowa jwa tlhaloganyo.

Tlhatlhobo-Potlana ya Maemo a Tlhaloganyo

Leina: _____ Letlha la botsalo: _____

Dingwaga tsa thuto: _____ Letlha la tlhatlhobo: _____

Nako	Nepagetse	Fosagetse
Letlha la gompieno ke?	☐	☐
Kgwedi eno ke efe?	☐	☐
Ngwaga ono ke ofe?	☐	☐
Letsatsi leno ke lefe la beke?	☐	☐
Setlha seno ke sefe?	☐	☐

Maduo: ___

Lefelo		
Lefelo leno ke lefe	☐	☐
Phaposi eno ke ya ga mang?	☐	☐
Re mo toropong efe?	☐	☐
Re mo nageng efe?	☐	☐
Re mo porofenseng efe?	☐	☐

Maduo: ____

Monagano wa ka bonako

Botsa motho gore a o ka leka tlhaloganyo ya gagwe. Jaanong e re “bolao”, “setulo”, “pitsa” ka bonya le gone kwa godimo. Fa o sena go fetsa go bua mafoko a mararo, kopa motho go boeletsa mafoko – lefoko lantlha le le nepagetseng le le boeleditsweng. Le tlaa fa motho maduo a gagwe (0-3).

Bolao	☐	☐
Setulo	☐	☐
Pitsa	☐	☐

Maduo: ____

Tlhagiso

A) monna o na le R20.00 gore a duele thekisi ka yona. Letsatsi le letsatsi o dirisa R3.00 go duela thekisi. Morago ga go duela ka letsatsi la ntlha, o tlaasalelwa ke R17.00. go tlaa sala bokae morago ga go dirisa thekisi mo letsatsing le le latelang? Le letsatsi le le latelang morago ga go duela thekisi. ... (ga tlhano/malatsi a matlhano)

R14.00	☐	☐
R11.00	☐	☐
R8.00	☐	☐
R5.00	☐	☐
R2.00	☐	☐

Maduo: _____

B) fa malatsi a beke, o a balela kwa morago, go fa sekao (pele ga LaTshipi ke Lamatlhatso, le pele ga Lamatlhatso ke Labotlhano, jj …)

Labotlhano	☐	☐
Labone	☐	☐
Laboraro	☐	☐
Labobedi	☐	☐
Mosupologo	☐	☐

Maduo: _____

Diegisa go gopola mafoko

Kopa motho gore a gopole mafoko a mararo a o ba boleletseng ona mo nakong e e fetileng.

Bolao	☐	☐
Setulo	☐	☐
Pitsa	☐	☐

Maduo: _____

Go fa maina

Bontsha motho kopi mme o botse gore ke eng. Boeletsa ka pene.

Kopi	☐	☐
Pene	☐	☐

Poeletso

Kopa motho go boeletsa polelo e e latelang

“E seng se le fa e le sele”	☐	☐

Taelo ya maemo a mararo
Fa motho pampiri e e sa kwadiwang sepe o bo o re: “Tsaya pampiri eno e e mo seatlengsa gago, o bo o e mena ka halofo o bo o e baya fa fatshe.”

Go tsaya	☐	☐
Go mena	☐	☐
Go baya	☐	☐

Go buisa
Motlhatlhobi a re: “Ntebe mme o dire se ke se dirang: a be a tsholetsa seatla sa gagwe sa mofa ka metsotswana e le meraro. Fa maduo a a nepagetseng fela fa motho a tsholetsa setla sa gagwe sa moja.

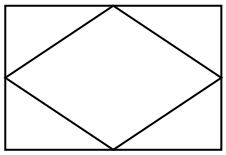
☐ ☐

Go tlhaloganya
Motho o tshwanetse go bodiwa ka gore: “Fa o ne o a sa itse leina la me, o ne o tlaa batlisisa gore leina la me ke mang jang?”

☐ ☐

Go tshwantsha
Fa motho dithobanyane mme o ba kope go aga dipopego tse di latelang ka tsela e di beilweng ka yona ka teemano mo teng.

☐ ☐



Maduo otlhe:_____

Lenane la Ditshupo la Hopkins la 25 (HSCL-25)

Lenane la Ditshupo la Hopkins la 25

_____LETLHA _____NOMORO YA SERIALE

Fa tlase go na le mafoko a makgwere kgotsa mathata abatho bangwe ba ka nnang le ona. Tsweetswee buisa nngwe le nngwe ya ona ka kelotlhoko mme o sweets gore mafoko ano a go tshwentse go le go kana kang mo bekeng e e fetileng, go akaretsa gompieno. Tsweetswee baya letshwao mo teng ga kholomo e e boammaaruri.

thatathata	thata	Go le gonnye	Le ka gope	Karolo 1 **Ditshupo tsa tlhobaelo**	
				Go tshoga ntle ga lebaka	1
				Go ikkutlwa o tshogile	2
				Go o otsela kgotsa go nna bokoa s	3
				Go roroma ka fa teng	4
				Pelo e itaya ka bonako	5
				Go roroma	6
				Go ikutlwa o tshwenyegile	7
				Tlhoko e e opang	8
				Go ikutlwa o tshogile thata kgotsa o tshwenyegile thata kgapetsa kgapetsa	9
				O sa repa	10
				Karolo ya 2 **Ditshupo tsa maikutlo a a ngodiegang**	
				Go ikutlwa o le bokowa le o lapile	11
				Go ikutlwa o le molato ka ga dilo	12
				Go lela ka bonako	13
				Go latlhegela ke kgatlhegelo ya thobalano le monate	14
				Go ikutlwa o sa batle go ja dijo	15
				G o kgone go robala, go sa robale sentle	16
				Go latlhegelwa ke tsholofelo ya isago	17
				Go ikutlwa o hutsafetse	18
				Go ikutlwa o le esi	19
				Go ikutlwa jaaka e kete o ka ipolaya	20
				Go ikutlwa o imelwa	21
				Go tshwenyega thata	22
				Go sa kgatlhegele dilo	23
				Go sa batle go dira sepe	24
				Go ikutlwa o sa itumele kgotsa o sa ratiwe	25

Potsopatlisiso e Khutshwane ya Setlhabi ya Wisconsin (mofuta wa thutopatlisiso o o retlhabolotsweng)

Setlhabi sa HIV
Potsopatlisiso e khutshwane ya setlhabi

Nomoro ya referense ya molwetse. _____

Letlha:_____

Go molwetse yo o rategang,

Tsweetswee buisa dipotso tse di fa tlase. Re rata gore o re bolelele ka ga setlhabi sepe se o ka tswang o na le sona gape o kaye gore o na le setlhabi fa kae mo setshwantsong sa mmele se se filweng.

22. E rile o sena go amogela temogo (tupo) ya gago, a setlhabi e ne e le nngwe ya ditshupo tsa gago? Tsweetswee tshwaya lebokoso le le nepagetseng.

☐ Ee ☐ Nnyaa

23. Fa karabo ya gago ya poso ya ntlha e ne e le Ee, tshwaya nngwe ya mabokoso a a latelang.

☐ Setlhabi e ne e le sona lebaka le le nkisitseng kwa ngakeng.

☐ Setlhabi se ne se le teng mme fela e ne e se sona lebaka le le dirileng gore ke ye kwa ngakeng

24. A o kile wa nna le setlhabi ka ntlha ya bolwetse jwa gago jwa ga jaana?

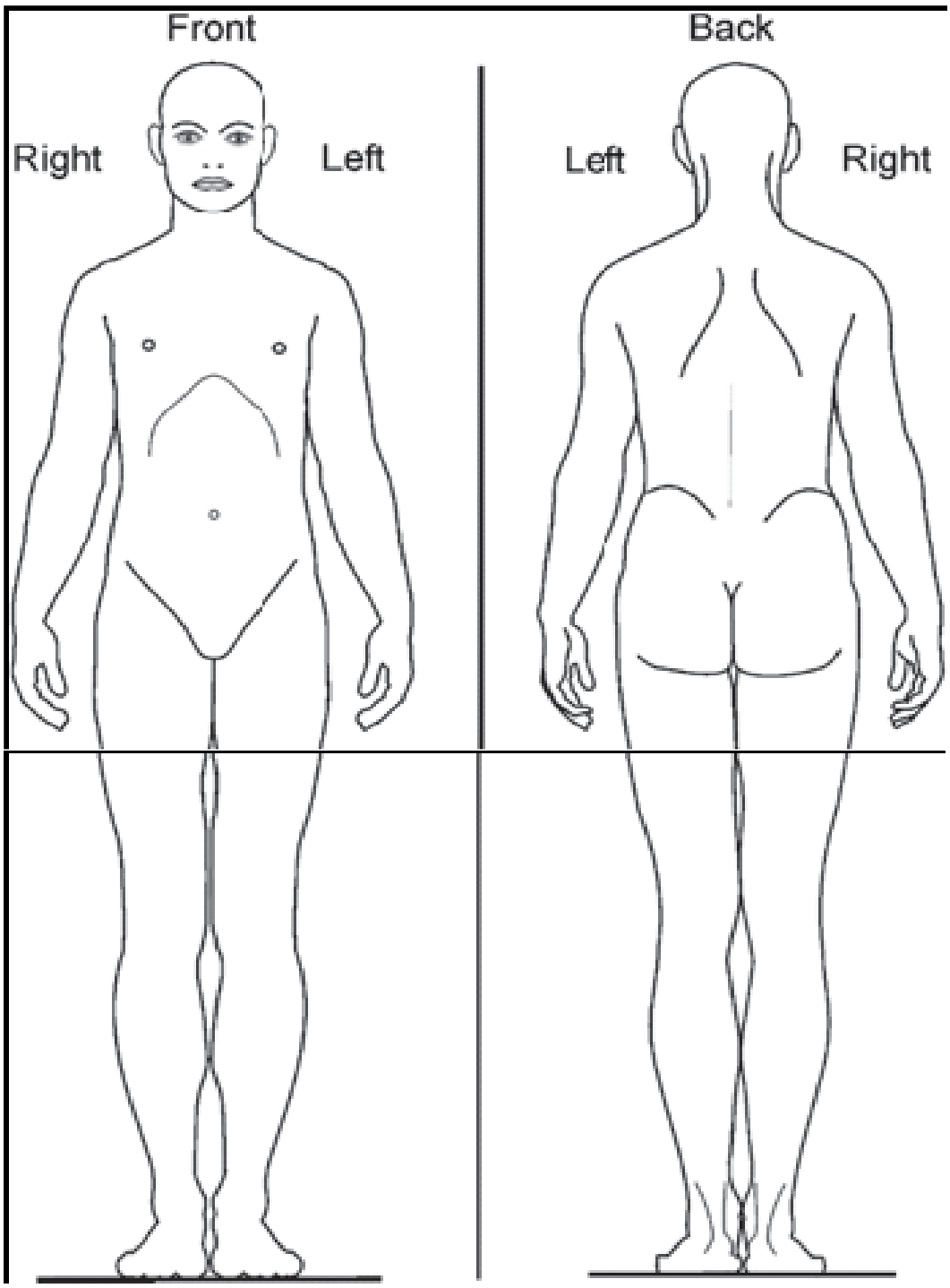
☐ Ee ☐ Nnyaa

25. Mo matshelong a rona otlhe, bontsi jwa rona ba nnile le ditlhabi ka gangwe le gape (tse di jaaka ditlhogo tse di opang, mesifa e e botlhoko le meno a a thunyang). A o kile wa nna le setlhabi se sengwe ntle ga mefuta eno ya ditlhabi tsa letsatsi le letsatsi mo kgweding e e fetileng? Tsweetswee tshwaya lebokoso le le nepagetseng.

☐ Ee ☐ Nnyaa

Fa o arabile ka gore Ee, tsweetswee tswelela go ya kwa potsong ya 5 e e mo tsebeng e e latelang.

26. Bontsha mo setshwantshong seno gore setlhabi sa gago se fa kae ka go tshasa mmala lefelo le le botlhoko. Kwala tlhaka “S” fa setlhabi se le fa godimo fela kgotsa tlhaka “D” fa setlhabi se le kwa tengteng mo mmeleng wa gago, go bapa le lefelo le o le tshasitseng mmala. Gape bontsha gore setlhabi se **segolo** se fa kae mo setshwantshong.



27. Setlhabi se “sengwe”, kwa ntle ga setlhabi sa gago se “segolo” se o se takileng mmala mo setshwantshong se botlhokwa le gone re rata gore tsweetswee o se tlhalose o dirisa sekale se es fa tlase. Tsweetswee tshwaya lebokoso le le nepagetseng go tlhalosa go utlwala ga setlhabo se:

- ☐ Go botlhoko go se gonene (Nnye)
- ☐ Go botlhoko thata (Fa gare)
- ☐ Go botlhoko thatathata (Masisi)

28. Ke mafoko afe a sele a o ka a dirisang go tlhalosa setlhabi seno “se sele”, se e seng setlhabi sa gago se segolo?

_____

_____

Ditshwaelo tsa tlaleletso:

_____

_____

EQ-5D

Potso-Patlisiso Ya Botsogo

(Thanolo Ya Setswana)
(Best available)

Ka go tshwaya (√) mo setlhopheng sengwe le sengwe sa dikarabo se se fa tlase, tsweetswee supa gore ke diele dife tse di tlhalosang thata seemo sa gago sa botsogo gompieno.

Go itsamaisa

Ga ke na mathata a go itsamaisa ❑
Ke na le mathata mangwe a go itsamaisa ❑
Ke nna fela mo bolaong ❑

Go itlhokomela mo mmeleng

Ga ke na mathata a go itlhokomela ❑
Ke na le mathata mangwe go itlhapisa kana go ikapesa ❑
Ga ke kgone go itlhapisa kana go ikapesa ❑

Dilo tse ke di dirang malatsi otlhe *(dikai; tiro, go ithuta, tiro ya mo gae, ditiro tsa balelapa kana tsa go itlosa bodutu)*

Ga ke na mathata a go dira dilo tse ke di dirang malatsi otlhe ❑
Ke na le mathata mangwe go dira dilo tse ke di dirang malatsi otlhe ❑
Ga ke kgone go dira dilo tse ke di dirang malatsi otlhe ❑

Setlhabi/Go sa ikutlwe sentle

Ga ke na setlhabi kana go sa ikutlwe sentle ❑
Ke na le setlhabi kana go sa ikutlwe sentle ka selekanyo se se fa gare ❑
Ke na le setlhabi kana go sa ikutlwe sentle mo go tseneletseng ❑

Go tshwenyega/Kgatelelo ya maikutlo

Ga ke na matshwenyego kana kgatelelo ya maikutlo ❑

Ke na le matshwenyego kana kgatelelo ya maikutlo ka selekanyo se se fa gare ❑

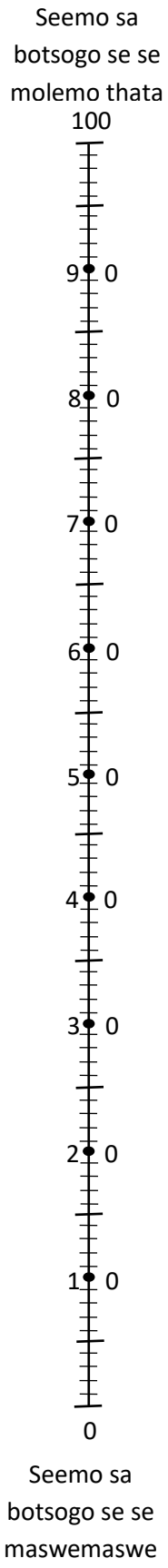
Ke na le matshwenyego kana kgatelelo ya maikutlo tse di kwa godimo ❑

Go thusa batho go bolela kafa seemo sa bone sa botsogo se siameng kgotsa se leng maswe ka teng, re tshwantshitse sekale (se se tshwanang le sedirisiwa se se metang mogote) se mo go sone seemo se se gaisang se se ka akanngwang se tshwailweng 100 mme seemo se se maswe se se ka akanngwang se tshwailweng 0.

Re tla eletsa gore o supe mo sekaleng se gore seemo sa gago sa botsogo se siame kana se maswe go le kae gompieno, go ya ka wena. Tsweetswee dira se ka go sega tselana go tswa mo bokoseng e e fa tlase go ya kwa lefelong lepe fela mo sekaleng le o bonang le supa go siama kana bomaswe jwa seemo sa botsogo jwa gago gompieno

Seemo sa gago sa botsogo

gompieno



APPENDIX 8

PILLAY P, WADLEY AL, CHERRY CL, KARSTAEDT AS, KAMERMAN PR

Psychological factors associated with painful vs. non-painful HIV-associated sensory neuropathy

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ORIGINAL PAPER

Psychological Factors Associated With Painful Versus Non-Painful HIV-Associated Sensory Neuropathy

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Abstract HIV-associated sensory neuropathy (HIV-SN) is a common, and frequently painful complication of HIV, but factors that determine the presence of pain are unresolved. We investigated: (i) if psychological factors associated with painful (n = 125) versus non-painful HIV-SN (n = 72), and (ii) if pain and psychological factors affected quality of life (QoL). We assessed anxiety and depression using the Hopkins Symptoms Checklist-25. Pain catastrophizing and QoL were assessed using the Pain Catastrophizing Scale and Euroqol-5D, respectively. Presence of neuropathy was detected using the Brief Neuropathy Screening Tool, and pain was characterised using the Wisconsin Brief Pain Questionnaire. Overall, there was a high burden of pain, depression and anxiety in the cohort. None of the psychological variables associated with having painful HIV-SN. Greater depressive symptoms and presence of pain were independently associated with lower QoL. In those participants with painful HIV-SN, greater depressive symptom scores were associated with increased pain intensity. In conclusion, in a cohort with high background levels of psychological dysfunction, psychological factors do not predict the presence of pain, but both depression and presence of pain are associated with poor quality of life.

Keywords Depression · Pain intensity · Quality of life · Painful HIV-associated sensory neuropathy

Introduction

HIV-associated sensory neuropathy (HIV-SN) is a frequent complication of both HIV infection and its treatment, affecting up to 60% of ambulatory people living with HIV/AIDS (PLWHA) [1]. Unfortunately, the decrease in incidence and prevalence of the neuropathy that was anticipated with widespread introduction of less neurotoxic antiretroviral drug treatments appears to have been minuscule in Sub-Saharan Africa [2–4], in comparison to the reductions reported by recent US cohorts, which are however, equivocal [5–7]. Indeed, there are reasons to believe there may be differences between the populations, which include, genetic [8–10] and behavioural [11] differences.

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Pain is a common feature of HIV-SN and causes significant physical, psychological, and social dysfunction (e.g., reduced physical independence, increased risk of depression, and increased risk of being unemployed) [5]. But, pain is not a universal symptom of the neuropathy, with 20–40% of individuals with HIV-SN being asymptomatic or having symptoms other than pain [3, 5, 12, 13]. Moreover, in individuals who have a painful neuropathy there is significant heterogeneity in the intensity of pain experienced, with patients reporting intensities anywhere between 'slight pain' and 'worst pain ever experienced' [5, 14–16]. The presence of pain in HIV-SN, and the intensity of that pain have been positively associated with disease-related factors such as greater nadir CD4 T cell count [5], past exposure to neurotoxic antiretroviral drugs [5], and increased viral load [17]. However, neither the presence of pain nor the variability in pain intensity is entirely explained by the extent of the neuropathology, as assessed by intraepidermal nerve fibre density [17–19] and somatosensory nerve function [13, 20].

There is, however, growing evidence that psychological factors, including anxiety, depression and pain catastrophizing, are associated with the presence and intensity of pain, clinical outcomes, and quality of life in individuals with chronic pains such as chronic low back pain and arthritis [21, 22]. Consistent with these findings from other causes of pain, a small study by Lucey and colleagues [23] reported that the intensity of the pain reported by patients with HIV-SN was positively associated with pain catastrophizing. And, in the large, multi-centre CHARTER cohort (https://www.charternntc.org), prevalent and incident painful HIV-SN has been associated with worsening depressive symptoms [5, 24]. The presence and intensity of painful HIV-SN was also positively correlated with worse quality of life [13, 25], but it appears that the severity of depressive symptoms may be a greater determinant of lower quality of life than is pain intensity [25].

Neuropathic pain is typically chronic and PLWHA with access to medical care have a near-normal life expectancy [26, 27]. Therefore, the impact of painful neuropathy on quality of life and long-term well-being of the affected individual, their support network and the healthcare system is substantial. Proven pharmacological agents for the treatment of painful HIV-SN are lacking [28, 29]. It is therefore particularly important that other, potentially modifiable risk factors for the pain and its adverse effects are identified. Consequently, we recruited a cohort of black HIV-positive South Africans with either painful or non-painful HIV-SN to investigate whether (i) psychological factors associate with having painful HIV-SN (primary objective 1), and (ii) whether pain and psychological factors affect quality of life in this population (primary objective 2). As a secondary objective, we also completed a sub-group analysis of the painful HIV-SN group only to determine whether psychological factors predict the intensity of their neuropathic pain (secondary objective 1).

Methods

One hundred and ninety-seven black African HIV-positive adults on combination antiretroviral therapy (cART) were recruited at Chris Hani Baragwanath Hospital, Johannesburg, South Africa. The Chris Hani Baragwanath Hospital is located in Soweto, Johannesburg. Soweto is a diverse community where multiple African languages are spoken and isiZulu is the predominant language. Thus, most participants in our study spoke isiZulu as their first language [44%, (87/197)], followed by isiXhosa [18%, (36/197)]. Recruitment was conducted at a single site on a convenience sample of outpatients attending a general HIV clinic for collection of treatment and routine care. Recruitment ran from May 2013 to January 2014. The study was approved by the Human Research Ethics committee (Medical) of the University of the Witwatersrand, South Africa (clearance numbers: M121018 and M090671). Written, informed consent was obtained from all participants.

All HIV-positive adults (≥18 years of age) with a distal symmetrical polyneuropathy (see "Neuropathy Screening"), and with a Mini-Mental State Examination (MMSE) score ≥24 [30] (see "Questionnaires"), were eligible for inclusion in the study. We screened 372 HIV-positive adults for the presence of HIV-SN. One hundred and seventy-one participants were excluded (166 participants were HIV-SN free, 4 participants scored <24 on the MMSE and we were unable to complete a bilateral assessment for HIV-SN in one participant due to a previous limb injury). Of the 372 HIV-adults screened, one hundred and ninety-seven black African HIV-positive adults who presented with HIV-SN were recruited to the study. Thereafter, participants were stratified into painful or non-painful HIV-SN groups (see Neuropathy Screening). Participants had the following demographic and anthropometric data recorded: sex, age, height, weight, ethnicity, date of HIV diagnosis, years of formal education, other potential causes of neuropathy [diabetes, alcohol consumption (units of alcohol consumed), vitamin B_{12} deficiency, treatment for current tuberculosis infection and diabetes]. The following haematological data were collected from participants' medical records: initial CD4T-cell count, current CD4 T-cell count, HIV viral load and Hepatitis B status. Participants' medical records were also reviewed to ascertain the type/s and dose/s of analgesic medications and cART regimens prescribed.

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Neuropathy Screening

A diagnosis of painful HIV-associated sensory neuropathy (HIV-SN) was made based on the bilateral presence of at least two signs (decreased vibration sense in the great toe, absent ankle reflex or decreased pin-prick sensitivity) and pain in the distribution of the neurological deficit in the legs and feet. From the Brief Peripheral Neuropathy Screen (BPNS), only those patients who presented with "pain, aching or burning" in the distribution of the neurological deficit in the legs and feet were classified as having 'pain'. The presence of paraesthesias and numbness were not classified as 'pain'. The diagnosis of non-painful HIV-SN was based on the bilateral presence of at least two signs (decreased vibration sense in the great toe, absent ankle reflex or decreased pin-prick sensitivity) in the feet in the absence of neuropathic pain in the lower limbs.

Vibration sense was assessed using a 128 Hz tuning fork, with a recording of 10 s or less deemed abnormal [31]. Pin-prick sensitivity was assessed using a size 4 disposable metal safety pin. We wanted to determine whether patients could differentiate between the blunt and sharp ends of the pin, allowing us to determine the patients' ability to detect a cutaneous pain sensation. Having established that the patient could determine the difference between sharp and blunt on another part of the body (usually the dorsum of the hand, if sensation was intact), we proceeded with performing the test on the patients' feet. We did not test specific anatomical sites on the foot but focused on assessing the entire foot which encompassed three specific dermatomes: L4, L5 and S1. We gently and alternately placed the 'sharp and blunt' ends of the safety pin on the patients' great toe and asked them to identify the stimulus each time. We then repeated the procedure on the patients middle toes and the smallest toe. Working upwards centripetally from the toe in 2 cm intervals up to the ankle region, we performed three repetitions each of the 'sharp' and 'blunt' stimuli on each toe separately. If patients identified all the stimuli correctly, we recorded the result as normal. If the patients identified any of the stimuli incorrectly, we recorded the result as abnormal and mapped out which area of the foot and dermatome were involved in the incorrect responses. The safety pin was then discarded after the test procedure was complete. We did not perform any specific catch tests. We used a similar method of pin-prick sensitivity testing as performed by Singleton et al. in their study on the validation of the Utah Early Neuropathy Scale (UENS) [32]. When symptoms were present, participants were asked to rate the severity of the symptom(s) on a 10-point numerical pain rating scale (NRS), ranging from 1 (mild pain) to 10 (most severe pain).

All neuropathy assessments were performed by a single study investigator with six years of experience using the BPNS tool. The study investigator received formal training from Dr. Catherine Cherry (CCL-primary clinician involved in the validation of the BPNS tool for use in clinical studies).

Questionnaires

With the aid of an on-site study interpreter who was fluent in local South African languages, participants were asked to complete a series of questionnaires that assessed pain intensity, pain distribution, cognitive function, coping skills related to pain, pain related anxiety and depression, and health related quality of life. Prior to using these questionnaires, all questionnaires that were not already translated were translated and assessed using the MAPI institute's guidelines for translating and validating questionnaires [33]. Briefly, questionnaires were dual forward and back translated into four indigenous South African languages (isiZulu, Setswana, Sesotho and isiXhosa). Thereafter, the questionnaires were pilot tested in a sample of 50 individuals recruited from the same study population used in the present study. All study questionnaires were therefore available in English, isiZulu, Setswana, Sesotho and isiXhosa and all patients were given the option to choose which language version of the questionnaire they preferred to complete.

The Mini-Mental State Examination (MMSE)

The MMSE quantifies cognitive function and screens for cognitive loss [30]. It tests the participants' orientation, attention, calculation, recall, language and motor skills. The total score for the MMSE is out of 30 and a score of 24 or more is deemed normal. The MMSE has been used in similar populations in South Africa [34, 35].

Pain Catastrophizing Scale (PCS)

The PCS assesses catastrophic thinking related to pain [36]. It consists of 13 descriptions of thoughts and feelings related to pain. Participants were asked to indicate the level to which they experience these thoughts and feelings on a 5-point rating scale ranging from 0 (not at all) to 4 (always). The questionnaire consists of three subscales: rumination, magnification and helplessness. The total score for the questionnaire is calculated by adding the scores from each item answered and dividing this value by the total number of items answered (13). Since the PCS questionnaire relates specifically to current pain burden, only participants in the painful HIV-SN group completed this questionnaire. The PCS has not been validated in the population we were using it in. By restricting the use of the PCS to only those people in pain we precluded the completion of an informative factor

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analysis of the translated questionnaires, however we did assess internal reliability of the translated questionnaires and the English version of the PCS. All scales showed excellent internal consistency as measured by McDonald's coefficient ω (total) [37]: Nguni translations (isiXhosa and isiZulu, n = 44) ω = 0.95 (95% CI 0.93–0.98), Sotho-Tswana translations (Sesotho and Tswana, n = 21) ω = 0.94 (95% CI 0.91–0.97), and the Original English (as completed by non-first language English speakers, n = 60) ω = 0.95 (95% CI 0.93–0.97). The full analysis script for the internal reliability analysis is provided in supplementary document 1.

EQ-5D-3L

The EQ-5D assesses health related quality of life [38, 39]. It consists of two sections; the first section consists of descriptive questions and the second contains a visual analogue scale (VAS). The first descriptive section is made up of five subscales: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Participants' are asked to rate their own state of health on that particular day for each subscale. The scale ranges from 1 (no problems) to 3 (severe problems). In the second section, participants are asked to rate how good or bad their current state of health is by drawing a line on the VAS where, 0 (worst state of health imaginable and 100 (best state of health imaginable). The use of the EQ-5D has been validated in a South African population by the EuroQol group who developed the questionnaire [39].

The Hopkins Symptoms Checklist-25 (HSCL-25)

The HSCL-25 assesses global psychological function [40]. It consists of two subscales; anxiety and depression. The first scale consists of ten questions, which assess anxiety related symptoms, and the latter consists of 15 questions, which assess depression related symptoms. Participants are asked to rate how much the symptoms in the two subscales bothered or distressed them using a 4-point rating scale ranging from 1 (not at all) to 4 (extremely). Participants responses are summed and divided by the number of answered items (in each of the two sections) to generate a score for each of the sections. For the anxiety section, the sum of the responses is divided by 10 and for the depression section by 15; the total of the responses is divided by 25. A value >1.75 in each of the sections is considered symptomatic. The HSCL-25 has been used in a South African HIV-positive population previously [41]. All patients were given the opportunity to complete the HSCL-25 in the language version of their choice and thus patients completed either the English, isiZulu, Setswana, Sesotho or isiXhosa version of the questionnaire. Although the English version of the HSCL-25 has been used in a South African population [41], it has not been validated in this population. Therefore, we validated the English version of the HSCL-25 (supplementary data 2).

The Wisconsin Brief Pain Questionnaire (WBPQ)-Adapted Version

The WBPQ assesses pain intensity and interference of pain in the patient's life [42]. Using the WBPQ, patients rate the severity of their pain and the degree to which their pain interferes with common aspects of feelings and function. Pains other than SN-related pains were determined using the following question from the original WBPQ: "Throughout our lives, most of us have had pain from time to time (such as headaches, muscle aches and tooth aches). Have you had pain other than these everyday kinds of pain during the last month?" For our purposes, we used only two questions from the WBPQ: (i) the pain body chart diagram, and (ii) the numerical pain rating scale for pain in the past month. In the adapted version of the questionnaire, patients were then specifically asked: (i) on the body chart diagram provided, could you please colour in all the places where your pain occurs, and (ii) aside from the pain in your 'feet', how bad is your 'other' pain? Could you please rate this pain on the 10-point numerical pain rating scale from 1 (mild pain) to 10 (most severe). To ensure the patient did not confuse the two types of pain being assessed, during the rating procedure, the onsite translator reminded the patient (in the patient's home language) that this question was specific to their 'other body pain' and not to the pain in their feet (if this symptom was present at this location as well). If patients presented with several additional pain sites (not SN-related), they gave an overall score of the intensity of their pain. The use of the WBPQ for the assessment of pain in ambulatory HIV-positive South Africans has been validated by our laboratory [43].

Statistical Analysis

Descriptive statistics are presented as mean (SD) for parametric data, median (interquartile range, IQR) for non-parametric data, and percentages for frequency data. Univariate analyses comparing participants with painful HIV-SN and non-painful HIV-SN included: Fisher's Exact Test (sex, current tuberculosis infection, vitamin B_{12} deficiency, Hepatitis B infection, Type II Diabetes and number of patients with pains outside the distribution of HIV-SN), Mann–Whitney two-sample statistic (initial CD4 T-cell count, current CD4 T-cell count, time since HIV diagnosis, formal education, units of alcohol consumed, number of pains outside the distribution of HIV-SN, HSCL-25 subscales, intensity of non-painful symptoms and EQ-5D-3L

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VAS), Student's unpaired t-test (age, height and weight), Chi squared test (antiretroviral treatment regimen and intensity of pains outside the distribution of HIV-SN) and Chi squared test for trend (EQ-5D-3L sub-scales).

Variables yielding p-values < 0.1 on univariate analysis, and those previously shown to be associated with painful HIV-SN [5, 17, 19, 23, 44, 45], were included in multivariate regression analyses.The multivariate analyses used differed according to the properties of the data being analysed. All regression modeling was performed using R3.1.2 [46] with the MuMIn [47] and the rms packages [48]. The analyses were carried out as follows:

Primary Objectives 1 and 2

(1) Logistic regression analysis was used to examine the relationship between pain status and psychological factors. We also included clinical, demographic, and physical factors in the modelling process. The full model included: time since HIV diagnosis, current CD4 T-cell count, presence of pains outside the distribution of HIV-SN, vitamin B_{12} deficiency, weight, and height. Model selection was based on step-wise regression, using Akaike information criterion (AIC) as the basis for comparing models.

(2) General additive model (GAM) regression modelling was used to assess predictors of health-related quality of life (EQ-5D-3L VAS score) in (1) the full cohort of 197 participants (full model included: age, presence of painful HIV-SN, having other pains, sex, education, HSCL-25 depression score, mean HSCL-25 anxiety score), and (2) within the painful HIV-SN group only (full model included: age, intensity of neuropathic pain, having other pains, sex, education, HSCL-25 depression score, mean HSCL-25 anxiety score). The modelling assumed an inflated beta distribution of the dependent variable (VAS score expressed as a proportion), which describes [0, 1] bounded continuous probability distributions and thus negates the need to transform the data to stabilize residual variance. Model selection was based on step-wise regression, using Generalized Akaike information criterion (GAIC) as the basis for comparing models.

Secondary Objective 1 (Painful HIV-SN Group Only)

(3) Ordinal logistic regression was used to assess associations with pain intensity (pain intensity of the neuropathy using the 0–10 NRS) in participants with painful HIV-SN (full model included: number of pain sites outside the distribution of HIV-SN, mean HSCL-25 depression score, mean HSCL-25 anxiety score, total PCS score, and whether currently on treatment for TB).

All data analysed in the multivariate analyses, the analysis scripts, and the outputs from the scripts are hosted on a public GitHub repository (http://kamermanpr.github.com/painful.HIVSN), with a back-up hosted in a public Figshare repository [49].

Results

One hundred and twenty-five (125/197) patients with painful HIV-SN and seventy-two (72/197) patients with non-painful HIV-SN were recruited. Of the 197 patients, 53% (105/197) completed the English version of the questionnaire set, 25% (49/197) completed the IsiZulu version, 11% (22/197) completed the isiXhosa version, 7% (14/197) completed the Sesotho version and 4% (7/197) completed the Setswana version.

Factors That Associated With Having Painful HIV-SN (Primary Objective 1)

Univariate Analyses

Physical and clinical characteristics of the predominantly female, middle-aged cohort are shown in Table 1. All the participants were currently on antiretroviral therapy and had well preserved CD4 T-cell counts. The painful HIV-SN group had, on average, lower median current CD4 T-cell count, a shorter time since HIV diagnosis at their assessment for this study, and a lower prevalence of vitamin B_{12} deficiency compared with the pain free HIV-SN group. Among participants with painful HIV-SN, 85% (106/125) reported their 'SN-related' pain as "moderate" to "severe" in intensity [11-point Numerical Pain Rating Scale score (NRS): 4–10], with a median pain intensity of 7 (IQR 1–10) out of 10. All participants with painful HIV-SN presented with at least one additional pain site (pains outside the distribution of HIV-SN/not SN-related), compared with only a quarter of patients in the non-painful HIV-SN group. Moreover, patients in the painful HIV-SN group were more likely than the non-painful HIV-SN group to report the pain at these other sites (pains outside the distribution of HIV-SN/not SN-related) as "moderate" to "severe". Participants in the painful HIV-SN group were more likely to report experiencing numbness and/or paraesthesias in the distribution of their neuropathy than were individuals in the non-painful HIV-SN group but the

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Table 1 Physical and clinical characteristics of the study cohort

Characteristic	Entire cohort (n = 197)	Painful HIV-SN (n = 125)	Non-painful HIV-SN (n = 72)	p-value (Painful vs. non-painful)
Age (years)[a]	43.2 (12.1)	42.4 (11.3)	44.6 (13.4)	p = 0.23[d]
Initial CD4 T-cell count (cells/mm^3)[b]	142 (94–142)	137.5 (92–231)$^{(n=114)}$	149 (96–221.5)$^{(n=64)}$	p = 0.59[g]
Current CD4 T-cell count (cells/mm^3)[b]	424 (256–674)	405 (227–674)$^{(n=125)}$	463.5 (352–651)$^{(n=72)}$	p = 0.03[g]
Time since HIV diagnosis (months)[b]	120 (72–162)	108 (60–144)$^{(n=104)}$	144 (120–186)$^{(n=55)}$	p < 0.0001[g]
Height(cm)[a]	163.3 (8.8)	162.4 (8.5)$^{(n=115)}$	164.8 (9.2)$^{(n=67)}$	p = 0.09[d]
Weight (kg)[a]	71.7 (19.8)	73.5 (22.0)	68.6 (14.5)	p = 0.13[d]
Current TB infection, n (%)	26 (13)	14 (11)	12 (17)	p = 0.28[e]
Standard units of alcohol consumed[b]	13.5 (2–42)	12.2 (2–29.7)$^{(n=32)}$	13.5 (6–42)$^{(n=25)}$	p = 0.29 [g]
Vitamin B_{12}deficiency, n (%)	25 (13)	11 (9)	14 (19)	p = 0.04[e]
Hepatitis B infection, n (%)	15 (7)	9 (7)	6 (8)	p = 0.78[e]
Type II diabetes, n (%)	10 (5)	4 (3)	6 (8)	p = 0.17[e]
Treatment regimen, n (%)				p = 0.78[f]
Non D4T-based	166 (84)	106 (85)	60 (83)	
D4T-based	24 (12)	14 (11)	10 (14)	
Other	7 (3)	5 (4)	2 (3)	
Number of patients with other pain sites, n (%)	143 (73)	125 (100)	18 (25)	p < 0.0001[e]
Number of other pain sites[b]	3 (1-9)	3 (1-9)	2 (1-4)	p = 0.02[g]
Intensity of other pains, n (%)				p = 0.02[f]
Mild	23 (11)	19 (15)	4 (6)	
Moderate	63 (32)	51 (41)	12 (17)	
Severe	57 (29)	55 (44)	2 (3)	
Prevalence of symptoms, n (%)				
Pins and needles	116 (59)	106 (85)	10 (14)	p < 0.001[e]
Numbness	115 (58)	102 (82)	13 (18)	p < 0.001[e]
Intensity of symptoms (0–10 NRS score)[c]				
Pins and needles	5 (1–10)$^{(n=106)}$	5 (1–10)$^{(n=98)}$	4.5 (2–10)$^{(n=8)}$	p = 0.13[g]
Numbness	6 (1–10)$^{(n=102)}$	6 (1–10)$^{(n=90)}$	5 (2–10)$^{(n=12)}$	p = 0.43[g]

[a] Data shown as mean (SD) for parametric data

[b] Data shown as median (IQR) for non-parametric data

[c] Numerical pain rating Scale (NRS) scores shown as median (IQR)

[d] Unpaired t-test (parametric data)

[e] Fishers exact test

[f] Chi squared test

[g] Mann-Whitney two-sample statistic (non-parametric data)

severity of these other symptoms, when present, did not differ between the two groups.

Psychological and demographic factors (sex, education, and depressive and anxiety-like symptoms) for the painful and non-painful HIV-SN groups are presented in Table 2. There were no significant differences between the two groups in the proportion of females (~75%) or years of education completed (~6 years). A similarly high proportion of patients in the painful HIV-SN (88%, 110/125) and non-painful HIV-SN (93%, 67/72) groups reported a clinically relevant burden of depressive and anxiety-like symptoms (>1.75 on each of the HSCL-25 sub-scales). Consistent with this finding, ~60% of participants in both groups reported "moderate" or "severe" depression or anxiety on the EQ-5D-3L (Table 3).

Multivariate Analyses (Supplementary data 3)

Presence of additional pains outside the distribution of HIV-SN/not SN-related [Odds Ratio (OR) 3.47, 95% Confidence Interval (CI) 6.7×10^{-29} to 1.78, p = 0.63)], time since HIV diagnosis (OR 2.02, 95% CI 0.08–0.49,

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Table 2 Demographic and psychological characteristics of the study cohort

Characteristic	Entire cohort (n = 197)	Painful HIV-SN (n = 125)	Non-painful HIV-SN (n = 72)	p-value (Painful vs. non-painful)
Female sex, n (%)	149 (76)	94 (75)	55 (76)	p = 1.00[c]
Formal education (years)[a]	6 (0–10)	6 (0–10)	7 (0–10)	p = 0.13[d]
HSCL-25 (average sub-scale score)[a]				
Anxiety symptoms	2.60 (2.1–3.4)	2.60 (2.1–3.4)	2.60 (1.0–4.0)	p = 0.97[d]
Depressive symptoms	2.53 (2.0–3.1)	2.53 (2.0–3.1)	2.53 (1.0–3.8)	p = 0.70[d]
PCS[b]	–	31.2 (14)	–	–

[a] Data shown as median (IQR) for nonparametric data

[b] Data shown as mean (SD) for parametric data

[c] Fishers exact test

[d] Mann-Whitney two-sample statistic (nonparametric data)

Table 3 Summary of quality of life outcomes from the EQ-5D for painful and non-painful SN

Characteristic	Entire cohort (n = 197)	Painful HIV-SN (n = 125)	Non-painful HIV-SN (n = 72)	p-value (Painful vs. non-painful)
EQ-5D-3L, n (%)				
Mobility (MO)				p = 0.0020[c]
No problems	165 (84)	96 (77)	69 (96)	
Moderate problems	27 (14)	24 (19)	3 (4)	
Severe problems	5 (2)	5 (4)	0	
Self-care (SC)				
No problems	183 (93)	114 (92)	69 (96)	p = 0.22[c]
Moderate problems	14 (7)	11 (8)	3 (4)	
Severe problems	0	0	0	
Usual activities (UA)				p = 0.03[c]
No problems	170 (86)	102 (82)	68 (94)	
Moderate problems	26 (13)	22 (18)	4 (6)	
Severe problems	1 (1)	1 (1)	0	
Pain/discomfort (PD)				p < 0.0001[c]
No problems	89 (45)	31 (25)	58 (81)	
Moderate problems	74 (38)	62 (50)	12 (17)	
Severe problems	34 (17)	32 (25)	2 (3)	
Anxiety/depression (AD)				p = 0.65[c]
No problems	79 (40)	51 (41)	28 (39)	
Moderate problems	80 (41)	48 (38)	32 (44)	
Severe problems	38 (19)	26 (21)	12 (17)	
EQ-5D-3L (VAS)[a]	80 (50–100)	65 (40–80)	100 (50–100)	p < 0.0001[b]

[a] Data shown as median (IQR) for nonparametric data

[b] Mann-Whitney two-sample statistic (nonparametric data)

[c] Chi squared test for trend

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$p < 0.001$) and body mass [OR = 1.89, 95% CI 0.76–4.64, $p = 0.16$)] were retained in the final logistic regression model of predictors for having painful HIV-SN. The model had good predictive value ($R^2 = 0.76$, Somers D = 0.87, area under the receiver operating characteristic curve = 0.93). However, only time since HIV diagnosis was independently associated with painful HIV-SN. A longer time since HIV diagnosis associated with a reduced likelihood of having pain.

Factors Associated With Quality of Life (Primary Objective 2)

Univariate Analyses

Data from the EQ-5D-3L are reported in Table 3. When examining scores from the five subscales of the questionnaire, there was no difference between the painful and non-painful groups for the depression/anxiety subscale, but there were differences for the three remaining EQ-5D-3L items, with a greater proportion of participants with painful HIV-SN reporting having "moderate" or "severe" problems with mobility ($p = 0.002$), "moderate" problems with their usual activities ($p = 0.03$), and, as anticipated, "moderate" or "severe" pain and discomfort ($p < 0.0001$). Consistent with these three EQ-5D-3L items, results from the EQ-5D-3L VAS differed between the two groups; painful HIV-SN participants had a lower median VAS score for their perceived state of health (65 IQR: 0-100 vs. 100 IQR: 50-100). We also measured pain catastrophizing in the patients with painful HIV-SN. The mean PCS score for the painful HIV-SN group was 31.2 with a standard deviation of 13.8, where a score > 30 indicates a clinically relevant level of catastrophizing [36].

In the full cohort, Hopkins depression mean score ($r = -0.26$, $p < 0.002$), Hopkins anxiety mean score ($r = -0.17$, $p = 0.01$) and intensity of pains outside the distribution of HIV-SN/not SN-related ($r = -0.54$, $p < 0.001$) all correlated with quality of life VAS scores. Other factors considered, including age ($r = 0.02$, $p = 0.78$), level of education ($r = -0.12$, $p = 0.07$) and presence of pains outside the distribution of HIV-SN/not SN-related ($r = -0.23$, $p = 0.21$) did not correlate with quality of life VAS scores on univariate analysis.

Within the painful HIV-SN group, Hopkins depression mean score ($r = -0.34$, $p < 0.001$), Hopkins anxiety mean score ($r = -0.25$, $p = 0.004$), PCS mean score ($r = -0.29$, $p = 0.001$), presence of pains outside the distribution of HIV-SN/not SN-related ($r = -0.23$, $p = 0.008$) and intensity of other pains ($r = -0.41$, $p < 0.001$) all significantly correlated with quality of life VAS scores. Other predictors including age ($r = 0.06$, $p = 0.47$) and level of education ($r = 0.03$, $p = 0.72$) did not correlate with quality of life VAS scores on univariate analysis.

Multivariate Analyses (Supplementary data 4 and 5)

Using general additive model regression analysis, we identified that having painful HIV-SN (mu coefficient = −0.91, t-value = −4.80, $p < 0.001$) and greater HSCL-25 depression scale scores (mu coefficient = −0.33, t-value = −3.14, $p = 0.002$) predicted lower EQ-5D-3L quality of life VAS score. And in the painful HIV-SN cohort, greater HSCL-25 depression subscale scores were associated with lower EQ-5D-3L VAS scores (mu coefficient = −0.35, t-value = −2.90, $p = 0.004$).

Factors that Associated With Increased Pain Intensity (Secondary Analysis 1)

Univariate Analyses

On univariate analysis for associations with pain intensity of the SN, Hopkins depression mean score ($r = 0.21$, $p = 0.01$), presence of pains outside the distribution of HIV-SN/not SN-related ($r = 0.18$, $p = 0.04$) and intensity of pains outside the distribution of HIV-SN/not SN-related ($r = 0.22$, $p = 0.01$) all correlated with intensity of HIV-SN related pain. Other psychological factors, including: sex, education, PCS score, EQ-5D VAS score and Hopkins mean anxiety score did not correlate with pain intensity in this cohort (all $p > 0.05$).

Multivariate Analyses (Supplementary Data 6)

Independent predictors of increasing pain intensity of the SN, included increasing levels of depression (OR 1.95, 95% CI 1.14–3.33, $p = 0.01$), additional pains outside the distribution of HIV-SN/not SN-related (OR 1.40, 95% CI 1.00–1.97, $p = 0.04$) and being treated for TB infection (OR 0.33, 95% CI 0.12–0.94, $p = 0.03$) (supplementary data 6). However, the model had poor predictive value ($R^2 = 0.09$, Somer's D = 0.25, area under the ROC curve = 0.63).

Discussion

We recruited individuals with painful or non-painful HIV-SN to determine: (1) if psychological factors associate with having painful HIV-SN, (2) if pain and psychological factors affect quality of life, and (3) if psychological factors predict the intensity of their pain. We report that in this black, African cohort of PLWHA that: (1) depression, anxiety and pain catastrophizing did not associate with the presence of

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painful HIV-SN, (2) increased depressive symptoms and presence (but not intensity) of pain independently associated with reduced quality of life, and (3) greater depressive symptoms independently associated with increased pain intensity in patients with painful HIV-SN.

Factors Associated With Having A Painful Rather than Non-Painful HIV-SN

Having additional pains outside the distribution of HIV-SN was retained in the final model of associations with painful HIV-SN, however this was not an independent risk factor ($p = 0.63$). As our study was cross-sectional, we cannot comment on the order in which additional pains outside the distribution of HIV-SN and painful SN occurred. However, the data do suggest that those with painful HIV-SN may be predisposed to having pain of any sort. Indeed, it may be that these individuals are generally more sensitive/susceptible to pain and other symptoms, including numbness and paraesthesias. The presence of multiple pain sites could also be indicative of central sensitisation of the central nervous system [50]. Nevertheless, our data provide clear evidence of a high burden of pain among individuals with HIV-SN. Eighty-five percent (85%) of those in the painful HIV-SN group reported that the pain of their neuropathy was moderate to severe in intensity. Furthermore, all 125 individuals in the painful HIV-SN group had at least one additional pain site, and these sites were on average also rated as moderate to severe in intensity. Thus, painful HIV-SN was just one part of a larger pain burden in this group.

Depression and anxiety did not associate with having a painful SN in our cohort, unlike previous studies [5, 13, 51]. Indeed, we found a similarly high burden of depressive and anxiety-like symptoms in those with HIV-SN, regardless of whether pain was present. The use of different tools to screen for depression by us (HSCL-25) and other investigators (Becks Depression Inventory [5, 23, 25]; and Centre for Epidemiologic Studies Depression Scale [CES-D] [51] prevents direct comparisons of the scores reported across studies. Overall, however, we found 93% of the non-painful and 88% of the painful HIV-SN group had clinically relevant levels of depressive symptoms, compared with 13% of non-painful and 20% of the painful HIV-SN group in the study by Ellis et al. [5]. The high level of depressive symptoms in our cohort is not remarkable given the high levels of depression reported in the population from which our study cohort was drawn [52–54]. Certainly, only by conducting our research in a unique setting have we been able to demonstrate that high levels of background depression in the community may erode the often reported link between depression and chronic pain [55–57].

Effect of Pain and Psychological Factors on Quality of Life

As in other studies, patients with painful HIV-SN had a lower overall rating of quality of life than SN patients without pain [5, 13, 25]. Analysis of subscale data from the EQ-5D-3L also identified that patients with painful HIV-SN were more likely to have problems with their mobility and usual activities, as expected.

In multivariate analyses we found depressive symptoms and presence of pain associated with reduced quality of life. These data fit with reports from other investigators, where coincident pain and depression have associated with reduced quality of life in HIV-positive patients [58, 59]. Of note, we found that the presence of pain, but not its intensity associated with reduced quality of life. Indeed, this is not the first time that factors other than the intensity of pain have associated more strongly with quality of life. In US patients with HIV-SN, depression affected quality of life more than pain intensity [25]. Neuropathic pain has associated with reduced quality of life in individuals with HIV-SN [5, 13, 23, 25]. Severe neuropathic pain was associated with reduced quality of life and pain function scores as well as higher pain interference scores [5, 13]. However, Ellis and colleagues also found that mild neuropathic pain associated with a profoundly reduced quality of life [5]. Similarly, high levels of depressive symptoms and the high pain burden (neuropathic pain and the presence of additional pains) observed in our cohort may have made patients more sensitive to any pain, regardless of its intensity. This may explain why we did not find any association between pain intensity and quality of life but rather an association between the presence of pain and reduced quality of life. One of the limitations of using depression instruments in pain studies is that the inclusion of somatic/pain symptoms in various depression instruments makes its quite difficult to ascertain whether there is truly a depressive component or just an artificial inflation due to the confounding from somatic/pain symptoms. One of the most commonly used rating scales for depression in chronic pain patients is the Becks Depression Inventory (BDI). The BDI's factor structure has a strong somatic component which can be very difficult in a chronic pain assessment. We used the HSCL-25 questionnaire that does include a small number of somatic symptoms, which includes 'feeling low in energy', 'difficulty falling asleep, staying asleep', and 'poor appetite'. However, the HSCL-25 does not have a large pain symptom component and only includes a single question on 'headaches' in the anxiety section of the questionnaire. The use of a depression instrument containing somatic/pain symptoms is a limitation in our study and highlights the need for improved depression tools in the setting of chronic pain patients.

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Effect of Psychological Factors on Pain Intensity

Although, depressive symptoms did not associate with having a painful SN, they did associate with pain intensity. These data fit with other reports that higher depression scores associate with increased pain intensity in US HIV-positive patients [59, 60].

Anxiety level did not associate with pain intensity here. Anxiety has not been assessed in relation to intensity of pain in HIV-SN previously, but greater anxiety has been associated with greater pain intensity in arthritis, migraine and back pain in US cohorts [21].

Having additional pains outside the distribution of HIV-SN was independently associated with greater pain intensity in this cohort. This may be suggestive of a spatial summation effect or may suggest that depression and anxiety (also associated with pain intensity) worsen with an increased load of chronic conditions. Indeed, the observed association between depressive symptom burden and pain burden intimates possible dysfunction in brainstem serotonergic and noradrenergic pathways and/or diffuse noxious inhibitory control system [61].

Catastrophizing has associated with pain intensity in other chronic pain conditions [62] including intensity of HIV-SN pain [13, 23] but did not associate with pain intensity here. Levels of pain catastrophizing were very high in this cohort: the mean (SD) PCS score was 31.2 (13.8) versus 19.6 (13.7) in Lucey's U.S cohort [23] and a score >30 indicates a clinically relevant level of catastrophizing [36]. Thus, similar to our findings for depression, universally high prevalence of pain catastrophizing in this population confounds the detection of an association between catastrophizing and pain.

Conclusion

In this cohort of black African PLWHA attending a public healthcare facility, we found a high pain burden in patients with SN, demonstrated by painful SN being only one of multiple pains experienced by these patients. In this first assessment of psychological factors in a non-US or European cohort with HIV-SN, we found high levels of depression, and depression associated with increased pain intensity and reduced quality of life. Painful HIV-SN is difficult to treat pharmacologically [29, 63] and recent commentary suggests that reducing pain intensity may not be a realistic therapeutic goal for chronic pain conditions [64] but improving quality of life and function may be more valuable. This suggests that a greater emphasis on assessing and treating depressive symptoms in patients with SN could be advantageous and warrants further investigation.

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Compliance with Ethical Standards

Conflict of interest All authors (PP, ALW, CLC, ASK and PRK) declare no conflict of interest.

Ethical Approval All procedures performed in the study were in accordance with the ethical standards of the Wits Human Research Ethics Committee (Medical) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Written informed consent was obtained from all individual participants included in the study.

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HIV-ASSOCIATED SENSORY NEUROPATHY IN THE ERA OF TENOFOVIR-BASED HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) Prinisha Pillay A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy Johannesburg, South Africa, March 2018 DECLARATION I, Prinisha Pillay, declare that this thesis is my own work, except where otherwise specified. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree of examination at this or any other University. _____ day of _____ 2018 Chapter 1 Introduction 1.1 HIV-related pain 1.1.1 Characteristics of HIV-related pain Pain is highly prevalent in people living with HIV/AIDS (PLWHA) (between 75% to 91%) (Narasimooloo et al. 2011; Parker et al. 2017) and this pain is often undertreated in PLWHA (Bouhassira et al. 1999; Parker et al. 2014, Turk et al. 2010; Forna et al. 2007). There are various causes of pain experienced by PLWHA (Parker et al. 2014, Kamerman & Mitchell 2012) which may encompass nociceptive and neuropathic components (Collins and Harding, 2007). Possible causes for the pain experienced by these individuals include: a direct effect of HIV on the peripheral or central nervous system, opportunistic infections that may arise, presence of tumours or cancer, exposure to neurotoxic antiretroviral therapies (stavudine, didanosine and zalcitabine), and other iatrogenic causes such as chemotherapeutic agents and procedural pain. Also, pre-existing co-morbid disease may contribute to the burden of this pain (Dobalian et al. 2004, Keswani et al. 2002; Parker et al. 2014). A recent systematic review of pain in PLWHA found that these individuals often rate their pain as 'moderate to severe' in intensity (Parker et al. 2014). This finding has huge implications for patient's quality of life and may be the basis for further psychological distress in these individuals. 1.1.2 Prevalence of HIV-related pain at different anatomical sites in PLWHA There are numerous pain sites reported by PLWHA and these data are consistent across African populations, but differ slightly when compared to those from abroad. A summary of the various anatomical sites where pain has been reported as more prevalent in PLWHA is provided in Table 1.1. Wahab and colleagues (2011) found that of the 40% of individuals in their cohort who reported moderate to severe pain, lower limbs (40.9%), head and neck region (31.8%), and abdomen (31.8%) were the major regions affected by pain. Congruently, Mphalele and colleagues (2012) who investigated pain in ambulatory HIV-positive individuals in both rural and urban settings also found that the head region (26% in rural vs. 39% in urban) and abdomen (30% in rural vs. 20% in urban) were frequent pain sites reported by individuals in their cohort. Other pain sites reported by Mphalele and colleagues (2012) include chest pain (19% in rural vs. 30% in urban), genital (15% only in rural group) and pain in the feet (33% only present in urban group) and these sites are not unique to African populations only (Del Borgo et al. 2001, Nair et al. 2009). The consistency of these particular pain sites in HIV- infected individuals is seen in the Indian population as well, as Nair and colleagues (2009) also found in their South Indian cohort that headache (28.8%) and the soles of the feet (25%) were common sites affected by pain. 1.1.3 Factors associated with HIV-related pain 1.1.3.1 Pain and HIV disease markers Several studies have assessed the relationship between CD4 T-cell counts and the prevalence of pain (Aouizerat et al. 2010, Martin et al. 1999, Peltzer et al. 2008, Powers et al. 2007, Richardson etal.2009, Rosen et al. 2008, Van as et al. 2009). In their study on pain in ambulatory HIV-positive individuals with and without intravenous drug use, Martin and colleagues (1999) reported a significant correlation between number of pain sites and CD4 T- cell counts in those individuals not using intravenous drugs (Martin et al. 1999). This infers that as CD4 T-cell counts decrease symptom burden increases. Aouizerat (2010) also reported a significant correlation between decreased CD4 T-cell counts and increased pain prevalence. In contrast, Ellis and colleagues (2010) reported a higher nadir CD4 T-cell count as well as previous, but not current, exposure to neurotoxic ARVs as risk factors for painful HIV-associated sensory neuropathy (HIV-SN) in their cohort. The latter finding has previously been reported by another study (Cherry et al. 2002). In their review of HIV-related pain and its management, interrogated the association between CD4 T-cell counts and pain prevalence in HIV-positive individuals and concluded that pain prevalence in HIV is not associated with CD4 T- cell counts. Also, spurious associations between pain prevalence and increased CD4 counts may be evident in successful cART treatment, where there is a distinctive pattern of increases in CD4 T-cell counts and simultaneous decreases in viral loads. Several sub-Saharan African studies also reported no consistent correlation between CD4 T- cell counts and measures of pain (Peltzer et al. 2008, Powers et al. 2007, Rosen et al. 2008, Van as et al. 2009). In addition, with successful cART, an increase in CD4 T-cell count should be paralleled by a decrease in pain in these patients, which is not the case in many studies

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