THE INCIDENCE OF PERIPHERAL NEUROPATHY IN HIV-POSITIVE INDIVIDUALS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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DECLARATION

I declare that this dissertation is my own unaided work, unless otherwise specified. It is being submitted for the degree of MSc (MED) at the University of the Witwatersrand, Johannesburg.

This work has not been submitted before for any degree or examination in any other university.

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ABSTRACT

Peripheral sensory neuropathy is a common neurological complication of antiretroviral therapy, typically occurring within 6-months of starting Highly Active Antiretroviral Therapy (HAART) which includes stavudine. Therefore, the primary aim of the study was to determine the 6-month incidence of ATN in patients free of neuropathy and beginning stavudine-based HAART for the first time. Also, we examined whether initiating stavudine-based HAART altered the symptoms of patients who had a pre-existing, virus-mediated distal symmetrical polyneuropathy (HIV-DSP). Seventy-five HIV-positive patients were screened for neuropathy, at the Chris-Hani Baragwanath Hospital, using the AIDS Clinical Trials Group neuropathy screening tool. The bilateral presence of atleast one sign (decreased vibration sense in the great toe or absent ankle reflex) and one symptom (pain, paraesthesia or numbness) in the feet was indicative of neuropathy. On recruitment, 52 patients presented without neuropathy and 13 patients presented with HIV-DSP. After 3-months of follow-up (n=46), 23% (10/46) of patients had developed peripheral neuropathy, and by 6-months (n=44), 41% (18/44) of patients had developed neuropathy. Greater disease severity was the only risk factor significantly associated with the development of neuropathy. Eleven (61%) of the 18 patients that developed neuropathy, developed painful symptomatic neuropathy, and only 6 (55%) of these patients were receiving treatment for symptom relief. In patients with HIV-DSP, numbness was the most common symptom reported at baseline and was the only

symptom to reduce in frequency across the 6-months. In conclusion, we found that the development of neuropathy is common in the first 6-months of patients initiating stavudine-based HAART.

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LIST OF ABBREVIATIONS

ACTG	Aids Clinical Trials Group
AIDS	Acquired immunodeficiency syndrome
ARV	antiretroviral therapy
ATN	antiretroviral toxic neuropathy
AZT	zidovudine
DNA	deoxyribonucleic acid
ddI	didanosine
d4t	stavudine
EFV	efavirenz
FTC	emtricitabine/tenofovir
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
HIV-DSP	human immunodeficiency virus-associated distal symmetrical polyneuropathy
HIV-SN	human immunodeficiency virus-associated sensory neuropathy
LPV/r	lopinavir/ritonavir
NNTRI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine

TDF	tenofovir
TB	tuberculosis
WHO	World Health Organisation
3TC	lamivudine

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Conference presentation

Pillay P., Dinat N. and Kamerman P. R. The incidence of peripheral neuropathy in HIV-positive individuals on highly active antiretroviral therapy (HAART). 4th Pan African Pain Congress, 11-13 March 2011, International Convention Centre, Cape Town, South Africa (oral presentation).

Chapter 1

INTRODUCTION

1. HIV-associated sensory neuropathy (HIV-SN): An overview

HIV-associated sensory neuropathy is a common neurologic complication of HIV-infection and its treatment, and is seen in about 35% of HIV-infected patients (McArthur *et al.* 2005;Ferrari *et al.* 2006). HIV-associated sensory neuropathy can either result as a consequence of HIV-infection, in which case it is often called HIV-associated distal symmetrical polyneuropathy (HIV-DSP), or as a consequence of the use of some classes of dideoxynucleoside drugs, such as stavudine, in which case it is often called antiretroviral toxic neuropathy (ATN) (Cornblath *et al.* 2006;Ferrari *et al.* 2006).

HIV-DSP is thought to occur primarily due to virus-dependant macrophage infiltration into peripheral nerves and the release of pro-inflammatory cytokines, which results in axonal degeneration and neuronal death (Cornblath *et al.* 2006;Ferrari *et al.* 2006), whilst ATN is thought to result primarily from mitochondrial toxicity associated with nucleoside reverse transcriptase inhibitor (NRTI) usage, although underlying sub-clinical virus-induced damage is probable (Keswani *et al.* 2002;Cornblath *et al.* 2006;Ferrari *et al.* 2006). Nevertheless, clinically, HIV-DSP and ATN are indistinguishable, presenting with the same signs and symptoms. Signs typically include, absent ankle reflexes and decreased vibration sense, while typical symptoms include pain, numbness and paraesthesias in the distal extremities, typically starting in the feet (Gonzalez-Duarte *et al.* 2007;Wadley *et al.*

2011). It has been suggested that subtle differences in presentation between HIV-DSP and ATN may include ATN being much more painful and having a more rapid onset and progression, compared with HIV-DSP (Keswani *et al.* 2002;Gonzalez-Duarte *et al.* 2007;Cherry *et al.* 2008). However, because of their similar clinical features, and possibly overlapping pathogenesis, the main criterion for differentiating between HIV-DSP and ATN is the relationship between the onset of peripheral neuropathy and nucleoside analogue initiation (Dalakas 2001;Pardo *et al.* 2001;Verma 2001;Keswani *et al.* 2002;Cherry *et al.* 2008). Because symptoms of ATN may improve or resolve within a period of 8 weeks of stopping nucleoside analogue use or if dosages are lowered, (Brew 2003;Gilman 2007;Gonzalez-Duarte *et al.* 2007) clinicians need to be certain of the type of neuropathy, as an early change in therapy to a less neurotoxic regimen may improve symptoms in patients with ATN (Moyle *et al.* 1998;Sacktor *et al.* 2009).

The main aim of my project was to assess the incidence of neuropathy in HIV-positive individuals initiating HAART, and therefore this literature review will focus on describing the incidence and prevalence of ATN and the relationship between HAART and ATN. However, to give a more general perspective of HIV-SN, I will also provide a brief description of the characteristics and epidemiology of HIV-DSP. Furthermore, since incidence and prevalence are two commonly used terms in this dissertation, a brief definition of each term will help to understand these topics more

clearly. Prevalence data provides us with a figure for a certain factor at a specific point in time (Shields *et al.* 2003). Thus, as an example related to this study, it provides the measure of the burden of neuropathy at a particular time in a certain population, whilst, incidence provides a measure of the risk of occurrence or development of a certain condition, within a specified time (Shields *et al.* 2003). Thus, as an example related to this study, it will provide us with the risk of development of neuropathy within the allotted time. Incidence data may be superior to that of prevalence data, as it provides health policy makers with a more accurate measure of the development of a condition within a specified time period.

2. HIV-associated distal symmetrical polyneuropathy (HIV-DSP)

2.1 Characteristics and pathophysiology of HIV-DSP

HIV-associated distal symmetrical polyneuropathy develops gradually and has a slow progression (Simpson *et al.* 1995;Pardo *et al.* 2001;Gilman 2007). It is also more common in the late stages of HIV-infection, stages III and IV of the World Health Organisation (WHO) disease staging guidelines (WHO 2007), when patients present with lower CD4 T-cell counts and AID-defining illnesses (Griffin *et al.* 1994;Parry *et al.* 1997;Wulff *et al.* 2000). These late WHO stages are characterised by the presence of the following conditions: Stage III severe bacterial infections (pneumonia and meningitis), unexplained chronic diarrhoea for longer than one month and current tuberculosis (TB) infection, Stage IV HIV wasting syndrome, Kaposi's sarcoma,

extrapulmonary TB, chronic isosporiasis and recurrent severe bacterial pneumonia (WHO 2007). High plasma viral load (Hitchcock *et al.* 2008) and the presence of underlying conditions such as diabetes mellitus (Verma *et al.* 2004) are also common risk factors associated with the development of HIV-DSP. A summary of the characteristics of HIV-DSP are provided in Table 1.

The cause of HIV-DSP probably involves a dysimmune response following infiltration of peripheral nerves with infected macrophages and direct toxicity of HIV-viral proteins, such as the gp120 envelope protein, on nerve fibres (Keswani *et al.* 2002). Studies have shown that the development of HIV-DSP occurs following activation and infiltration of macrophages into the nerve fibres and dorsal root ganglion and the release of pro-inflammatory cytokines around the dorsal root ganglion (Yoshioka *et al.* 1994;Rizzuto *et al.* 1995;Nagano *et al.* 1996;Bradley *et al.* 1998). Animal studies have shown direct effects and indirect effects, via immune activation, of gp120 on nerve cells (Oh *et al.* 2001;Keswani *et al.* 2003;Melli *et al.* 2006;Wallace *et al.* 2007a;Wallace *et al.* 2007b). This direct and indirect toxicity produces the most distinct pathological feature of HIV-DSP, axonal degeneration of long axons in distal regions. This axonal damage follows a “dying back” pattern of degeneration (Holland *et al.* 1997;Polydefkis *et al.* 2002).

Table 1. Characteristics of HIV-DSP and ATN.

Neuropathy	Onset	Mechanism and characteristic pathological feature	Signs	Symptoms	Risk factors	References
HIV-DSP	Slow progression	Immune dysfunction: Macrophage mediated axonal degeneration and neuronal death, reduction in density of unmyelinated fibres	-Absent ankle reflexes -decreased vibration sense in the feet (great toe) -decreased pin-prick and temperature sensation in a stocking-glove distribution	-Numbness -Paraesthesias -Burning sensations in the feet	-high plasma viral load -low CD4 T-cell count -increased age -late stage of HIV-infection; WHO stage III or IV (AIDS)	So *et al.* 1988;Nagano *et al.* 1996;Berger *et al.* 1999;Tagliati *et al.* 1999;Schifitto *et al.* 2002;Luciano *et al.* 2003;Ferrari *et al.* 2006;Gonzalez-Duarte *et al.* 2007;Smyth *et al.* 2007;Hitchcock *et al.* 2008
ATN	Rapid and more aggressive	Toxic neuropathy: Damage to mitochondria of cells, reduced density of unmyelinated fibres	Same as HIV-DSP above	Same as HIV-DSP above	-increased age -exposure to antiretroviral medication such as stavudine, didanosine and zalcitabine -high plasma viral load -exposure to isoniazid therapy -increased alcohol consumption	Lewis *et al.* 1995;Maschke *et al.* 2000;Moore *et al.* 2000;Scarsella *et al.* 2002;Morgello *et al.* 2004;Lichtenstein *et al.* 2005;Wester *et al.* 2005;Skopelitis *et al.* 2006;Gonzalez-Duarte *et al.* 2007;Robinson-Papp *et al.* 2009;Sacktor *et al.* 2009

2.2 Prevalence and incidence of HIV-DSP

The studies by So and colleagues (1988) and Schifitto and colleagues (2002) were the only two studies that were performed in the pre-HAART era. The findings of both these studies indicate that the prevalence and incidence of HIV-DSP during the pre-HAART era was high (So *et al.* 1988;Schifitto *et al.* 2002). A summary of the findings of these studies is provided in (Table 2).

The studies by Hitchcock and colleagues (2008), Maritz and colleagues (2010) and Forna and colleagues (2007) were all performed in African populations. The prevalence of HIV-DSP amongst these studies ranged from 13% to 37%. However, Hitchcock and colleagues (2008) were only assessing patients for the presence of pain of neuropathic origin in HIV-positive individuals, and therefore their results may underestimate the true prevalence of HIV-DSP.

Table 2. Prevalence and incidence of HIV-DSP.

Rate of HIV-DSP	Reference	n	CD4 T-cell count (cells/mm^3)	Male (%)	Race (%)
Prevalence					
35%[c]	So *et al.* 1988	40	Not reported	Not reported	Not reported
32%	Berger *et al.* 1999	106	437.0 (304)[a]	59	Caucasian: 4 Black: 19 Hispanic: 66 Caribbean: 1
38%	Tagliati *et al.* 1999	166	211.0 (216)[a]	75	Caucasian: 30 Black: 15 Hispanic: 39 Other: 16
13%	Forna *et al.* 2007	1029	126.0 [0-343][b]	26	Not reported
13%	Smyth *et al.* 2007	94	181.0 [10-840][b]	98	Not reported
18%	Hitchcock *et al.* 2008	354	111.0 (70.8)[a]	Not reported	Not reported
37%	Maritz *et al.* 2010	331	249 [153-437][b]	21	Not reported
Incidence					
52%[c]	Schifitto *et al.* 2002	121	190.2 (169.6)[a]	75	Caucasian: 52

[a]Mean (SD)
[b]Median [Q1-Q3]
[c]Studies conducted in the pre-HAART era. All other studies were conducted in the era of HAART, but on patients not on treatment.

Hitchcock and colleagues (2008) reported treatment for TB as a risk factor for HIV-DSP. Though, Maritz and colleagues (2010) did not find TB treatment as a risk factor for HIV-DSP in their cohort, they reported a history of previous TB infection as a risk factor for ATN. Additional risk factors reported by Hitchcock and colleagues (2008), and which were not common to the other African studies were male sex, high plasma viral load and low CD4 T-cell count. Indeed, even though Forna and colleagues (2007) reported similarly low mean CD4 T-cell counts in their cohort, to that reported by Hitchcock *et al.* (2008), CD4 T-cell count was not identified as a risk for the development of HIV-DSP. Maritz and colleagues (2010) reported a much higher prevalence of HIV-DSP in comparison to Forna and colleagues (2007), even though their cohort had a significantly higher mean CD4 T-cell count. The lack of correlation between low CD4 T-cell count and risk of HIV-DSP was also seen in the study by Smyth and colleagues (2007) in Caucasians. Interestingly, both these studies reported a prevalence of HIV-DSP of 13%, despite Forna and colleagues (2007) having a sample size 9 times that of Smyth and colleagues (2007). The presence of mycobacterium avium complex was the only risk factor identified by Smyth and colleagues (2007). The presence of this risk factor classifies an individual as WHO disease stage IV (WHO 2007), and thus, this finding probably is merely a surrogate measure for worsening disease severity. Increased age as a risk factor for the development of HIV-DSP, was not only reported by the two African studies conducted by Maritz and colleagues (2010) and Forna and colleagues (2007), but also

by studies in the USA, these include Tagliati and colleagues (1999) and Berger and colleagues (1999). For both these studies, in the USA, the cohorts consisted of a large number of Hispanic participants; however race was not identified as a risk factor in either study. A unique finding of the study by Berger and colleagues (1999) was that increased alcohol consumption was a risk factor for the development of HIV-DSP, whilst, Tagliati and colleagues (1999) reported low heamoglobin levels as a risk factor unique to their study cohort. Despite So and colleagues (1988) reporting the second highest ever prevalence of HIV-DSP (35%), their study only identified a single risk factor associated with the development of HIV-DSP, which was longer disease progression.

Schifitto and colleagues (2002) were, to my knowledge, the only study that investigated the incidence of HIV-DSP. After following up patients for two and a half years (30 months), Schifitto and colleagues (2002) reported a HIV-DSP rate of 211.6 cases per 1000 patient-years. They identified AIDS and low CD4 T-cell count as risk factors associated with the development of HIV-DSP.

From the studies that have been reviewed here (Table 2), the prevalence of HIV-DSP, ranges from 13% to 38%. There seems to be a pattern amongst African studies where higher mean CD4 T-cell counts are associated with a higher prevalence of HIV-DSP (Maritz *et al.* 2010) and low CD4 T-cell counts associated with a lower prevalence of

HIV-DSP (Forna *et al.* 2007;Hitchcock *et al.* 2008). The increased risk of TB treatment for the development of neuropathy is also evident in these African populations, and probably reflects exposure to isoniazid therapy for TB in these regions of high TB and HIV co-infection. Also, our knowledge of the incidence of HIV-DSP is limited, as only one study has investigated the incidence of HIV-DSP.

For the remainder of the introduction, I will focus on the prevalence, incidence and risk factors for ATN.

3. Antiretroviral toxic neuropathy (ATN)

Antiretroviral toxic neuropathy is an HIV-associated sensory neuropathy that typically develops within 4 to 6 months of starting antiretroviral therapy. The signs and symptoms associated with ATN are exactly the same as HIV-DSP, but have a more rapid onset, associated with commencing HAART (Cornblath *et al.* 2006). Table 1 provides a comparison between ATN and HIV-DSP.

The following sub-sections will provide a brief summary on the introduction of HAART and its effect on the prevalence and incidence of ATN. The mechanism employed by NRTI's that are frequently used in HAART regimens which cause ATN is also examined. Lastly, a brief description of the most prominent NRTI, stavudine, and its effects on the incidence of ATN is provided.

3.1 HAART and ATN

With increased access to HAART, which effectively decreases viral loads and slows disease progression (Brodt *et al.* 1997), there has been a reduction in the development of conditions such as opportunistic infections (Forrest *et al.* 1998;Palella *et al.* 1998) and HIV-related neurological conditions such as HIV-associated dementia (Sacktor *et al.* 2009). However, by increasing survival times and increasing exposure to potentially neurotoxic agents, the introduction of HAART is generally thought to have increased the prevalence and incidence of other neurological complications of HIV such as ATN (Bacellar *et al.* 1994).

However, despite the dogma that HAART has resulted in an increase in ATN prevalence, several studies have raised the question of whether HAART may reduce the risk of HIV-SN. Maschke and colleagues (2000) showed that HAART decreases the prevalence of HIV-SN. They compared a pre-HAART cohort (1995-1996) to a HAART cohort (1997-1998) and found a significant decrease in the prevalence of HIV-SN from 42.5% to 34.4%. They attributed this decrease to the improvement of immune status by HAART. The study by Villelabeitia-Jaureguizar and colleagues (2006) further supports the idea that HAART is beneficial to HIV-positive individuals and is associated with a low incidence of ATN. The study focused on determining the incidence of symptomatic peripheral neuropathy associated with HAART and the true prevalence rate of subclinical neuropathy following several

years of treatment. The presence of neuropathy in the study was confirmed both clinically and electromyographically. They reported an incidence of ATN of 2% (n=108) in their cohort. In addition to possibly decreasing the incidence of ATN, antiretroviral therapy also may improve symptoms of pre-existing HIV-DSP. The study by Martin and colleagues (2000) showed that HAART can improve physiological measures of neuropathy in patients who tolerate HAART regimens well. Similarly, Moyle and colleagues (1998) suggest that in some instances HAART may improve peripheral neuropathy, whilst in other individuals it may be neurotoxic; what separates these individuals is unclear. This is supported by Sacktor and colleagues (2009), who reported that a small group of patients in their cohort, who presented with pre-existing HIV-DSP, showed improvement in both their signs and symptoms of neuropathy after initiating stavudine-based HAART for 6-months. For those patients who presented (on recruitment) with foot numbness, 22% no longer had numbness after 6-months of HAART and for those who had signs of neuropathy at presentation (on recruitment), 23% no longer presented with these signs after 6-months of therapy. In summary, the abovementioned studies indicate that not only is HAART beneficial in reducing the symptoms of existing neuropathy but it also reduces the risk of developing ATN.

However, the findings of Maschke and colleagues (2000) and Villelabeitia-Jaureguizar and colleagues (2006), conflict with those of Smyth and colleagues

(2007) and Bacellar and colleagues (1994) who reported an increase in the prevalence and incidence of ATN, respectively, with the use of HAART. Smyth and colleagues (2007) reported an increase in not only the presence but severity of symptoms associated with ATN in a subgroup of participants in their study who presented with asymptomatic neuropathy before starting stavudine-based HAART. In support, Sacktor and colleagues (2009) also reported the development of symptomatic ATN in 38% of HIV-positive patients, within 3-months of starting stavudine-based HAART. Indeed, the study by Bacellar and colleagues (1994) showed an increase in the incidence of ATN in an upward linear trend between the years 1988 and 1992. In addition, they reported that stavudine, didanosine and zalcitabine were significantly associated with a higher incidence of neuropathy and also that sensory neuropathy demonstrated the highest yearly rate of increase in incidence amongst several other neurologic complications which were also investigated (Bacellar *et al.* 1994).

3.2 NRTI's and mitochondrial dysfunction in ATN

The NRTI's stavudine, didanosine and zalcitabine are associated with the development of ATN (Moyle *et al.* 1998). Studies have shown evidence of mitochondrial dysfunction with the use of NRTI's as the possible cause of ATN (Lewis *et al.* 1995;Moyle *et al.* 1998;Dalakas 2001). Dalakas and colleagues (2001) showed pathological evidence of mitochondrial abnormalities in sensory nerves of those patients with dideoxynucleoside-induced neuropathy. They found a large

number of abnormal mitochondria in both the axons and Schwann cells and evidence of mitochondrial DNA reduction, and thus concluded that this mitochondrial DNA damage may be the reason for ATN. Nevertheless, Moyle and Sadler (1998) suggest that ATN development in HIV-positive patients on therapy may have a multi-factorial origin, and whether or not HIV-positive patients develop ATN, the severity of the neuropathy and how well the patient recovers when they stop the nucleoside analogue is dependent on pre-existing risk factors instead of the nucleoside analogue itself.

3.3 Stavudine

As discussed in the previous section, the three most frequent drugs associated with the occurrence of peripheral neuropathy include stavudine, didanosine and zalcitabine. However, the drug zalcitabine had a very severe toxicity profile and was thus quickly removed from treatment regimen guidelines. In addition, the drug didanosine is more suitable to second line regimens and is therefore more commonly used in second line therapy. As a result, stavudine became the most frequently used drug in first line therapy. Therefore, the rest of this sub-section will focus the NRTI stavudine.

Stavudine was the fourth NRTI developed for use in HIV disease and has been linked to the development of ATN in many studies (Simpson *et al.* 1995;Skowron 1995;Wester *et al.* 2005;Sacktor *et al.* 2009). Until recently, the backbone of first line

HAART in South Africa and most African countries (Akileswaran *et al.* 2005) consisted of this neurotoxic drug, which was administered under the previous antiretroviral treatment guidelines in South Africa (Guidelines 2004). The previous and current South African National Department of Health's ARV treatment guidelines are summarised in Table 3.

The link between stavudine and HIV-SN appears to be dose related (White 2001). Skowron and colleagues (1995) found that a stavudine dose of 0.5mg/kg/day was associated with an incidence of ATN of 21%, and when this dose was increased to 12mg/kg/day, the incidence of ATN increased to 73%. The most common symptoms of the neuropathy in these patients was bilateral numbness and pain in the lower legs and feet (Skowron 1995). Simpson and Tagliati (1995) and Gottlieb and colleagues (1995), reported that a dose of 20mg of stavudine daily in their cohorts was associated with an incidence of ATN of 15% and 17%, respectively. In addition, when this dose was increased to 40mg daily, Simpson and Tagliati (1995) found that the incidence increased to 21%, whilst Gottlieb and colleagues (1995) reported their incidence rate increased to 23%. Thus, both these studies further support the dose-dependant relationship of stavudine and the risk of ATN suggested by Skowron and colleagues (1995). The recommended and approved dosage for stavudine under the previous South African National Department of Health guidelines in 2004, was 40mg every 12 hours or 30mg every 12 hours if the patients weigh <60kg (Guidelines

2004). These guidelines were revised in 2006 by the WHO and the new guidelines stated that a dose of 30mg of stavudine twice daily was to be used for all adult and adolescent patients regardless of their body weight (WHO 2006). The dose-dependent relationship between stavudine and the development of ATN in the above-mentioned studies is summarised in Table 4.

Table 3. South African National Department of Health's previous and current ARV treatment guidelines.

Previous guidelines (2004-2009)		
Regimen	**Drugs**	**Eligibility**
1a	d4t/3TC/EFV	• All men and women on injectable contraception + condoms
1b	d4t/3TC/NVP	• Women who are unable to guarantee reliable contraception while on therapy
2	AZT/ddI/LPV/r	• Patients who continue to fail virologically despite demonstrated adherence
Current guidelines (2010)		
1	TDF + 3TC/FTC + EFV/NVP	• All new patients needing treatment, including pregnant women
	d4t/3TC/EFV/NVP	• Currently on d4t-based regimen with no side effects
	AZT + 3TC + EFV/NVP	• Contraindication to TDF-renal disease
2	TDF + 3TC/FTC + LPV/r	• Failing on a d4t-based or AZT-based 1st line regimen
	AZT + 3TC + LPV/r	• Failing on TDF-based 1st line regimen

d4t, stavudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; AZT, zidovudine; ddI, didanosine; LPV/r, lopinavir/ritonavir; TDF, tenofovir; FTC, emtricitabine/tenofovir.
Source: (Guidelines 2004;Guidelines 2010)

Table 4. The dose-dependant relationship between stavudine and the development of ATN.

Drug Dosage (stavudine)	Peripheral neuropathy (%)	Reference
0.5mg/kg/day	21%	Skowron 1995
12mg/kg/day	73%	
20mg twice daily	15%	Simpson *et al.* 1995
40mg twice daily	21%	
20mg twice daily	17%	Gottlieb *et al.* 1995
40mg twice daily	23%	

3.4 Prevalence and incidence of ATN

Summaries of the studies examining the prevalence and incidence of ATN are provided in Table 5 and Table 6, respectively.

3.4.1 Prevalence of ATN

With the exception of the studies by Sithinamsuwan and colleagues (2008) and Maschke and colleagues (2000), all of studies reviewed in Table 5 reported increasing age as a significant risk factor associated with the development of ATN. Whilst, age was not identified as a risk factor in the studies by Sithinamsuwan and colleagues (2008) and Maschke and colleagues (2000), both these studies identified low CD4 T-

Table 5. Prevalence of ATN

Reference	Prevalence	n	CD4 T-cell count (cells/mm^3)	Age (years)	Male (%)	Race (%)
Affandi *et al.* 2008	34%	96	34 [9-98][h]	32.0 (7.6)[g, m] 29.0 (6.7)[g, n]	97	Indonesian cohort
Cherry *et al.* 2009a	42%[i] 19%[j] 34%[k]	100[i] 98[j] 96[k]	417 [0-1447][i, h] 328 [7-1326][j, h] 281 [11-1014][k, h]	46.0 (26-75)[h, i] 42.0 (23-75)[h, j] 30.0 (17-56)[h, k]	90[i] 84[j] 86[k]	Caucasian: 85[i] Malay: 15[j], 65[k] Chinese: 69[j], 30[k] Indian: 11[j] Asian: 10[i]
Cherry *et al.* 2009b	49%[a] 55%[b]	76[a] 71[b]	317.0 (282)[a, g] 432.0 (244)[b, g]	44.9 (6.5)[g] 46.9 (10.3)[g]	74.0[a] 14[b]	Caucasian: 13[a], 97[b] Black: 86[a], 0[b] Hispanic: 1[a], 0[b] Asian: 0[a], 1[b]
Ellis *et al.* 2010	43%	1539	120.0 [30-246][h]	46.0 (7.8)[g, m] 40.0 (8.3)[g, n]	43	Non-Caucasian: 60 Caucasian: 40
Maschke *et al.* 2000	43% (1995-1996)[c] 34% (1997-1998)[d]	334[c] 401[d]	214.0 (212)[c, g] 335.0 (267)[d, g]	38.6 (9.5)[c, g] 40.7 (10.6)[d, g]	34[c] 29[d]	Not reported
Maritz *et al.* 2010	60%	216	348 [263-455][h]	35.0 (30-43)[h]	21	Not reported
Morgello *et al.* 2004	53%	99	228.0 (29)[g]	45.3 (0.7)[g]	84	Black: 44 Hispanic: 23 Caucasian: 32
Pettersen *et al.* 2006	29%	221	139.3 (20.2)[g]	47.2 (0.9)[g, m] 43.9 (0.8)[g, n]	92	Caucasian: 91

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Reference	Prevalence	n	CD4 T-cell count (cells/mm^3)	Age (years)	Male (%)	Race (%)
Smyth *et al.* 2007	44% (2001)[e] 42% (2006)[f]	140[e] 100[f]	464.0 [1-1700][e, h] 422.0 [5-1447][f, h]	39.9 (9.2)[e, n] 48.3 (9.2)[e, m] 43.7 (9.7)[f, n] 50.0 (9.8)[f, m]	95[e] 90[f]	Not reported
Sithinamsuwan *et al.* 2008	28%	100	132.0 [24-320][h]	36.0 (30-47)[h]	46	Thai cohort
Skopelitis *et al.* 2006	36%	100	322.5 [40-1271][h]	39.0 (22-73)[h]	86	Caucasians: 98 Black: 2
Wadley *et al.* 2011	57%	395	383 [27-1091][h]	39.0 (8)[g]	25	Black: 100
Watters *et al.* 2004	50%	70	122[l]	group 1, ≥50[o] group 2, 20-40[p] group 3, ≥50[q]	Not reported	Not reported

[a]results from cohort (Johns Hopkins University)
[b]results from cohort (Monash University)
[c]results for cohort studied in (1995-1996)
[d]results for cohort studied in (1997-1998)
[e]results for cohort studied in 2001
[f]results for cohort studied in 2006
[g]Mean (SD)
[h]Median [Q1-Q3]
[i]results for cohort (Melbourne, Australia)
[j]results for cohort (Kuala Lumpur, Malaysia)
[k]results for cohort (Jakarta, Indonesia)
[l]CD4 T-cell count-range not reported
[m]neuropathy group
[n] neuropathy-free group
[o]HIV-1 seropostive patients
[p]HIV-1 seropositve patients
[q]HIV-1 negative patients

cell count as a risk factor significantly associated with increased ATN. More recently, Ellis and colleagues (2010) also identified low CD4 T-cell count as a risk factor for ATN. Unique to their study, Maschke and colleagues (2000) reported longer disease progression as a risk factor for ATN. The results of all three of these studies indicate that current or historical disease severity contributes to the risk of developing ATN, thus supporting the idea that patients with underlying, sub-clinical, neuropathy caused by HIV-infection are at risk of ATN. As mentioned previously, Maschke and colleagues (2000) were the only investigators that showed that HAART may decrease the prevalence of ATN. But this finding conflicts with those of several other studies. These studies include, Ellis and colleagues (2010), Cherry and colleagues (2009a), Smyth and colleagues (2007) and Skopelitis and colleagues (2006). All these studies report high prevalence figures for ATN, ranging from 36% to 55%. Indeed, all the abovementioned studies also reported the use of stavudine and didanosine as a risk factor significantly associated with the development of ATN. Morgello and colleagues (2004) investigated the prevalence of HIV-SN in the era of HAART; however their study was dissimilar to most of the tabulated studies in terms of patient selection criteria as well as risk factors. Patients in their cohort had to present with the following in order to be entered into the study: a CD4 T-cell count of <50cells/mm^3 for at least three months, a condition indicative of advanced HIV disease and be at risk of near term mortality. The study found no significant association between long-established risk factors such as low CD4 T-cell count and ARV use and the

development of ATN. Instead, they reported male sex and a higher mean CD4 T-cell count for patients who developed ATN as a significant risk for ATN. The lack of correlation between the development of neuropathy and established risk factors for HIV-DSP such as low CD4 T-cell count and high viral load could be as a result of the introduction of HAART. The widespread use of HAART has seen a change in the usual pattern of risk factors commonly associated with neuropathy, as conditions not dependent on immunologic or virologic status, such as neurotoxic antiretroviral drugs, alcohol abuse and diabetes mellitus may be becoming more important in the development of ATN.

Although, the study by Cherry and colleagues (2009b) did not identify the use of stavudine as a significant risk factor for the development of ATN, they reported an increase in the presence and severity of symptoms in previously asymptomatic patients, who were exposed to stavudine. This finding corresponds to the study by Wadley and colleagues (2011), a more recent African study, where the prevalence of symptomatic ATN was 57%; even though patients in the cohort were exposed to a reduced dose of stavudine (e.g. 30mg twice daily, rather than 40mg twice daily). Cherry and colleagues (2009b) also identified increased height as a risk factor significantly associated with the development of ATN. Both, Affandi and colleagues (2008) and Wadley and colleagues (2011) also reported increased height in stavudine exposed individuals as a risk factor for the development of ATN. The findings of

these studies, which include a broad range of ethnic groups, indicate that screening for taller and older individuals and selecting these individuals for alternative regimens, or to be more closely monitoring for side effects is a worldwide approach to mitigating the impact of ATN. The studies by Maritz and colleagues (2010) and Wadley and colleagues (2011) reported the highest and second highest prevalence figures recorded for ATN, 60% and 57%, respectively. Both these studies were performed in African cohorts highlighting the fact that ATN is a common problem in African patients who are exposed to stavudine-based HAART.

In summary, from the above studies, it is evident that the most frequent risk factor associated with the prevalence of ATN is age. Furthermore, the presentation of less apparent risk factors such as height, higher mean CD4 T-cell count and male sex is now more evident in patients developing ATN and with this information investigators are now more aware of these risk factors and may examine them in their respective study populations. As mentioned previously, it is possible that the introduction of HAART has changed the usual pattern of risk factors associated with the development of neuropathy. In addition, the identification of stavudine as a risk factor associated with development of ATN further supports the WHO recommendations for the removal of stavudine in all first line regimens. This is especially important, since, until recently, stavudine formed the backbone of ARV treatment in South Africa and

both the South African studies (Maritz *et al.* 2010 and Wadley *et al.* 2011) reported the highest prevalence figures recordered for ATN.

3.4.2 Incidence of ATN

Sacktor and colleagues (2009) investigated the benefits and the risks of stavudine therapy for HIV-associated neurologic complications. They found a significant improvement in HIV-positive individuals with cognitive impairment after the initiation of stavudine-based therapy and their study also reported the largest incidence of ATN (38%). However, they were not able to identify any risk factors associated with the development of ATN in their study. This could be as a result of patients in the cohort being very ill and presenting with HIV-associated dementia. Two other studies were also not able to identify risk factors for the development of ATN in their studies, these include: Reliquet and colleagues (2001) and Jamisse and colleagues (2007). Reliquet and colleagues (2001) attributed the lack of risk factor identification in their study to their low sample size (n=59), whilst Jamisse and colleagues (2007) whose sample size consisted of a 100% female participants, placed more emphasis on the toxicity of the NNRTI (non-nucleoside reverse transcriptase inhibitor) nevirapine, which was the main aim of their study and therefore, did not investigate further into the toxicity of stavudine. Similar to the findings from most prevalence studies; Pettersen and colleagues (2006), Wester and colleagues (2005), Scarcella and colleagues (2002) and Moore and colleagues (2000) all identified the

use of stavudine and didanosine as risk factors for the development of ATN. In addition, Scarcella and colleagues (2002) reported an increase in the number of drugs used in a treatment regimen as an additional risk factor for the development of ATN, and Wester and colleagues (2005) reported the presence of a pre-existing neuropathy and the concurrent use of stavudine and didanosine as a risk factor for the development of severe ATN.

Table 6. Incidence of ATN

Reference	Incidence	Mean duration of follow-up (months)	n	CD4 T-cell count (cells/mm^3)	Male (%)	Race (%)
Bacellar *et al.* 1994	5%	84	2641	Not reported	100	Caucasian: 79 Hispanic: 6 Black: 14 Other: 1
Forna *et al.* 2007	36%	11	894	126.0 [0-343][c]	26	Not reported
Fichtenbaum *et al.* 1995	27%[e] 13%[f]	48	103	59.0 [2-295][c]	95	Caucasian: 82 Black: 15 Hispanic: 3
Jamisse *et al.* 2007	18%	10	146	231.0 [160-286][c]	100[d]	Not reported
Lichtenstein *et al.* 2005	13%	132	2515	162.0 (49.2)[b]	Not reported	Not reported
Moore *et al.* 2000	10%	10	1116	270.0 [0-1539][c]	72	Caucasian: 23 Black: 76 Other: 1
Pettersen *et al.* 2006	17%	44	221	140.0 (23.5)[b]	92	Caucasian: 91
Reliquet *et al.* 2001	12%	6	59	198.0[a]	68	Caucasian cohort
Robinson-Papp *et al.* 2009	34%	20	53	290.0 (280)[b]	67	Caucasian: 22 Black: 11 Hispanic: 67
Sacktor *et al.* 2009	38%	6	102	129.0 (79.0)[b]	28	Not reported
Scarsella *et al.* 2002	21%	24	86	Not reported	Not reported	Not reported

Continued on page 25

Reference	Incidence	Mean duration of follow-up (months)	n	CD4 T-cell count (cells/mm^3)	Male (%)	Race (%)
Wester *et al.* 2005	28%	24	153	96.0 [33-165][c]	41	Not reported

[a]CD4 T-cell count-range not reported
[b]Mean (SD)
[c]Median [Q1-Q3]
[d]cohort consisted of 100% female participants
[e]zalcitabine-induced toxic neuropathy
[f]didanosine-induced toxic neuropathy

Bacellar and colleagues (1994) was the only study to highlight the significant association between male sex and the use of stavudine and didanosine as a risk factor for the development of ATN. In addition to white race, Lichtenstein and colleagues (2005) also reported low CD4 T-cell count, increased viral load and diabetes mellitus as risk factors for the development of ATN and was the only study to identify these risk factors in their cohort. The only other study to report race as a risk factor was Robinson-Papp and colleagues (2009). They reported the Hispanic race as a risk factor for the development of ATN. Unique to their study, Robinson-Papp and colleagues (2009) identified intravenous drug use as a risk factor for the development of ATN. They were also the only study to report lower plasma viral loads in their group of participants that developed ATN in comparison to the group that did not develop ATN. Interestingly, both Robinson-Papp and colleagues (2009) and Bacellar and colleagues (1994), reported a significant association between higher mean CD4 T-cell counts and the development of ATN in their cohorts. Bacellar and colleagues (1994) reported two distinct groups that developed neuropathy; the first group had mean CD4 T-cell counts <100cells/mm^3 and the second group which developed neuropathy had mean CD4 T-cell counts >500cells/mm^3. The association between the first group and development of ATN is possibly due to the effect of immunosuppression and greater viral-mediated nerve damage prior to starting HAART. A study performed by Lichtenstein and colleagues (2008) reported that the initiation of HAART at CD4 T-cell counts ≥350 cells/mm^3 decreased the incidence of

ATN (Lichtenstein *et al.* 2008). This finding is in conflict with that of Bacellar and colleagues (1994) as they reported a yearly rate of increase in the incidence of ATN of 84% for individuals with mean CD4 T-cell counts >500cells/mm^3. Even so, Bacellar and colleagues (1994) reported the lowest incidence of ATN; 5% in their cohort of 2641 participants in comparison to Sacktor and colleagues (2009), an African study which reported the highest incidence of ATN; 38% in a cohort of 102 participants. This high incidence of ATN further highlights the fact that ATN is a common problem in African patients on HAART.

Fichtenbaum and colleagues (1995) report risk factors associated with the development of ATN that are not common to any of the other tabulated studies. They reported low serum cobalamin levels, heavy alcohol consumption and a history of symptoms of peripheral nerve dysfunction as risk factors for the development of ATN. The possible reason for the identification of these risk factors could be the use of the ARV zalcitabine in this particular study, whilst cohorts in the other tabulated studies were exposed mainly to therapy which included stavudine and didanosine. Zalcitabine, was one of the earlier antiretroviral drugs, and was discontinued because of its toxicity (Fischl *et al.* 1995;Nuesch *et al.* 2006).

Although the abovementioned studies all report on the incidence of ATN, only the studies carried out by Sacktor and colleagues (2009) and Forna and colleagues (2007)

may be applicable to the South African population, as they were carried out in Uganda; a sub-Saharan African country. However, even those studies may not be relevant to the South African population as, first line treatment regimens in Uganda at the time of the studies consisted of the coformulation drug known as Triommune (Sacktor *et al.* 2009) which consisted of fixed dosages of the drugs, stavudine, lamivudine and nevirapine. In South African first line regimens, fixed dosages for the abovementioned drugs are not used, instead these drugs are administered based on the patients initial weight, at the time of initiating HAART (Guidelines 2010). Furthermore, at the time of the present study, efavirenz was the preferred first line drug used together with stavudine and lamivudine in South Africa and is only replaced by nevirapine in women of child bearing age not on contraception (Guidelines 2004). Moreover, the HIV-1 subtypes found in South Africa and Uganda are different (Janssens *et al.* 1997). The HIV-1 subtypes A and D are more common in Uganda whilst the subtypes B and C are commonly found in South Africa (Janssens *et al.* 1997;Van Harmelen *et al.* 1997). According to the study by Janssens and colleagues (1997), there is still much that is not known about the biological characteristics of the different HIV-1 subtypes (Janssens *et al.* 1997) and the possible differences in the biological characteristics of the subtypes may have implications for the spread and virulence of HIV-1, (Janssens *et al.* 1997;Van Harmelen *et al.* 1997). If this is the case, then the findings from the studies based in Uganda may not be applicable to our study cohort and there still remains a dearth of incidence data for

ATN in a South African population. The lack of incidence data for patients starting stavudine-based regimens means that the impact of changing the treatment regimen to include less neurotoxic drugs than stavudine on incidence of ATN will be difficult to assess. Therefore, the primary aim of my study was to determine the incidence of ATN in HIV-positive individuals who were beginning stavudine-based HAART for the first time, and to identify risk factors for developing ATN in this population. To our knowledge, the present study is the first study aimed at examining the incidence of ATN in a South African population. Based on reports that most cases of ATN develop within the first 3 to 6-months of initiating therapy, we examined the incidence over a 6-month period only (Cherry *et al.* 2002;Keswani *et al.* 2002;Cherry *et al.* 2008). Also, we examined whether initiating ARV therapy improved the symptoms of a small sample of patients starting ARV therapy who had a pre-existing HIV-DSP.

Chapter 2

METHODS

1. Ethical Approval

Ethical approval for the study protocol was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, clearance number: M090671 (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. Patient recruitment

The study was carried out at the Greenhouse Pharmacy, Chris-Hani Baragwanath Hospital, Soweto, South Africa. Patients were referred to the Greenhouse Pharmacy from the Nthabiseng HIV Clinic or the Obstetric ARV Clinic of Chris-Hani Baragwanath Hospital to collect their antiretroviral medication. Patient recruitment commenced in November 2009 and ended in April 2010. The final patient exited the study in October 2010.

We recruited 65 patients to the study. Individuals eligible for participation in the study had to have had no previous exposure to any ARV drugs (ARV naïve), be 18 years of age or older, have had a confirmed diagnosis of HIV, be attending either the Nthabiseng HIV Clinic or the Obstetric ARV Clinic at Chris-Hani Baragwanath Hospital, and have met the South African National Department of Health's criteria for initiating antiretroviral therapy at the time of the study (Guidelines 2004). The guidelines for initiating HAART included the following

medical criteria: CD4 T-cell count <200 cells/mm^3, irrespective of WHO HIV disease stage, or WHO stage IV disease irrespective of CD4 T-cell count. Patients were classified as WHO stage IV disease if they presented with the following conditions: HIV-wasting syndrome, Kaposi's sarcoma, extrapulmonary tuberculosis and chronic isosporiasis (WHO 2007). Patients who met the above-mentioned Department of Health treatment initiation criteria, and who had successfully completed two weeks of ARV counselling at the Nthabiseng Clinic were referred to the Greenhouse Pharmacy to begin HAART, where they were recruited for participation in the study.

During the time of the study there were two first line ARV treatment regimens that were recommended for use in the South African public health sector (Guidelines 2004). First line therapy (regimen 1a) for ARV naïve patients consisted of the following drugs: stavudine (d4t), lamivudine (3TC) and efavirenz (EFV). Women of child-bearing age who were unable to guarantee reliable contraception were initiated on first line therapy (regimen 1b) where nevirapine (NVP) was substituted for EFV. Patients with no side effects on stavudine-based regimens remained on stavudine as long as it was well tolerated. Tenofovir (TDF) was substituted for stavudine, in those patients who developed a side effect (e.g. peripheral neuropathy, hepatic steatosis) to stavudine-based therapy. Those patients who continued to fail virologically despite demonstrated adherence to their treatment, or who presented with lactic acidosis were placed on second line

therapy (regimen 2) which consisted of zidovudine (AZT), didanosine (ddI) and lopinavir/ritonavir (LPV/r).

On the 1st of April 2010, the South African government adopted new guidelines for the clinical management of HIV/AIDS (Guidelines 2010). Under the new guidelines, the drug stavudine was replaced with the less neurotoxic drug tenofovir as first line therapy. Since the aim of our study was to determine the incidence of neuropathy in HIV-positive individuals initiating stavudine-based HAART regimens, we were no longer able to recruit participants to the study as treatment facilities such as the Greenhouse Pharmacy had begun substituting stavudine with tenofovir, thus forcing the closure of participant recruitment.

3. Procedures

3.1 First assessment

The first assessment took place immediately before initiation of ARV therapy. At the first assessment we measured the height and weight of all the patients. Thereafter, demographic data such as population group (Black, White, Coloured and Indian) and country of birth was recorded. The contact details of the patients were also recordered and included their personal cell phone number, fixed line (home) telephone number, and the contact number of a family member or friend that could be reached should their contact numbers be unavailable. From the patient's medical records we recorded the following clinical data on their disease history: the date the patient was first diagnosed with HIV, CD4 T-cell count when

first diagnosed with HIV, initial CD4 T-cell count when starting ARV therapy, presence of AIDS-defining illnesses when starting treatment, disease staging at the time of starting antiretroviral therapy (using WHO guidelines). In addition, we also recorded information from the patient's medical records on possible alternative causes of peripheral neuropathy, namely, history of alcoholism, TB infection and isoniazid therapy for TB, diabetes mellitus, syphilis infection, hepatitis B infection, hepatitis C infection and vitamin B_{12} deficiency. We also recorded any other medication (e.g. pain medication) that the patient was taking concurrently.

Thereafter, the patient was assessed for the presence of peripheral sensory neuropathy using the AIDS Clinical Trials Group Neuropathy (ACTG) Screening tool, also referred to as the Brief Peripheral Neuropathy Screen (BPNS), which is a validated screening tool for HIV-SN (Cherry *et al.* 2005). The ACTG screening tool was validated using both physiologic and pathologic testing as the reference standards (Cherry *et al.* 2005;Mehta *et al.* 2011). The ACTG screening tool consists of three sections, and assesses clinical signs and symptoms of peripheral sensory neuropathy. The first section assesses the patient's subjective experience of "pain, aching or burning", "pins and needles" and "numbness", in their left and right feet or legs. If a symptom was present, the patient was asked to rate the severity of the symptom on a numerical pain rating scale, ranging from one (mild) to ten (most severe).

The second section assessed the patient's vibration sense in their feet. In order to first familiarize the patients 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 patient's second (middle) finger. The patient 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 patient could comfortably identify this sensation, the patient was then informed that the procedure would be repeated by placing the 128Hz tuning fork on the distal interphalangeal joint of the great toe. To assess vibration sense in the feet, the 128 Hz tuning fork was struck against the palm of the investigators hand and then placed onto the distal interphalangeal joint of the patient's great toe, the patient 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 patient stopped feeling the vibration/buzzing of the tuning fork. Vibration sense was recorded in both feet, and a recording of ten seconds or less was deemed indicative of a deficit in vibration sense.

The third section of the screening tool assessed the deep tendon reflex of the ankle. With the patient seated on a chair, and their foot resting against, but not supported by, the palm of the investigator's hand, a patellar reflex hammer was firmly tapped against the patient's Achilles tendon; the reflex was felt by the

investigator as a plantar flexion of the foot. If no reflex was elicited, the patient was asked to clench their fists to reinforce the reflex, while the reflex was tested again. The reflex was tested in both legs, and recorded as either present or absent for both limbs. A diagnosis of the presence or absence of peripheral sensory neuropathy was made based on the bilateral presence of signs and symptoms from the assessment. The requirements for a positive diagnosis, which we used in the study were consistent with those of several validation studies for the ACTG screening tool (Cherry *et al.* 2005;Ellis *et al.* 2005;Simpson *et al.* 2006). In the study by Ellis and colleagues (2005) which performed a clinical evaluation of the neuroscreen (a neurocognitive screening component together with the ACTG screening tool), they found that the presence of atleast one sign (e.g. either decreased vibration sense or absent ankle reflexes in both feet) and the presence of one or more symptoms was sufficient to diagnose the presence of symptomatic peripheral neuropathy (Ellis *et al.* 2005). The study reported sensitivity and specificity values > than 55% for the ACTG tool, with a higher sensitivity value in comparison to its specificity. In addition, Simpson and colleagues (2006) reported that asymptomatic peripheral neuropathy may be diagnosed where there is the presence of both signs of neuropathy but no symptoms (pain, paraesthesia or numbness) of neuropathy (Simpson *et al.* 2006). Thus, patients who presented with at least one sign and one symptom were classified as having symptomatic peripheral sensory neuropathy. While patients who had at least one sign but no symptoms of peripheral sensory neuropathy were classified as having

asymptomatic peripheral sensory neuropathy. Each screening, together with the recording of clinical data from patient's files took about 20 minutes to complete.

3.2 Second and third assessment

The second and third assessments took place at 3-months and 6-months, respectively. Patients visited the Greenhouse Pharmacy every 3-months for a period of 6-months after starting treatment. On their second and third assessment visits, we re-screened the patients for the presence of neuropathy using the ACTG screening tool and we also recorded their weight. We then reviewed the patient's medical records to obtain the following clinical information: most recent CD4 T-cell count, any adjustments to their ARV treatment regimens, side-effects and adverse effects to ARV treatment, interim illnesses and concurrent medications being taken.

In addition to the three physical assessments, patients also were telephoned every two weeks in order to check on their well being, the development of new symptoms or changes to their previous symptoms, to confirm ARV adherence, to discuss any complications as a result of ARV treatment and to remind them of the date of their next scheduled visit the Greenhouse Pharmacy. In the case of patients who had been transferred to other facilities after a period of three months through the down referral system, requests for the abovementioned information from patient files at the various facilities were sent to the clinicians treating the patients.

Patients were also encouraged to contact the investigator should they experience any unusual symptoms or develop new symptoms whilst on the ARV medication.

4. Data analysis

Mean (SD) was used to describe continuous parametric data and median (interquartile range) was used to describe continuous non-parametric data. Categorical data was summarised by using number (%). For each variable, differences between those subjects that developed neuropathy and those that did not develop neuropathy, were assessed by the Students t-test for continuous parametric data, the Mann-Whitney U-test for continuous non-parametric data and by the χ^2 or Fishers exact test for dichotomous non-parametric variables. Using the Kruskal-Wallis test for non-parametric data, we assessed change in neuropathic symptom severity from baseline to three months and three months to six months in patients with pre-existing HIV-DSP. Statistical significance level was set at $P<0.05$.

Chapter 3

RESULTS

1. Patient flow and demographics

From November 2009 through to April 2010, 75 HIV-positive patients were recruited and 65 patients were enrolled into the study at the Greenhouse Pharmacy, Chris-Hani Baragwanath Hospital, Soweto. Ten patients were excluded because they did not initiate therapy on stavudine-based regimens. All 65 patients were HIV-positive, ARV naïve and started HAART on the day of recruitment for participation in the present study. All 65 patients were 18 years or older and identified themselves as Black Africans; 61 (94%) were South African, 2 (3%) were from Lesotho and 2 (3%) were from Mozambique. Fifty-two patients were free of peripheral neuropathy on initial assessment and 13 patients had pre-existing neuropathy. Of those patients free of neuropathy, 44 patients exited the study at 6-months and of those patients with pre-existing neuropathy, 8 patients exited the study at 6-months. Patient flow is summarised in Figure 1.

Demographic and baseline clinical characteristics for the study population are shown in Table 1. A significantly greater proportion of female patients were seen in the group free of neuropathy in comparison to those with pre-existing neuropathy. Despite having mean CD4 T-cell counts of <200cells/mm^3, 69% of the 'free of neuropathy' group and 61% of the 'pre-existing neuropathy' were at WHO disease stage I or II. Non-HIV-related neuropathy risk factors such as diabetes mellitus were not common in our cohort.

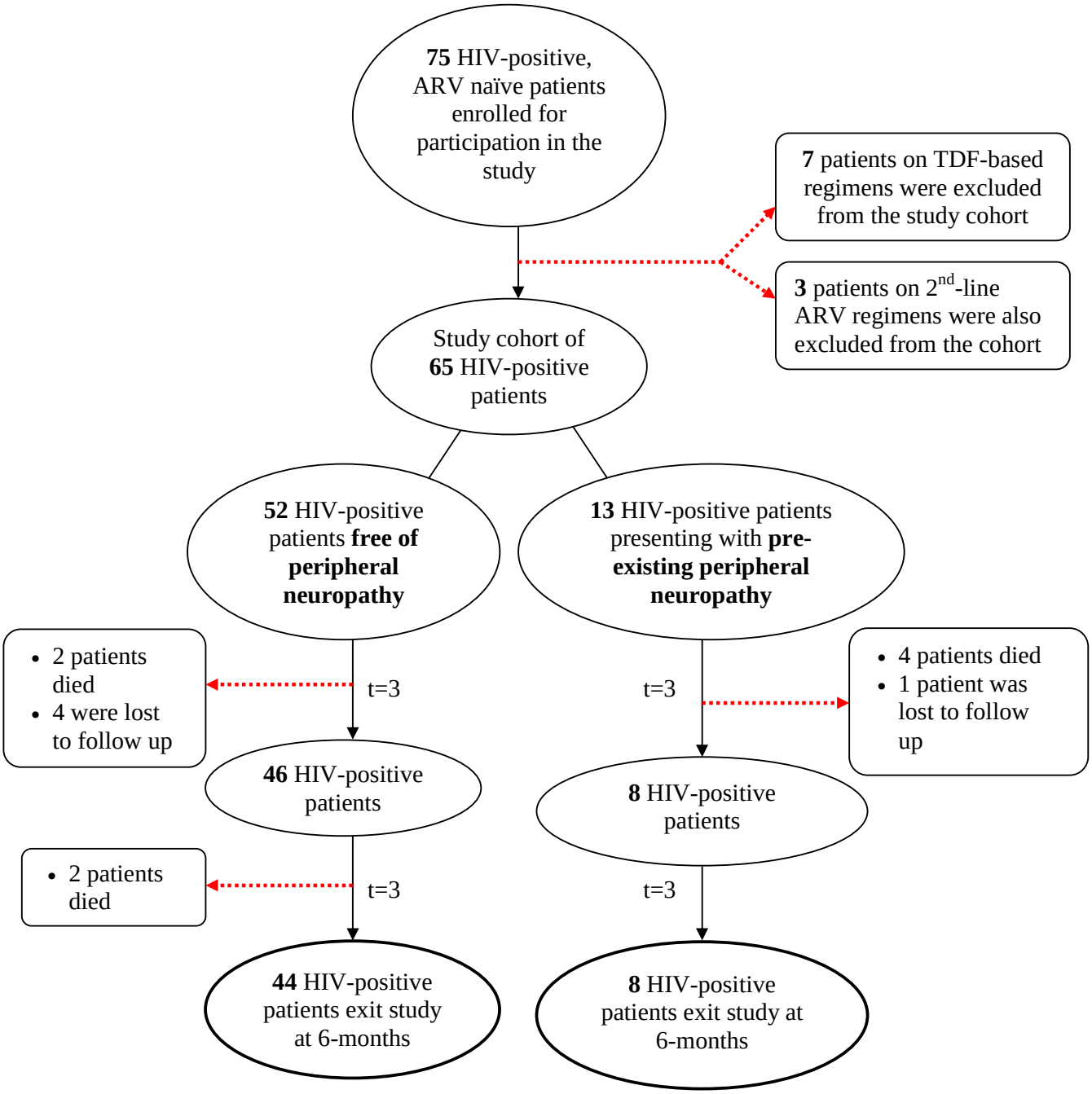

Figure 1. A flow diagram which provides an overview of the study cohort from the initial recruitment of 75 HIV-positive, ARV naïve patients, to the 3-month (t=3) assessment of 54 patients (46 patients without neuropathy and 8 patients with pre-existing neuropathy) and the 6-month (t=6) assessment of 52 patients (44 patients without neuropathy and 8 patients with pre-existing neuropathy).

Table 1. Demographic and baseline clinical characteristics of the study population (n=65 HIV-positive patients).

	Free of neuropathy (n=52)		Pre-existing neuropathy (n=13)		P-value
Female (n, %)	40	(77)	3	(23)	<0.001
Initial weight (kg), Mean (SD)	64.5	(15.7)	65.3	(13.5)	0.712
Age in years, median (range)	35.6	(18-57)	38.2	(22-57)	0.431
Height (cm), Mean (SD)	166.3	(7.0)	169	(8.3)	0.228
Baseline CD4 T-cell count (cells/mm^3), median (min-max)	154	(11-414)	157	(24-403)	0.844
Population group (n, %)					
Zulu	13	(25)	4	(31)	0.897
Swati	9	(17)	1	(8)	
Tswana	8	(15)	2	(13)	
South Sotho	6	(12)	2	(13)	
Other	16[a]	(31)	3[b]	(23)	
WHO staging (n, %)					
Stage I	15	(29)	2	(15)	0.307
Stage II	21	(40)	6	(46)	
Stage III	11	(21)	1	(8)	
Stage IV	5	(10)	4	(31)	
Current TB-infection (n, %)	9	(17)	2	(15)	1.000
Previous TB-infection (n, %)	7	(13)	3	(23)	0.405
Current Syphilis infection (n, %)	6	(12)	1	(8)	1.000
Vitamin B_{12} deficiency (n, %)	6	(12)	2	(15)	0.655
Hepatitis B-infection (n, %)	2	(4)	2	(15)	0.175
Hepatitis C-infection (n, %)	2	(4)	2	(15)	0.175
Outcome at 6-months (n, %)					
Alive	44	(84)	8	(61)	0.075
Dead	4	(8)	4	(31)	
Lost to follow up	4	(8)	1	(8)	

TB, tuberculosis.

[a]Other, n (%) Xhosa, 4 (7); Tsonga, 2 (4); Sotho, 2 (4); Shaangaan, 3 (5); Venda, 1 (2); North Sotho, 2 (4); Pedi, 1 (2); Ndawo, 1 (2).

[b]Other, n (%) Shaangaan, 2 (13); Xhosa, 1 (8); Pedi, 1 (8).

2. Neuropathy-free group

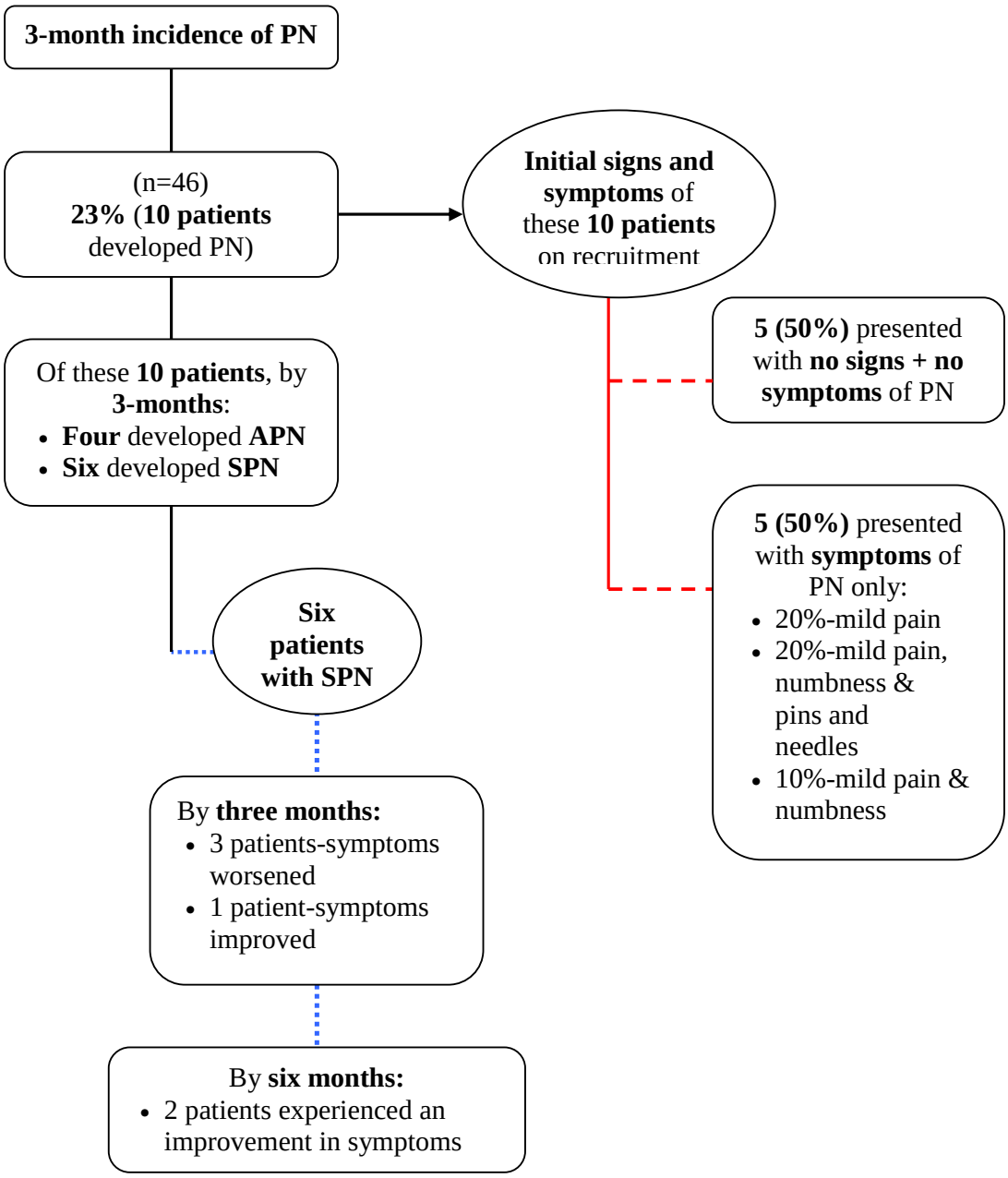

Figure 2. A flow diagram which describes the incidence of neuropathy and symptom development in patients who initiated stavudine-based HAART.

The six month cumulative incidence of neuropathy (n=44) was 41%, with 8 more HIV-positive patients developing peripheral neuropathy in the 3-month period between 3 and 6-months of therapy at a rate of 69.5 cases per 100 person-years. Of the 8 patients that developed neuropathy between 3 and 6-months, 4 (50%) patients presented with no signs and no symptoms of neuropathy on recruitment, whilst 4 (50%) patients presented with symptoms but no signs of neuropathy on recruitment. Of the 4 patients with symptoms on recruitment, 2 (25%) patients had a combination of mild pain, numbness and pins and needles, 1 (12.5%) patient had mild numbness and 1 (12.5%) patient had moderate pins and needles. Of these 8 patients, by 6-months, 3 of these patients developed asymptomatic peripheral neuropathy and 5 patients developed symptomatic peripheral neuropathy. For the 5 patients with symptomatic peripheral neuropathy, by 6-months, symptoms worsened in 4 patients and 1 patient experienced an improvement in symptoms.

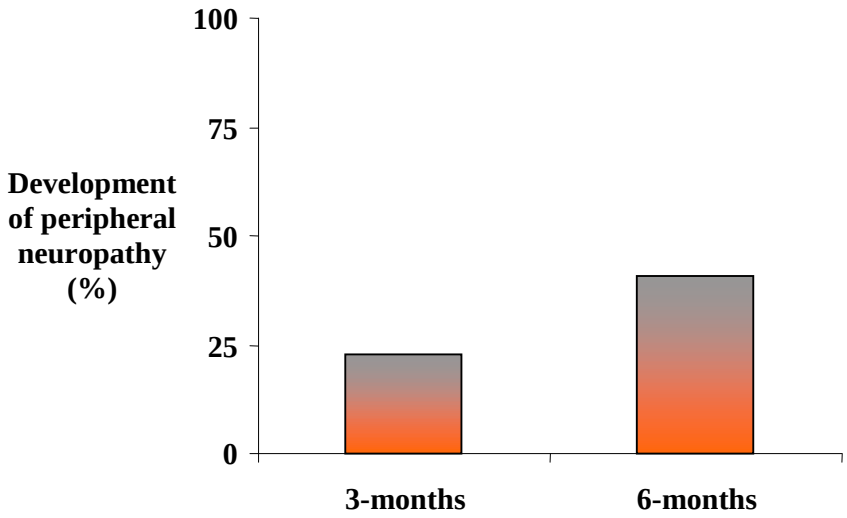

Figure 3. Cumulative percentage of HIV-positive patients who were neuropathy-free at the time of initiating stavudine-based therapy, who developed a neuropathy, 3-months and 6-months after initiating therapy.

As shown in Table 2, in univariate analysis for possible risk factors associated with the development of neuropathy at 3-months, none of the risk factors considered were significantly associated with peripheral neuropathy development. The risk factors considered were: patient age, height, current and previous exposure to isoniazid therapy for TB treatment, disease staging and vitamin B_{12} deficiency. Since, CD4 T-cell counts were not available for all patients at 3-months of follow up; it was not assessed as a possible risk factor at 3-months. However, we assessed the baseline CD4 T-cell counts of those patients who developed neuropathy and those who remained neuropathy-free and found no significant difference between the two groups.

The characteristics of the study population, at 6-months of follow up are shown in Table 3. Cumulatively, 18 (41%) patients developed peripheral neuropathy over the 6-month period of therapy. In univariate analysis for possible risk factors associated with the development of peripheral neuropathy at 6-months of follow up, the only risk factor significantly associated with the development of peripheral neuropathy was disease staging ($p=0.04$), with patients developing neuropathy being at a more advanced disease stage on average than those who had not developed neuropathy. The CD4 T-cell counts, which were available at 6-months of follow up, were not significantly different between the two groups.

Table 2. Characteristics of HIV-positive patients who were initiated onto stavudine-based therapy and either developed peripheral neuropathy or remained neuropathy free at 3-months of follow-up (n=46).

	Neuropathy free (n=36)	**Neuropathy (n=10)**	**Test statistic; P-value**
Female (n, %)	27 (75)	8 (80)	Fishers exact; P=1.00
Weight (kg), Mean (SD)	63.6 (13.0)	69.7 (21.8)	Mann Whitney; U=172.00; P=0.84
Age in years, Median (range)	36.1 (18-54)	34.9 (27-45)	Mann Whitney; U=165.5; P=0.70
Height (cm), Mean (SD)	165.9 (6.8)	166.1 (8.5)	Unpaired t-test; t=0.06; P=0.94
CD4 T-cell count (cells/mm^3), (baseline) Median (min-max)	152.9 (11-414)	160.3 (68-414)	Mann Whitney: U=176.00; P=0.92
CD4 T-cell count (cells/mm^3), Median (min-max)	na[a]	na[a]	
Current isoniazid exposure (n, %)	5 (14)	3 (30)	Fishers exact; P=0.34
Previous isoniazid exposure (n, %)	6 (17)	0 (0)	Fishers exact; P=0.31
WHO disease staging (n, %)			
I	13 (36)	1 (10)	χ^2;P=0.19
II	13 (36)	6 (60)	
III	6 (17)	3 (30)	
IV	4 (11)	0 (0)	
Vitamin B_{12} deficiency (n, %)	4 (11)	2 (20)	Fishers exact; P=0.59

na[a], not available at 3-months of ARV therapy

Table 3. Characteristics of HIV-positive patients, who were initiated onto stavudine-based therapy and either developed peripheral neuropathy or remained neuropathy free at 6-months of follow-up (n=44).

	Neuropathy free (n=26)	**Neuropathy (n=18)**	**Test statistic; P-value**
Female (n, %)	14 (54)	14 (78)	Fishers exact; P=0.12
Weight (kg), Mean (SD)	63.2 (13.9)	69.8 (17.3)	Mann Whitney; U=186.5; P=0.26
Age in years, Median (range)	34.9 (18-55)	37.9 (28-50)	Mann Whitney; U=178.5; P=0.19
Height (cm), Mean (SD)	165.3 (6.6)	166.8 (8.2)	Unpaired t-test; t=0.66; P=0.50
CD4 T-cell count (cells/mm^3), Median (min-max)	191.7 (34-415)	227.7 (62-607)	Mann Whitney; U=206.5; P=0.51
Current isoniazid exposure (n, %)	4 (15)	4 (22)	Fishers exact; P=0.70
Previous isoniazid exposure (n, %)	3 (12)	2 (11)	Fishers exact; P=1.00
WHO disease staging (n, %)			
I	11 (42)	3 (17)	χ^2;P=0.04
II	7 (27)	10 (55)	
III	4 (15)	5 (28)	
IV	4 (15)	0 (0)	
Vitamin B_{12} deficiency (n, %)	3 (12)	3 (17)	Fishers exact; P=0.67

2.1 Symptoms

Symptoms reported by patients who were initially free of neuropathy prior to initiating stavudine-based therapy and developed a symptomatic neuropathy within 6-months of therapy is shown in Figure 3. Of the 18 (41%) patients that developed peripheral neuropathy, 7 (38%) were asymptomatic; presenting with decreased vibration sense in the great toe and absent ankle reflexes only. Pain was the symptom experienced by all patients who had developed symptomatic peripheral neuropathy. In addition, there was significant difference in the severity of the symptom pain across the six months of follow-up, with an increase in pain severity from baseline Median (min-max), 1.8 (0-5) to 4.0 (5-10), $p<0.01$. Furthermore, nine out of the 11 patients that developed painful symptomatic peripheral neuropathy experienced moderate to severe pain. Five (45%) patients developed pins and needles in their feet which were often accompanied by cramps. In addition, for the 26 (59%) patients who remained classified as neuropathy free within the 6-month period, 9 (35%) developed symptoms of neuropathy but did not present with any signs of neuropathy. Three (12%) of these patients had pain in the feet, 2 (8%) had pain together with pins and needles, 1 (4%) patient had pins and needles only and 1 (4%) patient had numbness only. Two (8%) patients also presented with a combination of pain, pins and needles and numbness, and pain and numbness, respectively.

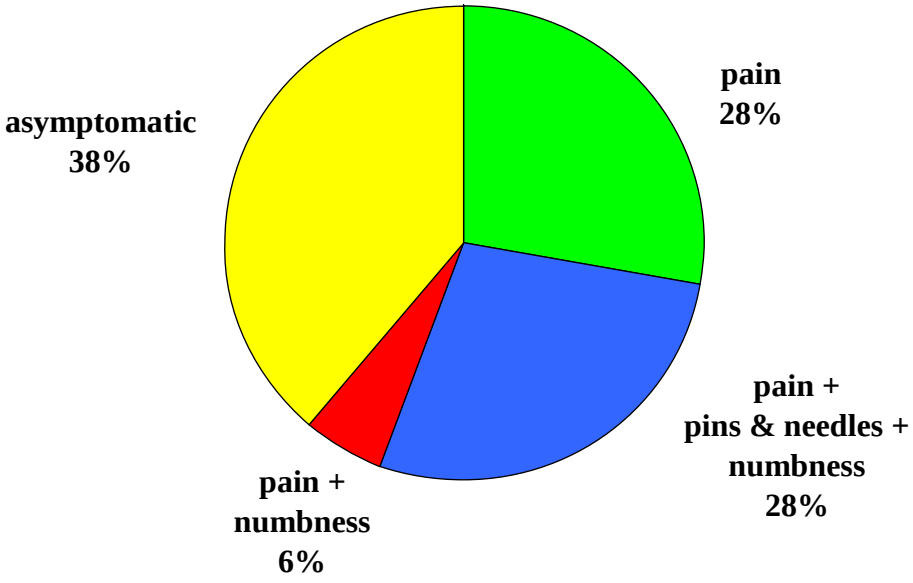

Figure 4. Symptoms reported by patients who developed peripheral sensory neuropathy within 6-months of initiating stavudine-based therapy.

2.2 Treatment

Six (54%) of the 11 patients that developed symptomatic peripheral neuropathy were receiving treatment for symptom relief, whilst 5 (46%) received no analgesic treatment for the symptom pain. As illustrated in Figure 4, two (18%) patients received amitriptyline only, whilst 4 (36%) patients received combinations of the various medications which included; amitriptyline, paracetamol, ibuprofen and codeine phosphate, all used to treat the neuropathic symptom pain. In those patients who had no signs of neuropathy and who were therefore classified as neuropathy free,

but who did develop symptoms, 7 (78%) developed pain in their feet, but only 3 (43%) received treatment for pain in their feet. Two (29%) of these patients received amitriptyline together with codeine phosphate and 1 (14%) patient received amitriptyline and paracetamol, despite no evidence for this being neuropathic pain, other than the site of the pain.

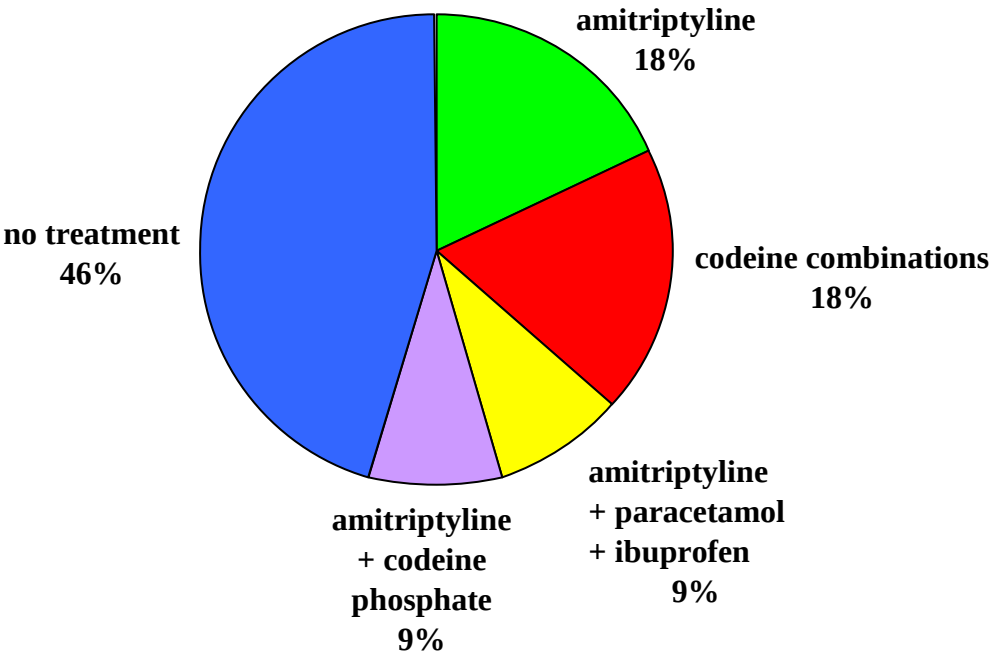

Figure 5. Treatment for the neuropathic pain in patients who developed a symptomatic neuropathy within 6-months of initiating stavudine-based HAART.

2.3 Drug regimen switches

The ARV drug regimen switches for six HIV-positive patients initiated onto stavudine-based HAART are shown in Table 4. From the 6 patients whose drug regimen was switched; patients 42, 65 and 59 were switched to treatment regimens that replaced stavudine with tenofovir. Amongst the above-mentioned patients, pain was a symptom experienced by all three of these patients, whilst the presence of cramps in the feet occurred in two of these patients. Stavudine was also replaced by tenofovir in patient 52, who experienced pain, pins and needles and severe numbness in the feet; this patient was on a treatment regimen that included nevirapine instead of efavirenz. Patients 7 and 13 had efavirenz replaced by lopinavir/ritonavir after falling pregnant and did not present with any severe or long-lasting symptoms. The ARV drug regimens of the patients were often switched after a period of 3-months or longer as CD4 T-cell counts and viral loads were carefully monitored during this period before a regimen was changed.

Table 4. Antiretroviral drug regimen changes in HIV-positive patients who developed symptomatic peripheral neuropathy and were switched within 6-months of treatment to alternate regimens mainly due to the symptom pain.

Patient no.	Initial ARV regimen	Regimen change (new regimen)	Time to regimen change	Reason for change
42	d4t/3TC/EFV	TDF/3TC/EFV	± 4 months	Severe pain and cramps in feet and hands
52	d4t/3TC/NVP	TDF/3TC/NVP	5 months	Pain, pins and needles and severe numbness in feet
65	d4t/3TC/EFV	TDF/3TC/EFV	3 months	Pain and burning in the feet and development of severe cramps
59	d4t/3TC/EFV	TDF/3TC/EFV	5 months	Admitted to hospital due to convulsions as a result of hypocalcaemia and also experienced pain in the feet.
7	d4t/3TC/EFV	d4t/3TC/LPV/r	± 4 months	Fell pregnant whilst on ARVs
13	d4t/3TC/EFV	d4t/3TC/LPV/r	5 months	Fell pregnant whilst on ARVs

d4t, stavudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir; LPV/r, lopinavir/ritonavir.

3. Pre-existing neuropathy

3.1 Symptoms

Figure 5 illustrates the symptoms experienced by those patients who had pre-existing neuropathy at baseline. The most common symptom experienced by those patients with pre-existing neuropathy at baseline was numbness (n=6; 75%). One patient only experienced numbness, whilst 5 (63%) patients had either a combination of pins and needles and numbness (n=2; 25%) or a combination of numbness, pain and pins and needles (n=3; 37%). Pain was experienced by 62% of the patients, of which 25% experienced pain as their only symptom.

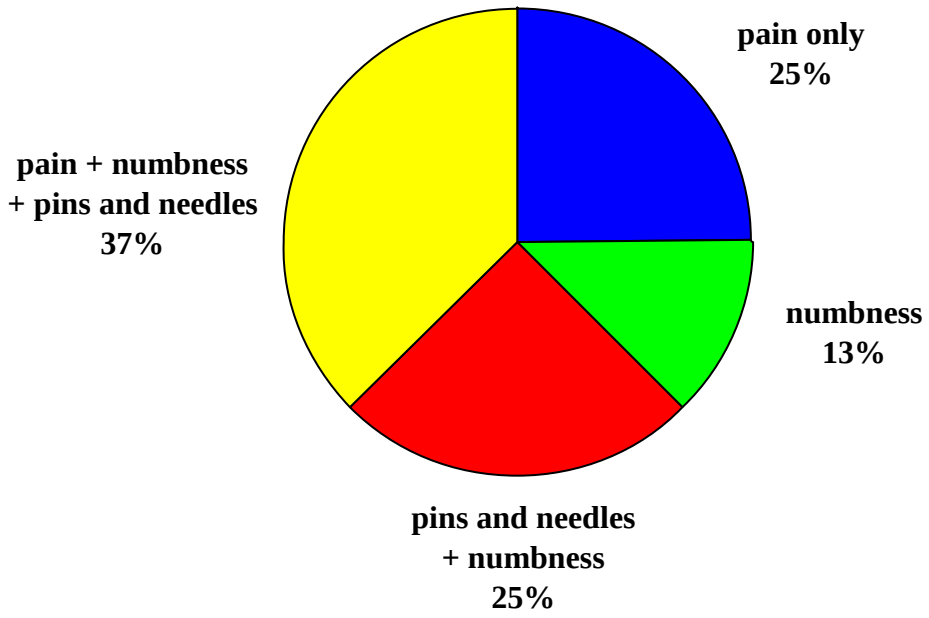

Figure 6. Symptoms reported by patients who had a pre-existing peripheral neuropathy at baseline and were initiated onto stavudine-based HAART.

3.2 Change in symptoms

Figure 6 illustrates the change in symptoms within 6-months of stavudine-based therapy, in those patients with pre-existing neuropathy. By 3-months of follow up, 2 (25%) more patients had developed a combination of all three symptoms, the presence of pins and needles together with numbness in 2 (25%) patients still persisted as well as numbness in 1 (13%) patient. However, from the 2 (25%) patients who had pain only at baseline, both developed pins and needles as well as numbness. By 6-months, a few patients experienced reduced symptoms; 1 patient initially presenting with numbness was free of this symptom at 6-months. From the 2 patients who had pins and needles together with numbness at 3-months; 1 patient was free of these symptoms at 6-months, whilst the symptoms persisted in the other patient at 6-months. For the 5 (63%) patients who had a combination of all three symptoms; 2 (25%) patients experienced symptom reduction at 6-months whilst 2 (25%) patients still had the presence of pain and pins and needles and 1 patient still had pain and numbness in the feet at 6-months.

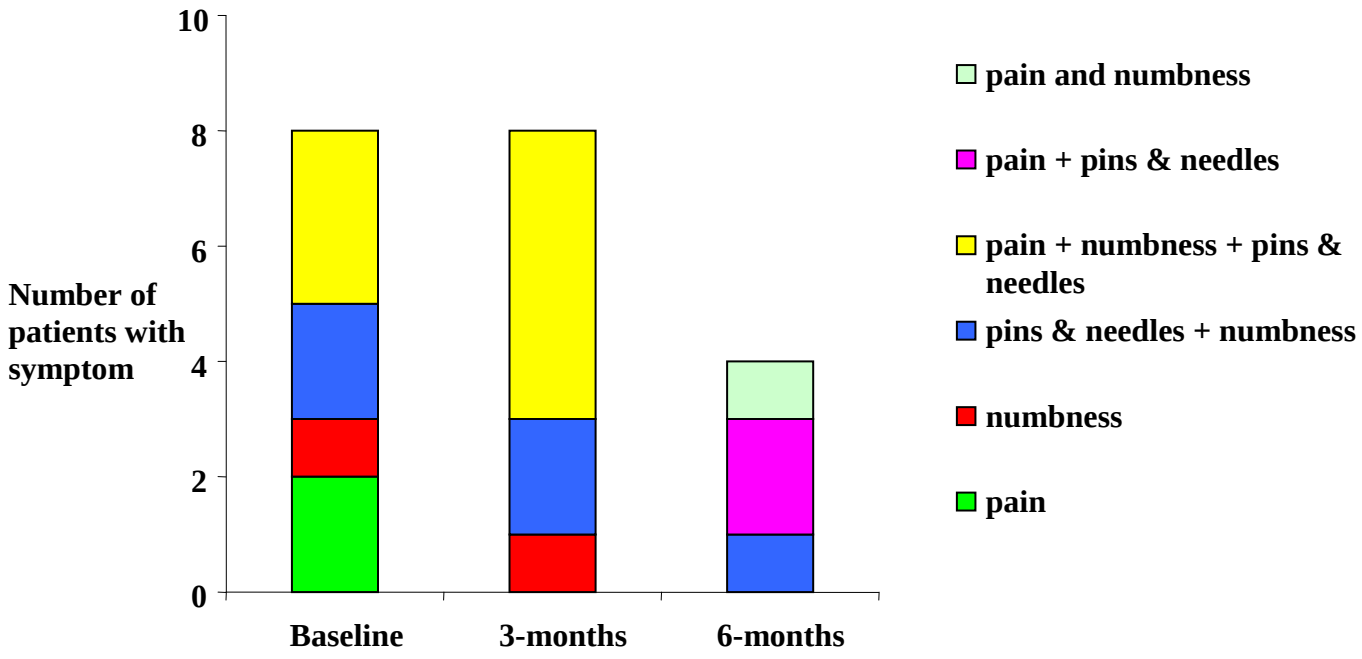

Figure 7. Symptoms reported by patients with pre-existing neuropathy who either developed new symptoms or experienced a reduction in previous symptoms after 3-months and 6-months of stavudine-based HAART, (n=8).

The severity of the symptoms experienced by those patients who presented with pre-existing neuropathy and initiated stavudine-based therapy is shown in Table 5. Three (38%) patients experienced a decrease in the severity of the symptoms pain and pins and needles; from an average rating of 5 (min-max; 2-8) to 2 (min-max; 3-4) and 6 (min-max; 2-10) to 1 (3), respectively. However, there was no significant difference in severity of these symptoms overall between baseline and 6-months. However, the severity of the numbness patients reported did decrease significantly over the course

of the 6-months, but this attenuation was not linear; the symptom initially worsened, on average, in patients over the first 3-months and then subsided by 6-months.

Table 5. Neuropathic symptom severity over 6-months in patients who had pre-existing peripheral neuropathy and initiated stavudine-based HAART (n=8).

Symptom	Baseline median (min-max)	3-months median (min-max)	6-months median (min-max)	Test statistic; P-value
Pain	5 (1-10)	4 (2-10)	2 (3-10)	Kruskal-Wallis; KW=1.9; P=0.38
Numbness	5 (2-10)	8 (3-10)	1 (2-3)	Kruskal-Wallis; KW=10.2; P=0.006*
Pins and needles	4 (1-10)	4 (2-10)	2 (1-10)	Kruskal-Wallis; KW=1.4; P=0.49

*Significant

3.3 Treatment

As illustrated in Figure 7, only 1 patient who presented with painful HIV-DSP was not receiving medication for symptom relief. The most common analgesic administered was amitriptyline; either by itself or in combination with codeine phosphate or codeine phosphate as well as ibuprofen. Paracetamol and diclofenac were not commonly administered to patients and only 1 patient received this combination.

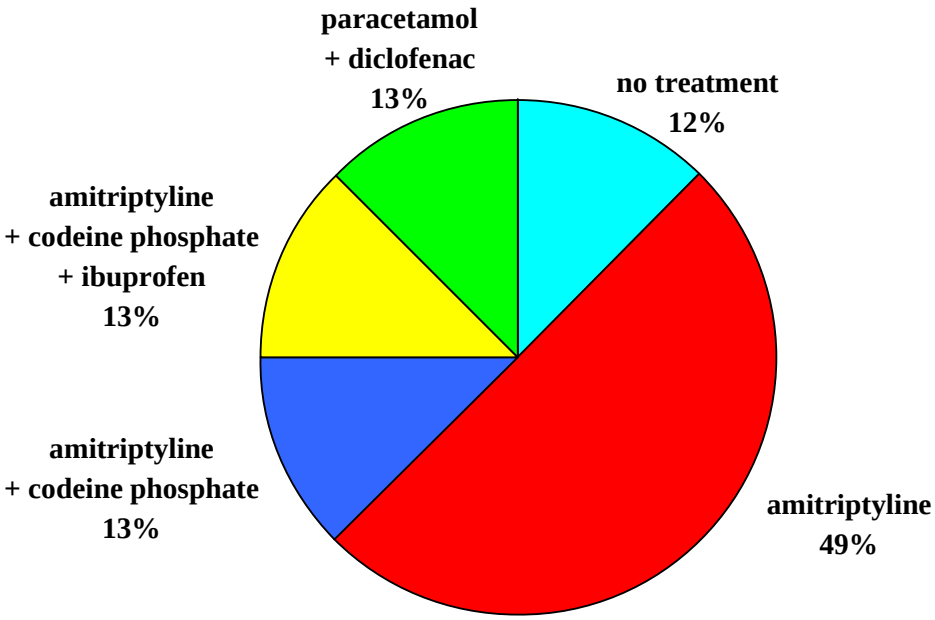

Figure 8. Treatment of neuropathic pain in patients who had pre-existing peripheral neuropathy at baseline, (n=8).

Chapter 4

DISCUSSION

We investigated the 6-month incidence of neuropathy in patients on stavudine-based HAART, and described symptoms experienced by those patients who developed symptomatic peripheral neuropathy. We found that in HIV-positive South Africans who were free of peripheral neuropathy prior to the initiation of stavudine-based HAART, the 6-month incidence of ATN was 41%. Of these, 16% were asymptomatic, whilst 25% developed symptomatic peripheral neuropathy. The high rate of neuropathy in individuals in the first 6-months after starting stavudine-based therapy emphasises the possible importance of the recent introduction of less neurotoxic, tenofovir-based regimens, in reducing this iatrogenic neurological complication. Moreover, the results highlight the undertreament of the symptoms of ATN, yet not HIV-DSP. It has been established through prevalence studies that symptomatic peripheral neuropathy is a common problem in South African patients (Maritz *et al.* 2010;Wadley *et al.* 2011). Our finding of an incidence of ATN of 41% emphasises the rapid onset of peripheral neuropathy in the South African population exposed to stavudine-based therapy. In addition, we also found that there was a significant difference between the group of patients who were initially free of neuropathy and the group with pre-existing HIV-DSP in terms of gender. This finding highlights the possibility that males are possibly more likely to present with pre-existing HIV-DSP in comparison to females and is supported by another African study (Hitchcock *et al.* 2008) which also found male sex a risk factor associated with the prevalence of HIV-DSP. However, upon comparison of other frequent risk factors

between the two groups, namely, age, height, CD4 T-cell count, current and previous TB-infection, Vit B_{12} deficiency and the presence of Hepatitis B or C, none of these risk factors showed any significant associations.

Importantly, all those patients who developed symptomatic peripheral neuropathy within 6-months, had pain as a symptom. Also, there was a significant difference in pain severity across the six months of follow-up, with an increase in the severity of pain by six months of follow up, and 82% (9 of 11) of these patients experiencing moderate to severe pain.

Pain affects the quality of life in patients with neuropathy (Cocito *et al.* 2006), making it difficult for many of these individuals to perform activities of daily living (e. g walking to their place of work or attending a clinic visit). Yet, only 55% (6 of 11) patients who developed ATN were receiving analgesic treatment for symptom relief. This indicates that the presence of pain is frequently underestimated or under-diagnosed, which leads to the undertreament of this symptom. Interestingly, most patients with painful HIV-DSP were receiving analgesic therapy. There is no clear rationale to explain the difference in analgesic therapy between the two groups of patients, those with HIV-DSP and those with ATN. Overall, it is important that patients with painful HIV-SN are carefully monitored for the detection of this symptom and timely treatment to ensure the suffering of these patients is reduced.

Indeed, pain was an important factor determining drug regimen switches from stavudine-based therapy to tenofovir-based regimens, with a switch taking place in 36% (4 of 11) of patients with symptomatic painful neuropathy. Early switching may affect long-term management of HIV-infection, and successful symptom treatment may delay drug switching.

Our study reports the highest incidence of ATN, 41%, in comparison to the findings of the studies evaluated in Table 6 (pg 24), where the lowest incidence was reported by Bacellar and colleagues (1994), 5% and the highest incidence by Sacktor and colleagues (2009), 38%. However, our sample size (n=52) was the lowest from all these studies and it is possible that this may have been the origin for the higher incidence of ATN observed on our study. Indeed, a pattern of a high incidence of ATN amongst studies performed in Africa in noticeable and places emphasis on the revision of guidelines (WHO 2009) which suggest stavudine be removed from first line therapy and in resource limited settings where the elimination of stavudine in first line is not yet possible, patients at a higher risk for the development of ATN, should be pre-selected for alternate less neurotoxic regimens.

In the univariate analysis for possible risk factors associated with the development of ATN at three and six months, we found no significant associations between ATN development and the following risk factors: weight, height, gender, age, baseline

CD4 T-cell count, CD4 T-cell count at six months, previous and current exposure to isoniazid therapy and Vit B_{12} deficiency. However, we found that disease staging was the only risk factor associated with the development of neuropathy in our cohort. Though, at baseline, there was no significant difference in disease staging between the two groups (i.e. those that developed neuropathy vs. those without neuropathy). The finding that disease stage of those individuals who developed neuropathy within 6-months had progressed to a more advanced stage compared to patients without neuropathy is thus difficult to explain, especially in the face of similar CD4 T-cell counts. Possibly patients who develop ATN have other co-morbidities which increases the risk of neuropathy and disease progression, but were not assessed by us. Still, this finding is important as it shows that patients, who progress to intermediate and late disease stages, for whatever reason, may be at a higher risk for the development of peripheral neuropathy when commencing stavudine-based therapy. Intermediate to late disease staging as a risk factor for the development of HIV-DSP has been well documented in many studies (Cornblath *et al.* 1988;de la Monte *et al.* 1988;Husstedt *et al.* 1993;Bacellar *et al.* 1994;Maschke *et al.* 2000). However, the finding of progression to intermediate and late disease staging as a risk factor associated with the development of ATN in our cohort may be unique to our study. This finding conflicts with those of two other studies which both reported a reduction in disease progression in HIV-positive patients on HAART (Brodt *et al.* 1997;Palella *et al.* 1998). However, for both these studies, HIV-positive patients were stratified

into 3 groups; each group received different types of ARV therapy; ranging from monotherapy with zidovudine, two drug therapy with reverse transcriptase inhibitors, and any combination therapy which included one protease inhibitor. Our cohort was universally exposed to stavudine-based HAART, and both these studies did not identify the exact NRTI that their cohort was exposed to, which makes it difficult to ascertain whether their cohort was indeed exposed to stavudine-based HAART and therefore does not provide a base for comparison between our studies. Indeed, the introduction of HAART has lead to a reduction in both opportunistic infections and HIV-related neurological conditions, such as HIV-associated dementia (Sacktor *et al.* 2009) and has many benefits for patients such as reducing plasma viral loads and slowing down disease progression (Brodt *et al.* 1997;Sacktor 2002). Another possibility for the increased disease progression observed in patients that developed neuropathy in our cohort could be failure of adherence to HAART. Since moderate to severe pain was a symptom experienced by many of the patients on stavudine-based therapy that developed peripheral neuropathy, the possibility of these patients not adhering completely to HAART as a result of the occurrence of these neuropathic symptoms does exist. This possibility is supported by several studies (Holzemer *et al.* 1999;Murri *et al.* 2000;Ammassari *et al.* 2001;Duran *et al.* 2001). These studies all found that in patients who reported the presence of neuropathic symptoms and had a greater intensity of these symptoms; these patients all displayed a much poorer adherence to HAART regimens. The effectiveness of HAART regimens does depend

on strict adherence (Stone 2001). Two studies have shown that where there is a reduced adherence to HAART regimens, this leads to increased disease progression (Hogg *et al.* 2000;Bangsberg *et al.* 2001). However, this remains a postulation as there are two limitations with regards to patient HAART adherence. The first is that we had to rely on patients themselves confirming adherence and had no method of ascertaining whether they were indeed adhering to the regimen, and secondly, the test for viral load is expensive and as a result was only performed on a regular basis in those patients who were very ill. We were however able to record CD4 T-cell counts for the patients at 6-months of therapy and when comparing the CD4 T-cell counts of the whole group of patients at baseline to the CD4 T-cell counts at 6-months of therapy, we found that there was a significant difference ($p=0.03$). Therefore, this increase indicates that patients were possibly adhering well to the treatment and treatment for these patients was indeed working. Hence, since treatment was working well for both groups of patients, the reason for certain patients in our cohort developing neuropathy whilst others remained neuropathy free is still unclear.

Pain was common in our cohort on stavudine-based therapy who developed symptomatic ATN. This finding is consistent with those of several other studies (Forna *et al.* 2007;Sithinamsuwan *et al.* 2008;Wadley *et al.* 2011). Indeed, pain in the feet is a common problem experienced by African patients on stavudine-based HAART (Forna *et al.* 2007;Wadley *et al.* 2011). In an effort to reduce rates of

toxicity, in 2006, WHO guidelines for stavudine-based treatment were changed from 40mg to twice daily 30mg doses of stavudine for all HIV-positive patients initiating HAART, irrespective of their weight (WHO 2006). However, in our cohort, where patients were universally exposed to twice daily 30mg doses of stavudine, a high incidence of peripheral neuropathy still persisted. Recent WHO guidelines (WHO 2009) indicate that stavudine should be phased out of first line regimens and replaced with the more tolerable, from a neurological perspective, tenofovir. We anticipate that this switch in therapy may reduce the incidence of ATN, however further investigation is needed to determine whether this does occur because several studies have now shown that even when stavudine no longer is used, neuropathy rates remain high (Smyth *et al.* 2007;Ellis *et al.* 2010).

We also monitored a small sub-group of patients with pre-existing peripheral neuropathy prior to initiating stavudine-based HAART and described their symptom experience and symptom treatment after starting stavudine-based therapy. For this sub-group of patients, numbness 75% (6 of 8) was the most common symptom reported at baseline and was the only symptom to reduce in frequency across the 6-months and thus accounted for significant reduction in symptom burden in this sub-group of patients. Numbness of the feet in patients with HIV-DSP is well documented (So *et al.* 1988;Cornblath *et al.* 2006;Ferrari *et al.* 2006). In addition, 38% (3 of 8) of these patients had all three symptoms of neuropathy at baseline which increased to

63% (5 of 8) by three months, but had fallen to 50% (4 of 8) by 6-months. These findings indicate that even on neurotoxic regimens, patients with numbness do have a reduction in the symptom, as previously reported in the study performed by Sacktor and colleagues (2009). These findings also show that the initiation of stavudine-based HAART in patients with pre-existing neuropathy may lead to an initial increase in the presence and severity of other symptoms, but that a few of these patients do experience symptom reduction and relief by 6-months of therapy. What separates these patients is unclear; it may possibly be that some patients are at a more advanced level of immunosuppression in comparison to others; however, further investigation is needed. These findings also emphasise that proper monitoring of patients with pre-existing neuropathy is vital as many of these patients already present with a variety of symptoms and exposure to stavudine-based HAART may either complicate pre-existing symptoms or provide symptom relief in others (Sacktor *et al.* 2009). Of the patients with symptoms, 88% (7 of 8) were receiving analgesic treatments for the presence of symptomatic neuropathy and the most common analgesic administered was amitriptyline; a tricyclic antidepressant commonly used in the treatment of neuropathic pain (Dallocchio *et al.* 2000). Although amitriptyline has not been shown to be effective in treating HIV-SN (Kieburtz *et al.* 1998), there was a reduction in symptom burden by 6-months after it peaked at 3-months and amitriptyline was often prescribed at the patient's second visit. However, this treatment is not without its own dose limiting side effects which include: dizziness, drowsiness and dry mouth.

(Dallocchio *et al.* 2000). Thus, patient and doctor communication is key in ensuring that the patient not only receives the most appropriate treatment but is also aware of the risks associated with the treatment.

A significant strength of our study is that it was a longitudinal study which allowed us to collect data prospectively and monitor for incident cases of ATN. Also, by conducting similarly designed studies on patients put on tenofovir-based regimens, we can make direct calculations of relative risk of tenofovir and stavudine-based therapies. There are however, a few limitations to this study. The most evident being the moderate sample size. As a result of the change in the South African National Department of Health's treatment guidelines for ARV therapy on the 1st of April 2010, which substituted the more tolerable tenofovir for stavudine in all first line regimens (Guidelines 2010), the recruitment of new patients to our study was closed as we were investigating the incidence of neuropathy for stavudine-based therapy only. Thus, our cohort only consisted of 65 HIV-positive patients. Secondly, although the tool we used to assess for the presence of neuropathy was a validated tool for HIV-SN (Cherry *et al.* 2005); it was a rather simple tool. We assessed ankle reflexes and vibration perception which mostly reflects damage to larger fibres (Ziegler *et al.* 1988;Tavee *et al.* 2009), whereas assessment of pin prick sensation, which reflects damage to smaller fibers (Tavee *et al.* 2009), may have been superior. However, this tool was developed and has been validated for use specifically in detecting HIV-SN

(Cherry *et al.* 2005), thus, we are confident that we have not underestimated the rate of HIV-SN.

In conclusion, we found that ATN occurs in 41% of HIV-positive patients who initiate stavudine-based HAART. Thus, in settings where stavudine-based regimens are still being used first line, clinicians would potentially need to prioritise such patients for the use of less toxic regimens, but rather, now, we find that it is more important that these patients probably should be monitored more closely to check for the onset of symptoms. Symptomatic peripheral neuropathy often presenting with moderate to severe pain is still a common problem in African patients initiating stavudine-based HAART. The timely detection and treatment of this symptom will aid in reducing the suffering of these patients. Even though some patients with pre-existing symptomatic neuropathy initially experience an increase in symptom severity, after 6-months of stavudine-based treatment a small percentage do experience symptom relief. Lastly, since treatment regimen guidelines have recently been updated in South Africa, and with many HIV-positive individuals now initiating first line regimens containing the less neurotoxic drug tenofovir, there is hope that this will reduce the incidence of ATN, however, further investigation to determine whether this reduction does occur is important. Thus, a study is underway to determine whether tenofovir-based HAART does reduce the incidence of ATN in the South African population.

Chapter 5

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