The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

HIV-associated sensory neuropathy in an African cohort; a longitudinal study of risk factors predisposing to antiretroviral induced painful neuropathy

Johan J van der Watt, MBChB

University of Cape Town

Thesis Presented for the Degree of Doctor of Philosophy

Department of Medicine, Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

January 2013

Principal supervisor:

Prof. Jeannine M Heckmann, PhD

Co-supervisors:

Dr Collet Dandara, PhD

Prof. Robert J Wilkinson, PhD

Declaration

I, Johan J van der Watt, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the University of Cape Town to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: …….

Johan Jacobus van der Watt

January 2013

University of Cape Town

Published works by the author incorporated into the thesis

Van der Watt JJ, Harrison TB, Benatar M, Heckmann JM. Polyneuropathy, anti-tuberculosis treatment and the role of pyridoxine in the HIV/AIDS era: a systematic review. Int J Tuberc Lung Dis 2011;15:722-8.

Van der Watt JJ, Wilkinson KA, Wilkinson RJ, Heckmann JM. Plasma cytokines in early incident painful antiretroviral-associated HIV neuropathy. Submitted BMC Infectious Diseases 2013.

Statement of contributions to jointly authored works contained in the thesis

No jointly authored works.

University of Cape Town

Acknowledgements

I wish to thank the following individuals:

Prof Jeannine Heckmann for her patience, support, academic experience and for posing challenging questions throughout the study period.

Dr Collet Dandara for guidance with and facilitation of the genetics component of the project.

Prof Robert Wilkinson for guidance with and facilitation of the immunology component of the project.

Dr Katalin Wilkinson for guidance with and facilitation of the immunology component of the project.

Dr Henri Carrara for guidance with the statistical analyses.

Drs Michael Benatar and Taylor Harrison for general guidance and advice.

Dr Michael Rybak for carrying out the vitamin B6 HPLC analysis.

Prof Joshua Taljaard and Ms Amelia Theron for carrying out the C-reactive protein and albumin analyses.

Dr Jeanne Rousseau for assistance with DNA sequencing.

Drs Chad Centner, Nate Sowa and John-Randel Vermaak for assistance with field and laboratory work.

The McHAART study team for their support.

Study participants and staff at Crossroads Community Health Clinic.

The funders of this project:

University of Cape Town Neurology Research Fund
Carnegie Corporation
The Clinical Infectious Diseases Research Initiative (Wellcome Trust 084323)

University of Cape Town

Abstract

Introduction

Distal sensory polyneuropathy (DSP) amongst human immunodeficiency virus (HIV)-infected patients is frequently a painful and disabling condition. HIV-associated DSP is either a consequence of HIV (HIV-DSP) or antiretroviral-induced toxic neuropathy (ATN). The purpose of this research was to investigate the characteristics of HIV-associated DSP over the first 24 weeks of starting cART in an African community-based cohort. Apart from studying the longitudinal association of candidate risk factors based on previous reports, we focused on two additional aspects. Firstly, the *N-acetyltransferase 2* (*NAT2*) genotype, which determines an individual's acetylation status, influences the risk for isoniazid (INH)-associated neuropathy. As INH may predispose to vitamin B6 deficiency and the frequency of HIV/TB co-infection is high, we investigated *NAT2* genotypes and deduced acetylation-phenotypes, vitamin B6 deficiency and the incidence of ATN amongst HIV-infected South Africans. Secondly, incident painful ATN usually develops within 12 weeks of starting combination antiretroviral therapy (cART). As the timing coincides with the manifestations of the immune reconstitution inflammatory syndrome, we hypothesized that immune dysregulation involving previously identified pain-associated cytokines, may correlate with incident ATN. Therefore, we investigated the longitudinal association of plasma cytokine and their soluble receptor concentrations with incident painful ATN within the first 12 weeks of cART initiation.

Materials and methods

In a longitudinal observational cohort, adults about to initiate cART at a Cape Town community-based clinic were clinically screened for peripheral neuropathy by clinicians at a pre-cART visit and 2-, 4-, 12- and 24 weeks after cART initiation. Two validated instruments were used to define DSP as the presence of ≥1 neuropathic symptom and ≥1 neuropathic sign (symptomatic DSP) or ≥2 signs without symptoms (asymptomatic DSP). ATN was defined as new symptoms (±signs) or worsening in existing symptomatic DSP since cART initiation. *NAT2* genotyping was performed using polymerase chain reaction restriction fragment length polymorphism analysis, sequencing and computational haplotype-predicted acetylation phenotype (rapid, intermediate or slow). Fasting vitamin B6 levels [pyridoxal-5'-phosphate (PLP) and 4-pyridoxic acid (4-PA)] were quantified by high performance liquid chromatography. A nested case-control study was included to assess the hypothesis that ATN could be associated with immune dysregulation. Cytokines and soluble receptors were quantified using multiplex immunometric assays with a particular focus on candidate cytokines previously associated with painful neuropathies [tumour necrosis factor-α (TNF-α), interleukin (IL)-1,

University of Cape Town

IL-2, IL-6]. High-sensitivity C-reactive protein (hs-CRP) and albumin levels were measured by immunoturbidimetric assays. Anthropometric data, drug and supplement use, and fasting lipid profiles were obtained at baseline, 12- and 24-week visits. Previous medical history, CD4 T-cell counts, week 24 viral loads and white cell counts were obtained from chart reviews.

Results

One hundred and eighty-four individuals were assessed at baseline. The median age was 33 (interquartile range 26-39); 70% were female. Sixty-three per cent were classified as either World Health Organization stage 1 or 2 HIV infection. The median CD4 T-cell count prior to cART initiation was 158 cells/mm^3 (interquartile range 114-196).

Baseline symptomatic DSP frequency was 17%. Individuals with current/previous INH exposure (n=69) had a higher frequency of DSP (p=0.015). Other factors associated with DSP pre-cART were increasing age, triglycerides >0.9 mmol/L and hs-CRP >5 g/L.

A stavudine-containing regimen was started in 57%, zidovudine in 11%, and tenofovir in 32% based on the government-sponsored guidelines at the time. One hundred and forty-four individuals completed 24 weeks follow-up (22 lost to follow up, 18 excluded). The majority of individuals with symptomatic DSP pre-cART experienced improvement in neuropathic symptoms on cART (54%) and half of those with asymptomatic DSP showed complete resolution of their neuropathic signs by 24 weeks.

Incident ATN occurred in 34% of individuals within 24 weeks of starting cART and did not differ significantly between cART regimens. The peak incidence in ATN occurred between 4 and 12 weeks after cART. NAT2 acetylation phenotypes were predicted in 165 participants: 16% rapid, 52% intermediate and 32% slow. The presence of at least one slow allele positively associated with the development of new neuropathic symptoms (p=0.036), an association that was strengthened by a history of INH exposure. Overall, vitamin B6 levels showed no association with NAT2 phenotypes. However, vitamin B6 deficiency (PLP<25 nmol/L) was present in 53% of individuals at baseline and 55% at 12 weeks despite standard of care B6 supplementation.

Overall, candidate cytokine and hs-CRP concentrations increased at 2 weeks compared with baseline levels, irrespective of ATN status, returning to baseline concentrations again at 12 weeks. Interestingly, individuals who developed ATN had higher pre-cART levels of IL-1 receptor (R)-antagonist and showed greater increases in soluble (s) IL-2Rα sIL-2Rα and sTNFRII at week 2 and sIL-6R at week 12. In those developing ATN, ratios of proinflammatory- vs anti-inflammatory cytokines were significantly higher for TNF-α/IL-4 at 2 weeks and IFN-γ/IL-10 at 12 weeks and showed greater CD4 T-cell count reconstitution at 24 weeks (p=0.002).

University of Cape Town

Conclusion

HIV-DSP and ATN remain clinically significant in Africa and at higher frequencies compared to populations from developed countries. The incidence of symptomatic ATN peaked between 4 and 12 weeks after initiating cART. The development of neuropathic signs on the other hand, increased steadily over the 24-week period suggesting, to some degree, a separate pathogenetic mechanism for asymptomatic DSP. Despite widespread vitamin B complex supplementation there was a high prevalence of suboptimal circulating plasma PLP levels with at least half of the cohort identified as being vitamin B6 deficient. Genotype-deduced slow acetylators are prevalent in this population and the slow acetylation-phenotype is overrepresented in individuals developing ATN suggestive of a contributory role.

All individuals had a transient but significant burst in both pro- and anti-inflammatory plasma cytokine concentrations, as well as hs-CRP levels, within 2-4 weeks after starting cART. In addition, those who developed ATN within 12 weeks of starting cART showed evidence of a higher set point of subclinical inflammation, albeit in the systemic compartment, even before cART was started. These results suggest that those who developed ATN have insults driving chronic subclinical inflammation preceding cART. Furthermore, the observed cytokine burst, altered and higher ratios of "pain-associated" cytokines and concomitant increases in soluble cytokine receptors soon after starting cART, may all give rise to neuropathic symptoms via complex direct and indirect signalling mechanisms.

We propose a synergistic, multifactorial model for the development of DSP where HIV-DSP and ATN are part of a continuum of a similar pathological process. Increased age, immune dysregulation, previous tuberculosis, slow NAT2 acetylation status and micronutrient deficiencies may all contribute to increased oxidative stress via several mechanisms through which these factors, in association with NRTIs and immune reconstitution, may contribute to the development of neuropathy.

Preventative strategies should be directed at multiple levels to maintain adequate redox homeostasis; in addition to possibly earlier initiation of cART, careful attention to optimizing nutritional status by vitamin supplementation prior to commencing cART may provide a simple measure to reduce cellular oxidative stress known to augment inflammation, thereby preventing nerve injury. In Southern Africa where HIV/tuberculosis co-infection is high, public health care policies should implement the recommendations of the developed world regarding pyridoxine supplementation (10-25 mg), prior to commencing cART in all individuals.

Keywords: HIV, distal sensory polyneuropathy, antiretroviral therapy, vitamin B6, *NAT2*, cytokines

University of Cape Town

Table of Contents

University of Cape Town

University of Cape Town

University of Cape Town

University of Cape Town

List of Figures

University of Cape Town

University of Cape Town

List of Tables

University of Cape Town

University of Cape Town

University of Cape Town

University of Cape Town

University of Cape Town

University of Cape Town

Abbreviations

3TC	lamivudine
4-PA	4-pyridoxic acid
ABC	abacavir
ADSP	asymptomatic distal sensory polyneuropathy
AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
ATN	antiretroviral toxic neuropathy
ATP	adenosine triphosphate
AZT	azidothymidine (zidovudine)
BH4	tetrahydrobiopterin
BMI	body mass index
bp	base pairs
BP	blood pressure
BPNS	Brief Peripheral Neuropathy Screen
cART	combination antiretroviral therapy
CCR5	C-C chemokine receptor 5
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CRP	C-reactive protein
CSF	cerebrospinal fluid
CXCR4	C-X-C chemokine receptor
d4T	2'-3'-didehydro-2'-3'-dideoxythymidine (stavudine)
ddC	2'-3'-dideoxycytidine (zalcitabine)
ddI	2'-3'-dideoxyinosine (didanosine)
DNA	deoxyribonucleic acid
dNRTI	dideoxynucleoside reverse transcriptase inhibitor
dNTP	deoxyribonucleotide triphosphate
DSP	distal sensory polyneuropathy
EDTA	ethylenediaminetetraacetic acid
EFV	efavirenz
GCH1	guanosine triphosphate cyclohydrolase
GM-CSF	granulocyte-macrophage colony-stimulating factor
gp	glycoprotein
Hb	haemoglobin
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
hs	high-sensitivity
IFN	Interferon
IHDS	International HIV dementia scale
IL	interleukin
IL-1RA	interleukin-1 receptor antagonist
IL-1RI	type I interleukin-1 receptor
IL-1RII	type II interleukin-1 receptor
INH	isoniazid
IQR	interquartile range
IRIS	immune reconstitution inflammatory syndrome

University of Cape Town

LDL	low-density lipoprotein
MAP	multiple analyte profiling
MCV	mean corpuscular volume
min	minutes
mRNA	messenger ribonucleic acid
mtDNA	mitochondrial deoxyribonucleic acid
mTNS	modified version of the Total Neuropathy Score
NAT2	N-acetyltransferase type 2
Nef	negative regulatory factor
NRTI	nucleoside reverse transcriptase inhibitors
NVP	nevirapine
OR	odds ratio
PAR	proteinase-activated receptors
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
pg	picogram
PI	protease inhibitor
PLP	pyridoxal-5'-phosphate
QST	quantitative sensory threshold testing
RANTES	regulated upon activation, normal T-cell expressed and secreted
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
ROS	reactive oxygen species
rTNS	reduced version of the Total Neuropathy Score
SDSP	symptomatic distal sensory polyneuropathy
sIL	soluble interleukin
sIL-1RI	soluble interleukin-1 receptor I
sIL-1RII	soluble interleukin-1 receptor II
sIL-2Rα	soluble interleukin-2 receptor-α
sIL-4R	soluble interleukin-4 receptor
sIL-6R	soluble interleukin-6 receptor
SNAP	sensory nerve action potentials
SNP	single nucleotide polymorphism
sTNFRI	soluble tumour necrosis factor receptor I
sTNFRII	soluble tumour necrosis factor receptor II
Tat	trans-activator of transcription
TB	tuberculosis
TBE	tris-borate EDTA electrophoresis buffer
TDF	tenofovir disoproxil fumarate (tenofovir)
Th1	T-helper 1
Th2	T-helper 2
TNF-α	tumour necrosis factor-α
TNS	Total Neuropathy Score
UCT	University of Cape Town
UNAIDS	United Nations Programme on HIV/AIDS
VAS	visual analogue scale
Vpr	viral protein R
WCC	white cell count
WHO	World Health Organization

University of Cape Town

Chapter 1 Background

University of Cape Town

1.1 Introduction

By the end of 2011, 34 million adults and children were estimated to be living with human immunodeficiency virus (HIV) worldwide according to the United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) (WHO, 2011). Sub-Saharan Africa remains the region most heavily affected by this pandemic. It is estimated that 2.5 million adults and children were newly infected with HIV and 1.7 million deaths due to acquired immunodeficiency syndrome (AIDS)-related illnesses (UNAIDS, 2011). Currently, there is no cure or vaccine against infection by HIV. However, dramatic scientific progress over the past 30 years since the discovery of HIV/AIDS has not only advanced our understanding of this deadly pathogen, but also controlled the spread of HIV by the success of combination antiretroviral therapy (cART), introduced in 1996. As a result, patients have experienced suppression in their viral load with an improved lifespan and prognosis. HIV infection has become a chronic illness rather than a fatal disease. Despite the success of cART there is unfortunately also increasing recognition of early and more chronic drug-specific toxicities and complications. Up to 70% of individuals infected with HIV develop neurologic complications of the central or peripheral nervous system (Sacktor, 2002). While the incidence of many HIV-associated neurological complications such as dementia and opportunistic infections has decreased on cART, the prevalence of neuromuscular disorders has increased (Morgello et al., 2004, Schifitto et al., 2002, Schifitto et al., 2005).

Distal sensory polyneuropathy (DSP) is probably one of the most common neurologic complications of HIV infection (Barohn et al., 1993, Tagliati et al., 1999). The exact pathogenesis of HIV-associated DSP remains ill-defined and is likely multifactorial (Moyle and Sadler, 1998). It is now clear that some HIV treatments have inherent neurotoxic potential, in particular the nucleoside reverse transcriptase inhibitor (NRTI) class, and more specifically the dideoxynucleoside reverse transcriptase inhibitors (dNRTIs) or d-drugs - stavudine, didanosine, and zalcitabine (Moyle et al., 1998, Boulle et al., 2007). Despite WHO recommendations to reduce and phase out the use of these agents, they remain in use due to wide availability, low cost and limited alternatives in resource-limited settings (Hung et al., 2008).

The clinical importance of DSP in people living with HIV is the painful symptoms, which remain difficult to treat, cause significant functional impairment, and negatively impact on an

University of Cape Town

individual's quality of life (Ellis et al., 2010, Hays et al., 2000). HIV management is constantly evolving and the expanding range of different antiretroviral drugs augments the likelihood for toxicities and drug–drug interactions (Harrison and Smith, 2011).

The classification, clinical presentation and prevalence of HIV-associated DSP will now be discussed further.

1.2 Classification, clinical presentation and prevalence and of HIV-associated DSP

HIV-associated DSP includes two clinically identical distal sensory neuropathies. It can arise either as a consequence of the HIV infection (HIV-DSP) or as a treatment-induced toxicity related to cART, termed antiretroviral toxic neuropathy (ATN). In many cases, HIV-DSP and ATN may coexist, such that an individual with existing HIV-associated DSP may experience worsening of neuropathic symptoms after commencing cART. There are limited data on the evolution of neuropathic symptoms after the initiation of cART in those who have symptoms pre-cART. A reduction of neuropathic intensity after cART initiation has so far only been observed in an anecdotal report (Pialoux et al., 1997) and a small prospective study of three patients (Markus and Brew, 1998).

Onset or worsening of DSP following initiation of a d-drug and subsequent improvement following d-drug withdrawal, suggests ATN. The natural history of particularly neuropathic symptoms, in relation to cART, is therefore valuable in the aetiological diagnosis in HIV-associated DSP. However, frequently and particularly in cross-sectional studies, the distinction between HIV-DSP and ATN has been made simply by the presence or absence of cART exposure (Maritz et al., 2010), a categorization that lacks accuracy. A further limitation in the literature is that DSP, with or without symptoms, are often grouped together. This impacts on prevalence data as well as the study of risk factors. These will be discussed further later.

1.2.1 Prevalence of HIV-associated DSP

Prior to the availability of cART, the estimated prevalence of DSP amongst ambulatory HIV-infected individuals was found to be around 14%. However, depending on the stage of HIV infection, the prevalence varied from less than 2% in those individuals with early HIV

University of Cape Town

disease (Barohn et al., 1993), to 35% amongst hospitalized patients (So et al., 1988). Nearly all patients dying of AIDS have pathological abnormalities detectable in peripheral nerves (de la Monte et al., 1988).

After the introduction of cART, the prevalence of HIV-associated DSP increased, probably due to the development of ATN (Bacellar et al., 1994). The prevalence of ATN associated with NRTI use has ranged from ≈20% to over 60% (Schifitto et al., 2002, Morgello et al., 2004, Hung et al., 2008, Cherry et al., 2009, Schifitto et al., 2005, Affandi et al., 2008). Most prospective studies estimate a one-year incidence of ATN at 20-50% (Hung et al., 2008, Schifitto et al., 2002, Schifitto et al., 2005, Simpson et al., 2006). Although data are often incomplete soon after cART initiation, it is thought that incident ATN usually peaks within the first 90 days after starting a cART regimen containing dNRTIs (Arenas-Pinto et al., 2008).

1.2.2 Anatomical considerations

An anatomical classification of peripheral nerves will be briefly discussed as an introduction to clinical symptomatology of HIV-associated DSP. As illustrated in Figure 1.1, peripheral nerve fibres can be classified according to size, which correlates with the degree of myelination. Large diameter nerve fibres are heavily myelinated and include A-α fibres, which mediate motor strength, and A-β fibres mediating touch and vibratory sensation. Medium-sized fibres, known as A-γ fibres, are also myelinated and innervate muscle spindles. Small fibres include thinly myelinated A-δ fibres and unmyelinated C fibres, which innervate skin (somatic fibres) and involuntary muscles, such as cardiac and smooth muscles (autonomic fibres). Together, small nerve fibres mediate thermal and nociceptive sensation as well as autonomic function (Vinik et al., 2008).

The sensation of pain is transmitted as an action potential in unmyelinated (C-fibres, burning or dull second pain, slow transmission) and thinly myelinated (A-δ fibres, pricking or sharp first pain, fast transmission) primary afferent neurons. Nociceptors respond best to potentially noxious stimuli [mechanical (touch or pressure), thermal (hot or cold), or chemical (endogenous or exogenous)] and are usually silent in the absence of stimulation.

University of Cape Town

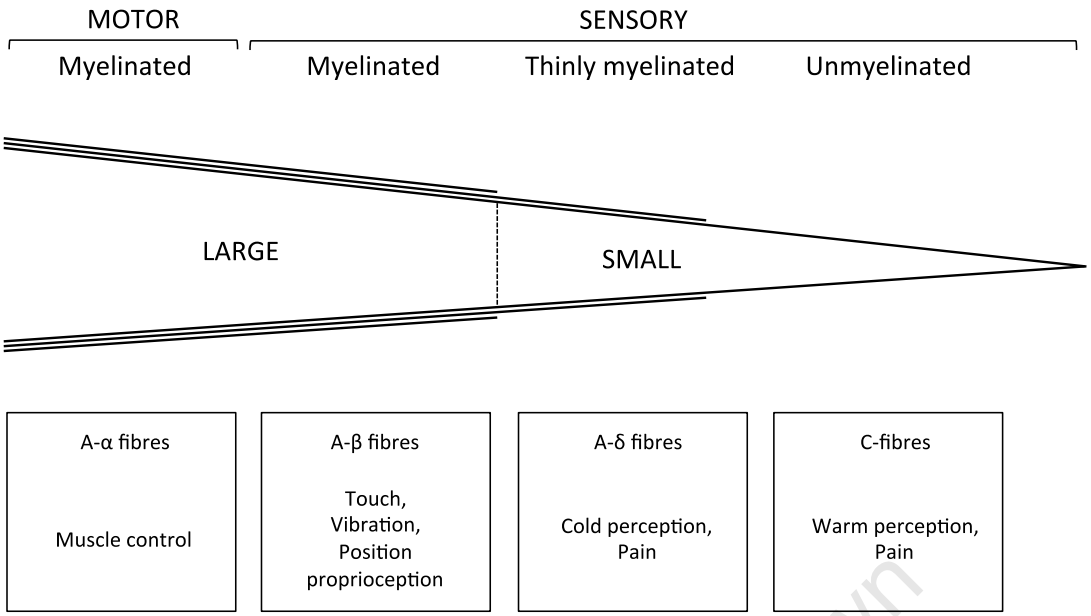


Figure 1.1: Anatomical classification of peripheral nerve fibres (Adapted from Vinik et al., 2008)

Even though small nerve fibres comprise thermal and nociceptive sensation along with autonomic functions, small fibre neuropathy typically refers to somatic neuropathy alone and the overlap with the term 'painful neuropathy' is often used.

1.2.3 Clinical presentation of HIV-associated painful neuropathies

1.2.3.1 Symptoms

The clinical presentation of symptomatic DSP is consistent with that of small fibre dysfunction and consists of neuropathic pain, paraesthesiae and dysesthesiae (Verma et al., 2004). Sensory symptoms are bilateral, symmetrical in distribution and of gradual onset. In the early stages, the patient describes neuropathic symptoms, such as 'aching' or 'burning' pain, 'numbness', 'tingling' or 'pins and needles' in the soles of both feet or toes (Cornblath and McArthur, 1988).

In a length-dependent fashion it ascends from the lower extremities over weeks to months (generally at least 4 weeks) to involve the feet and lower legs bilaterally (Fuller et al., 1993); hands are much less commonly involved (Keswani et al., 2002, Verma et al., 2004, Watters et al., 2004). This pattern of distal onset and proximal progression of the pain in the upper and/or lower extremities is referred to as a "glove and stocking" length-dependent neuropathy. As the neuropathy progresses, sleep disturbances may occur and the ability to

University of Cape Town

perform activities of daily living may become increasingly difficult (Dorsey and Morton, 2006). Individuals may avoid wearing socks or shoes, and those suffering from severe symptoms may find difficulty in walking or standing for prolonged periods (Harrison and McArthur, 1995). Weakness is not a presenting symptom, unless the individual has advanced HIV disease (Keswani et al., 2002).

The most disabling aspect of DSP is related to neuropathic pain. In a large South African cross-sectional study, approximately half of the individuals with signs of DSP suffered from pain (Maritz et al., 2010). Of those with neuropathic symptoms, 90% had positive sensory symptoms (pain and/or paraesthesiae) graded as at least moderately-severe [Visual Analogue Scale (VAS) ≥4/10] in 70% of these individuals. The pain may be both spontaneous and dependent on stimuli, including hyperalgesia, i.e. lowered pain threshold to noxious stimuli and allodynia, i.e. pain induced by non-painful stimuli such as rubbing (Keswani et al., 2002).

ATN and HIV-DSP share identical clinical features. However, some authors have suggested that in HIV-DSP, symptom onset is slow and insidious. Paraesthesiae and numbness often precede burning or aching painful symptoms (Moyle and Sadler, 1998). In contrast, ATN is more likely to be sub-acute in onset and rapidly progressive with pain as an early feature, particularly in individuals with moderate to severe immunosuppression (Berger et al., 1993, Moyle and Sadler, 1998).

1.2.3.2 Neuropathic signs in HIV-associated DSP

The neurological examination may show symmetrical neuropathic signs with or without altered sensory function in a distal to proximal gradient. Although all sensory modalities may be affected it is most evident in those related to small fibre axons, namely pain and temperature, and less common in large fibre axons, viz. proprioception and vibration (Brew, 2003). Hence, examination often reveals reduced pinprick and thermal sensation in a distally symmetrical distribution, predominantly in the toes and feet and may even show hyperalgesia or allodynia in the affected area (Keswani et al., 2002, Gonzalez-Duarte et al., 2008). Vibration sensibility may be reduced at the toes but proprioception is often normal. Other neuropathic signs of DSP include absent or depressed ankle reflexes relative to the knees and even generalized hypo- or areflexia (Gonzalez-Duarte et al., 2008). Muscle strength may be affected in advanced cases, with weakness generally limited to foot intrinsic muscles (Wulff

University of Cape Town

et al., 2000). An antalgic gait may result as a consequence of pain or increased sensitivity at the soles of the feet (Verma et al., 2005).

Some individuals with a clinical diagnosis of DSP have minimal neurological signs when compared to the severity of neuropathic symptoms, suggesting predominant involvement of small nerve fibres. In contrast, others with clinical signs of DSP have no symptoms (Verma, 2001). While it has been suggested that DSP may be asymptomatic in its early stage (England et al., 2005), Schifitto et al. demonstrated in a longitudinal analysis (the DANA cohort) that asymptomatic DSP was not a significant predictor of subsequently developing symptomatic DSP (Schifitto et al., 2002).

1.2.3.3 Differential diagnosis of distal sensory polyneuropathies

In the general HIV-uninfected population, predominantly sensory polyneuropathy may be found in a range of medical conditions, including diabetes and a pre-diabetic state, vitamin deficiencies such as vitamin B12 deficiency, hepatitis C, immune-mediated and connective tissue disorders such as Sjögren's disease, dysthyroidism, dyslipidaemia, chronic renal failure, celiac disease, drug exposure including isoniazid (INH)-, statin- and chemotherapy, industrial toxins and alcohol, rare hereditary diseases, and paraneoplastic or paraproteinemic syndromes (Pardo et al., 2001, Tavee and Zhou, 2009).

Although HIV-infected individuals may develop other peripheral nerve manifestations, they rarely present as a symmetrical length-dependant pure sensory neuropathy. However, care should be taken with the history as immune-mediated vasculitic and inflammatory neuropathies may initially manifest as mononeuritis multiplex or asymmetrical distal painful neuropathy before progressing to a symmetrical polyneuropathy (Brew, 2003).

University of Cape Town

1.3 Clinical relevance of painful neuropathy

Pain associated with DSP substantially affects quality of life. Patients with painful DSP report considerable interference in sleep, recreational activities, mobility, social relationships and mood (Benbow et al., 1998, Galer et al., 2000, Hays et al., 2000). Approximately 50-70% of individuals experiencing chronic pain report sleep disturbances, such as insomnia, middle of night awakening, and restlessness (Morin et al., 1998, Gore et al., 2005). These individuals are also found to have higher levels of anxiety than people without pain (McWilliams et al.,

2003). Over 20% of individuals suffer from major depression due to the chronicity of pain, loss of function, emotional burden, and reduced quality of life (Fishbain et al., 1997). Patients with co-morbid depression also tend to report higher levels of pain, are less active and report greater functional disability (Gore et al., 2005).

The pain associated with HIV-associated DSP has been found refractory to most currently used drugs. This may be due to unique mechanisms of pain compared to other predominantly small fibre neuropathies, such as diabetic neuropathy or post-herpetic neuralgia. Also, a high placebo response observed in individuals participating in many HIV trials may have contributed to the futility of the trials.

Generally, four major classes of pharmacotherapeutic agents are used for symptomatic relief of neuropathic pain: antidepressants (amitriptyline, duloxetine, venlafaxine) (Kieburtz et al., 1998, Shlay et al., 1998), anti-epileptic drugs (carbamazepine, gabapentin, lamotrigine, pregabalin) (Hahn et al., 2004, Simpson et al., 2010, Simpson et al., 2003, Simpson et al., 2000), disease modifying agents (nerve growth factor, acetyl-L-carnitine) (McArthur et al., 2000, Youle et al., 2007) and topical agents (capsaicin, lignocaine) (Kieburtz et al., 1998, Kemper et al., 1998, Estanislao et al., 2004a). However, evidence based data showing analgesic efficacy superior to placebo in the context of painful HIV-associated DSP exist only for smoked cannabis, nerve growth factor and high dose (8%) topical capsaicin (Simpson et al., 2008, Clifford et al., 2012, McArthur et al., 2000). Although there is no evidence for efficacy, many physicians use non-steroidal anti-inflammatories, paracetamol and opioids to treat neuropathic pain (Simpson and Tagliati, 1995, Gonzalez-Duarte et al., 2008). Careful attention should also be given to treating an underlying depression.

University of Cape Town

1.4 Pathological features of HIV-associated DSP

DSP is associated with degeneration of long axons in their distal regions (Pardo et al., 2001). This pattern of distal axonal degeneration is often termed ‘dying back’, a term that carries the implication that the distal regions of axons degenerate first, and over time the process progresses in a centripetal fashion. Although the density of both small and large myelinated fibres is reduced, a distinctive feature of HIV-associated DSP is the early loss of unmyelinated fibres (Pardo et al., 2001, Mah et al., 1988, de la Monte et al., 1988). In ‘dying

back' neuropathies the changes during the phase of frank axonal breakdown are nonspecific, and are therefore sometimes also termed Wallerian-like degeneration.

1.4.1 'Dying back' or Wallerian degeneration

As illustrated in Figure 1.2, Wallerian degeneration describes an experimental model that refers to the process of axonal breakdown distal to any type of axonal injury. It is characterized by granular disintegration of the axonal cytoskeleton and a sequence of changes in both the myelin sheath and Schwann cell (Purves et al., 2001). The disintegration of the axonal cytoskeleton was traditionally thought to result from an atrophic process exclusively caused by failure to deliver nutrients to these distal parts (Waller, 1850). However, increasing evidence suggests that Wallerian degeneration is a regulated, active autodestructive programme akin to apoptotic cell death (Buckmaster et al., 1995, Ikegami and Koike, 2003, Coleman, 2005, Osterloh et al., 2012).

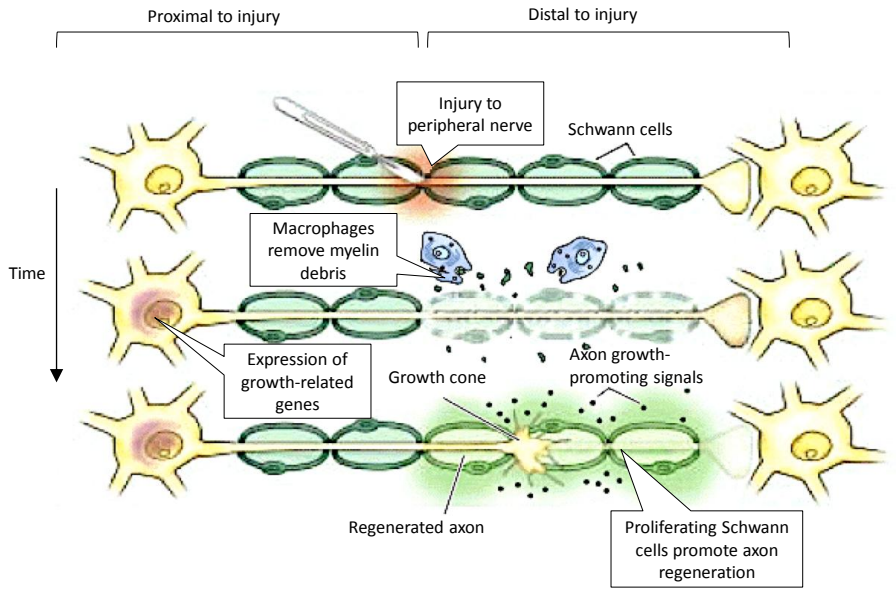


Figure 1.2: Wallerian degeneration (Purves et al., 2001)

The relationship between Wallerian degeneration and 'dying back' neuropathies has long since been a subject of debate. A hypothesis has therefore been developed for the mechanism of 'dying back' degeneration, linking it to Wallerian degeneration. Hereby, two alternative models based on the directionality of degeneration have been proposed to explain why several neuropathies show greater axonal degeneration at their distal ends. Both models are supported by experimental evidence (Beirowski et al., 2005).

The 'dying back' model proposes that degeneration of each axon starts at the distal end and moves retrogradely. Therefore, the initial episode of Wallerian-like degeneration in a given distal axon is followed at some subsequent time by another episode more proximally in the fibre, thereby sequentially amputating another portion of the axon in a distal to proximal fashion (Griffin and McArthur, 1998, Coleman, 2005). The focal lesion model suggests that focal lesions may trigger Wallerian degeneration of distal axons in an anterograde direction, while proximal axons remain intact. Wallerian degeneration can also be triggered by a focal block of axonal transport, therefore the "lesion" does not necessarily need to transect the axon (Griffin and McArthur, 1998, Coleman, 2005).

1.4.2 Histopathological features of HIV-associated DSP

Pathological abnormalities in peripheral nerves are frequently detectable in patients dying of AIDS (de la Monte et al., 1988, Mah et al., 1988). Two histopathological studies done on post mortem nerve biopsies revealed moderate to severe demyelination and axonal degeneration with relatively few epineurial or endoneurial perivascular mononuclear inflammatory infiltrates (T-cells and macrophages) in at least half, irrespective of whether they had symptomatic DSP (de la Monte et al., 1988, Mah et al., 1988). Immunohistochemistry showed evidence of macrophage activation with local release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) in areas of axonal degeneration in over 60% of specimens (Bradley et al., 1998, Rizzuto et al., 1995, Nagano et al., 1996, de la Monte et al., 1988, Tyor et al., 1995). In addition, the proximal ascending spinal sensory axons arising from the second-order neurons also show degeneration in patients dying of AIDS (Griffin and McArthur, 1998). Studies also reported inflammation in the dorsal root ganglia with prominent macrophage and T-lymphocyte infiltration, loss of neuronal cells and fibrosis (Rizzuto et al., 1995, Nagano et al., 1996, de la Monte et al., 1988, Jones et al., 2005). Viral inclusions were not observed (de la Monte et al., 1988). Clustering of lymphocyte satellite cells (Nagoette nodules) is frequently seen to accompany neurophagia and ganglion cell degeneration within the dorsal root ganglia (Keswani et al., 2002, Nagano et al., 1996).

Neuropathological studies suggest that both HIV-DSP and ATN share similar features. However, prominent mitochondrial abnormalities such as vacuolization with loss of cristae and disorganized matrix have been shown in association with exposure to NRTIs (Dalakas et al., 2001, Lehmann et al., 2011).

University of Cape Town

1.5 Diagnostic Methods to identify DSP

Many different outcome measures have been used to quantify or assess the qualitative severity of neuropathic symptoms in clinical trials. These include questionnaires on sensory symptoms, functional scales, quantitative clinical examinations, electrophysiological studies measuring sural sensory nerve action potentials (SNAP), computerized sensory examinations such as quantitative sensory threshold testing (QST), and epidermal nerve fibre density quantification on skin biopsy. Each of these measures has its strengths and weaknesses (Barohn et al., 1996, Devigili et al., 2008, Herrmann et al., 1999, Tagliati et al., 1999, Chavanet et al., 1989).

The closest to a gold standard for the clinical diagnosis of sensory neuropathy is a skin biopsy for the determination of epidermal nerve fibre density. It is a sensitive and valid marker of nerve fibre damage with a high diagnostic efficiency (Herrmann et al., 1999, Lauria et al., 2005). Epidermal nerve fibre quantification has been employed as a reference standard for validation of DSP instruments (Cherry et al., 2005) and has been used as an outcome measure in drug trials (Polydefkis et al., 2002), as well as in clinical practice (Devigili et al., 2008).

Reduced epidermal nerve fibre density is a sign of epidermal denervation. This occurs typically in a length dependent fashion in HIV-infected patients with neuropathic signs, whether they are symptomatic or asymptomatic. Immunocytochemical staining is done with antibodies against the panaxonal marker, protein-gene-product 9.5, in order to visualise the innervation of the epidermis (McArthur et al., 1998, Devigili et al., 2008). This technique also shows that small fibres penetrating the stratum spinosum layer undergo morphologic changes such as axonal swelling preceding the degeneration of small nerve fibres. It has been suggested that individuals with reduced leg epidermal nerve fibre densities, whether they have asymptomatic DSP or no clinical neuropathy, appear to be at an estimated 14-fold greater risk of conversion to symptomatic DSP (Herrmann et al., 2006).

Interestingly, even though symptomatic DSP presents clinically in a distal-to-proximal fashion, the epidermal nerve fibre density ratio between thigh and distal leg was shown to be similar irrespective of whether the patient had symptoms. This finding argues against an exclusively length-dependant epidermal nerve fibre loss and is consistent with the pathological observation of activated macrophages at several levels of the peripheral nervous

University of Cape Town

system which includes the dorsal root ganglia (Herrmann et al., 1999). However, some individuals with a clinical diagnosis of small fibre neuropathy may have what is currently regarded as normal epidermal nerve fibre densities (Tagliati et al., 1999, Lauria et al., 2005). Although the histological assessment of epidermal nerve fibre density requires a minimally invasive skin biopsy procedure, it requires significant resources including expertise to process and interpret histology.

Electrophysiological studies of nerve conduction may show an axonal, length-dependent, sensory polyneuropathy with small SNAPs (Keswani et al., 2002). Importantly however, normal nerve conduction studies do not necessarily exclude the diagnosis of HIV-DSP, as they primarily survey the function of large-diameter afferent nerve fibres and sural SNAPs may be normal in a small fibre neuropathy such as HIV-DSP. Electrophysiological studies such as nerve conduction and QST require specialized equipment (Lauria et al., 2005). Furthermore, a receiver operating characteristic curve analysis confirmed a significantly higher performance of skin biopsy compared to QST, although this study was flawed by the absence of an absolute gold standard (Devigili et al., 2008).

Due to these limitations, field or epidemiological studies rely on the availability and validity of clinical tools to diagnose DSP. The routine use of specialized equipment or techniques for fieldwork is not feasible in many research settings. Clinical epidemiological research in peripheral neuropathy requires easily performed, low-cost, non-invasive, reliable and validated measures of peripheral nerve function. A practical screening tool for detecting HIV-associated DSP in many settings would be a simple, standardized set of clinical criteria. This allows DSP screening where specialized investigations are unavailable. However, there is poor agreement on the clinical definition for small fibre neuropathy to be used in clinical research (Brew, 2003). The use of a formal case definition across future research studies would ensure greater consistency in reporting of neuropathy rates and risk factors.

1.5.1 Proposed definitions of small fibre neuropathy

A study of the predictive value of any test used to diagnose small fibre neuropathy is affected by the absence of a generally accepted gold standard against which the performance of such a diagnostic test may be compared (Benatar, 2006). Without a gold standard, estimation of the specificity and sensitivity of a diagnostic test is not possible. In 2005, the Polyneuropathy Task Force of the American Academy of Neurology developed a case definition of distal

University of Cape Town

sensory polyneuropathy to standardise and facilitate clinical and epidemiological research studies. The proposed definition was based on peer-reviewed publications and expert opinion (England et al., 2005). The clinical definition consisted of neuropathic symptoms, including numbness, burning, paraesthesiae, dysesthesiae and allodynia, with the presence of signs including decreased pin sensation, decreased vibration sense, and attenuated deep tendon reflexes. The Task Force recognised that deep tendon reflex abnormalities may not be necessary for the diagnosis of small fibre neuropathy (England et al., 2005).

Different definitions for clinical research studies and epidemiologic studies were proposed, and definitions were ranked by estimated ordinal likelihood for disease. For field or epidemiologic studies such as the study that we have undertaken, the definition with the highest likelihood for sensory neuropathy included a combination of clinical neuropathic symptoms, decreased or absent ankle reflexes and decreased distal sensation (either pin or vibration) (England et al., 2005). For clinical studies the recommendations were to use a combination of neuropathic symptoms, signs, and electrodiagnostic findings. Abnormal electrodiagnostic studies increase the likelihood of the presence of distal symmetrical polyneuropathy and provide a higher level of specificity to the case definition (England et al., 2005). However, Benatar argued that the definition proposed by the Polyneuropathy Task Force was based on diagnostic modalities that overlapped with the reference tests used in the studies (incorporation bias), thereby artificially increasing sensitivity and specificity of the tests (Benatar, 2006). Also, most of the best evidence available is restricted to diabetic peripheral neuropathy and therefore possibly not valid in another population or setting such as HIV-associated DSP (Benatar, 2006).

As an alternative, Devigili et al. proposed a diagnostic standard for small fibre neuropathy in individuals with normal large-fibre function and nerve conduction studies. It required abnormalities in two of the following three tests: distal small fibre sensory examination, thermal QST threshold, and distal leg epidermal nerve fibre density (Devigili et al., 2008). Similarly, due to the lack of an independent reference standard, these proposed diagnostic criteria also suffered from incorporation bias.

Despite these diagnostic problems, for this community-based research, two different validated clinical tools were employed and will now be discussed in further detail.

University of Cape Town

1.5.2 Clinical tools

1.5.2.1 Total Neuropathy Score (TNS)

The Total Neuropathy Score (TNS) is a validated composite measure of peripheral nerve function developed in diabetic neuropathy, and used in prospective studies of diabetic and toxic neuropathies (Cornblath et al., 1999). The TNS combines information obtained from grading symptoms, signs (reflexes, pinprick sensibility, vibration sense and motor function), nerve conduction studies and QST, and provides a comprehensive and easily obtained measure for detecting and quantifying DSP. Inter- and intrarater reliability of the TNS was excellent (0.966 and 0.986, respectively), with a strong positive correlation to other validated measures of neuropathy in patients with diabetes, including the neuropathy symptom score and the neurologic impairment score (Cornblath et al., 1999).

A reduced version of the TNS (rTNS) was developed specifically for the diagnosis of chemotherapy-induced painful sensory polyneuropathy (Cavaletti et al., 2003). This version did not include QST testing and also did not score motor or autonomic symptoms, but was validated against the same clinical tools as the original TNS and confirmed as an effective and faster alternative to the original TNS (Cavaletti et al., 2003). Furthermore it demonstrated good concordance with epidermal nerve fibre density measurement (ρ=-0.26, $p < 0.01$), supporting its reliability as a screening tool for DSP (Zhou et al., 2007).

Another modified version of the TNS was developed by the original authors based purely on clinical neurological assessment (mTNS) which has been utilised in several studies (Robinson-Papp et al., 2010, Maritz et al., 2010, Cavaletti et al., 2006, Ellis et al., 2005) (see Appendix A). Similar to the rTNS, it was validated against the same clinical tools as the original TNS, and was found to have good correlation with these aforementioned widely used scores (ρ=0.88, $p<0.01$) (Cavaletti et al., 2007).

1.5.2.2 Brief Peripheral Neuropathy Screen (BPNS)

The Brief Peripheral Neuropathy Screen (BPNS) (see Appendix A) was specifically developed for the clinical diagnosis of HIV-associated DSP and has been used as a screening tool in several AIDS Clinical Trial Group protocols (McArthur et al., 1998, Cherry et al., 2005). It is simple and rapid to administer and does not require any specialized equipment. The BPNS instrument consists of brief questions regarding neuropathic symptoms in the feet

University of Cape Town

or legs: 1) pain, aching, or burning; 2) pins and needles sensation; and 3) numbness. Individuals grade each of these symptoms bilaterally from 0 (absent) to 10 (most severe). It also includes an examination of distal lower extremity vibration and ankle reflexes but not pin sensibility. It is practical for use in most settings such as busy outpatient clinics, clinical trials, and even in resource-constrained communities, although its accuracy when performed by non-clinicians (e.g. trained study nurses) is less reliable (Simpson et al., 2006). By using the BPNS, a diagnosis of symptomatic DSP, defined as the presence of neuropathic symptoms and at least one neuropathic sign, has been shown to correlate well with decreased epidermal nerve fibre densities and increased thresholds on QST (Cherry et al., 2005).

1.6 Data on HIV-associated DSP in Africa

Limited data are available from African populations. A summary of studies performed in African populations is included in Table 1.1. The prevalence of symptomatic DSP in cART naïve individuals ranged from 11-43% in studies using validated tools to define neuropathy (Forna et al., 2007, Maritz et al., 2010, Mehta et al., 2011, Mullin et al., 2011, Oshinaike et al., 2012, Sacktor et al., 2009, Shurie and Deribew, 2010, Phillips et al., 2010).

In the post-cART era there has been 5 longitudinal studies reported, three of which were primarily aimed at investigating safety and tolerability of NRTI therapy and not the incidence of DSP (Forna et al., 2007, Hawkins et al., 2007, Hoffmann et al., 2008). Therefore, to date only two were performed using validated tools to diagnose DSP (Mehta et al., 2011, Sacktor et al., 2009). Reported incidences of neuropathic symptoms ranged between 12-38% at 12 and 6 months respectively. However, the incidence of ATN within the first 12 weeks of cART, as well as the impact of cART on pre-existing HIV-DSP has not been previously investigated in an African cohort.

University of Cape Town

Table 1.1: Summary of clinical studies on HIV-DSP performed in Africa (Adapted from Phillips et al., 2010)

Group	Study cohort	Design	Cohort size	DSP prevalence/ incidence	DSP definition	Risk factors for HIV-associated DSP
Pre cART: African studies						
Mehta et al. 2011	Ambulatory clinic patients, Kenya	Cross-sectional	199	11%	1 sign + symptoms[§]	
Maritz et al. 2010	Ambulatory patients, South Africa	Cross-sectional	331	23%	2 signs + symptoms[§]	
Mullin et al. 2011	Ambulatory clinic patients, Tanzania CD4 count <200	Cross-sectional	81	33%	1 sign + symptoms[§]	Advancing age, male sex
	Ambulatory clinic patients, Tanzania CD4 count ≥200	Cross-sectional	86	21%	1 sign + symptoms[§]	Advancing age, male sex
Forna et al. 2007	Outpatients commencing cART,home-based care programme, Uganda	Cross-sectional	1029	13%	1 sign or symptoms[§]	Advancing age, TB treatment
Oshinaike et al. 2012	Outpatients, Nigeria	Cross-sectional	181	37%	1 sign ± symptoms[§]	Advancing age
Sacktor et al. 2009	Outpatients commencing cART, Uganda	Cross-sectional	102	37%	Symptoms[§]	
	Outpatients commencing cART, Uganda	Cross-sectional	102	43%	1 sign[§]	
Shurie et al. 2010	Outpatients, Ethiopia	Cross-sectional	114	11%	1 sign ± symptoms[§]	Advancing age
Post cART: African studies						
Beadles et al. 2009[†]	cART exposed outpatients, Malawi	Retrospective	3341	13%	Not specified	
Boulle et al. 2007[†]	Outpatients commencing cART, South Africa	Retrospective	2679	6% (3 year)[¶]	Not specified	Advancing age, WHO stage, cART > 24 weeks
Van Oosterhout et al. 2005[†]	cART exposed outpatients, Malawi	Cross-sectional	264	56%	Symptoms	
Cettomai et al. 2010	cART exposed outpatients, Kenya	Cross-sectional	30	53%	1 sign + symptoms[§]	
Cherry et al. 2010	Ambulatory cART exposed patients, South Africa	Cross-sectional	300	62%	1 sign + symptoms[§]	
Mehta et al. 2010	cART exposed outpatients, Kenya	Cross-sectional	102	36%	1 sign + symptoms[§]	Advancing age
Maritz et al. 2010	cART exposed outpatients, South Africa	Cross-sectional	267	50%	1 sign + symptoms[§]	Advancing age, previous TB
Wadley et al. 2010	cART exposed outpatients, South Africa	Cross-sectional	395	57%	1 sign + symptoms[§]	Advancing age, increased height
Mullin et al. 2011	Outpatients cART exposed, Tanzania CD4 count <200	Cross-sectional	81	43%	1 sign + symptoms[§]	Advancing age, male sex
	Outpatients cART exposed, Tanzania CD4 count ≥200	Cross-sectional	78	41%	1 sign + symptoms[§]	
Shurie et al. 2010	Outpatients cART exposed, Ethiopia	Cross-sectional	204	48%	1 sign ± symptoms[§]	Advancing age
Oshinaike et al. 2012	Outpatients cART exposed, Nigeria	Cross-sectional	142	42%	1 sign ± symptoms[§]	Advancing age, d4T exposure
Forna et al. 2007[†]	Outpatients commencing cART, home-based care programme, Uganda	Longitudinal	894	36% (3 year)[¶]	1 sign or symptoms	Advancing age, TB treatment
Hawkins et al. 2007[†]	Outpatients commencing cART, Kenya	Longitudinal	1286	21% (1 year)[¶]	Not specified	Advancing age, low CD4+ count
Hoffmann et al. 2008[†]	Outpatients commencing cART, South Africa	Longitudinal	788	4%	1 sign ± symptoms	
Mehta et al. 2011	Outpatients commencing cART, Kenya	Longitudinal	150	12% (1 year)[¶]	1 sign + symptoms[§]	Females
Sacktor et al. 2009	Outpatients commencing cART, Uganda	Longitudinal	79	38% (6 month)[¶]	Symptoms[§]	
	Outpatients commencing cART, Uganda	Longitudinal	79	31% (6 month)[¶]	1 sign[§]	

[†] Studies investigating tolerability and clinical events on cART

[¶] DSP incidence calculated as cumulative incidence over the period

[§] Validated tool used to assess neuropathy

Table 1.2: Summary of clinical studies on HIV-DSP performed outside Africa (Adapted from Phillips et al., 2010)

Group	Study cohort	Design	Cohort size	DSP prevalence/ incidence	DSP definition	Risk factors for HIV-associated DSP
Pre cART: Non-African studies (prior to cART initiation)						
Evans et al. 2011	Outpatients commencing cART, USA	Cross-sectional	1923	23%	1 sign ± symptoms	
Post cART: Non-African studies						
Schifitto et al. 2002	Advanced HIV disease, early symptoms of HIV dementia, USA	Cross-sectional	272	55%	1 sign ± symptoms§	
Morgello et al. 2004	Advanced HIV disease, USA	Cross-sectional	187	53%	2 signs ± symptoms§	Advancing age, males
Schifitto et al. 2005	Advanced HIV disease, early symptoms of HIV dementia, USA	Cross-sectional	376	67%	1 sign ± symptoms§	
Cherry et al. 2006	Ambulatory patients, USA and Australia	Cross-sectional	147	52%	1 sign + symptoms§	Advancig age, dNRTI exposure (ddI, d4T)
Pettersen et al. 2006	Outpatients with neurological disease, Canada	Cross-sectional	221	46%	2 signs + symptoms§	Advancing age, low CD4+ count, peak viral load, alcohol,
Simpson et al. 2006	cART exposed patients, USA	Cross-sectional	101	52%	1 sign + symptoms§	Advancing age, caucasians
Smyth et al. 2007	Outpatients, Australia	Cross-sectional	100	42%	1 sign + symptoms§	Advancing age, dNRTI or PI exposure
Affandi et al. 2008	Outpatients, Indonesia	Cross-sectional	96	34%	1 sign + symptoms§	Advancing age, increased height, TNF-α genotype
Hung et al. 2008	cART exposed d-drug regimen, USA	Cross-sectional	252	23%	1 sign ± symptoms§	
Hung et al. 2008	cART exposed non-d-drug regimen, USA	Cross-sectional	250	29%	1 sign ± symptoms§	
Sithinamsuwan et al. 2008	Outpatients, Thailand	Cross-sectional	63	25%	1 sign or symptoms§	
Wright et al. 2008	Outpatients, Asia-Pacific countries	Cross-sectional	640	20%	1 sign + symptoms§	dNRTI exposure
Cherry et al. 2009	Outpatients, Australia	Cross-sectional	100	42%	1 sign + symptoms§	Advancing age, increased height, dNRTI or PI exposure
Cherry et al. 2009	Outpatients, Malaysia	Cross-sectional	98	19%	1 sign + symptoms§	Advancing age, increased height, dNRTI or PI exposure
Cherry et al. 2009	Outpatients, Indonesia	Cross-sectional	96	34%	1 sign + symptoms§	Advancing age, increased height, dNRTI or PI exposure
Ellis et al. 2010	Outpatients, USA	Cross-sectional	1539	57%	1 sign ± symptoms§	Advancing age, immunosuppression, dNRTI exposure, current cART use
Banerjee et al. 2011	Outpatients, USA	Cross-sectional	436	27%	1 sign ± symptoms§	Advancing age, increased height, diabetes, PI exposure, statin use, low nadir CD4+ count, increased triglycerides
Lichtenstein et al. 2005	Outpatients at multiple clinics, USA	Longitudinal (Retrospective)	2525	13%	Symptoms	Advancing age, peak viral load, low nadir CD4+ count, diabetes, caucasians, NRTI or PI exposure
Schifitto et al. 2002	Advanced HIV disease, early symptoms of HIV dementia, USA	Longitudinal	89	52% (1 year)¶	1 sign ± symptoms§	WHO stage, AIDS, low CD4+ count
Schifitto et al. 2005	Advanced HIV disease, early symptoms of HIV dementia, USA	Longitudinal	185	40% (1 year)¶	1 sign + symptoms§	dNRTIs associated with reduced risk
Simpson et al. 2006	cART exposed patients, USA	Longitudinal	28	21% (48 week)¶	1 sign + symptoms§	Caucasians
Hung et al. 2008	cART exposed d-drug regimen, USA	Longitudinal	194	27% (1 year)¶	1 sign ± symptoms§	
Hung et al. 2008	cART exposed non-d-drug regimen USA	Longitudinal	177	28% (1 year)¶	1 sign ± symptoms§	
Nakamoto et al. 2010	Outpatients, Hawaii	Longitudinal	61	31% (4 year)¶	2 signs ± symptoms§	Advancing age, low nadir CD4+ count
Evans et al. 2011	Outpatients commencing cART, USA	Longitudinal	1923	58% (7 year)¶	1 sign ± symptoms§	
Arenas-Pinto et al. 2008	Outpatients commencing cART, Europe and Australia	Longitudinal	3195	6% (3 year)¶	Symptoms	Advancing age, low CD4+ count, dNRTI exposure

¶ DSP incidence calculated as cumulative incidence over the period

§ Validated tool used to assess neuropathy

1.7 Pathophysiology of HIV-associated DSP

The pathophysiology of HIV-associated DSP is not completely understood, although it is likely multifactorial. Neuronal damage secondary to immune activation is the most generally accepted pathogenic mechanism, similar to that in HIV-associated dementia (Hoke and Cornblath, 2004, Gonzalez-Scarano and Martin-Garcia, 2005). This concept is supported by pathological studies that failed to show HIV replication in neurons, but in approximately 50% of cases demonstrated abundant macrophage activation and viral replication within the macrophages of peripheral nerves and dorsal root ganglia (see section 1.4) (Pardo et al., 2001, Gonzalez-Scarano and Martin-Garcia, 2005). Although prominent loss of unmyelinated sensory fibres are noted pathologically (see section 1.4), the nature and the site of initial injury to the nerves are unknown. In this section, the following will be discussed: 1) The direct and indirect mechanisms of nerve injury in HIV-DSP; 2) the pathophysiological aspects of ATN; and 3) the role of mitochondrial dysfunction in HIV-associated DSP (including HIV-DSP and ATN).

1.7.1 Direct mechanism of nerve injury in HIV-associated DSP

Although De la Monte et al. proposed that the neuropathy in AIDS results from direct HIV infection of peripheral nerves (de la Monte et al., 1988), there is limited evidence for the presence of HIV in peripheral nerve or dorsal root ganglion cells. Ho et al. cultured HIV from sural nerve homogenates in only one of three patients with AIDS-related peripheral neuropathy whereas HIV was cultured from the cerebrospinal fluid in all three (Ho et al., 1985). Similarly, Bailey et al. reported electron microscopic evidence of retroviral-like inclusions in myelinated sural nerve fibres in only one of six AIDS patients with DSP (Bailey et al., 1988). Many other pathologic studies have failed to identify HIV antigens or retroviral-like particles in the peripheral nerves or dorsal root ganglion cells (de la Monte et al., 1988, Rance et al., 1988, Mah et al., 1988). It is now widely accepted that almost all virus recovered from peripheral nerve tissue is likely from the resident monocytes/macrophages that account for about 10% of the cells in peripheral nerves (Brinley et al., 2001, Jones et al., 2005). These findings suggest that direct HIV infection of peripheral nerve is an unlikely cause of DSP, although viral products such as the major HIV envelope glycoprotein (gp)-120 has been proposed to have a major role in DSP pathogenesis (Herzberg and Sagen, 2001).

HIV entry into macrophages and T helper cells is mediated through interaction of the viral gp120 with the target CD4+ molecule and its chemokine coreceptors, C-C chemokine

University of Cape Town

receptor 5 (CCR5) and C-X-C chemokine receptor (CXCR4) (Zheng et al., 1999). It has been demonstrated *in vitro* that soluble gp120, in the absence of viral infection can bind to both CXCR4 and CCR5 receptors on axonal membranes. This ligation leads to axonal degeneration through two independent pathways; the first engages neuronal apoptosis indirectly by mediating the activation of Schwann cells, and the second uses direct local axonal toxicity by activation of the caspase pathway (Melli et al., 2006).

The mechanism of neuronal injury in dorsal root ganglion cell bodies is indirect. Gp120 ligation to the CXCR4 receptor on surrounding Schwann cells results in the upregulation of chemokines such as "regulated upon activation, normal T-cell expressed and secreted" (RANTES). In turn, Schwann cell secreted RANTES binds to CCR5 on the dorsal root ganglia neurons and induces neuronal TNF-α release. TNF-α then binds to TNF receptors and results in classical apoptotic neuronal cell death in sensory neurons (Hahn et al., 2008).

Direct application of gp120 exclusively to axons resulted in ligation of CXCR4 and CCR5 receptors followed by downstream changes in the mitochondrial transmembrane potential, cytochrome c release and caspase activation. This process results in degeneration of distal axons, independent from the cell body and without evidence of neuronal apoptosis proximally (Melli et al., 2006). In conclusion, several studies have shown that although HIV itself may not be the principal pathogenic agent in DSP, viral proteins such as gp120 as well as host chemokine and cytokine responses may participate in a multifaceted interaction ultimately leading to nerve fibre damage distally and neuronal loss proximally.

1.7.2 Indirect mechanism of nerve injury in HIV-associated DSP

As direct HIV infection of the peripheral nerve has not been convincingly demonstrated, research has focused on indirect mechanisms such as the action of chemokines and cytokines. Chemokines and cytokines can direct leukocyte trafficking during inflammatory responses and also have a role in neural development and the modulation of nervous system responses to injury and disease (Lu et al., 2002, Tran and Miller, 2003, Bhangoo et al., 2009). Inflammation is a principal characteristic of HIV pathogenesis and it is therefore not surprising that cytokines and chemokines appear to be predominant in the pathophysiological mechanisms of HIV-associated neural pathology.

HIV pathogenesis can be explained by a state of chronic immune dysregulation (Herbein et al., 2010) characterised by immune activation and the progressive decline in CD4 T-cells

University of Cape Town

(Herbein and Khan, 2008, Salazar-Gonzalez et al., 1997). Direct immune activation results in the generation of polyfunctional effector T-cells able to produce large amounts of pro-inflammatory and/or pro-apoptotic cytokines such as TNF-α, interleukin (IL)-1β, IL-6 and type I interferons. These factors sustain the generalized immune activation and facilitate the death of bystander CD4- and CD8 T-cells. Chronic immune activation can also suppress the immune system by inhibiting the normal functions of monocytes, B cells, natural killer cells and dendritic cells. In addition, it may limit the regenerative potential of T-cells as a result of the destruction of the architecture of lymphatic tissues such as bone marrow, thymus and lymph nodes (Vanderford et al., 2010).

Immunopathogenic factors characteristic of HIV immune dysregulation may contribute to the indirect mechanism of nerve injury. Firstly, gp120 may cause indirect nerve damage by promoting macrophage infiltration in peripheral nerves and the dorsal root ganglia (Wallace et al., 2007). Further, a degree of distal axonal degeneration may already exist in HIV-infected individuals due to inadequate nutritional state, exposure to alcohol and/or substance abuse or other nonspecific factors, promoting a leaky blood-nerve-barrier (Griffin and McArthur, 1998). As seen in Wallerian-like degeneration, macrophages are activated and recruited into the areas of nerve damage. In HIV infection, as a result of chronic immune activation and the leaky blood-nerve barrier, the macrophages may show exaggerated inflammatory responses in the distal and proximal segments of peripheral nerves (Keswani et al., 2002). These activated macrophages within the peripheral nerve cause local release of pro-inflammatory neurotoxic cytokines, such as IL-6 and TNF-α that result in nerve injury (Keswani et al., 2002, Dalakas et al., 2001). The release of chemoattractant molecules recruits more immune cells from the circulation into the damaged nerve, resulting in cycles of neuroimmune activation with further release of pro-inflammatory cytokines, nitric oxide and reactive oxygen species (ROS) (Trifilieff et al., 2000, Bergsteinsdottir et al., 1991, Taskinen and Roytta, 2000, Taskinen et al., 2000). This process can occur despite advanced systemic immunosuppression (Nagano et al., 1996, Wesselingh et al., 1994).

The secondary immune response as a result of nerve injury has multiple effects on peripheral nerves and has been extensively researched in animal models. It can directly increase nerve excitability by induction of ectopic electrogenesis (Leem and Bove, 2002, Sorkin et al., 1997), damage myelin (Muijsers et al., 1997, Said and Hontebeyrie-Joskowicz, 1992), and increase vascular permeability further compromising the blood-nerve barrier (Greenacre et

University of Cape Town

al., 1997, Uncini et al., 1999). Activated macrophages and denervated Schwann cells phagocytose myelin debris and secrete a variety of proteases and other enzymes, including matrix metalloproteases that damage the basal lamina of endoneurial blood vessels, increasing the blood-nerve barrier dysfunction (Shubayev et al., 2006). Exposure of peripheral nerve proteins or cryptic epitopes as a result of the neuro-inflammation may lead to priming of the immune system to these self-antigens. This in turn can result in a secondary bystander 'auto-immune' response (Watkins and Maier, 2002).

1.7.3 Pathophysiology of antiretroviral toxic neuropathy (ATN)

The exact pathogenesis of ATN is not yet established. Various explanations are offered for the neurotoxic effects of d-drugs. Mitochondrial dysfunction is associated with exposure to NRTIs, and believed to contribute to the pathogenesis of ATN. NRTIs inhibit mitochondrial DNA polymerase-γ, which is the only enzyme responsible for replication and repair of the mitochondrial genome (Lewis et al., 2003). The role of mitochondrial dysfunction in ATN development will be discussed in further detail in section 1.8.

From animal studies it is evident that peripheral sensory neurons can be strongly excited by chemokines and gp120 either by lowering the threshold for action potential generation without changing the membrane potential, or by depolarization (Oh et al., 2001). Apart from the effect of NRTIs on mitochondria, a recent study in rodents showed that dNRTI treatment led to an upregulation of CXCR4 and its ligand, stromal cell-derived factor-1, in sensory neurons and dorsal root ganglia (Bhangoo et al., 2009). As such, it is possible that exposure of dNRTI to CXCR4-positive neurons is directly linked to chronic neuronal hyperexcitability. Therefore, concurrent exposure to gp120 and d-drugs may act synergistically to activate chemokine signalling pathways that may result in mechanical hypernociception (Bhangoo et al., 2009).

Although some have suggested that ATN represents the unmasking of subclinical HIV-DSP (Simpson and Tagliati, 1995) or that asymptomatic DSP precedes symptomatic DSP (Cherry et al., 2003, Martin et al., 2000, Mehta et al., 2010), this was not demonstrated in a 2.5 year follow-up study (Schifitto et al., 2002). However, morphologic abnormalities such as axonal swelling as well as non-clinical objective measures of DSP, such as epidermal nerve fibre densities and QST have been noted in those with neuropathic signs prior to the onset of symptoms (Devigili et al., 2008, Lauria et al., 2003, Cherry et al., 2005).

University of Cape Town

Immune modulation may also play a role in the pathophysiology of ATN. With the initiation of cART there is a phase of immune restoration and in some patients this is early and rapid, resulting in immune activation and inflammatory responses that may cause clinical deterioration known as the "immune reconstitution inflammatory syndrome" (IRIS) (further discussed in section 1.9.4). In the context of HIV-associated peripheral nerve injury, the immune activation near peripheral nerves could increase nerve hyperexcitability and damage with further release of cytokines, free radicals, and lipid membrane derivatives (Schifitto et al., 2005). As incident symptomatic ATN peaks around 3 months (Arenas-Pinto et al., 2008), within the period in which IRIS also manifests, one of the hypotheses of this work is that an exaggerated cytokine response or immune reconstitution after cART initiation, contributes to the immunopathogenesis of ATN.

To summarize, ATN involves a possible 'double-hit' process whereby the proximal (dorsal root ganglion) and distal (distal intraepidermal nerve fibres) of the peripheral sensory nerves are damaged or sensitized directly by viral proteins such as gp120 and/or by HIV-associated chronic immune dysregulation. Sensory neurons may then be further compromised by NRTI-induced mitochondrial toxicity or possible NRTI-induced immune activation driving further release of cytokines.

The discussion will now focus on mitochondria and mitochondrial toxicity in relation to HIV-associated DSP.

University of Cape Town

1.8 Mitochondrial dysfunction and the relationship to HIV-associated DSP

Mitochondria are double-membrane intracellular organelles found in all human cells with the exception of mature red blood cells. These intracellular organelles host a number of essential biochemical reactions largely concerned with fuel oxidation and generation of adenosine triphosphate (ATP) (Scheffler, 2001). In addition, mitochondria are intimately involved in a number of cellular processes including cell differentiation, signalling, and division, ageing and programmed cell death or apoptosis (Kroemer and Reed, 2000, Kujoth et al., 2005, Scheffler, 2001).

Mitochondria contain their own unique circular deoxyribonucleic acid (DNA), referred to as mitochondrial DNA (mtDNA) (DiMauro and Schon, 2003, Wallace, 1999), which is maternally inherited. Most mitochondria contain at least two copies of mtDNA and the number of mitochondria in cells range from a few hundred to hundreds of thousands in tissues with high energy demands such as muscle, renal tubular cells and neurons (Chang et al., 2011, DiMauro and Schon, 2003, Robin and Wong, 1988).

Mitochondrial dysfunction contributes to cellular damage. Normal cell metabolism and physiological processes including cytokine signal transduction pathways generate oxygen free radicals and other ROS during oxidative phosphorylation. Under physiological conditions, approximately 1-2% of total molecular oxygen utilized by mitochondria is converted into ROS which include superoxide anions and hydrogen peroxidase (Van der Watt, 2011). Levels can increase dramatically during times of environmental stress. Mitochondria contain antioxidant enzymes that assist in modifying oxidative radicals into innocuous molecules, thereby protecting them against damage from ROS. Examples of antioxidant enzymes include manganese superoxide dismutase and glutathione peroxidase (Mates et al., 1999). These enzyme systems prevent the build up of hydrogen peroxide and limit the formation of potentially more reactive species.

Oxidative stress refers to the consequence of pathological states such as inflammation, where there is a disequilibrium between the production of ROS and the levels of antioxidant enzymes, favouring the oxidant environment (Betteridge, 2000). Oxidative damage is the result of a myriad of biochemical reactions that occur simultaneously. There are two main control mechanisms to mitigate or repair oxidative damage: antioxidant defence mechanisms such as enzymes and scavenger molecules, as well as extensive metabolic pathways to control ROS production. When the control mechanisms are overwhelmed, oxidative damage may lead to dysfunction of many cellular organelles. This damage may be cumulative and not amenable to repair, particularly in postmitotic cells such as neurons (Galasko and Montine, 2010).

Reactive oxygen species can cause damage or mutations to DNA or ribonucleic acid (RNA), including mtDNA (Wei and Lee, 2002). The accumulation of mtDNA mutations results in further increases in ROS, which in turn may increase tissue susceptibility to accumulating mtDNA (Finkel and Holbrook, 2000). At a critical point this will impact on the effective functioning of mitochondria. Mitochondrial ROS may also act as signalling molecules to

University of Cape Town

trigger pro-inflammatory cytokine production (Nakahira et al., 2011). Pro-inflammatory cytokines generate high levels of ROS, which can cause disruption in the normal balance between antioxidants and ROS, shifting cells into a state of oxidative stress. This cycle is believed to be a critical factor for the exponential increase in oxidative damage, accumulation of somatic mitochondrial mutations and subsequent loss of cellular functions during ageing and in diseases associated with mitochondrial cytopathies.

1.8.1 The effect of antiretroviral therapy on mitochondrial DNA

The antiretroviral activity of NRTI drugs is determined by their capacity to competitively inhibit the RNA-dependent DNA polymerase-γ activity of HIV reverse transcriptase, which synthesizes viral DNA from deoxynucleotide triphosphates (Nolan et al., 2003). NRTIs undergo triphosphorylation by intracellular kinases (Lewis et al., 2003) and the active tri- (or di-) phosphorylated NRTIs compete with endogenous deoxynucleotide triphosphates for incorporation by DNA polymerase-γ. When incorporated into the viral nucleic acid chain it prematurely terminates the replicating viral strand due to the absence of a 3' hydroxyl group for addition of the next nucleotide (Lewis et al., 2003, Kakuda, 2000).

The degree of toxicity associated with NRTI incorporation has been shown to correlate with several factors such as cellular uptake, metabolic activation, the rate and strength of binding with host DNA polymerase-γ as well as the kinetics of drug removal (Feng et al., 2001, Johnson et al., 2001). Accordingly, NRTIs have been ranked by their potential toxicity to inhibit DNA polymerase-γ: zalcitabine (ddC) > didanosine (ddI) > stavudine (d4T) > lamivudine (3TC) > tenofovir (TDF) > zidovudine (AZT) > abacavir (ABC), respectively (Lewis et al., 2003). Once the NRTI is incorporated into the mtDNA, it stalls further replication until it is removed by the proofreading exonuclease. Stavudine has the slowest rate of exonuclease removal compared to other NRTIs, suggesting hindrance of repair and compounding damage to mtDNA (Lewis et al., 2003).

Figure 1.3 is a schematic representation of possible mechanisms through which NRTIs can affect mtDNA and mitochondrial gene expression. Apart from their direct effect on mtDNA polymerase-γ [1], *in vivo* evidence suggests that NRTI exposure may influence the transcription of host mitochondrial RNA [2]. Mallon et al. reported significantly decreased mitochondrial gene expression in adipose tissue and blood monocytes after two weeks on stavudine- or zidovudine-containing regimens without any significant depletion in mtDNA

University of Cape Town

content or metabolic parameters such as serum lactate (Mallon et al., 2005a). *De novo* synthesis of pyrimidine nucleotides is coupled to optimal functioning of the mitochondrial respiratory chain (Ruckemann et al., 1998). Therefore, any cause of respiratory chain dysfunction leads to decreased mitochondrial ATP regeneration, which in turn impairs pyrimidine synthesis as well as its nucleotide phosphorylation (Gattermann et al., 2004), further compromising the cycle of mitochondrial dysfunction [3 and 4]. NRTIs may also compete with endogenous nucleosides for phosphorylation by mitochondrial thymidine kinase-2 and may even inhibit its activity in the mitochondria [5]. These factors reduce levels of endogenous nucleotides resulting in the decreased synthesis of mtDNA (Cote, 2005, Lynx et al., 2006). Mitochondrial DNA depletion has been reported in fat, muscle and nerve tissue of HIV-infected individuals experiencing drug-related symptoms of lipoatrophy, myopathy and DSP, respectively (Arnaudo et al., 1991, Dalakas et al., 2001, Shikuma et al., 2001).

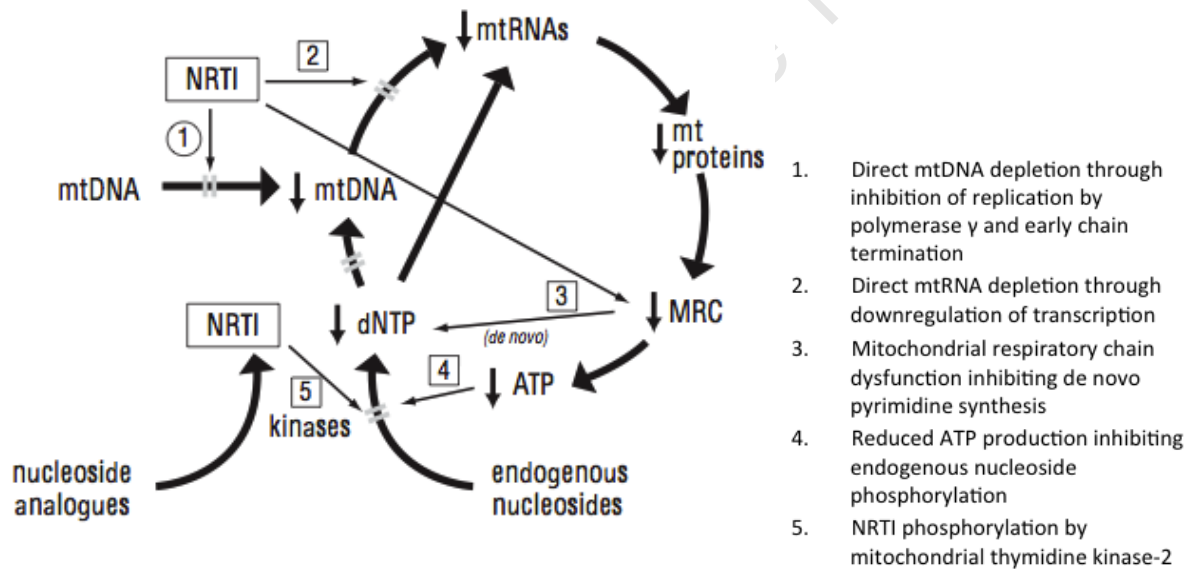


Figure 1.3: Mechanisms by which NRTI affect mtDNA and mtRNA (From Cote, 2005)

Much recent attention has been paid to quantifying mtDNA levels. However, the quality of mtDNA is also important, but technically challenging to evaluate. It is well documented that mtDNA mutations and deletions accumulates over time, and play a role in conditions associated with ageing (Payne et al., 2011, Gianni et al., 2004, Alexeyev et al., 2004). As summarized, NRTI therapy provides conditions permissive for the accumulation of mtDNA mutations, which has been reported longitudinally in peripheral leukocytes, axons and skeletal muscle of individuals exposed to NRTIs (Martin et al., 2003, Lehmann et al., 2011,

Maagaard et al., 2006). Large mtDNA deletions have been reported in some cases of NRTI-associated lactic acidosis (Bartley et al., 2001, Walker and Venhoff, 2001). Reduced mitochondrial gene expression has been demonstrated in adipocytes exposed to NRTIs without significant depletion of mtDNA (McComsey et al., 2008, Mallon et al., 2005b).

Although at this stage it is unknown whether these deletions occurred prior to HIV infection and/or its therapy, the presence of such deletions would, in all likelihood, diminish the mitochondria's capacity to compensate during NRTI therapy. This may possibly accelerate or exacerbate the development of symptoms related to mitochondrial toxicity.

1.8.2 Clinical manifestations of mitochondrial dysfunction

Certain principles govern the manifestation of mitochondrial cytopathies whether as a result of mitochondrial dysfunction or mtDNA mutations/deletions. Firstly, due to the high reserve of oxidative capacity inherent in most tissues, a critical threshold of dysfunctional mitochondria need to be present before a tissue manifests with mitochondrial failure (Van der Watt, 2011). Mitochondrial dysfunction will manifest earlier in the most metabolically active tissues such as nerves, muscle and liver. Secondly, within any cell or tissue there are heterogeneous populations of mtDNA with sequence variations known as heteroplasmy. Differences in the tissue distribution or tissue load of abnormal mtDNA will influence phenotypic manifestations (DiMauro and Schon, 2003). Further, the manifestation of mitochondrial cytopathies is not only determined by the functional magnitude of the genetic mutation, but also by environmental stressors applied to that tissue (Look et al., 2001). For example, the effects of silent genetic variants (e.g. somatic mtDNA mutations) may be unmasked only in the presence of significant environmental insults such as HIV infection or treatment with drugs that are potentially toxic to mitochondria. As such, a wide range of adverse events occurring in individuals with HIV infection, particularly those receiving NRTI-based therapy, have been suggested as relating to compromised mitochondrial function, either by accumulating mtDNA deletions or through mtDNA depletion (Lewis, 2005).

Mitochondrial cytopathies may result in the following clinical manifestations, in no particular order: peripheral neuropathy, myopathy, seizures, ataxia, fatigue, weight loss, nausea and vomiting, abdominal pain, cardiomyopathy, Fanconi syndrome, pancreatitis, hepatic steatosis and death (Carr and Cooper, 2000, Brinkman et al., 1998). Biochemically, mitochondrial

University of Cape Town

dysfunction can present as hyperlactatemia, compensated lactic acidosis or, rarely, as acute decompensated lactic acidosis with a high mortality (Lewis, 2005).

Despite well-documented *in vitro* studies on mtDNA polymerase-γ inhibition by NRTIs, there has been no consistent correlation between mtDNA levels and manifestations of mitochondrial toxicity. For example, one study found that stavudine therapy did not decrease peripheral blood mononuclear cell (PBMC) mtDNA or -RNA levels (Casula et al., 2004). Another found no difference in mtDNA levels in blood and subcutaneous fat between individuals with or without peripheral neuropathy or lipoatrophy (Lewis, 2005). Several factors may have contributed to the paucity of evidence implicating NRTI-related mitochondrial toxicity with mtDNA depletion. The majority of reports to date have studied relatively small observational cohorts, often through cross-sectional analysis, thereby limiting the conclusions that can be reached [reviewed in (Cote, 2005)]. Another issue is the potential time lag between the "event" resulting in mitochondrial damage and the clinical manifestation of the toxicity. Also, risk factors including mitochondrial haplotypes may render an individual more susceptible to NRTI-related mitochondrial damage. Overall, poor correlation between mtDNA levels and the clinical manifestation of mitochondrial toxicity strongly suggest additional risk factors to the development of mitochondrial damage and dysfunction.

1.8.3 Host factors influencing susceptibility to mitochondrial dysfunction

Not all individuals treated with NRTIs develop adverse effects related to mitochondrial toxicity, suggesting that host factors influence susceptibility towards mitochondrial dysfunction (Maagaard et al., 2006). These may include differences with respect to intracellular phosphorylation of NRTIs, drug–drug or drug–nutrient interactions, female sex, white race, older age, hepatitis B and C co-infection, nadir CD4 T-cell counts and altered renal function (Lichtenstein et al., 2003, Galli et al., 2003, Fleischer et al., 2004, Currier, 2007, Bonnet et al., 2003, Gervasoni et al., 1999).

Mitochondrial dysfunction is increasingly postulated as an important component in the pathogenesis of neural damage by HIV. Peripheral nerves are first damaged directly by viral proteins such as gp120 or sensitized by the aberrant inflammatory response associated with HIV infection. Neurons are then further compromised by NRTI-induced mitochondrial

University of Cape Town

toxicity. The susceptibility to mitochondrial damage may be dependent on factors previously described in the literature and is briefly outlined further in this section:

1.8.3.1 Sex

Lactic acidosis in stavudine-exposed patients has been reported more frequently in women (Bolhaar and Karstaedt, 2007, Moyle et al., 2002, Arenas-Pinto et al., 2008) with a particular increased risk in obese African women (Currier, 2007, Boulle et al., 2007). Women might also be more susceptible to NRTI-associated lipodystrophy (Gervasoni et al., 1999, Galli et al., 2003).

1.8.3.2 Age

Senescence has been the most consistent factor shown to be an independent predictor for developing HIV-associated DSP (see Table 1.1 and Table 1.2). Senescence is generally associated with cumulative somatic mtDNA mutations (Chan, 2006, Payne et al., 2011). As oxidative phosphorylation in the mitochondria contributes to the bulk of free radicals and other ROS, senescence is the result of accumulation of macromolecular damage from oxidative stress (Harman, 2001). Another theory of senescence also involves the mitochondria. The lysosomal-mitochondrial axis theory explains the accumulation of undegradable oxidative products of oxidative stress that contribute to the ageing of cells due to imperfect lysosomal degradation of damaged cells or structures (Brunk and Terman, 2002).

Accelerated senescence has recently been described in HIV-infected individuals on NRTI therapy. Rapid clonal expansion of somatic mtDNA mutations provides a plausible mechanism for accelerated ageing in NRTI-treated HIV infection (Payne et al., 2011). Further, clinical complications of NRTI therapy appear to be more common in older individuals (Smyth et al., 2007, Robinson-Papp et al., 2012). This may be due to older individuals harbouring a greater number of age-related somatic mtDNA mutations than younger individuals, which rapidly clonally segregate during NRTI therapy (Payne et al., 2011). For these reasons, the consequences of age-related mtDNA mutations might be even more challenging in the future as individuals treated with cART not only become older, but also have a long cumulative exposure period to NRTIs, especially in resource-poor settings where few more affordable options exist.

University of Cape Town

1.8.3.3 Genetic predisposition

Certain mitochondrial haplogroups have been associated with increased risk for mitochondrial dysfunction. Individuals belonging to mitochondrial haplogroup T, best characterized as a European mitochondrial lineage, had a marginally higher incidence of NRTI associated neuropathy (Hulgan et al., 2005). This was demonstrated in a cART exposed Caucasian case-control cohort where 17% of those who developed DSP (defined as the presence of at least one neuropathic symptom or sign) had mtDNA haplogroup T compared to 7% of those who did not develop DSP (Hulgan et al., 2005). African mtDNA generally have greater variation than that of Caucasian populations and thus present a more challenging classification. This may be one reason why fewer association studies have been performed and less is known about functional differences in African mtDNA variation. In a self-identified non-Hispanic black American cohort, the African mtDNA subhaplogroup L1c was an independent predictor of DSP (Canter et al., 2010). However, there are no published data from African cohorts to support these results.

The role of genetic predisposition was also demonstrated *in vitro* using fibroblasts from patients with Kearns-Sayre syndrome, a mitochondrial cytopathy due to mtDNA deletion mutations. Increased mtDNA mutations and depletion of mtDNA levels occurred with zidovudine treated fibroblasts (Wang et al., 1996). Similarly, subclinical Leber's hereditary optic neuropathy, a rare cause of blindness due to inherited mitochondrial mutations, manifested in HIV-infected individuals upon starting NRTI therapy (Mackey et al., 2003).

1.8.3.4 Metabolic syndrome

In recent years there has been increasing research focusing on the potential role of oxidative stress in the metabolic syndrome, defined as the presence of three or more of hypertension, dyslipidaemia, dysglycaemia and obesity. There is evidence to support the theory that a chronic inflammatory state may play an important role in metabolic syndrome manifestations such as hypertension, diabetes and obesity (Urakawa et al., 2003, Ford et al., 2003, Ceriello and Motz, 2004). The resultant oxidative stress is believed to be an early event in the pathology of these chronic diseases and not simply a consequence or an innocent bystander (Roberts and Sindhu, 2009).

The metabolic syndrome was an independent risk factor for small fibre neuropathy in a retrospective mixed neuropathy cohort (Zhou et al., 2011). Elements of the metabolic

University of Cape Town

syndrome (hypertriglyceridemia and diabetes) were found to be associated with HIV-associated DSP (Letendre et al., 2009, Banerjee et al., 2011, Ances et al., 2009). High triglyceride levels might lead to alterations in mitochondrial membrane permeability and energy metabolism, although the mechanism remains unclear. It is thought that hypertriglyceridemia activates mitochondrial K^+ ATP channels resulting in increased resting oxidative metabolism (Alberici et al., 2003). This promotes high levels of ROS generation and axonal injury. Further, hypertriglyceridemic individuals, with or without metabolic syndrome, have increased levels of serum lipoperoxides and low levels of antioxidant enzymes (Cardona et al., 2008).

Therefore, both HIV infection and cART may increase the risk for the metabolic syndrome as a result of inflammation and lipid abnormalities. With ageing, metabolic risk factors accumulate, further increasing the risk in a cART treated population (Letendre et al., 2009). Mitochondrial dysmetabolism may be central to all these factors.

1.8.3.5 Nutrition

Underlying nutritional deficiencies may predispose an individual to mitochondrial NRTI toxicity through two mechanisms (Look et al., 2001). Firstly, several nutrients are imperative elements of mitochondrial flavoproteins and cytochromes, and are therefore needed for mitochondria to function normally (Ames, 2004). Secondly, malnutrition increases the vulnerability to oxidative stress due to impaired antioxidant defence mechanisms. Furthermore, it has previously been shown that oxidative stress stimulates HIV replication *in vitro* (Allard et al., 1998) and that multivitamin supplements may reduce viral replication and delay the progression of HIV disease (Fawzi et al., 2004). Absorption and metabolism of micronutrients and vitamins (e.g. vitamin B6, B12 and iron) are abnormal in chronic HIV infection, and therefore the prevalence of deficiencies may be higher in HIV-infected individuals (van Lettow et al., 2003, Karyadi et al., 2000, Tang and Smit, 1998).

General poor nutritional status has been reported to increase the risk of ATN, although these factors are not well characterized (Keswani et al., 2002, Tagliati et al., 1999). Severe deficiencies of vitamin E, vitamin B6, thiamine, riboflavin, nicotinic acid and iron have been associated with peripheral neuropathy in HIV-infected individuals (Kumar, 2007). The association between vitamin B6 and DSP is reviewed in sections 1.9.1 and 1.9.3.

University of Cape Town

1.8.3.6 HIV infection

Decreased levels of mtDNA may be present in HIV infected individuals preceding the use of NRTI therapy or symptoms. In individuals with untreated HIV infection, samples from various tissues show reductions in mtDNA levels compared to age-matched HIV-negative individuals (Cote et al., 2002, McComsey et al., 2002, Morgello et al., 1995). For example, nerve and muscle biopsies from untreated individuals presenting with HIV-associated DSP or myopathy may have low mtDNA levels, abnormalities in mitochondrial ultrastructure or show *in vitro* respiratory chain functional abnormalities (Morgello et al., 1995). These findings are similar to those reported with NRTI therapy in nerve and muscle cells. Longitudinal measures of mtDNA in HIV-infected individuals showed reduced levels in PBMCs compared to HIV-negative individuals. For example, a 43% difference was noted prior to initiating NRTI therapy, with a more pronounced reduction after starting NRTIs in HIV-infected individuals compared to HIV-negative individuals (Cote et al., 2002).

HIV can directly influence mitochondrial function by activating mitochondrial pathways for apoptosis (Van der Watt, 2011). There are two main apoptotic signalling pathways known as the 'extrinsic' and the 'intrinsic' pathways of apoptosis (Tischner et al., 2010). HIV is implicated in both of these pathways. The extrinsic pathway is activated through exogenous factors such as viral proteins "Negative regulatory factor" (Nef), Trans-activator of transcription (Tat), and gp120, all of which may bind to cell membrane receptors such as the Fas receptor (or 'death receptor'). This ligation activates cytoplasmic caspase through a cytoplasmic signalling pathway resulting in cell apoptosis. The intrinsic pathway receives cues from factors within the cell to facilitate cytochrome C and other pro-apoptotic factors to be released from the mitochondria that in turn result in cytoplasmic caspase activation and apoptosis (Tischner et al., 2010). Activation stimuli for the intrinsic pathway include viral protein R (Vpr), gp-120, Nef and Tat as well as free radicals or cellular DNA damage. HIV induced apoptosis has been demonstrated in neurons, CD4 T-cells, haematopoietic cells and cardiac myocytes (Kaul and Lipton, 1999, Rasola et al., 2001).

Apart from these direct viral effects, persistent HIV-associated immune activation might also indirectly impact on mtDNA synthesis in cART naive individuals. Casula et al. found reduced levels of mtDNA in PBMCs after seroconversion (Casula et al., 2004). Increased rates of mitochondrial apoptosis induced by pro-inflammatory cytokines, such as TNF-α,

University of Cape Town

IFN-α and IFN-γ, IL-2 and IL-6, were postulated as a cause for the decreased mtDNA levels (Shedlock et al., 2008).

Alternatively, HIV-induced chronic inflammation and immune activation is capable of causing oxidative stress through an increase in ROS and a reduction in antioxidant levels (Bogden et al., 1990, Buhl et al., 1989, Dworkin et al., 1986, Eck et al., 1989). Studies in HIV-infected individuals have shown significantly higher oxidative stress determined by increased breath-alkane output and plasma lipid peroxide concentrations, as well as lower concentrations of key plasma antioxidants (glutathione, ascorbic acid, alpha tocopherol, beta carotene and selenium) compared to non-HIV infected individuals (Constans et al., 1995, Delmas-Beauvieux et al., 1996, Kalebic et al., 1991).

In summary, HIV can have a direct impact on mitochondrial function and can also indirectly affect mtDNA levels and mutation load via cytokine release in response to HIV infection. These mechanisms potentially increase the vulnerability of mitochondria to the effects of NRTI therapy.

1.8.3.7 Stage of HIV infection and comorbidities

Emphasis has also been placed on the association between stage of HIV infection and mitochondrial dysfunction. Advanced disease with nadir CD4 T-cell counts of less than 100 cells/mm^3 has been associated with lipoatrophy as well as DSP (Moyle, 2000, Lichtenstein et al., 2003). Studies have demonstrated significantly higher levels of serum TNF-α, soluble TNF receptor II, and interferon (IFN)-γ production with lower CD4 T-cell counts, particularly when less than 100 cells/mm^3 (Hestdal et al., 1997, Godfried et al., 1993). Therefore it has been speculated that mitochondrial dysfunction could be attributed to an increased susceptibility to cell damage due to advanced HIV with subsequent higher levels of these cytokines (Anderson et al., 2004).

Co-morbidities and concomitant medication may increase the risk for mitochondrial dysfunction. For example, HIV-infected patients co-infected with hepatitis C and treated with ribavirin together with ddI, have a substantially increased risk for lactic acidosis (Bani-Sadr et al., 2005, Butt, 2003, Moreno et al., 2004). Two cross-sectional community-based African studies have shown that tuberculosis (TB)-HIV co-infection and/or a history of previous TB infection in individuals on cART were independently associated with an increased risk of DSP (Forna et al., 2007, Maritz et al., 2010). Although there are many reasons why TB-HIV

University of Cape Town

co-infected patients might develop DSP, such as advanced HIV disease with poor nutrition and malabsorption, TB, anti-tuberculosis treatment or cART, DSP is mainly observed in patients prescribed INH with one of the d-drugs, d4T or ddI (Breen et al., 2006, Dean et al., 2002, Westreich et al., 2009). INH has been shown to generate free radicals *in vitro* (Sipe et al., 2004), thereby increasing the risk for mitochondrial dysfunction by increasing ROS.

1.8.3.8 Factors specific to neurons

In peripheral nerves, numerous factors may contribute to the increased susceptibility to oxidative stress and cell damage:

- Neurons are solely dependent on oxygen and glucose for energy and therefore generate ROS in abundance (Romero et al., 1991). As neurons rely mostly on aerobic energy production, processes that disrupt oxidative phosphorylation will have an effect on neuronal function (Brinkman et al., 1998). Impaired antioxidant defences through malnutrition or HIV infection can perturb the normal redox balance leading to oxidative stress and cell damage (Price and Thio, 2010).
- The metabolism of unmyelinated axons is energy-intensive. Even though unmyelinated axons have a higher density of mitochondria, they are at an energy disadvantage compared to myelinated axons due to the inefficient manner of action potential conduction (Lehmann et al., 2011, Ritchie, 1973, Wang et al., 2008). In the event of a local energy deficit (i.e. ATP depletion), Na^+/K^+ ATPase failure can lead to reverse flow of calcium through the Na^+/Ca^{2+} exchanger, which may lead to axonal degeneration (Lehmann et al., 2011).
- The peripheral nervous system is particularly sensitive to damage from oxygen radicals. This is as a result of the high levels of polyunsaturated lipids that comprise the uniquely specialized neuronal membranes (Romero et al., 1991). Data from animal models demonstrate that the oxygen tension of the peripheral nervous tissue is relatively low compared with other tissues. It is possible that the decreased oxygen partial pressure increases the risk for hypoxia-induced free radical generation. Nerves are at risk for the effects of nitric oxide- and ROS as antioxidant levels are lower than in other tissues (Romero et al., 1991). For example, the concentration of brain glutathione, a major cellular antioxidant, is 10-fold higher than that in sciatic nerve (Romero et al., 1991).

University of Cape Town

- It is believed that length-dependency of many peripheral neuropathies are to some extent a consequence of distal axons being the most distant from the cell body, akin to the last field of an irrigation system getting the least "nutrients" from the cell body (Lehmann et al., 2011). As the site of mitochondrial biogenesis of peripheral nerves are in the dorsal root ganglion cell bodies, somatic mtDNA mutations accumulate and increase as they move distally down the axon over time (Lehmann et al., 2011). Therefore, with ageing, the distal axons are probably more vulnerable to mitochondrial dysfunction.

To summarize, both NRTIs and HIV infection itself can directly or indirectly influence mitochondrial function in a number of tissues; directly by activating mitochondrial apoptotic pathways triggering cell death, or indirectly through immune activation and cytokine production. NRTIs can directly or indirectly interfere with mtDNA integrity as described in section 1.8.1. Variations in tissue vulnerability to oxidative stress, including antioxidant defence and nutrition, inflammatory response related to HIV infection and nerve injury repair mechanisms may all influence the neurotoxicity of NRTIs. Therefore, it is not surprising that the clinical manifestations of HIV pathology and NRTI-induced mitochondrial toxicity should overlap in a number of tissues with high energy demands, including peripheral nerves.

1.8.4 Can mitochondrial toxicity be measured?

With the widespread use of d-drugs in cART regimens, and the documentation of cases of fatal lactic acidosis, researchers looked for biomarkers that could possibly predict mitochondrial toxicity. Mitochondrial oxidative phosphorylation normally removes protons generated by the hydrolysis of ATP during the conversion of glucose-6-phosphate to pyruvate. Dysfunctional oxidative phosphorylation leads to proton accumulation and lactic acidosis. Therefore, blood lactate was investigated as a potential biomarker for mitochondrial dysfunction and extensively measured and monitored in large cohorts of individuals on cART regimens. A number of studies found a twofold increased risk of lactataemia with d4T- and ddI- versus AZT-based regimens [reviewed in (White, 2001)]. However, the data are inconsistent in ATN. Increased serum lactate levels were found in some patients with ATN (Estanislao et al., 2004b), but others have not found such an association (Maritz et al., 2010, Simpson et al., 2010, Simpson et al., 2006). One explanation for these findings may be that serum lactate lacks specificity and is influenced by technical and physiologic variability (Brinkman, 2001). To this end, early studies showed that more than 85% of HIV-infected

University of Cape Town

adults on NRTI therapy with hyperlactatemia were symptom-free (Boubaker et al., 2001, Brinkman, 2001, Gerard et al., 2000, John and Mallal, 2002, John et al., 2001, Moyle et al., 2002).

Studies have also used markers of oxidative stress to demonstrate mitochondrial dysfunction. Increased levels of prostaglandin F2-like products, termed F2-isoprostanes, were shown in NRTI-treated individuals with symptomatic hyperlactatemia, lactic acidosis, or lipoatrophy (McComsey and Morrow, 2003). However, DSP was not associated with increased systemic oxidative stress as assessed by quantification of F2-isoprostanes in plasma (Hulgan et al., 2006). It is possible that oxidative stress related to nerve damage or dysfunction may be localized and therefore not of sufficient magnitude to alter systemic lipid peroxidation profiles.

1.8.5 A summary of the destructive cycle of mitochondrial damage in the context of HIV-infection

Oxidative stress as a result of an imbalance between ROS and antioxidants may contribute to mtDNA damage, which in turn increases ROS production and leads to further oxidative damage. Ageing or cellular senescence results in ROS and free radical damage to the mtDNA (Payne et al., 2011). Furthermore, host factors may contribute to increased susceptibility to free radical mtDNA damage, possibly certain mtDNA haplogroups. Malnutrition and co-morbid infections such as TB, which commonly occur in HIV-infected individuals, leads to an increase in oxidative stress. There is increasing evidence that HIV itself can directly and indirectly affect mtDNA and its function (Morgello et al., 1995, Van der Watt, 2011), predisposing individuals to mitochondrial toxicity (Lewis et al., 2003). Finally, it is well accepted that NRTIs inhibit the function of mitochondrial DNA polymerase-γ, leading to mtDNA damage (Lynx et al., 2006). In addition, the rapid clonal segregation of mtDNA mutations after NRTI therapy leads to accelerated ageing and increased ROS.

Figure 1.4 shows the interactive effect of the three main factors resulting in mitochondrial damage. Cumulative effects of HIV-infection, oxidative stress and NRTIs on mtDNA damage eventually impair mitochondrial function to a critical threshold, which then further augments oxidative stress and mtDNA damage. NRTI-containing regimens in predisposed individuals such as older or malnourished individuals thus provide the context for multiple insults to the mitochondria (Figure 1.4).

University of Cape Town

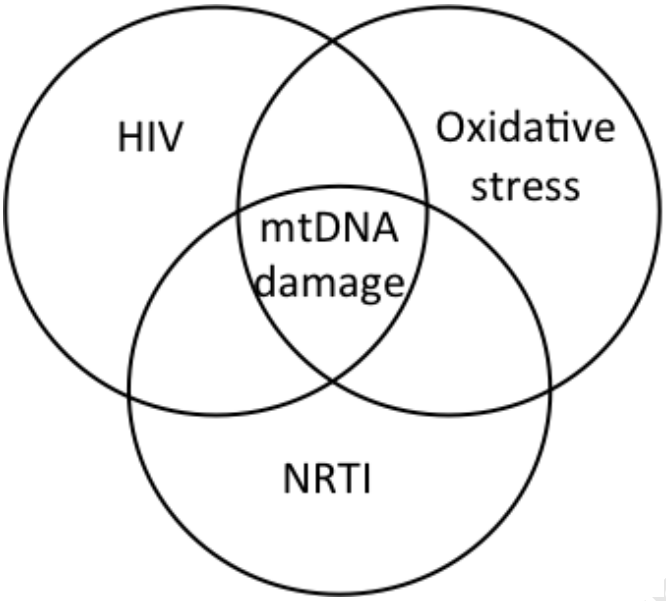


Figure 1.4: Cumulative effects of HIV-infection, oxidative stress and NRTIs on mtDNA damage (From Cote, 2005)

University of Cape Town

1.9 Risk factors for the development of HIV-associated DSP

Prior to the introduction of cART, studies examining the risk factors for HIV-DSP consistently found surrogate markers of advanced HIV disease such as low CD4 T-cell count, higher viral load, or *Mycobacterium avium* infection, to be associated with HIV-DSP (Woolley et al., 1997, So et al., 1988, Barohn et al., 1993).

In the cART era, identified risk factors have been less consistent. Factors associated with an increased risk of ATN have included increasing age, lower CD4 T-cell counts and higher HIV viral load at the time of cART initiation (representing more advanced HIV disease) as well as dNRTI exposure (Morgello et al., 2004, Maritz et al., 2010, Lichtenstein et al., 2005). A summary of clinical studies examining risk factors associated with the development of HIV-associated DSP in the cART era are summarized in Table 1.1 and Table 1.2. These data should be interpreted cautiously as various factors limit comparisons such as the use of varying diagnostic criteria and variable periods of observation. One of the most consistent risk factors for HIV-associated DSP has been increasing age. However, one study reported that 5-year increments of older age was only associated with the presence of DSP, but did not predict a worsening in TNS score over 48 weeks (Simpson et al., 2006). It may be argued that

a follow-up time period of 48 weeks was insufficient time to reveal any worsening in TNS score.

Earlier studies reported higher HIV load and lower CD4 T-cell counts to be predictors of DSP, particularly symptomatic DSP. Some found nadir CD4 T-cell count to be a predictor of HIV-DSP, but not the CD4 T-cell count at the time of the cross-sectional analysis (Lichtenstein et al., 2005). Furthermore, Simpson et al. failed to establish a correlation between CD4 T-cell count and HIV viral load and the presence or progression of DSP, although the authors acknowledged that selection bias might have influenced their findings (Simpson et al., 2006).

There are other risk factors that have limited or inconsistent supporting evidence. In first world setting these include white race, female sex, hepatitis C co-infection, plasma lactate level, impaired renal function and concomitant use of hydroxyurea, interferon-α or the protease inhibitor (PI) indinavir. In African cohorts, previous TB infection has recurrently been found to be a significant risk factor for DSP (Forna et al., 2007, Maritz et al., 2010).

It is increasingly evident that HIV-associated DSP is a product of multiple intersecting pathogenic processes (Brew, 2003). Many of the risk factors pertaining to the development of HIV-associated DSP show a definite connection with factors relating to increased risk of mitochondrial dysfunction (section 1.8.3). These factors include increased age, metabolic syndrome, stage of HIV and consequences of immune dysregulation, NRTI therapy, malnutrition and co-morbidities such as TB.

Particular attention will now be given to TB infection and treatment, malnutrition, NRTI therapy and HIV immune dysregulation as risk factors for the development of HIV-associated DSP. These are areas of interest for the different study aims.

1.9.1 Tuberculosis and low vitamin B6 as risk factors for HIV-associated DSP

TB remains a major cause of morbidity and mortality worldwide. Globally, 9.4 million new TB cases occurred in 2009 of which 15% were also HIV-positive. The majority of new cases occur in South-East Asia and sub-Saharan Africa with the incidence rate of new disease in sub-Saharan Africa estimated at 360 cases per 100 000 population, nearly twice that of South-East Asia (WHO, 2012). Thirteen of the 15 countries with the highest estimated TB incidence rates are in Africa. In certain areas of South Africa such as the Western Cape

University of Cape Town

Province, the TB incidence is >900 per 100 000 population (Wood et al., 2007). Southern Africa is the epicentre not only of a TB epidemic, but also of the HIV/AIDS epidemic. The African region accounted for 79% of HIV-positive TB cases, of which 31% were from South Africa (WHO, 2012). HIV/TB-co-infection probably accounts for the high rates of both new and recurrent TB disease as well as the morbidity and mortality associated with TB despite the availability of effective anti-TB chemotherapy (Harries et al., 2001).

Peripheral neuropathy is a well-known adverse event associated with anti-TB chemotherapy with a documented frequency ranging from 2% amongst HIV-negative subjects, to 14% amongst those with concomitant HIV-infection (Marks et al., 2009, Breen et al., 2006). However, our understanding of the pathophysiology of this complication is limited (Argov and Mastaglia, 1979). INH is considered the predominant culprit due to the interference with vitamin B6 metabolism causing deficiency in biologically active vitamin B6 (Snider, 1980, Van der Watt et al., 2011). However, inflammation related to TB itself may play a role. The role of TB inflammation, INH therapy and vitamin B6 will be discussed further:

1.9.1.1 TB inflammation

The presence of TB-associated DSP is not well described (Paterson, 1913, Money, 1959), but has been observed in a prospective cohort of individuals co-infected with HIV, where neuropathic symptoms develop prior to initiating INH therapy (Centner, 2012). Although this observation was based on patient recall, it is possible that the systemic inflammation accompanying TB infection drives oxidative stress and exacerbates HIV-related nerve injury and that the phenomenon of TB-associated DSP may be underreported due to INH-related neuropathy obscuring its incidence.

1.9.1.2 Isoniazid

Isoniazid is a pivotal agent in the treatment of TB in combination with other drugs or alone as a prophylactic agent. Following the introduction of INH in 1952, numerous observers noted the occurrence of a painful peripheral neuropathy temporally related to INH initiation. Since then it has been recognised as the main contributor to DSP in patients on anti-TB treatment (Biehl and Sklavem, 1953, Jones and Jones, 1953, Lubing, 1953, Mandel, 1959, Snider, 1980).

University of Cape Town

INH is metabolised extensively in humans (Hughes et al., 1954). The primary metabolic pathway is acetylation of the hydrazide functional group by the drug metabolizing enzyme, *N*-acetyltransferase type 2 (NAT2), which is under the genetic control of a polymorphic *NAT2* locus (Blum et al., 1991, Evans et al., 1960). The acetylation of acetylhydrazine to diacetylehydrazine is also mediated by the NAT2 enzyme (Figure 1.5) (Preziosi, 2007).

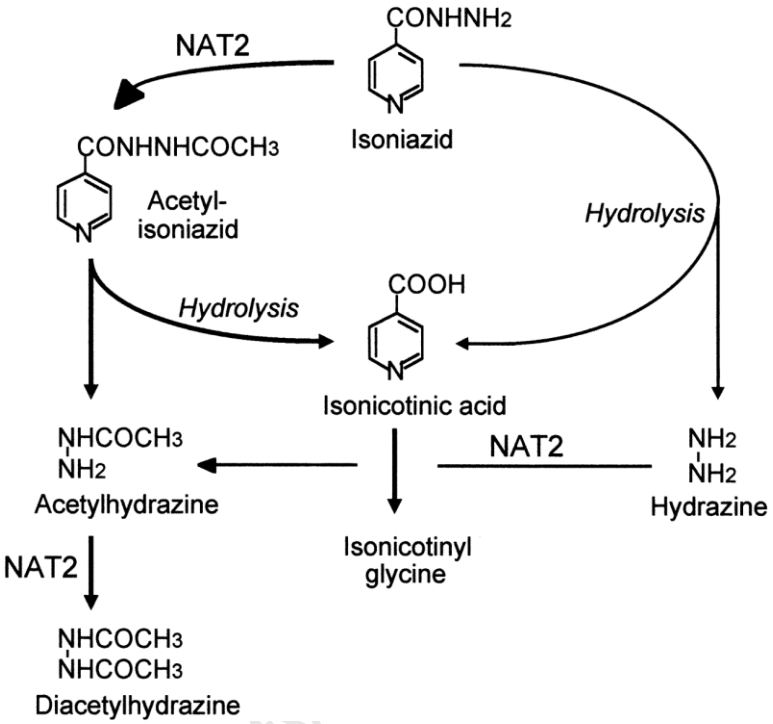


Figure 1.5: INH metabolism via NAT2

1.9.1.3 N-acetyltransferase 2

Human *NAT2* has been localized to the short arm of chromosome 8 (8p22). This gene has two exons but the coding region, spanning 870 base pairs (bp) is located in exon 2 and the functionally active NAT2 enzyme can be transcribed after transient heterologous transfection of exon 2 (Hein et al., 2000).

At least 52 different *NAT2* allelic variants have been identified (University of Louisville, 2011). The ancestral *NAT2*4* haplotype is associated with functionally rapid acetylation status and fourteen other *NAT2* haplotypes consist of a combination of one single nucleotide polymorphism (SNP) or more, occurring at positions 191, 282, 341, 434, 481, 590, 803, 845, and 857 of the 870 bp *NAT2* coding region. Seven of the nine SNPs (191 G>A, 341 T>C, 434 A>C, 590 G>A, 803 A>G, 845 A>C, and 857 G>A) result in amino acid changes (non-

synonymous), whereas two (282 C>T and 481 C>T) do not (synonymous). Except for 341 T>C, each of the nucleotide substitutions either adds or deletes an endonuclease restriction site (University of Louisville, 2011).

Individuals are rapid, intermediate or slow acetylators depending on their ability to acetylate certain NAT2 substrates. Acetylation status has traditionally been differentiated based on phenotyping tests, performed with different substrates including caffeine and INH (Kinzig-Schippers et al., 2005). More recently, genotyping enabled the identification of 52 *NAT2* alleles (University of Louisville, 2011). Individuals phenotyped as rapid acetylators carry two alleles associated with high acetylation activity (e.g. *NAT2*4* and *NAT2*12*), intermediate acetylators carry one allele associated with high activity, while slow acetylators have two alleles associated with low acetylation activity. Several combinations of slow acetylator alleles may give rise to the slow acetylation phenotype. Correlative studies between *NAT2* genotypes and INH pharmacokinetics have shown that INH plasma concentrations are higher (up to 6-fold higher at short intervals) in those individuals homozygous for low-activity associated alleles at the *NAT2* locus (Parkin et al., 1997, Kinzig-Schippers et al., 2005). The rate of acetylation is therefore considered a critical determinant of both therapeutic efficacy and toxicity, with slow acetylators more susceptible to toxicity, but also more likely to benefit therapeutically.

The frequency of acetylator phenotypes varies considerably among racial populations (Sabbagh et al., 2008, Hamdy et al., 2003). The geographical distribution of common functional variants of *NAT2* has been well studied in both European and East Asian populations (Upton et al., 2001). In most European Caucasian populations, the proportions of slow and fast acetylators are equal, whereas in Alaskan Inuits the majority (95%) are fast acetylators; Saudi Arabians are generally slow acetylators (95%). In contrast, data from Sub-Saharan Africa are limited (Dandara et al., 2003, Patin et al., 2006). Few reports suggest that indigenous Africans comprise of approximately 59% fast and 41% slow acetylators (Bach et al., 1976, Eidus et al., 1979).

Most reports used simplified protocols for *NAT2* allele detection, omitting analysis of several polymorphic positions (such as 191 G>A, 282 C>T, 341 T>C, and 803 A>G). These focused on a limited number of "indicator" SNPs, thought to be tightly linked with other SNPs and predictive of acetylation phenotype. Allele designation is then based on the presence of the "indicator" SNP. Such incomplete genotyping methods have the potential to incorrectly

University of Cape Town

assign different alleles as the same and may lead to misclassification of genotypes and deduced phenotypes (Cascorbi and Roots, 1999, Garcia-Martin, 2008). For example, the base change 341 T>C which defines the *NAT2*5* allelic cluster, does not always occur together with 481 C>T. Thus, 481 C>T should not be used to estimate the frequency of the *NAT2*5* allele cluster. Furthermore, the 481 C>T change may sometimes be associated with another allelic cluster such as *NAT2*6* (defined by 590 G>A). Therefore, while the method of detecting "indicator" mutations might have been suitable for Caucasian populations, in an African population, it is much more effective to detect individual SNPs. Nonetheless, the fact remains that DNA sequencing for the entire intron-exon organization of the *NAT2* gene will provide the most detailed information about genetic diversity and possible functional effects in a population.

The relevance of establishing *NAT2* functional polymorphisms is that certain phenotypes modify susceptibility to adverse drug reactions (Krishnamurthy et al., 1967, Preziosi, 2007, Matar et al., 2004). Although epidemiological studies have investigated the relationship between *NAT2* phenotype and individual risk for adverse events such as hepatotoxicity on INH (Ohno et al., 2000), *NAT2* genotype (as opposed to phenotype) has not been studied extensively in relation to the incidence of INH-associated DSP. In Hiratsuka et al., all of two cases of INH-associated painful neuropathy occurred in genotypically slow acetylators; however, as it formed part of an 'adverse event' group in the analysis, risk for INH-associated painful neuropathy was not established. Several intermediate and rapid acetylators, who did not experience any INH-related adverse effects, had higher concentrations of INH compared to slow acetylators (Hiratsuka et al., 2002). This observed association between slow acetylation and INH-related adverse reactions may be attributable to a metabolic process unrelated to absolute INH concentrations.

1.9.1.4 Effect of INH on vitamin B6 (pyridoxine) metabolism

Vitamin B6 naturally appears in three forms: pyridoxine, pyridoxal and pyridoxamine. They are enzymatically interconvertible, and are all phosphorylated to pyridoxal-5'-phosphate (PLP) via a phosphokinase (pyridoxal kinase) dependent on ATP, which is abundant in all tissues (Brin, 1978). Absorption by intestinal epithelial cells occurs by a carrier-mediated, pH-dependent mechanism that has saturable and non-saturable components. Absorbed dietary pyridoxine, pyridoxal and pyridoxamine are phosphorylated to enable metabolic trapping. PLP and pyridoxal are the main circulating forms, but they require dephosphorylation for

University of Cape Town

tissue uptake from the circulation (Kumar, 2007). The major catabolite of ingested vitamin B6 is 4-pyridoxic acid (4-PA) and results from oxidation of PLP by either NAD-dependent aldehyde dehydrogenase, found in all tissues, or FAD-dependent aldehyde oxidases, found in liver and kidneys. Pyridoxic acid, 4-PA, is excreted in the urine and indicates recent vitamin intake and does not reflect vitamin stores as it biologically inactive (Brin, 1978). Plasma PLP levels are reduced in renal disease, celiac disease, inflammatory bowel disease, rheumatoid arthritis and in individuals on anti-tuberculosis regimens that include INH (Chiang et al., 2005a, Kumar, 2007, Visser et al., 2004)

Sixty years ago it was recognised that INH-associated DSP was related to a deficiency in biologically active pyridoxine (Biehl and Vilter, 1954). The excretion of urinary pyridoxine doubled during INH treatment, which suggested that INH combines with pyridoxine to form a hydrazone that is excreted in the urine. Although the capacity of pyridoxine to form true hydrazones with INH is still being debated, it is accepted that INH forms a complex with pyridoxine, resulting in increased pyridoxine excretion (Preziosi, 2007).

There are, however, other mechanisms that could contribute to the observed neurotoxicity, including the inhibitory effect of INH on pyridoxine-dependent enzyme systems. The most widely supported view holds that INH combines with PLP via the Schiff base condensation between the aldehyde group of PLP and the free amine group of INH to form pyridoxal isonicotinoyl hydrazine (Buss and Ponka, 2003, Preziosi, 2007). This irreversible reaction prevents the utilisation of pyridoxal by nerve tissue. Pyridoxal sequestration may be extensive enough to provoke polyneuropathy, which can sometimes be reversed by withdrawing INH, administering pyridoxine, or both (Preziosi, 2007).

Others have proposed that INH inhibits phosphorylation of pyridoxine by inhibiting pyridoxal kinase, thereby reducing PLP production (Tower, 1956, Visser et al., 2004). There are multiple enzymatic reactions that depend on PLP as cofactor, including all transaminase reactions, deamination and dehydration of serine to produce pyruvate, desulfhydration of cysteine, decarboxylation of dihydroxyphenylalanine (dopa) to dopamine and 5-hydroxy tryptophan to serotonin, sphingolipid synthesis (an important constituent of myelin structure), and the synthesis of neurotransmitters such as gamma-amino butyric acid, noradrenaline and adrenaline. Consequently, PLP represents an essential enzyme for energy production supplying metabolites to the Krebs tricarboxylic cycle. Function and activity of nervous tissue depend almost exclusively upon energy derived from the oxidative metabolism of

University of Cape Town

glucose. When energy supplies fail, dysfunction and permanent structural damage rapidly follow (Tower, 1956, Marks, 1975). Vitamin B6 is also directly involved in heme synthesis, and vitamin B6 deficiency results in a shortage of heme. Heme deficiency decreases the activity and protein content of mitochondrial complex IV and activates nitric oxide synthetase, leading to increased levels of ROS (Scholnick et al., 1972, Atamna et al., 2001, Ames, 2004). Furthermore, although vitamin B6 is not classified as an antioxidant compound, it has been shown to have effective antioxidant properties, protecting cells against the damage from ROS, thereby slowing the progression of HIV immunodeficiency (Stocker et al., 2003).

Another theory is that vitamin B6 disrupts ion gradients, thus accounting for the periaxonal swellings observed in individuals with INH-associated painful neuropathy (Jacobs et al., 1979). However, the exact mechanism whereby vitamin B6 deficiency causes peripheral neuropathy is unclear.

1.9.1.5 Vitamin B6 supplementation

Humans cannot synthesise pyridoxine, and must therefore obtain this micronutrient from exogenous sources via intestinal absorption. Good sources of pyridoxine in the diet include spinach, carrots, eggs, flour, potatoes, peas, dairy products, meat and fish (Snider, 1980). For normal adults, the recommended daily allowance of pyridoxine is 1–2 mg/day (Snider, 1980, Steichen et al., 2006). Individuals considered to be at risk of developing vitamin B6 deficiency include HIV-infected or malnourished individuals, pregnant and lactating women, patients on pyridoxine antagonists such as cycloserine, hydralazine and penicillamine, alcoholics, diabetics and the elderly. It is recommended that individuals considered at risk for developing peripheral neuropathy due to INH therapy such as those with alcohol dependency, malnutrition, diabetes and HIV infection, receive daily pyridoxine supplementation (South African Department of Health, 2004, CDC, 2009, WHO, 2012). As INH is metabolised in the liver and the acetylation products are excreted by the kidneys, both hepatic and renal dysfunction influence the levels of INH and its metabolites, respectively, increasing the potential of vitamin B6 deficiency (Goldman and Braman, 1972).

The South African national guidelines for the management HIV-infected individuals recommend supplementation of one to two tablets of vitamin B complex preparation(s) if the individual has a poor appetite and/or financial constraints that compromise nutrition (South African Department of Health, 2004). The recommendation in TB/HIV co-infected

University of Cape Town

individuals implies that the amount of vitamin B6 within two tablets of vitamin B complex would be sufficient for INH-associated DSP prophylaxis (South African Department of Health, 2004). However, the amount of vitamin B6 varies according to the manufacturer, but this currently equates to 2–4 mg/day in government-sponsored programmes (Rossiter et al., 2012). South African guidelines do not recommend additional vitamin B6 supplementation in TB/HIV co-infected individuals without evidence of what is described as risk factors such as alcohol abuse, pregnancy, diabetes, epilepsy, or those with evidence of peripheral neuropathy (Van der Watt et al., 2011). However, HIV infection itself is considered a risk factor for INH-associated DSP in guidelines from developed countries, recommending vitamin B6 dosages greater than 10 mg/day (Chan and Iseman, 2002, WHO, 2009, CDC, 2003, JTC, 1998). A previous South African cross-sectional study reported that less than 10% of individuals with TB/HIV co-infection were receiving what might therefore be considered as adequate vitamin B6 supplementation (daily dosage 25 mg/day) (Maritz et al., 2010).

1.9.1.6 Neurotoxicity of INH not only related to vitamin B6 deficiency

Although the precise mechanism of INH neurotoxicity is still not established, the neurotoxic effects may not be limited to those mediated by a functional or overt vitamin B6 deficiency. Some metabolites such as isonicotinic acid, ammonia, hydrazine and other hydrazine derivatives may produce oxygen radicals, which lead to protein damage, and have been implicated in neurotoxicity (Timperio et al., 2005).

Hydrazine is particularly reactive, requiring minutes as opposed to hours to produce measurable quantities of radical species. Importantly, hydrazine metabolism and inactivation is also dependent upon acetylation, and therefore on an individual's acetylator status (Timperio et al., 2005). Production of oxygen radicals may be particularly critical when TB is treated with INH in the context of concomitant HIV infection, which increases oxidative stress in its own right (Allard et al., 1998, Israel et al., 1992).

1.9.2 NRTI exposure as risk factor for ATN

Currently, stavudine (d4T) is the most commonly used backbone antiretroviral drug in resource-limited settings (Bygrave et al., 2011). A WHO survey on cART-use in 23 developing countries undertaken in 2006 revealed that 69% of first-line regimens included stavudine, representing 53% of all patients on cART, or an estimated 851 000 individuals (Renaud-Thery et al., 2007). However, side effects related to its previously described

University of Cape Town

mitochondrial toxicity, including peripheral neuropathy, have led to the discontinuation of stavudine in developed countries in favour of a less toxic alternative. Tenofovir exhibits less side effects (Birkus et al., 2002) and has a long intracellular half-life that allows for formulation as a once daily regimen (together with lamivudine and efavirenz) (Grim and Romanelli, 2003). In randomized trials, tenofovir has demonstrated comparable or greater antiviral efficacy and favourable tolerability compared with other first-line NRTIs such as stavudine, zidovudine or abacavir (Gallant et al., 2004, Pozniak, 2008). Therefore, the WHO revised its cART guidelines in late 2009 to withdraw stavudine. Many countries in southern Africa adopted tenofovir in their first line cART regimens (Ford and Calmy, 2010). South Africa followed in April 2010. However, stavudine is still used in South Africa and elsewhere in resource limited settings and can thus still have a considerable impact on the prevalence of ATN. The incidence of treatment-related neurotoxicity appears to be dose-dependent, for example, in a retrospective study, stavudine at 80 mg/day vs 60 mg/day increased the odds of ATN by a factor of 1.7 (Lichtenstein et al., 2005).

However, not all studies have shown an association between the use of dNRTIs and the development of neuropathy. Although earlier studies intimated a relationship between duration of therapy to the development of neuropathy (Boulle et al., 2007, Moyle and Sadler, 1998), more recent publications are suggesting a different perspective. Arenas-Pinto et al. challenged the common supposition that DSP arises from cumulative exposure to NRTIs. They found that patients who developed symptomatic DSP tended to do so shortly after exposure to antiretroviral therapy, with the risk for DSP peaking at 90 days post exposure, after which the risk decreased (Arenas-Pinto et al., 2008).

Longitudinal studies have demonstrated that dNRTI exposure was associated with a decreased risk of developing DSP as viral load was controlled. A trend towards protection against symptomatic DSP was observed which seemed paradoxical, although it was thought that the restoration of immune function by dNRTIs possibly outweighed the drugs' potential neurotoxicity (Schifitto et al., 2005). Similar results were reported in another cohort where dNRTI use was associated with incident DSP in the first year of their use, but increasing duration of exposure was associated with a decreased incidence of DSP (Lichtenstein et al., 2005).

An African cross-sectional study found no difference in the proportion of individuals developing DSP on stavudine compared to those who have never been on stavudine (Maritz

University of Cape Town

et al., 2010). Neither did they find a significant difference in the median number of months on stavudine amongst those with DSP and those without. It is possible that treatment-naïve individuals with pain are being judiciously diverted from stavudine therapy masking an effect in a cross-sectional design. It was also suggested that if DSP did not develop within the first year of d-drug therapy, individuals continuing on d-drugs would have no greater risk of developing DSP or worsening in existing DSP, than those not receiving d-drug regimens (Arenas-Pinto et al., 2008). However, there are reports of an increased risk for developing ATN in the presence of pre-existing neuropathy. (Berger et al., 1993, Moyle and Sadler, 1998, Dalakas et al., 2001, Blum et al., 1996). Although data are limited, other classes of cART drugs, such as protease inhibitors (ritonavir, indinavir, saquinavir) have also been associated with the development of neuropathy although they are frequently used in combination with dNRTIs (Pettersen et al., 2006, Smyth et al., 2007).

Based on these findings, it appears that individuals susceptible to the development of d-drug associated DSP, usually develop it early which often results in discontinuation of d-drugs. The apparent "neuroprotective" effect of subjects on long-term dNRTI regimens may simply represent a form of survival bias in those subjects who tolerate the drug (Simpson et al., 2006). However, with cART, true neuroprotection may exist with recovery in immune function and potentially neurological function.

1.9.3 Malnutrition as risk factor for HIV-associated DSP

Globally, poor nutritional status and weight loss is common in people with HIV infection (van Lettow et al., 2003). These patients frequently suffer from deficiencies of several micronutrients such as vitamins A, -C and -E, B-complex, as well as zinc and selenium (van Lettow et al., 2003, Tang and Smit, 1998, Karyadi et al., 2000). Micronutrient deficiencies and weight loss may be the result of changing metabolic requirements, decreased appetite with reduced caloric intake (van Lettow et al., 2003, Villamor et al., 2008), as well as abnormal gut integrity affecting nutrient absorption (Anabwani and Navario, 2005, Beach et al., 1992). As discussed in section 1.8.3.5, several of these nutrients are fundamental components of mitochondrial flavoproteins and cytochromes, which mean they are imperative for adequate and proper functioning of mitochondria (Look et al., 2001). Pre-existing vitamin B6 deficiency could well be exacerbated by INH administration, increasing the risk for DSP, and the need for supplementation. Available experience suggests that micronutrient supplementation (with vitamin B6) may improve clinical outcomes, including

University of Cape Town

DSP (Fawzi et al., 2004, Kaiser et al., 2006, Villamor et al., 2008, Isanaka et al., 2012) and reduced production of oxidant compounds and reduce oxidative stress (Allard et al., 1998, Packer, 1993).

As discussed in section 1.7.2, inflammation and immune dysregulation likely contribute to HIV-associated DSP. Markers of immune activation such as C-reactive protein (CRP) have been found to inversely correlate with circulating vitamin B6 levels (Chiang et al., 2005b). There is some evidence from studies in rheumatoid arthritis to suggest that inflammation may be contributing to impaired vitamin B6 status (Chiang et al., 2005a). In this study it was thought that low plasma PLP levels (the most important catalytic form of vitamin B6) was unlikely to be due to insufficient intake or excessive vitamin B6 excretion. Rather, that higher demands of certain tissues during inflammation resulted in the removal of vitamin B6 coenzymes from the circulation (Chiang et al., 2005a).

Serum albumin is not only an objective measure of malnutrition, but also a negative acute-phase protein that decreases with inflammation (Moshage et al., 1987, Don and Kaysen, 2004). Therefore, hypoalbuminaemia may be related to HIV-associated malnutrition and/or the chronic inflammatory state in these individuals. Frequencies of hypoalbuminaemia in African populations are reported to range from 13-32% (Graham et al., 2007, Dao et al., 2011). Given that over 90% of PLP is bound to serum albumin while being transported, the binding of PLP to albumin will impact on the availability of PLP to tissues. Low serum albumin results in the dephosphorylation of plasma PLP into pyridoxal (Merrill and Henderson, 1987) and thereby influences plasma PLP concentrations.

To summarize, the premise that vitamin B6 deficiency has a defining role to play in the pathogenesis of HIV-associated DSP is broadly based on two theories; the antioxidant/oxidative stress theory resulting in mitochondrial dysfunction, and the nutritional/immunological theory which have related and overlapping complex mechanisms with a fine balance between redox homeostasis, T-lymphocyte functioning as well as axonal damage and repair mechanisms.

The imbalance in cellular redox homeostasis due to vitamin B6 deficiency leads to oxidative stress and subsequent overproduction of ROS. Increased ROS levels may mediate local damage and lead to further immune activation with inflammation such as described in section 1.7.2. Vitamin B6 may be utilized at an increased rate during systemic inflammation and

University of Cape Town

result in reduced concentrations in the plasma. Vitamin B6 deficiency also leads to reduced production and decreased activity of T-lymphocytes (Baum et al., 1991, Rall and Meydani, 1993, Willis-Carr and St Pierre, 1978). This leads to a reduced capacity of lymphocytes to respond to relevant stimuli affecting repair mechanisms to local damage. Furthermore, the reduced T-lymphocyte effector function as well as the increased ROS levels may accelerate HIV disease, leading to a cycle of enhanced immune activation and viral production with further micronutrient deficiency.

1.9.4 Inflammation and cytokines as risk factor for ATN

Cytokines are very potent small proteins produced by either immune (macrophages, B-cells or T-cells) or nonimmune cells (endothelial cells or Schwann cells). They function as cellular communicators at an autocrine or paracrine level. Most cytokines are pleiotropic in nature, and different cytokines may share similar functions (Vallejo et al., 2010). Cytokines produced by T-helper 1 (Th1) cells include IFN-γ, IL-2 and IL-12, and constitute pro-inflammatory cytokines. Those produced by T-helper 2 (Th2) cells include IL-4, IL-5, and IL-10 and are considered anti-inflammatory cytokines (Mosmann and Coffman, 1989). Some cytokines such IL-13 and TNF-α are common to both T-helper subsets.

During inflammation, cytokines are produced and released to act on other cells, often as part of a cytokine cascade (Chiang et al., 2007) aiding the immune system in destroying pathogens and healing damaged tissue. Importantly, although the physiological function of cytokines may be to restore homeostasis, its uncontrolled, prolonged or excessive production may lead to pathological responses and tissue injury. Selected cytokines and their functions are presented in an abridged form in Table 1.3 (Coico and Sunshine, 2009).

University of Cape Town

Table 1.3: Selected cytokines and their functions (Adapted from Callard and Gearing, 1994, Coico and Sunshine, 2009)

Cytokine	Produced by	Major function
IL-1β	Monocytes, T-cells, B cells, many other cell types	Mediates host inflammatory response to infection; stimulates acute-phase protein synthesis
IL-2	Naïve T-cells and Th1-cells	T-cell growth factor
IL-4	Th2 cells, mast cells, bone marrow stromal cells	Growth factor for B cells and Th2 CD4 T-cells; inhibits Th1 CD4 T-cells; promotes IgE and IgG
IL-5	Th2 cells, mast cells, B cells	Stimulates B cell growth and Ig secretion; growth and differentiation factor eosinophils
IL-6	T-cells, macrophages, endothelial cells, keratinocytes, mast cells	Induces acute-phase protein synthesis, T-cell activation and IL-2 production; stimulates B cell Ig production and hematopoietic progenitor cell growth
IL-7	Thymic stromal cells, bone marrow	Growth factor for pre-T and pre-B cells
IL-8	Monocytes, macrophages, fibroblasts, keratinocytes, endothelial cells	Mobilizes and activates neutrophils; promotes angiogenesis
IL-10	Th2 cells, macrophages, B cells	Inhibits production of Th1 cells and macrophage function
IL-12	B cells, macrophages, T-cells, dendritic cells	Activates natural killer cells and promotes generation of Th1 CD4 T-cells
IL-13	T-cells	Shares characteristics of IL-4, but does not affect T-cells; growth factor for human B cells
GM-CSF	T-cells, monocytes	Promotes growth of granulocytes and macrophages; growth of dendritic cells *in vitro*
IFN-γ	Th1 cells	Activates natural killer cells and macrophages; inhibits Th2 CD4 T-cells; induces expression of MHC class II on many cell types
TNF-α	Macrophages, mast cells	Involved in inflammatory responses; activates endothelial cells and other cells of immune and nonimmune systems; induces fever and septic shock

Abbreviations: IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; TNF, tumour necrosis factor; Ig, immunoglobulin; Th, helper T-cell NK, natural killer; MHC, major histocompatibility complex

University of Cape Town

1.9.4.1 Why study cytokines and painful HIV-DSP?

A substantial amount of data exists on the association between cytokines and pain. The first indications that cytokines may play a role in hyperalgesia stemmed from research using cytokine injections in the rat (Ferreira et al., 1988, Cunha et al., 1992). The intraneural or intraplantar application of pro-inflammatory cytokines induces behavioural signs associated with pain and hyperalgesia. This can be effectively reduced by treatment with anti-inflammatory cytokines. A pro-inflammatory cytokine profile has also been associated with pain in the setting of peripheral neuropathy in HIV-negative individuals, with higher blood messenger RNA (mRNA)levels of TNF-α and IL-2 levels in individuals with painful neuropathy compared to those with painless neuropathy (Uceyler et al., 2007).

Small but significant increases in pro-inflammatory cytokine activity were detected in chronic pain patients. Both blood and intrathecal concentrations of sTNFR were significantly elevated, as were cerebrospinal fluid (CSF) concentrations of IL-1ß. On the other hand, IL-10, which has anti-inflammatory properties, was reduced in both compartments in patients suffering with chronic pain (Backonja et al., 2008).

Although pro-inflammatory cytokines have been associated with severe HIV-associated dementia (Sevigny et al., 2004, Wesselingh et al., 1994, Tagliati et al., 1999), few reports exist assessing the role of cytokines in HIV-associated painful DSP. In a cross-sectional cohort, largely on cART, markers of immune activation in the plasma and CSF were studied; only CSF macrophage colony-stimulating factor levels predicted time to developing symptomatic DSP (Schifitto et al., 2005). Individuals with HIV-associated DSP compared to those without were found to have persistently higher levels of chemokine ligand 5 (CCL5) during the first 48 weeks after cART (Chew et al., 2011). Increased TNF-α- and reduced IL-4 mRNA levels were also found in peripheral nerve tissue from AIDS patients with DSP (Tyor et al., 1995). A study genotyping polymorphisms in several cytokine genes in individuals with or without symptomatic ATN, showed an association with the *TNFA*-locus. (Cherry et al., 2008).

Upon starting cART, immune reconstitution is accompanied by a rise in CD4 T-cell count and partial restoration of pathogen-specific immunity (Wilkinson et al., 2009). However, 8-43% of patients will experience pathological inflammatory responses that may cause clinical deterioration, termed immune reconstitution inflammatory syndrome (IRIS) (Muller et al.,

University of Cape Town

2010, Meintjes et al., 2008). Typically IRIS occurs during the first 12 weeks of cART initiation (Meintjes et al., 2009) and has been shown to be associated with increased circulating pro-inflammatory cytokines IL-6, IFN-γ and TNF-α, albeit in the setting of HIV-TB co-infection (Tadokera et al., 2011). The resultant inflammation not only causes symptoms of the subclinical infection to emerge, which can be severe, but also results in oxidative stress with subsequent cell damage. In this research project we hypothesize that an exaggerated cytokine response after cART initiation may contribute to the immunopathogenesis of early painful ATN via the indirect mechanism of axonal damage.

As discussed in section 1.7.2, axonal damage with exposure of cryptic epitopes may result in a secondary autoimmune response directed at the peripheral nerves. Alternatively, immune restoration and activation as a result of cART initiation with the release of cytokines can increase peripheral nerve hyperexcitability and injury. Subsequent release of cytokines, free radicals, and lipid membrane derivatives, are all potentially neurotoxic (Schifitto et al., 2005) and may also be associated with 'dying-back' degeneration of peripheral nerve fibres (Cherry et al., 2008, Pardo et al., 2001, Dalakas et al., 2001).

One common aspect to the manifestation of IRIS is a low CD4 T-cell count prior to treatment followed by a significant and rapid increase in CD4 T-cells after initiation of cART (Badri et al., 2002, Douek et al., 2001, Lawn et al., 2008). However, the pathogenesis of this amplified inflammatory response may be more complex. Individuals who develop IRIS may have perturbations in their T-regulatory cell profile and regulatory immune responses due to cytokine imbalances that may contribute to the manifestation of this syndrome both before and after cART is commenced (French, 2012, Oliver et al., 2010). Multiple cytokines may be part of the pathogenesis of IRIS and as various cytokines may in turn regulate the production of other cytokines, their combined effect is often greater than the function of a single component. However, the remainder of this review will be focused on those cytokines that have been implicated in the pathogenesis of painful neuropathy.

University of Cape Town

Tumour necrosis factor alpha

TNF-α is a multipotent pro-inflammatory cytokine that induces a wide variety of cellular responses, which includes apoptosis in some cells and proliferation in others (Ashkenazi 1999). Its main function appears to be the recruitment and activation of mononuclear cells and neutrophils to the site of inflammation (Schafers et al., 2003a, Schafers et al., 2003b, Kress, 2010). Although activated mononuclear phagocytes are the major cellular source of

TNF-α, T-cells can also produce TNF-α during inflammation (Woska and Magram, 2005). In the nervous system, microglia and astrocytes are believed to be the primary producers of TNF-α (Szelenyi, 2001, Hopkins and Rothwell, 1995) whereas in the peripheral nervous system, it is macrophages, Schwann cells, dorsal root ganglia and sensory neurons (Wagner and Myers, 1996, Schafers et al., 2002, Schafers et al., 2003b).

Several studies have shown TNF-α to be involved in the generation of inflammatory pain, neuropathic pain and hyperalgesia through its actions in the periphery and in the central nervous system (Aloe et al., 1997, Empl et al., 2001, Schafers et al., 2003b, Sommer et al., 1998, Wagner and Myers, 1996, Watkins et al., 1995). Injury-induced increases in TNF-α expression in the nerve microenvironment correlate with the development of allodynia and hyperalgesia in several neuropathic pain models (Schafers et al., 2003b). Endoneurial and intrathecal injection of TNF-α induces axonal degeneration, demyelination and thermal and mechanical hyperalgesia, and topical application evokes ectopic activity in nerve fibres (Sorkin et al., 1997, Reeve et al., 2000). In an animal model of chronic nerve constriction injury, inhibition of the synthesis and release, or functional neutralization of TNF-α results in reduced pain-associated behaviour (Schafers et al., 2001, Sommer et al., 2001, Sommer et al., 1998).

Soluble TNF-α receptors

The ability of TNF-α to influence cellular signalling is dependent on the activation of TNF receptors. An alternative measure of TNF-α activity is TNF receptor levels (Zangerle et al., 1994), which are relatively stable and therefore better indicators of inflammatory responses than TNF-α concentrations (de Beaux et al., 1996, Gardiner et al., 1995, Chikanza et al., 1993, Aziz et al., 1999). The longer plasma half-life of sTNFR and rapid biological inactivation of detectable systemic TNF-α may be the reason for this observation. There are two distinct TNF receptors, 55 kD (type I TNF receptor-TNFRI) and 75 kD (type II TNF receptor-TNFRII). Both are expressed by primary afferent nociceptors (Sommer et al., 1998) and both exist in soluble and cell surface membrane-bound forms. Neuropathic pain seems to be largely dependent on TNFRI signalling (Sommer et al., 1998) whereas TNFRII appears to play plays an important role in neurodegeneration (Arnett et al., 2001). However, both receptors are upregulated following experimental nerve lesions or systemic inflammation (Sommer et al., 1998, Sommer and Kress, 2004).

University of Cape Town

Interleukin-1

Interleukin-1 is a pleiotropic cytokine with diverse roles in immunity and inflammation, having both protective and pro-inflammatory effects. Biologically active IL-1 consists of two distinct forms called IL-1α and IL-1β, respectively. Principal functions of IL-1 include mediation of the host's inflammatory response to infections and other inflammatory stimuli, induction of acute-phase protein synthesis and proliferation of Th2 cells. It is produced by a large variety of cells including macrophages, fibroblasts, glial cells and neurons (Callard and Gearing, 1994), although activated monocytes and macrophages are the major source. Stimuli for IL-1 production include bacterial products such as lipopolysaccharides and other cytokines such as TNF-α.

In addition to its immune functions, IL-1 is involved in nociceptive behaviour (Bianchi et al., 1998) and appears to play a role in rat dorsal root ganglion neural regeneration after axotomy (Horie et al., 1997). In cultured dorsal root ganglion cells, IL-1β has been shown to induce the release of substance P (Morioka et al., 2002). This peptide induces vasodilation, increases vascular permeability (Lembeck and Holzer, 1979), activates bystander macrophages and evokes inflammatory responses resulting in hyperalgesia (Walsh et al., 1995). Whether administered centrally or peripherally, IL-1β has been found to induce hyperalgesia in several animal pain models (Bianchi et al., 1998). Subcutaneously, IL-1β is able to produce a dose-dependent increase in the sensitivity of rat paws to mechanical and thermal stimulation with the activation of small diameter cutaneous nerves (Fukuoka et al., 1994).

Interleukin-I receptors and antagonist

The capacity of IL-1 to influence cellular functions depends on the expression of the appropriate receptor. There are two membrane receptors that have been identified for IL-1. The type I IL-1 receptor (IL-1RI) is the major receptor for IL-1-mediated biologic responses. The type II IL-1 receptor (IL-1RII) does not transmit any cell signal and its major function is to act as a decoy receptor that competitively inhibits IL-1 binding to the type I signalling receptor (Cunningham and De Souza, 1993). IL-1RI knockout mice do not respond to intraperitoneal or intracerebroventricular injected IL-1 (Touzani et al., 2002). In the same way, IL-1RI inhibition through neutralizing antibodies administered to the lateral ventricle abrogates the behavioural effects of intracerebroventricular applied IL-1β (Cremona et al., 1998a). In contrast, inhibition of IL-1RII potentiates this effect (Cremona et al., 1998b). More importantly, inflammatory hyperalgesia is prevented by local administration of endogenous

University of Cape Town

IL-1 receptor antagonist (IL-1RA), and neutralizing antibodies to IL-1RI reduce pain-associated behaviour in mice after chronic constrictive injury (Sommer et al., 1999).

Interleukin-6

Interleukin-6 is a pro-inflammatory cytokine and a terminal differentiation factor for B cells. It also stimulates acute-phase protein production by hepatocytes, T-cell activation and IL-2 production (Callard and Gearing, 1994). IL-6 is synthesized by a variety of cells including T-cells, monocytes, macrophages, fibroblasts, endothelial cells and keratinocytes (Callard and Gearing, 1994).

There is considerable evidence in animal models reporting roles for IL-6 in nociception and inflammatory hyperalgesia (Cunha et al., 1992, Oka et al., 1995). Intraplantar, intrathecal or intracerebroventricular injection of IL-6 induces allodynia or hyperalgesia in rats. Increased IL-6 mRNA transcripts are detected in the dorsal root ganglion after chronic constriction injury. In behavioural studies, IL-6 knockout mice showed reduced thermal hyperalgesia and mechanical allodynia (Ramer et al., 1998, Zhong et al., 1999) and less hyperalgesia after carrageenan inflammation or nerve constriction (Xu et al., 1997). Neutralizing endogenous IL-6 with antibodies inhibits lipopolysaccharide-induced hyperalgesia (Sommer, 2001, Ramer et al., 1998).

IL-6 has been associated with non-HIV small fibre neuropathies. Uceyler et al. reported an increase of IL-6 mRNA transcripts in skin biopsies of individuals with length-dependent small fibre neuropathy (Uceyler et al., 2007).

This concludes the background summary covering all aspects of this research. The research aims will now be briefly outlined.

University of Cape Town

1.10 Study Aims

High prevalence and incidence rates in a population at risk, various diagnostic and clinical management issues, and limited data especially within the first 12 weeks after cART initiation makes HIV-associated DSP research a priority. A prospective approach starting prior to cART initiation would offer the opportunity to assess the impact of various risk factors and offer a description of the natural clinical evolution of the disease within the first 24 weeks of starting treatment.

Specific aim 1

To determine the incidence of painful ATN following the initiation of antiretroviral therapy and to examine the impact of cART on pre-existing HIV-DSP.

The purpose of this research was to focus on painful ATN and specifically that which occurs in the first 24 weeks of starting cART. The first aim was to determine the incidence of symptomatic ATN in a community based cohort by recruiting ambulatory individuals at the point of cART initiation, and studying them over the first 24 weeks when the incidence of symptomatic ATN is reportedly the highest.

Specific aim 2

To investigate the impact of certain risk factors (such as age and prior TB infection) previously identified in this population in a cross-sectional study, on the development of ATN.

The second aim was to examine the impact of certain risk factors identified in a previous cross-sectional study in this population on the development of ATN, to understand the pathogenesis of ATN with the ultimate objective of identifying an intervention without compromising HIV control.

There are two additional etiological aspects we wished to study in relationship to incident neuropathy a) an early "inflammatory" component and b) the independent risk of TB therapy. This work is particularly relevant in Africa, which carries a disproportionate burden of the HIV and TB epidemic, and where avoidance of cheaper cART is not always feasible.

University of Cape Town

Specific aim 3

To correlate selected markers of immune dysregulation, with a particular focus on candidate cytokines previously associated with painful neuropathies (TNF-α, IL-1, IL-2, IL-6) with incident painful ATN within the first twelve weeks of initiating cART.

The third aim of this research included a nested case-control-study to assess the hypothesis that ATN could represent an IRIS phenomenon in which previously identified "pain-associated" cytokines may be involved in this immune dysregulation. The objective was to investigate the impact of ART-initiation on immune dysregulation or reconstitution, and how this correlates with the development of painful symptoms within the first 12 weeks when the incidence of IRIS as well as ATN peaks.

Specific aim 4

To evaluate NAT2 status as a surrogate marker for INH-acetylation status and serum pyridoxine levels (at baseline and 12 weeks) with markers of inflammation, and how these measures correlate with incident painful ATN.

A history of TB infection was associated with an increased risk for DSP in African cohorts and INH-associated painful neuropathy is preventable with adequate vitamin B6 supplementation. In addition, the hyper-inflammatory state accompanying HIV-infection may contribute to a critically low vitamin B6 level in certain tissue compartments. Therefore, the fourth aim was to determine whether vitamin B6 deficiency and the *NAT2* acetylation phenotype are risk factors for ATN.

University of Cape Town

Chapter 2 Subjects and Methods

University of Cape Town

2.1 Ethical approval

The Research Ethics Committee of the University of Cape Town (UCT) approved the study with reference number REC REF 221/2008 (See Appendix A).

2.2 Study design and study site

The study design was a prospective analytical observational cohort with a baseline cross-sectional component. The study was undertaken at Crossroads Community Health Centre in Crossroads, a township approximately 15 km within the city of Cape Town, South Africa, from May 2009 until December 2010. This institution runs a dedicated clinic for an estimated 6000 HIV-infected individuals. In Crossroads Township, services are limited to shared water outlets and toilet facilities. Unemployment is high in the area and is estimated to be between 25-50%. Most people living in the area are indigenous Africans. The primary language is isiXhosa (O'Connor et al., 2011).

University of Cape Town

2.3 Recruitment

Ambulatory individuals were recruited from the clinic by two clinicians (Drs JJ vd Watt and C Centner) and a trained field worker, fluent in English, isiXhosa and isiZulu, who would also act as an interpreter between study staff members and participants if necessary.

HIV-infected individuals not on cART and scheduled to commence cART within seven days were referred from the clinic doctors. After being counselled, individuals received an information pamphlet (see Appendix C). Consent was given for study participation, blood sampling for biochemistry, vitamin B6, cytokine and DNA analysis and storage of samples. Clinic folders were reviewed hereafter to screen for any exclusion criteria. Individuals were enrolled into the study consecutively as they were found to be eligible to start cART within the following days to one week. Study appointment dates were coordinated with the clinic in order to perform baseline visits within one week prior to commencing cART. Participants were instructed to be nil per mouth for a minimum of eight hours prior to each study visit and were reminded telephonically one day before the visit.

2.4 Eligibility and exclusion criteria

Individuals were eligible if 18 years or older and met criteria for cART initiation in the South African government-sponsored HIV treatment programme, i.e. a CD4 T-cell count <350 cells/mm^3 and/or WHO stage IV AIDS-defining illness.

Exclusion criteria included active opportunistic infection, systemic illness, known inflammatory conditions, current pulmonary TB on therapy for less than one month, severe diarrhoea (> six stools per day), co-morbid neurological disease that may confound the diagnosis of DSP, diabetes mellitus, exposure to glucocorticoids within the past six months, or pregnancy.

2.5 Clinical study procedure and assessments

Clinical examinations were performed at baseline (the visit prior to cART initiation), 2-, 4- 12- and 24 weeks thereafter. Assessments at baseline, week 12 and week 24 were conducted at Groote Schuur Hospital and the 2- and 4 week assessments were performed at Crossroads Clinic. Study procedures at each visit are summarized in Table 2.1.

University of Cape Town

Table 2.1: Summary of study procedures at each visit

Procedure	Baseline	Week 2*	Week 4*	Week 12	Week 24
Informed consent	x				
Clinic Folder review					
Medical and drug history	x	x	x		x
Laboratory data collection	x				x
Clinical assesssment					
Neurological assessment					
BPNS [1]	x	x	x	x	x
mTNS	x		x	x	x
IHDS	x	x	x	x	x
Anthropometry	x			x	x
Blood pressure	x			x	x
Blood collection					
Metabolic paramaters [2]	x			x	x
NAT2 genotype	x				
PLP and 4-PA	x			x	
Albumin	x				
Inflammatory biomarkers					
Cytokines	x	x	x	x	
Soluble receptors	x	x	x	x	
C-reactive protein	x	x	x	x	

* Only applies to individuals without DSP at baseline and not on TB therapy

[1] Modified at Week 2: Only neuropathic symptoms assessed without neurological examination

[2] Fasting glucose, insulin, total cholesterol, HDL, LDL, triglycerides, lactate (baseline only)

University of Cape Town

2.5.1 Neurological assessment

Two clinicians (Drs JJ vd Watt and C Centner) performed the neurological assessment. For peripheral neuropathy, a combination of the BPNS and mTNS methods was used. The International HIV dementia scale (IHDS) was used as a screening tool for HIV dementia.

2.5.1.1 Neuropathic symptoms

The BPNS consists of brief questions regarding symptoms of DSP: 1) pain, aching, or burning; 2) pins and needles sensation; and 3) numbness in the feet or legs. Individuals

graded each of these symptoms bilaterally on a VAS (translated into isiXhosa) from 0 (absent) to 10 (severe). The single highest score of the three modalities as determined by the VAS was defined as the Total Sensory Severity score (see Appendix A). This score was used to establish a sensory severity grade, called Total Sensory Grade, which was important in defining the natural progression of DSP:

0 if the Total Sensory Severity score = 0
1 if the Total Sensory Severity score was 1 – 3
2 if the Total Sensory Severity score was 4 – 6
3 if the Total Sensory Severity score was 7 or 8
4 if the Total Sensory Severity score was 9 or 10

In the mTNS, sensory symptoms in all modalities are assessed as a single entity and scores represent the level of anatomical involvement. A higher score is obtained for more proximal involvement (0 for no symptoms; 1 – soles/toes; 2 – extends up to ankle; 3 – extends up to knee; and 4 – more proximal involvement).

Both clinical tools required symptoms and signs to be symmetrically present, and individuals with asymmetry were excluded from the analysis. In addition, if neuropathic symptoms were absent at baseline, individuals were asked if symptoms had been experienced in the past.

2.5.1.2 Neuropathic signs

The BPNS includes the examination of two neurological signs, namely vibration perception and deep tendon reflexes. Vibration perception was evaluated using a 128 Hz tuning fork (RAGG, England), opposing the two ends and applying it to the dorsum of the hallux interphalangeal joint of both feet, proximal to the nail bed. Vibration sense was defined as normal if vibration felt for at least 10 seconds (see Appendix A). If vibration sense was <10 seconds on the feet, the procedure was repeated proximally at the level of the ankle joint, followed by the knee joint.

Deep tendon reflexes were examined with the individual sitting on the side of the examining table, relaxing the lower limbs. A long-handed reflex hammer (Eschmann, England) was used. Ankle reflexes were compared to knee reflexes. The ankle reflex was elicited by holding the relaxed foot with one hand and striking the Achilles tendon with the hammer and

University of Cape Town

noting plantar flexion. Reflexes were defined as absent, normal, hypoactive or hyperactive with or without clonus.

The mTNS examination includes the following four evaluations: (1) pinprick sensibility, (2) vibration perception, (3) tendon reflexes and (4) distal strength. Each of the four elements was graded from 0 (normal) to 4 (most abnormal). For elements (1) and (2), grades represented the level of anatomical involvement as described for sensory symptoms. Tendon reflexes (3) were simultaneously graded according to severity and level of involvement (0 for all normal; 1 for ankle reflexes reduced; 2 for ankle reflexes absent; 3 for ankle reflexes absent and others reduced; and 4 for all reflexes absent). Ankle- and toe plantar- and dorsiflexion (4) were graded according to the Medical Research Council muscle power grading (0 – 4). In addition to the BPNS and mTNS, proprioception at the big toe was assessed (normal, reduced or absent). Neuropathy findings were recorded on a neuropathy data form (see Appendix A).

2.5.1.3 IHDS Cognitive function screening

The IHDS was applied as a screening tool for HIV-associated cognitive involvement (shown in Appendix D). It has been validated as a sensitive screening test for HIV dementia and consists of three subtests: timed finger tapping (motor speed), timed alternating hand sequence test (psychomotor speed), and memory recall (Sacktor et al., 2005). A total score out of 12 was calculated for each individual, with each of the three subtests contributing 4 points to the total score. The cut-off of <10 out of 12 was applied for defining HIV dementia.

2.5.2 Other clinical measures

Digital blood pressure (BP) readings were recorded in triplicate with the use of an Omron M6 Comfort BP monitor (Omron Healthcare, Kyoto, Japan). Systolic and diastolic BP measures were obtained from seated individuals. Anthropometric data on weight, height and waist-to-hip ratio were collected for all individuals. Field workers conducted interviews to complete a questionnaire regarding social and medical history.

2.5.3 Blood collection and storage

Fasting venous blood was collected between 08h00 and 09h00 to reduce variability (diurnal variations or food ingestion) (Geiss et al., 1997). Capillary blood was collected for fasting lactate level by finger prick without a tourniquet and analysed on an Accutrend Lactate

University of Cape Town

system (Roche, Mannheim, Germany). Thereafter intravenous canulation was performed and venous blood was collected for:

a) Metabolic parameters viz. glucose, insulin, lipid profile (baseline, 12- and 24 weeks)
b) DNA for *NAT2* genotyping (baseline)
c) PLP and 4-PA for vitamin B6 levels (baseline and 12 weeks)
d) Albumin (baseline)
e) High-sensitivity (hs)-CRP, cytokine and soluble receptor screen (baseline, 2-, 4- and 12 weeks)

Venous samples were collected by venepuncture using the appropriate vacuum blood collection tubes (Greiner Bio-One, Kremsmunster, Germany). After blood collection, samples were immediately placed on ice until centrifuged. Samples were centrifuged at 3300 g[1] for ten minutes (min). The supernatant fraction of each tube aliquoted into 1.5 mL Eppendorf tubes labelled with the appropriate study identification number, date and visit number, and stored immediately in a -80°C freezer. (Laboratory materials and methods will be discussed further in Chapter 3).

2.5.4 Clinic folder reviews

Data on general medical history and laboratory risk factors were extracted from the clinic medical records. General medical history included the date of HIV diagnosis, WHO clinical stage, previous or current TB infection, current prescribed vitamin supplementation (vitamin B complex or vitamin B6) or any analgesia (all verified during history taking). Changes in cART regimens were recorded as well as any evidence of cART-related adverse effects.

Data from routine blood testing performed at the clinic were collected from clinic folders (and confirmed on electronic records from the National Health Laboratory Services). These consisted of baseline measurements of mean corpuscular volume (MCV), haemoglobin (Hb),

[1] The relationship between Revolutions Per Minute and Relative Centrifugal Force (expressed in units of gravity) is as follows: $g=(1.118 \times 10^{-5})\ RS^2$. Where g is the relative centrifugal force, R is the radius of the rotor in centimetres, and S is the speed of the centrifuge in revolutions per minute.

University of Cape Town

white cell count (WCC), creatinine and alanine aminotransferase (ALT), and baseline and 24-week measurements of CD4 T-cell counts. Plasma HIV RNA viral load was used as a measure of virological control 24 weeks after commencing cART according to South African guidelines. All viral load levels <200 RNA copies/mL were considered undetectable. Data from clinic folder reviews were recorded on an examination form (see Appendix E).

2.6 Study definitions and criteria for diagnosis

2.6.1 Clinical definitions

Clinical definitions used in defining neuropathy at baseline are summarized in Table 2.2. At baseline assessment, the diagnosis of DSP required at least two of four key components (sensory symptoms, vibration sense, pin sensibility, and tendon reflexes) to be abnormal:

a) An individual was defined as having symptomatic DSP (SDSP) if sensory symptoms were present and at least one of the three remaining key components were abnormal.
b) An individual was considered to have asymptomatic DSP (ADSP) if sensory symptoms were absent and at least two of three remaining key components were abnormal with a minimum mTNS score of 3.

The primary baseline analysis compared individuals with DSP to those without DSP, and those with symptomatic DSP to those without DSP.

Table 2.2: Baseline neuropathy definitions

Neuropathy status	Sensory symptoms[a]	Neuropathic signs[b]	Neuropathy score
DSP			
Symptomatic (SDSP)	Any one	Any one	
Asymptomatic (ADSP)	None	At least two	mTNS score ≥ 3
Symptoms only	Any one	None	

[a] Pain, numbness or paraesthesiae (according to the BPNS or mTNS score)

[b] Abnormal pin sensibility, vibration sensibility, deep tendon jerks or muscle strength (according to the BPNS or mTNS score)

Follow-up DSP definitions are summarized in Table 2.3. Incident symptoms were defined as no sensory symptoms at baseline and the development of any sensory symptoms after

University of Cape Town

baseline. Incident symptomatic DSP was defined as no symptomatic DSP at baseline, with development of symptomatic DSP after baseline. For worsening or improving symptoms or signs in individuals with DSP pre-cART, we compared the number and severity of symptoms and signs in each individual between the initial and follow-up visits (see Table 2.3).

The primary outcome measure for the longitudinal study was a diagnosis of ATN either at 2-, 4-, 12- or 24 weeks after initiating cART. ATN was defined as individuals who developed any new neuropathic symptoms (with or without neuropathic signs), or worsened in existing symptomatic DSP. The follow-up analysis compared individuals with ATN to those who had been exposed to cART but had not developed ATN during the 24-week follow-up period. A caveat in the definition of ATN is that worsening of neuropathic symptoms may be part of the natural progression of HIV-DSP; the addition of cART as an additional neurotoxic insult does not necessarily imply that the worsening was due to cART. Furthermore, the population at risk for incident symptoms and that at risk for worsening symptoms (which would be individuals with symptomatic DSP at baseline) are mutually exclusive and with our definition of ATN, individuals with symptomatic DSP will be in both comparative groups. Therefore, an analysis was also performed looking at individuals who developed neuropathic symptoms, comparing them to individuals who never had or developed neuropathic symptoms, and excluding individuals with symptomatic DSP at baseline.

University of Cape Town

Table 2.3: Follow-up neuropathy definitions

Category	Definition
Incident symptoms	No sensory symptoms at all prior study visits (including baseline); AND Any one abnormal sensory symptom at specified follow-up study visit.
Incident SDSP	No SDSP at all prior study visits (including baseline); AND SDSP at specified follow-up study visit.
Worsened symptoms since baseline*	Increase in BPNS Total Sensory Grade at specified follow-up study visit compared to baseline.
Worsened signs since baseline*	Increase in the sum of mTNS scores relating to neuropathic signs at specified follow-up study visit compared to baseline.
Worsened SDSP since baseline*	SDSP at baseline; AND SDSP at specified follow-up study visit; AND Worsened symptoms since baseline.
ATN	Incident symptoms; OR Worsened SDSP since any prior study visit.
Improved symptoms since baseline*	Decrease in BPNS Total Sensory Grade at specified follow-up study visit compared to baseline.
Improved signs since baseline*	Decrease in the sum of mTNS scores relating to neuropathic signs at specified follow-up study visit compared to baseline.

* The definition compares the specified follow-up study visit to baseline.
It can be applied at any specified follow-up study visit compared to any prior study visit,
e.g. week 24 compared to week 12.

University of Cape Town

To investigate risk factors for the development of HIV-associated DSP overall, a comparison was performed between those who either had symptomatic DSP at baseline or developed ATN to those who remained symptom-free. This analysis was performed to investigate the hypothesis that symptomatic DSP at baseline and the development of ATN are part of a continuum of the same disease process. This group of individuals will be referred to as 'HIV-SN'.

Transition of DSP states over the 24-week follow-up period was examined by dividing individuals into four baseline DSP groups, namely no DSP, asymptomatic DSP, symptomatic DSP and symptoms only (neuropathic symptoms without any neuropathic signs).

2.6.2 Vitamin B6 definitions

Vitamin B6 deficiency was defined as a plasma PLP level <25 nmol/L. Individuals received pyridoxine supplementation in two forms:

a) Vitamin B complex supplementation: Pyridoxine doses in vitamin B complex preparations may vary according to the manufacturer, but this currently equates to 2-4 mg/day in South African government-sponsored programmes (Rossiter et al., 2012). This will further be referred to as 'vitamin Bco supplementation'.
b) Vitamin B6 supplementation: A dosage of 25 mg/day, further referred to as 'vitamin B6 supplementation'.

2.6.3 Metabolic definitions

Abnormal fasting serum triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol were defined in line with the National Cholesterol Education Program Adult Treatment Panel III guidelines and WHO definitions (Lorenzo et al., 2007, Grundy et al., 2005):

a) Elevated triglycerides (≥1.7 mmol/L)
b) Low HDL cholesterol levels (<1.15 mmol/L)
c) Elevated LDL cholesterol (≥3.0 mmol/L)
d) Elevated total cholesterol (≥5.0 mmol/L)

Normal body mass index (BMI) was defined as 20.0-25.0 kg/m^2.

Abnormal waist-to-hip ratio was defined as >0.90.

Metabolic Syndrome was defined if at least three of the following criteria from the National Cholesterol Education Program Adult Treatment Panel III were present (Grundy et al., 2005):

a) Elevated mean arterial BP of at least 100 mmHg
b) Elevated fasting triglycerides (≥1.7 mmol/L)
c) Reduced HDL cholesterol (<1.15 mmol/L)
d) Obesity with elevated BMI (>25 kg/m^2)
e) Elevated fasting plasma glucose (≥5.6 mmol/L)

Diabetes was defined as a fasting glucose of ≥7 mmol/L.

University of Cape Town

2.7 Data capturing

Data were captured by JJvdW via an electronic version of the examination form designed using Microsoft Excel 2007 (Microsoft Corporation, Redmond, Washington, USA) and stored in an Excel database. The electronic form was designed to perform the following functions:

a) Allow the user to input the result of an examination.
b) Validate the data as it is entered using validation rules that were customised for each field.
c) Automatic calculation of results of all pre-defined scores and definitions and final calculations of the BPNS and mTNS scores.
d) Convert the data to an Excel database.
e) Look up results of a particular examination for a particular individual from the database and to view the data in the format that it was recorded as.

All uploading of new data, data corrections and changes to the electronic form and database were documented in an audit trail in the relevant Excel workbooks.

University of Cape Town

2.8 Statistical analysis

Data management and statistical analysis were performed using STATA version 12.0 (Statacorp, College Station, Texas, USA). All statistical analyses were performed by JJvdW and supervised by a statistician, Dr Henri Carrara, School of Public Health and Medicine, University of Cape Town. Descriptive statistics were used to describe the study sample and any change in variables over time. Analyses were both cross-sectional and longitudinal in nature.

2.8.1 Cross-sectional analysis

2.8.1.1 Univariate analysis

In the univariate analysis variables were evaluated for their association with DSP. All variables were investigated and will be presented and grouped in the following way in all relevant chapters:

a) **Clinical:** Age, weight, height, BMI, waist-to-hip ratio, systolic and diastolic BP, TB history
b) **Immunological/Haematological:** WHO HIV stage, CD4 T-cell count, white cell count, hs-CRP, haemoglobin and MCV
c) **Biochemical:** Albumin, ALT and creatinine
d) **Metabolic:** Fasting glucose, insulin, triglycerides, total cholesterol, HDL, LDL and lactate
e) **Pyridoxine:** PLP and 4-PA

All variables were tested for normality and log-transformed when necessary. Untransformed data are displayed in the tables and figures. For normally distributed data, t-tests were used to compare continuous variables, and Pearson's chi-squared (χ^2) test was used to compare differences in categorical variables. A non-parametric statistical approach was used for data that remained non-normally distributed after transformation; Wilcoxon rank-sum test was used to compare continuous variables, and Fisher's exact test to compare differences in categorical variables. Differences were considered statistically significant at p-value ≤ 0.05. All reported p-values are two-tailed. The odds ratio (OR) was estimated by generalized linear models and were calculated to determine univariate risk factors associated with DSP status. Factors significantly associated in the primary analysis (parametric, non-parametric and proportional) were examined by individual logistic regression. All variables were explored for an association, but only variables showing significance level of $p<0.25$ in the primary analysis were included in the multivariate analysis, described in section 2.8.1.2.

Spearman's correlation coefficients were used for bivariate correlation analyses. The strength of the relationship was defined by rho (ρ) as: 1) less than 0.3 – weak, 2) 0.3 to 0.5 – moderate, 3) 0.6 to 1.0 – strong. Categorizing continuous variables by biological meaningful cut-off values or by population medians were additionally used as a measure of variation in the data. Individuals with missing data for a specific variable were only excluded from the

University of Cape Town

analysis for the specific variable. Individuals who did not complete follow-up at 24 weeks were excluded from the 24-week analysis.

To evaluate the association of vitamin B6 and hs-CRP with DSP status, we used two parallel strategies: 1) to test differences between individuals categorized as DSP and no DSP, 2) to test correlations between vitamin B6, hs-CRP and other variables, such as triglycerides or albumin, independent of DSP category. The association between *NAT2* and DSP status was investigated using three different genetic models described in section 3.1.3.6.

2.8.1.2 Multivariate analysis

Using a stepwise backward approach to variable selection, we fitted a multivariate logistic-regression model to study baseline continuous or categorical variables found to be significantly associated with DSP in the univariate analysis.

Variables with a p-value ≤0.25 were included in the initial multivariate model. Variables not independently statistically significant (i.e. with a p-value >0.05) were excluded from the multivariate model by stepwise backward logistic regression. Therefore, the final model included only those variables independently associated with DSP status. All models included age and gender. Analyses included all individuals with blood samples available at the specified time of assessment. The model with the best fit was determined with the Hosmer-Lemeshow test for goodness of fit. Adjusted odds ratios and 95% confidence intervals (CIs) were estimated from the final model. The coefficient of determination (R^2) was used in the statistical models to describe the proportion of variability in the data set that was accounted for by the specific model. Explanatory variables were assessed for collinearity. Nonmonotonic continuous variables were dichotomized for the multiple logistic regression analysis. All reported p-values are two-sided. No correction was made for multiple comparisons in the cytokine analysis as specific biomarkers were selected in a pre-established hypothesis.

It was decided not to use a forward logistic regression approach. The disadvantage of using forward logistic regression is the possible statistical removal of a mediating or confounding variable that could increase the magnitude of the relationship between the independent and dependent variable (Menard, 2001, MacKinnon et al., 2000). Although both forward and backward stepwise methods often produce identical results, backward elimination is more

University of Cape Town

likely to uncover associations since all variables are initially included in the model (Menard, 2001).

2.8.2 Longitudinal analysis

Shift tables were used to describe DSP group transitions over time. As data were collected at four time points, it was only known that neuropathy occurred within a certain time interval. The data was therefore interval censored, therefore the assumption was made that neuropathy developed at a halfway point between two time points. The incidence rate of ATN was calculated as the ratio of the number of individuals who had developed ATN to the number of individuals at risk during a specific time interval. Only the first detected episode of ATN was considered for calculating the incidence rate. In order for the incidence rates to be accurately calculated, exposure for each time interval was dependent on whether or not the individual was examined at the end of that interval. For example, if the individual had a 12-week follow-up visit, but was not seen at week 2, the individual was not included in the 0-2 week interval exposure group.

Different methods were used to assess the serial changes in the variables of interest over time. First the longitudinal trends in the variables were examined graphically. Additionally, as the data consisted of repeated observations on the same individuals taken at specific time points (cross-sectional time series analysis), repeated measures analysis with a random effects model was performed. The model describes the trend over time while taking account of the correlation that exists between serial measurements. It also describes the variation in the baseline measurement and in the rate of change over time. To analyze the longitudinal patterns of different laboratory measurements, we used time as a within-individual factor and group (DSP status) as the between-individual factor (Genser et al., 2007). Random slopes were included in the model to address heterogeneity in measurements over time by adding dummy variables for the three time points after baseline, and creating interactions between group and time dummies. Data were expressed as a regression coefficient and represents the rate of change of one variable as a function of changes in the other, compared to baseline. No adjustment was made for multiple comparisons.

Cox proportional hazards regression, an aspect of time-to-event (survival) analysis, was used to assess the relationship between two or more continuous or categorical variables and ATN or incident symptoms. HIV viral load, white cell count, haemoglobin, MCV, ALT and

University of Cape Town

creatinine were not routinely performed at the local clinic after 24 weeks on cART. Therefore, these measurements were not included in the week 24 random effects model.

To determine the within-individual stability of the immunological data, Spearman's rank correlations were calculated between time points. The immunological data are presented as median and interquartile range (IQR) values, since their distribution was not normal.

2.8.3 Sample size for symptomatic DSP

We calculated sample size estimate using the proportions known to have developed symptomatic DSP from a cross-sectional study from a similar population (OpenEpi v2; http://www.openepi.com/SampleSize/SSPropor.htm). From a previous cross-sectional cohort (n=270) we expected 67% to have been eligible to enter the study i.e. would not have developed symptomatic neuropathy pre-cART. If Crossroads clinic services 6000 people who are HIV-infected (as per clinic manager) and about 3000 are already on cART. Every month approximately 30 individuals commence cART, therefore we estimate that the population size of 360 will be eligible for the study in one year of recruitment.

The hypothesized frequency of developing symptomatic DSP on cART in this population would be 36% ±5% (with a 95% CI) based on a previous South African cross-sectional study (Maritz et al., 2010). Therefore, with 90% confidence level the sample size should be 148.

University of Cape Town

Chapter 3 Laboratory Materials and Methods

University of Cape Town

3.1 NAT2 genotyping

3.1.1 DNA isolation

Genomic DNA was prepared from 3-5 mL of peripheral whole blood collected from each individual, in separate ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes. The buffy coat was prepared by centrifuging whole blood at 3300 g for 10 min at room temperature. The samples were divided into aliquots and stored at -80°C until analysed. Genomic DNA was isolated from the thawed samples using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). All extractions were performed according to the manufacturer's instructions (see Appendix F).

3.1.2 Determination of DNA concentration and integrity

The quantification of the purified nucleic acid solution was determined spectrophotometrically at 260 and 280 nm, by measuring the absorbance of a diluted sample aliquot placed in a beam of light with a 1 cm path length (NanoDrop 1000 software, NanoDrop Technologies, Wilmington, Delaware, USA) (Sambrook et al., 1989). Good quality high molecular weight genomic DNA was extracted. Stock solutions were diluted in sterile distilled water to a final working concentration of 50 ng/μL and stored at -20°C. The integrity of the DNA samples was ascertained by subjecting 150 ng of each sample with 5 μL loading buffer [40% sucrose, 0.25% bromophenol blue (Merck, Germany)] to agarose gel electrophoresis for 30 min at 120 V, on a 1% agarose low electroendosmosis gel (Roche Diagnostics, Indiana, USA) using Tris-Borate EDTA electrophoresis buffer (TBE)(891.8 mM Tris base; 889.4 mM boric acid; 19.9 mM EDTA, pH 8), and incorporating ethidium bromide (Sigma-Aldrich, Missouri, USA) (0.5 μg/mL) for visualization under ultraviolet light [UVIpro UVIGold Transiluminator (UVItec Limited, UK)].

3.1.3 Determination of NAT2 genotype

NAT2 genotype was determined using a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay adapted from the method by Doll et al. (Doll et al., 1995).

University of Cape Town

3.1.3.1 Polymerase chain reaction (PCR)

NAT2 was amplified by PCR using 150 ng of genomic DNA in a 100 µL reaction, each containing 0.04 µmol deoxyribonucleotide triphosphate (dNTP), 0.01 µmol $MgCl_2$, 2.5 units *Taq* DNA polymerase I and 1µg of each primer 5'GGCTATAAGAACTCTAGGAAC-3' (forward) and 5'AAGGGTTTATTTTGTTCCTTATTCTAAAT-3' (reverse) (Integrated DNA Technologies, Coralville, Iowa, USA) in corresponding reaction buffer (Promega, Madison, Wisconsin, USA) and distilled water. The mixture was subjected to thermal cycling conditions described in Table 3.1.

Table 3.1: Thermal cycling conditions for *NAT2* PCR, allele-specific PCR and cycle sequencing

Cycle	Process	*NAT2* PCR		Allele-specific PCR		Cycle sequencing	
		Temperature	Duration	Temperature	Duration	Temperature	Duration
1 x	**Pre-denaturation**	94.0 °C	5 min	94.0 °C	5 min	98.0 °C	5 min
30 x	**Denaturation**	94.0 °C	1 min	94.0 °C	1 min	96.0 °C	30 sec
	Annealing	55.0 °C	1 min	57.5 °C	30 sec	50.0 °C	15 sec
	Extension	72.0 °C	1 min	72.0 °C	1 min	60.0 °C	4 min
1 x	**Final extension**	72.0 °C	5 min	72.0 °C	5 min	-	-

Following the PCR, a 4 µL aliquot was mixed with 3 µL loading buffer, and electrophoresed on a 1% agarose gel in TBE buffer containing 0.5 µL/mL ethidium bromide for 30 min at 120 V. To determine the molecular mass of the band observed, 500 ng of the Gene Ruler 100 bp DNA Ladder-Plus Molecular Weight Marker (Fermentas International Inc., Hanover, USA) was included as a size standard. DNA was visualized and photographed under ultraviolet light. The resulting 895 bp PCR-product was subjected to RFLP analysis as described below.

3.1.3.2 Restriction fragment length polymorphism (RFLP) analysis

The presence or absence of eight separate nucleotide substitutions (all except 341 T>C) was determined by RFLP analysis. Digestion of 20 µL of PCR product was done in a total volume of 40 µL using the appropriate buffers and restriction enzymes for each reaction obtained from Promega/Fermentas Laboratories. Digestions were performed in four separate reactions. The restriction enzymes use in each reaction, their recognition and cleavage sites as well as their incubation conditions are summarized in Table 3.2.

University of Cape Town

Table 3.2: Restriction enzymes used for determination of seven polymorphisms of *NAT2*, recognition and cleavage sites and incubation conditions

Digest	SNP	Restriction enzymes	Recognition site	Temperature	Duration
1	191 G>A	*Msp* I	5'-C↓CGG-3'	37 °C	3 hours
	434 A>C	*Msp* I	5'-C↓CGG-3'	37 °C	3 hours
	481 C>T	*Kpn* I	5'-GGTAC↓C-3'	37 °C	3 hours
2	590 G>A	*Taq* I	5'-T↓CGA-3'	37 °C	2 hours
	857 G>A	*Bam* HI	5'-G↓GATCC-3'	65 °C	2 hours
3	282 C>T	*Fok* I	5'-GGATG $(N)_9$↓-3'	37 °C	3 hours
	845 A>C	*Dra* III	5'-CACNNN↓GTG-3'	37 °C	3 hours
4	803 A>G	*Dde* I	5'-C↓TNAG-3'	37 °C	3 hours

Nucleotide substitutions 191 G>A, 434 A>C, and 481 C>T were detected by digesting PCR product with the restriction enzymes *Msp*I (10 units) and *Kpn*I (10 units). *Msp*I cuts *NAT2* at bases 189 and 96 and *Kpn*I cuts at base 480 resulting in fragments of 415, 291, 96, and 93 bp (Figure 3.1). The 191 G>A substitution causes the loss of the *Msp*I site, yielding a 384-bp fragment instead of 291- and 93-bp fragments. An additional *Msp*I site is added by nucleotide substitution 434 A>C, yielding bands of 416, 244, 93, 66 and 47 bp. Nucleotide substitution 481 C>T leads to the loss of the *Kpn*I restriction site resulting in the replacement of the 415- and 291-bp fragments with a 706-bp fragment.

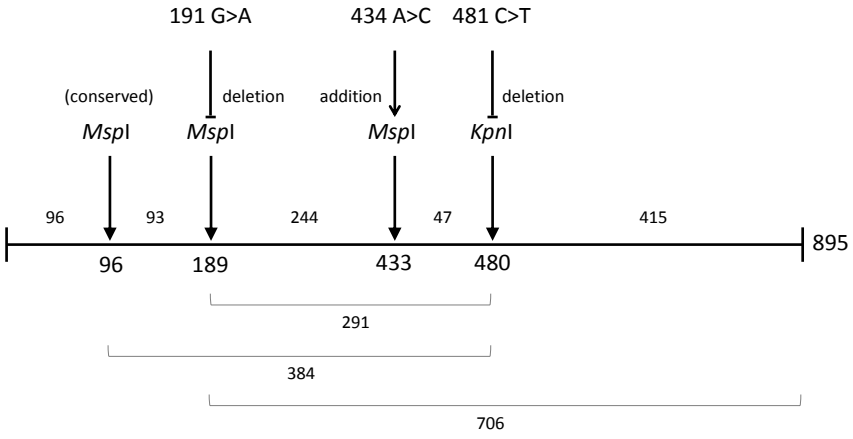


Figure 3.1: Predicted cleavage of *NAT2* by restriction enzymes *Msp*I and *Kpn*I (Digest 1)

University of Cape Town

Nucleotide substitutions 590 G>A and 857 G>A were detected by digesting the PCR product with restriction enzymes *Taq*I (10 units) and *Bam*HI (10 units). The 590 G>A substitution causes a loss of one of the *Taq*I sites at base 588, resulting in a 396-bp fragment instead of the 226- and 170-bp fragments (Figure 3.2). Nucleotide substitution 857 G>A causes the loss of the *Bam*HI restriction site resulting in a 137-bp fragment instead of the 98- and 39-bp fragments (Figure 3.2).

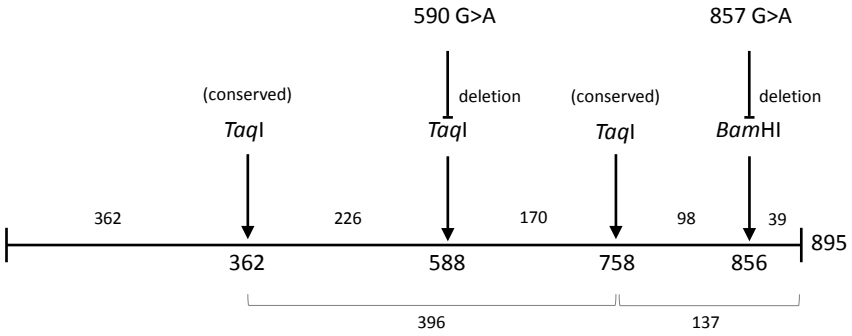


Figure 3.2: Predicted cleavage of *NAT2* by restriction enzymes *Taq*I and *Bam*HI (Digest 2)

The 282 C>T and 845 A>C substitutions were detected by digestion of the PCR product with the restriction enzymes *Fok*I (10 units) and *Dra*III (10 units). Nucleotide substitution 282 C>T causes the loss of one of the *Fok*I sites resulting in a 697-bp fragment instead of the 268- and 429-bp fragments (Figure 3.3). A new *Dra*III site id added by the 845 A>C substitution, cutting the 198-bp fragment into 153- and 45-bp fragments (Figure 3.3).

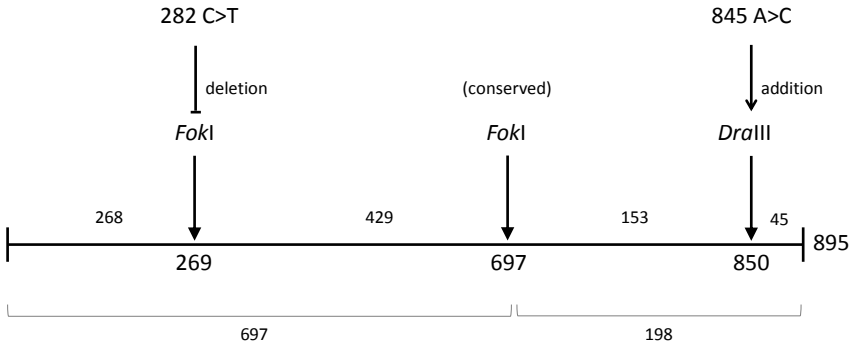


Figure 3.3: Predicted cleavage of *NAT2* by restriction enzymes *Fok*I and *Dra*III (Digest 3)

Nucleotide substitution 803 A>G was detected by digesting the 20 µL *NAT2* PCR product with the restriction enzyme *Dde*I (10 units). The 803 A>G substitution results in an additional *Dde*I restriction site, cutting a 119 bp fragment to 96- and 23-bp fragments (Figure 3.4).

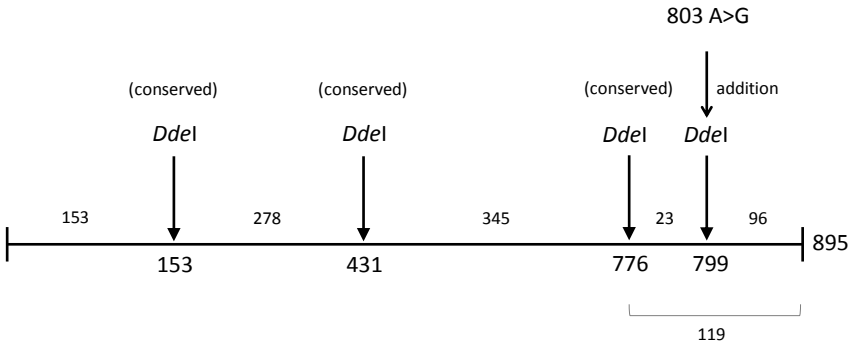


Figure 3.4: Predicted cleavage of *NAT2* by restriction enzyme *Dde*I (Digest 4)

For better separation of similarly-sized bands, digested samples were loaded on a 2.5% agarose gel containing 0.5 µL/mL ethidium bromide, run at 80 V for 2 hours, and visualized and photographed under ultraviolet light. In the presence of any unclear bands, the agarose gel was submerged in 0.5 µL/mL ethidium bromide staining solution for 15 min. The gel was then resubmerged in distilled water for 30 min to remove background staining, and thereafter visualized and photographed under ultraviolet light.

3.1.3.3 Allele-specific PCR amplification

Nucleotide substitution 341 T>C was detected by using allele-specific PCR due to the lack of a known restriction site near base 341. In order to distinguish between a C or a T at nucleotide 341, genomic DNA was amplified using two sets of primers: one primer set was specific for *NAT2* alleles with 341 T (5'-CTCCTGCAGGTGACCA**T**-3' and 5'-GGAGACGTCTGCAGGTATG-3'), whereas the other primer set was specific for *NAT2* alleles with 341 C (5'-CTCCTGCAGGTGACCA**C**-3' and 5'-GGAGACGTCTGCAGGTATG-3').

Amplification of *NAT2* (100 ng DNA) was carried out by PCR in a 100 µL reaction, each containing 0.04 µmol deoxyribonucleotide triphosphate (dNTP), 0.01 µmol $MgCl_2$, 2.5 units *Taq* DNA polymerase I and 1 µg of each primer in corresponding reaction buffer and distilled water. The optimal annealing temperature was determined with a temperature gradient experiment as the temperature at which the desired product yield was the highest (brightest

University of Cape Town

band) and showed the least amount of non-specific amplification (other bands). The mixture was then subjected to thermal cycling conditions as summarized in Table 3.1. PCR products were loaded onto a 1% agarose gel containing 0.5 µl/mL ethidium bromide, run at 100 V for 40 min, and visualized and photographed under ultraviolet light. Only primer set T amplified *NAT2* alleles homozygous for 341 T, *NAT2* alleles homozygous for 341 C were amplified only by primer set C, and heterozygotes were amplified by both sets of primers.

3.1.3.4 NAT2 sequencing

Forty-four PCR products samples were randomly selected and sequenced to confirm the genotyping obtained by RFLP. *NAT2* amplification was carried out by PCR using a forward primer (5´-GTCACACGAGGAAATCAAATGC-3`) and a reverse primer (5´-GTTTTCTAGCATGAATCACTCTGC-3`) as described in section 3.1.3.1. Sequencing reactions were done using the ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems, Foster City, California, USA). Reaction volumes are listed in Table 3.3 and reactions were performed according to thermal cycling conditions in Table 3.1.

Table 3.3: Reaction volumes for DNA sequencing

Reagent	Volume (µl)
Big dye	2
Buffer	4
H_2O	10
Primer	1
Purified PCR product	3
Final volume	20

Products of the cycle sequencing reaction were then purified by ethanol precipitation prior to resolution on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) as recommended by the manufacturer. Results were collected using ABI PRISM Data Collection Software v1.1 (Applied Biosystems). *NAT2* sequence chromatograms were visually inspected and analysed using software programme Chromaspro version 1.5 and aligned with the *NAT2* reference sequence (GenBank Accession number X14672.1) for SNP identification. Chromatograms were analysed for heterozygous changes and ambiguous sequence positions, and then aligned using ClustalW, available in BioEdit Sequence Alignment Editor version 7.0.9.0 (Figure 3.5).

University of Cape Town

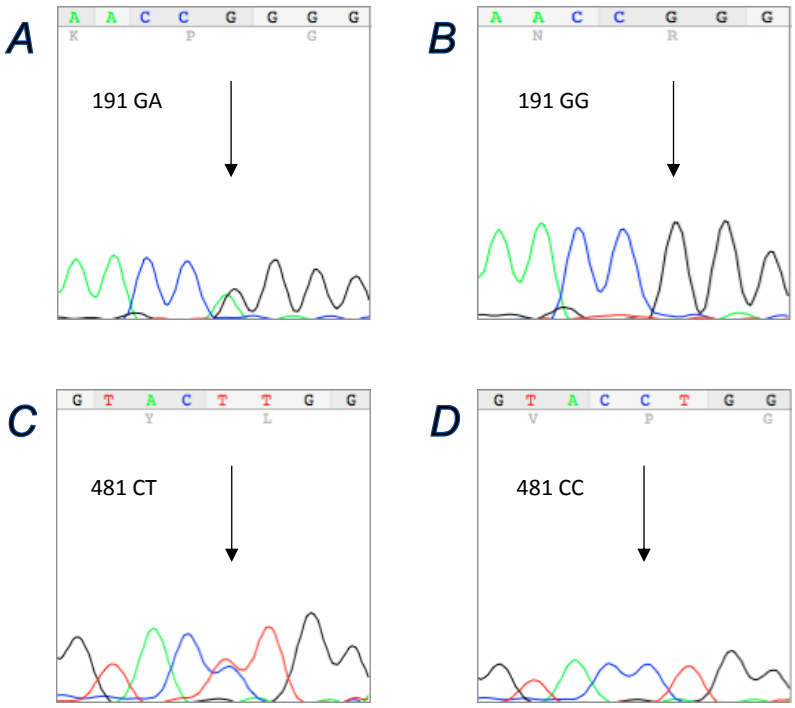


Figure 3.5: Chromatogram examples demonstrating SNP 191 heterozygous (A) and homozygous (B) and SNP 481 heterozygous (C) and homozygous (D)

Positions that differed from the reference sequence in both the forward and reverse sequences for a particular individual were investigated in the literature to determine their relevance to the structure and function of *NAT2* and its protein product. With a novel variant, the *NAT2* haplotype was translated into its corresponding amino acid sequence. The web-based tools PolyPhen-2 and PANTHER were used to predict the effect of the polymorphism on protein activity by measuring the evolutionary constraint acting on the site where it appeared.

3.1.3.5 Haplotype reconstruction

PHASE v2.1.1, a statistical programme for haplotype reconstruction was used to clarify haplotype ambiguity. The programme predicts an individual's acetylation status using a default model for recombination rate variation (Stephens et al., 2001, Stephens and Donnelly, 2003, Li and Stephens, 2003).

The PHASE method from Agundez et al. was applied (Agundez et al., 2008). We performed 20 independent runs with 1000 iterations, 500 burn-in iterations, and a thinning interval of 1. Of the 20 runs we selected one that showed the best consistency of results across all runs. The consistency of the results obtained was also investigated by applying the PHASE algorithm

University of Cape Town

repeatedly with default and varying values for the number of iterations, burn-in iterations and the thinning interval.

3.1.3.6 Genotype classification and genetic models

SNP frequencies were evaluated for Hardy-Weinberg equilibrium using the χ^2 test for goodness of fit to detect differences between observed and predicted frequencies of homozygous and heterozygous SNPs. The Hardy-Weinberg law states that both allele and genotype frequencies of a large, randomly mating population remain constant from generation to generation provided migration, mutation, and selection do not take place (Lunetta, 2008). Therefore, Hardy-Weinberg equilibrium is the stable distribution of frequencies of the genotypes AA, AB, and BB in the proportions p^2, 2pq, and q^2, respectively (where p and q represent the frequencies of the alleles A and B) (Lunetta, 2008). The genotype of each individual was determined as per standard *NAT2* nomenclature scheme (University of Louisville, 2011).

Three exploratory genetic models were applied for testing association between *NAT2* genotype and DSP status. In the dominant model, dominance of one of the alleles is inferred by treating the heterozygote and one of the homozygote genotypes as a single category. This dichotomization of the SNP genotypes implies that heterozygotes have the same risk or mean phenotype as one of the homozygotes (Lewis, 2002).

	AA	***AB + BB***
Cases	a	b + c
Controls	d	e + f

The general genetic model retains the three genotypes and makes no distinction about how the risk differs between heterozygotes or homozygotes. A single SNP with alleles A and B will generate six different genotypes (AA, AB and BB) between cases and controls (Lewis, 2002).

	AA	***AB***	***BB***
Cases	a	b	c
Controls	d	e	f

University of Cape Town

In the additive model, the risk conferred by an allele is increased r-fold for heterozygotes and 2r-fold for homozygotes, although it is acknowledged that genes do not generally act in a simple additive manner, but rather through complex networks involving gene to gene and gene to environment interactions (Colhoun et al., 2003).

	AA	*AB*	*BB*
Cases	a	b	c
Controls	d	e	f

Additive model: r-fold increased risk for AB, 2r increased risk for BB

3.1.3.7 NAT2 phenotype

We inferred from *NAT2* genotypes the distribution of rapid, intermediate and slow acetylation phenotypes. Individuals homozygous for slow bearing haplotypes were classified as slow acetylation phenotype. Individuals homozygous for rapid haplotypes were classified as rapid acetylation phenotype. Heterozygote individuals bearing a rapid and a slow haplotype were considered intermediate acetylators, because they present a mean intermediate NAT2 activity significantly different from that of rapid or slow homozygotes (Cascorbi et al., 1995).

University of Cape Town

3.2 Cytokine and soluble cytokine receptor quantification

3.2.1 Multiplex multiple analyte profiling

Levels of plasma cytokine concentrations were quantified on a BioPlex Multiplex platform (Bio-Rad Laboratories, Hercules, California, USA) using MILLIPLEX xMAP High Sensitivity Human Cytokine kits (Millipore, Billerica, Massachusetts, USA). The Millipore Multiplex Multiple Analyte Profiling (MAP) system permits the simultaneous quantitative analysis of up to 100 different proteins and peptides in 12 µL or less of sample – thus the term "multiplexing". The system uses a liquid suspension array of 100 sets of 5.6 µm polystyrene microspheres (beads), each internally dyed with different ratios of two spectrally distinct fluorophores, red and infrared. This red/infrared mixture assigns it a unique spectral signature.

Each set of beads is then conjugated with a different capture molecule (Figure 3.6). Capture molecules can include DNA, enzyme substrates, antigens, antibodies and receptors. The

conjugated beads are then mixed with a sample and incubated in a microplate-well to react with specific cytokines. To detect and quantitate each captured analyte, a fluorescently labelled reporter antibody that specifically binds the analyte is added. The reporter antibody is biotinylated, enabling a fluorescent protein, streptavidin phycoerithrin, to bind.

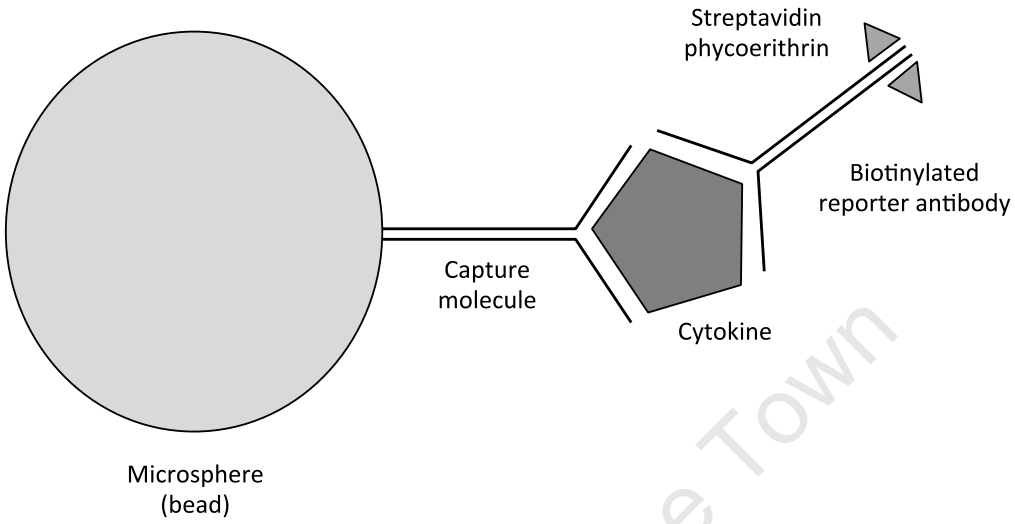


Figure 3.6: Multiple analyte profiling assay principle

University of Cape Town

Following incubation, the contents of each microplate well are drawn into the Bio-Plex array reader, and precision fluidics, based on the principle of flow cytometry, align the beads in single file through a flow cell. This allows the particles to be measured discretely. Here two lasers excite the beads individually. As the bead passes through the detection chamber, the red laser excites both the internal red and infrared dyes, allowing the proper classification of the bead (i.e. classification channel reading). The green laser excites any orange fluorescence associated with the binding of the analyte (i.e. reporter channel reading). Since both the classification and the reporter readings are made on each individual particle, the system is able to precisely determine multiplexed assay. High-speed digital signal processors and Bio-Rad software record the fluorescent signals simultaneously for each bead, translating the signals into data for each assay.

3.2.2 Method

Assays were performed by JJvdW on plasma samples processed as previously described. As we anticipated low systemic cytokine levels, measuring picogram (pg) levels of cytokines was critical. We therefore decided to quantify cytokine concentrations by using a high

sensitivity assay. This assay simultaneously measures levels of the following human cytokines: Granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13 and TNF-α.

Plasma cytokine receptor concentrations of soluble IL (sIL)-1 receptor I (sIL-1RI), sIL-1 receptor II (sIL-1RII), sIL-2 receptor-α (sIL-2Rα), sIL-4 receptor (sIL-4R), sIL-6 receptor (sIL-6R), sTNF receptor I (sTNFRI) and sTNF receptor II (sTNFRII) were quantified using MILLIPLEX xMAP Human Soluble Cytokine Receptor Panel. IL-1RA was quantified by using a customised MILLIPLEX xMAP Human Cytokine/Chemokine Immunoassay.

Milliplex kits were stored at 2-8°C until used for quantification. The assays were carried out according to the manufacturer's instructions. In brief, 25 µL pre-mixed antibody-conjugated beads were prepared and aliquoted into a 96-well pre-wetted filter plate before addition of either 50 µL of standard or control solution in the designated wells or 50 µL of undiluted plasma. For the soluble cytokine receptor and IL-1RA quantification, 25 µL antibody-conjugated beads were added to a pre-wetted filter plate after addition of either 25 µL of standard or control in the designated wells or 25 µL of undiluted plasma. The fluorescent beads for this study were obtained from Millipore (Billerica, Massachusetts, USA). The filter plate was sealed with a plate sealer, covered with the lid and incubated with agitation on a plate shaker overnight (16-18 hours) at 4°C for maximum sensitivity.

Samples were run in single wells only to allow all samples for each assay to be run on a single plate and thus avoid plate-to-plate variability. Two controls per plate and eight calibrated standards with known concentrations of the cytokines of interest (measured in duplicate) were added to aid in the in interpretation of results (Figure 3.7). Subsequent steps involved washes interspersed by the addition of 50 µL biotinylated detector antibody and later 50 µL streptavidin-phycoerythrin solution. Incubation with agitation on a plate shaker followed for 30 min at room temperature (20-25°C). For the soluble receptor and IL-1RA analysis, this step involved washes interspersed by the addition of 25 µL biotinylated detector antibodies with 25 µL streptavidin-phycoerythrin solution. Incubation with agitation on a plate shaker followed for 30 min at room temperature.

University of Cape Town

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pg/mL Standard (Background)	16 pg/mL Standard	QC-1 Control	Etc.								
B	0 pg/mL Standard (Background)	16 pg/mL Standard	QC-1 Control									
C	0.13 pg/mL Standard	80 pg/mL Standard	QC-2 Control									
D	0.13 pg/mL Standard	80 pg/mL Standard	QC-2 Control									
E	0.64 pg/mL Standard	400 pg/mL Standard	Sample 1									
F	0.64 pg/mL Standard	400 pg/mL Standard	Sample 1									
G	3.2 pg/mL Standard	2000 pg/mL Standard	Sample 2									
H	3.2 pg/mL Standard	2000 pg/mL Standard	Sample 2									

Figure 3.7: Well plate layout with calibrated standard- and control samples

University of Cape Town

For analysis, the plate was placed on the XY platform of the Bio-Rad Luminex 100 Bio-Plex Liquid Array Multiplexing System (BioRad Laboratories Ltd, Missasauga, Ontario, Canada) after adding 100 μL sheath fluid to each well. STarStation v3.0 software was used for data acquisition and analysis. Standard curves were generated for each analyte, and the mean fluorescence intensity value of each analyte in each well was converted into a concentration using the linear portion of the standard for all detected values. Concentrations lower than the detection limit of the kit's sensitivity were extrapolated from the standard curve. Concentrations were plotted over time using Prism version 6 (GraphPad Software, Inc., La Jolla, California, USA).

3.3 Vitamin B6 quantification

Plasma vitamin B6 levels were quantified at the United States Centers for Disease Control and Prevention (CDC) under the direction of Dr Michael Rybak. A reversed-phase high-performance liquid chromatography (HPLC) method with fluorometric detection was used determination of PLP and 4-PA in plasma (Rybak and Pfeiffer, 2004).

Fasting plasma samples were collected in EDTA tubes at baseline and 12-week visits. The samples were immediately placed on ice after collection to protect samples from exposure to direct ultraviolet-light. After blood collection, tubes were centrifuged at 3300 g for 10 min The supernatant fraction of each tube aliquoted into labelled 1.5 mL Eppendorf tubes and stored immediately in a -80°C freezer for long-term storage. Prepared samples were sealed in plastic containers and shipped to Atlanta (Georgia, USA) on dry ice prior to analysis.

3.4 High-sensitivity CRP and albumin quantification

Metropolis Pathology Laboratories (Cape Town, South Africa) performed serum hs-CRP and albumin quantification. Albumin levels were measured by an immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). High-sensitivity CRP levels were measured by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics). Prepared aliquoted serum samples were sealed in plastic containers and transported on ice to the laboratory for analysis.

University of Cape Town

Chapter 4 Clinical Results

University of Cape Town

4.1 Baseline results

4.1.1 Demographic characteristics

The baseline study sample comprised of 184 participants. Initially, 190 individuals who were eligible to initiate cART and elected to participate were enrolled. Two individuals were excluded from the study, as they did not commence cART within two weeks of the baseline (pre-cART) assessment. Three individuals withdrew their consent to participation prior to the baseline assessment. One individual was excluded due to asymmetrical neuropathic signs on examination.

The baseline (pre-cART) characteristics of the 184 remaining individuals are summarized in Table 4.1 for continuous variables and Table 4.2 for categorical variables. The baseline assessment was performed a median of 1 day prior to initiation of cART (IQR 1-5 days). The study population was predominantly female (70%), with an age range of 19-63 years and a median age of 33 years (IQR 26-39 years).

Sixty-nine individuals (38%) had a history of previous or current treatment for TB (indicating INH exposure). Of these, 39 completed TB treatment before study entry and 30 started TB treatment before study entry, continuing with treatment during the study period. Eight developed TB after cART initiation and started TB treatment after the baseline assessment (six between week 4 and week 12, and two after week 12). Fifty-two individuals (29%) reported consumption of alcohol during the year prior to enrolment.

University of Cape Town

Table 4.1: Baseline characteristics in individuals with and without DSP (continuous data)

Variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=184)	DSP (N=41)	No DSP (N=143)	
Clinical						
Age	years		33 (26 - 39)	38 (29 - 43)	31 (26 - 37)	***0.001*** [β]
Weight	kg		61 (54 - 71)	61 (53 - 71)	61 (55 - 71)	0.488 [β]
Height	metre		1.61 (1.56 - 1.67)	1.62 (1.55 - 1.70)	1.61 (1.56 - 1.66)	0.348 [β]
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.2 (20.3 - 27.7)	22.9 (20.2 - 26.1)	23.2 (20.4 - 28.0)	0.430 [ε]
Waist : Hip ratio		*< 0.90*	0.86 (0.81 - 0.90)	0.87 (0.83 - 0.93)	0.86 (0.81 - 0.89)	0.073 [β]
Systolic BP	mmHg	*120 - 140*	110 (103 - 122)	110 (100 - 122)	111 (104 - 121)	0.431 [β]
Diastolic BP	mmHg	*80 - 90*	72 (66 - 80)	71 (66 - 78)	73 (67 - 80)	0.215 [ε]
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	158 (114 - 196)	162 (105 - 199)	156 (114 - 196)	0.447 [β]
White cell count	$x10^9/L$	*4.0 - 11.0*	5.3 (4.1 - 6.5)	5.2 (3.9 - 6.0)	5.4 (4.1 - 6.8)	0.455 [β]
C-reactive protein	mg/L	*< 5.0*	2.8 (0.9 - 6.6)	3.7 (1.2 - 11.9)	2.7 (0.9 - 5.9)	0.199 [β]
Haemoglobin	g/dL	*11.6 - 15.6*	11.8 (10.4 - 12.6)	12.1 (9.6 - 12.9)	11.7 (10.6 - 12.6)	0.569 [β]
MCV	fL	*80.0 - 100.0*	92.4 (88.5 - 96.1)	93.2 (89.8 - 96.9)	92.1 (87.9 - 95.9)	0.075 [β]
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 43)	38 (34 - 43)	39 (35 - 43)	0.286 [β]
ALT	IU/L	*10 - 41*	19 (15 - 26)	23 (18 - 29)	18 (14 - 26)	0.107 [β]
Creatinine	µmol/L	*53 - 115*	64 (55 - 73)	65 (55 - 75)	64 (55 - 73)	0.908 [β]
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.5 - 4.9)	4.7 (4.4 - 4.9)	0.859 [β]
Fasting insulin	µU/mL	*0.2 - 9.4*	5.2 (2.5 - 8.6)	4.3 (1.8 - 7.6)	5.5 (2.5 - 8.9)	0.155 [β]
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.3)	4.1 (3.0 - 4.4)	3.7 (3.2 - 4.2)	0.602 [β]
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.7 - 1.2)	1.0 (0.7 - 1.4)	0.8 (0.7 - 1.1)	***0.035*** [β]
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.75 - 1.13)	0.94 (0.74 - 1.25)	0.95 (0.75 - 1.12)	0.659 [β]
LDL	mmol/L	*1.0 - 3.0*	2.3 (1.9 - 2.9)	2.5 (1.8 - 2.9)	2.3 (1.9 - 2.9)	0.420 [β]
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	2.1 (1.2 - 2.8)	2.5 (1.7 - 3.3)	0.099 [β]
Pyridoxine						
PLP	nmol/L	*> 25.0*	23.8 (16.5 - 38.4)	25.5 (16.5 - 53.7)	23.8 (15.7 - 36.8)	0.392 [β]
4-PA	nmol/L	*unknown*	17.4 (11.9 - 23.3)	20.5 (12.1 - 28.6)	16.7 (11.6 - 22.4)	0.089 [ε]

[β] Student's *t*-test

[ε] Wilcoxon rank-sum test

Table 4.2: Baseline characteristics in individuals with and without DSP (grouped data)

Variable	No. (%)			p-value
	Total (N=184)	DSP (N=41)	No DSP (N=143)	
Female sex	129 (70%)	27 (66%)	102 (71%)	0.500 §
Age > 40 years	42 (23%)	16 (40%)	26 (18%)	***0.004*** §
Previous/Current TB	69 (38%)	22 (54%)	47 (33%)	***0.015*** §
Time of TB				0.128 ¶
Currently	30 (16%)	11 (27%)	19 (13%)	
< 1 year ago	13 (7%)	4 (10%)	9 (6%)	
1 year ago	5 (3%)	2 (5%)	3 (2%)	
2 years ago	5 (3%)	1 (2%)	4 (3%)	
> 2 years ago	16 (9%)	4 (10%)	12 (8%)	
Vit Bco supplement	179 (97%)	40 (98%)	139 (97%)	1.000 ¶
Vit B6 supplement	15 (8%)	8 (20%)	7 (5%)	***0.011*** ¶
WHO clinical stage				0.612 ¶
Stage 1	57 (32%)	11 (28%)	46 (33%)	
Stage 2	58 (32%)	11 (28%)	47 (34%)	
Stage 3	58 (32%)	16 (40%)	42 (30%)	
Stage 4	7 (4%)	2 (5%)	5 (4%)	
CD4 T-cell count				0.929 §
< 100 cells/mm ^{3}	30 (17%)	6 (15%)	24 (17%)	
100 - 200 cells/mm ^{3}	108 (60%)	24 (60%)	84 (60%)	
> 200 cells/mm ^{3}	42 (23%)	10 (25%)	32 (23%)	
Metabolic syndrome	22 (12%)	4 (10%)	18 (13%)	0.622 §
Body Mass Index				0.901 §
< 20 kg/m ^{2}	37 (21%)	9 (23%)	28 (20%)	
20 - 25 kg/m ^{2}	77 (43%)	16 (41%)	61 (44%)	
25 - 30 kg/m ^{2}	37 (21%)	9 (23%)	28 (20%)	
> 30 kg/m ^{2}	28 (16%)	5 (13%)	23 (16%)	
Alcohol last year	52 (29%)	8 (20%)	44 (31%)	0.160 §
IHDS score < 10	33 (24%)	10 (45%)	23 (20%)	***0.011*** §

¶ Fisher's exact test

§ χ^2 test

University of Cape Town

4.1.2 HIV staging and severity

Seventy five per cent of individuals (n=138) were diagnosed with HIV after 2007. Nearly two thirds (n=115, 63%) were classified as either WHO stage 1 or 2 HIV infection, and only 4% (n=7) were classified as WHO stage 4. None of the individuals had any overt clinical

opportunistic infections as such cases were excluded from participating in the study at enrolment (n=7).

The CD4 T-cell count prior to cART initiation ranged from 4 to 448 cells/mm^3, with a median of 158 cells/mm^3 (IQR 114-196). The majority of individuals (60%) had a CD4 T-cell count between 100 and 200 cells/mm^3 and only 17% had counts <100 cells/mm^3. Baseline HIV viral loads were not routinely done at the local clinic.

4.1.3 Nutritional and metabolic parameters

The median BMI was 23.2 kg/m^2 (IQR 20.3-27.7) and 77 (43%) had normal values. There were less individuals underweight (21%) compared to overweight or obese individuals (36%) (Table 4.2) The height of this cohort ranged from 1.43 to 1.84 m, with a median height of 1.61 metres (IQR 1.56-1.67). Waist-to-hip ratios were used to assess body fat distribution. Forty-eight females (37%) had a waist-to-hip ratio greater than 0.85 and 19 males (35%) had a ratio greater than 0.90.

Baseline levels of serum albumin were done in 151 consecutive individuals (82%). Serum albumin <38 g/L was found in 68 (45%). There was no correlation between BMI and serum albumin (ρ=0.10 p=0.22). Of those with albumin <38 g/L, 23 (35%) were eutrophic and 19 (29%) overweight or obese. Of 83 (55%) with serum albumin ≥38 g/L, 40 (48%) were eutrophic and 32 (39%) were overweight.

Anaemia has been widely reported to predict a poorer prognosis independent of the CD4 T-cell count in HIV-infected individuals, both in terms of progression to AIDS and in survival (Chene et al., 1997, Apolonio et al., 1995, Hoover et al., 1995). At baseline, 40 (22%) had a haemoglobin <10 g/dL, 123 (69%) had a haemoglobin between 10 and 14 g/dL, and 15 (8%) >14g/dL. The majority of individuals (86%, n=153) had a normal MCV; 16 (9%) had an MCV >100 fL and four individuals (2%) had a microcytic anaemia (with an MCV <80 fL and haemoglobin <10 g/dL).

Levels of plasma PLP and 4-PA as measures of vitamin B6 were investigated in 159 consecutively selected individuals who were followed up for at least 12 weeks. Detailed results on vitamin B6 concentrations are discussed in Chapter 8.

University of Cape Town

Eight individuals (5%) were prediabetic and had fasting serum glucose levels >5.5 mmol/L. Median serum fasting glucose was 4.7 mmol/L (IQR 4.4-4.49). Fasting serum insulin levels >15 μU/mL, as a measure for insulin resistance (McLaughlin et al., 2003), were present in seven individuals (4%).

Overall, the baseline fasting median values and IQR for all metabolic blood biochemistry outlined in Table 4.1 were within normal ranges. The median triglyceride level was 0.87 mmol/L, the median total cholesterol 3.74 mmol/L and the median HDL cholesterol 0.94 mmol/L. Thirty (16%) of the individuals had low HDL cholesterol levels, eight (5%) had elevated triglycerides, 10 (6%) had elevated total cholesterol, and 30 (16%) had elevated LDL cholesterol. Twenty-two individuals (12%) were classified as having metabolic syndrome at baseline assessment as defined in section 2.6. Point-of-care lactate levels ranged from 0.8 to 6.1 mmol/L, with 89 individuals (61%) having elevated lactate levels (>2.0 mmol/L).

4.1.4 Supplements and concomitant drug administration

Nearly all individuals (97%) were prescribed vitamin B complex supplements at the time of enrolment. It was not documented why five individuals did not receive vitamin B complex supplementation. Of 30 individuals on TB treatment at the time of enrolment, only 12 (40%) received additional vitamin B6 supplementation in a dosage of 25 mg/day. Cotrimoxazole was prescribed for 97% of individuals at baseline.

Eight individuals (4%) reported using analgesia (paracetamol and/or topical methyl salicylate) for existing neuropathic pain. These agents did not provide any relief in symptoms to any of these individuals. None of the individuals received amitriptyline during the study period.

4.1.5 Frequency and clinical characteristics of DSP at baseline (pre-cART)

The frequency of DSP at baseline was 22% (n=41). Baseline DSP status was categorised as follows:

- Symptomatic DSP, 31 (17%)
- Asymptomatic DSP, 10 (5%)

University of Cape Town

- No DSP, 143 (78%) – those individuals did not meet the criteria of symptomatic DSP or asymptomatic DSP and included seven (4%) with neuropathic symptoms in isolation.

Data regarding symptom and sign frequency for individuals with DSP are presented in Table 4.3. Paraesthesiae and burning or aching pain were prevalent symptoms associated with DSP, affecting 73% of individuals. Muscle cramps as a neuropathic symptom were more prevalent in individuals with DSP (63%), although reported by 38% of those without DSP. Although most individuals with DSP at baseline experienced moderate symptoms (VAS=4-6), six (15%) reported mild (grade 1) symptoms, and in 10 (24%) the symptoms were graded as moderately severe (grade 3 or 4; VAS=7-10).

Table 4.3: Baseline neuropathic symptoms and signs

Variable	No. (%)			p-value
	Total (N=184)	DSP (N=41)	No DSP (N=143)	
Neuropathic symptoms				
Pain	30 (16%)	26 (63%)	4 (3%)	***<0.001*** ¶
Paraesthesiae	29 (16%)	26 (63%)	3 (2%)	***<0.001*** ¶
Numbness	22 (12%)	19 (46%)	3 (2%)	***<0.001*** ¶
Cramps	80 (43%)	26 (63%)	54 (38%)	***0.003*** §
*Positive Sensory**	35 (19%)	30 (73%)	5 (4%)	***<0.001*** ¶
Neuropathic signs				
Altered vibration sensibility	23 (13%)	15 (37%)	8 (6%)	***<0.001*** ¶
Altered pin sensibility	49 (27%)	31 (76%)	18 (13%)	***<0.001*** §
Altered proprioception	17 (9%)	9 (22%)	8 (6%)	***0.001*** ¶
Absent/reduced deep tendon reflexes	65 (35%)	28 (68%)	37 (26%)	***<0.001*** §
Reduced muscle strength	6 (3%)	6 (15%)	0 (0%)	***<0.001*** ¶

* Pain and/or paraesthesiae

¶ Fisher's exact test

§ χ^2 test

The most common neuropathic sign in individuals with DSP was altered pinprick sensibility in a stocking distribution, affecting 76% of the group. Eighteen individuals (13%) without the clinical definition of DSP also had altered pinprick sensibility. Twenty-eight individuals with DSP (68%) had abnormal ankle reflexes whereas 37 without DSP (26%) had abnormal ankle reflexes in isolation. Of those with abnormal ankle reflexes, nearly half (41%) had absent ankle reflexes. Fifteen individuals (37%) had abnormal vibratory sensibility. Weakness of the extensors was only present in six individuals (3%) and was mild; those with weakness also

University of Cape Town

had abnormal ankle reflexes, five had abnormal vibratory sensation and four had altered pinprick sensibility. Although 17 individuals (9%) had abnormal proprioception, this included eight individuals (6%) in whom the sign was isolated and therefore categorized as no DSP. The presence of at least one neuropathic sign in over 25% of individuals categorized as no DSP suggests a definition that is perhaps too stringent.

4.1.6 Risk factor investigation

4.1.6.1 DSP risk factors in cART naïve individuals at baseline

A comparison of baseline characteristics between individuals with DSP pre-cART and individuals without DSP are illustrated in Table 4.1 page 89. The presence of DSP [either (i) two signs or (ii) one symptom and one sign] in this cART-naïve cohort was associated with the following:

a) Age >40 years (p=0.004)
b) As individuals on TB treatment receive vitamin B6 supplementation, current or previous TB treatment (p=0.015) and vitamin B6 supplementation (p=0.011) were both associated with DSP.
c) Increased fasting triglycerides (p=0.035). Although individuals with DSP had higher fasting triglycerides compared to those without DSP, the 75th percentile values of both DSP and no-DSP groups were within normal fasting triglyceride ranges. Of eight individuals with raised fasting triglyceride levels, only two had DSP at baseline.
d) Total IHDS score <10 (p=0.011)

Clinical features including sex (p=0.50), weight (p=0.49), height (p=0.35), BMI (p=0.43), systolic BP (p=0.43) and diastolic BP (p=0.22) were not significant predictors of DSP at baseline. There was a trend towards higher waist-to-hip ratios in individuals with DSP compared to those without, however the 75th percentile values of both groups were still within normal range.

The presence of DSP was not associated with advanced HIV disease. While there was no difference between the two groups in terms of WHO HIV stage (p=0.61) or baseline CD4 T-cell count (p=0.45), there was a trend towards higher hs-CRP levels in individuals with DSP at baseline compared to those without (3.7 vs 2.7 mg/L, p=0.19).

University of Cape Town

Apart from triglycerides, other fasting lipids and metabolic factors did not associate with DSP at baseline, including total cholesterol (p=0.60), glucose (p=0.86), lactate (p=0.10), and the metabolic syndrome (p=0.62). Haematological factors including white cell count (p=0.46), haemoglobin (p=0.57) and MCV (p=0.08) were not significant predictors of DSP at baseline. Biochemical factors including ALT (p=0.11), albumin (p=0.29) and creatinine (p=0.91) did not associate with DSP at baseline. No difference was detected in alcohol exposure between the two groups in general (p=0.30).

Univariate odds ratios and prevalence ratios (with a 95% CI) were calculated to determine univariate risk factors associated with DSP. Factors significantly associated with DSP in the primary analysis (parametric, non-parametric and proportional) were examined by individual logistic regression. All variables were explored for an association with DSP, but only variables primarily showing a significance level of p<0.25 in the primary analysis were included in the multivariate analysis and are shown in Table G-1 (Appendix G).

A multivariate logistic regression analysis was performed as described in section 2.8.1.2 to identify independent predictors of DSP at baseline. Results are reflected in Table 4.4. Variables included in the multivariate model were age, sex, waist-to-hip ratio, diastolic BP, previous/current TB, alcohol exposure, ALT, hs-CRP, MCV, fasting insulin and triglycerides, lactate and 4-PA. The IHDS score and vitamin B6 supplementation were not included due to the possibility of a high covariance with DSP and TB treatment respectively. The wide confidence intervals make interpretation of the model difficult and introduce a degree of uncertainty in associations found. Furthermore, other associations cannot be ruled out.

University of Cape Town

Table 4.4: Multivariate analysis of baseline DSP risk factors

Model	Odds Ratio	Odds Ratio 95% confidence interval	Variable p-value	Pseudo R^2	Model p-value
DSP vs No DSP				0.31	***<0.001***
*Age **	1.14	1.07 - 1.22	***<0.001***		
Triglycerides > 0.9 mmol/L	4.97	1.39 - 17.81	***0.010***		
C-reactive protein > 5 mg/L	4.55	1.36 - 15.18	***0.010***		
Previous/Current TB	4.00	1.23 - 13.01	***0.020***		

* Per one year increase

4.1.6.2 Risk factors for symptomatic DSP in cART naïve individuals at baseline

To investigate risk factors associated with symptomatic DSP at baseline, a comparison between individuals with symptomatic DSP to those without DSP was performed (Table 4.5 for continuous variables and Table 4.6 for categorical variables).

Univariate analysis revealed that variables associated with symptomatic DSP at baseline included:

a) Age >40 years (p=0.009).
b) Previous or current TB treatment (p=0.009).
c) Higher waist-to-hip ratio (p=0.022), a factor that only showed a trend towards an association with DSP at baseline.
d) As individuals on TB treatment receive vitamin B6 supplementation, current or previous TB treatment (p=0.009) and vitamin B6 supplementation (p=0.001) were both associated with symptomatic DSP.
e) Increased fasting triglycerides (p=0.026). Although individuals with symptomatic DSP had higher fasting triglycerides compared to those without DSP, the interquartile ranges of both groups were within normal limits.

Therefore, the factors associated with symptomatic DSP were similar to those shown to be associated with DSP overall.

University of Cape Town

Table 4.5: Baseline characteristics in individuals with symptomatic DSP vs those without DSP (continuous data)

Variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=174)	SDSP (N=31)	No DSP (N=143)	
Clinical						
Age	years		33 (26 - 39)	37 (29 - 43)	31 (26 - 37)	***0.008*** [β]
Weight	kg		61 (54 - 71)	60 (53 - 71)	61 (55 - 71)	0.618 [ε]
Height	metre		1.61 (1.56 - 1.67)	1.62 (1.55 - 1.68)	1.61 (1.56 - 1.66)	0.565 [β]
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.2 (20.4 - 27.8)	23.2 (20.8 - 25.9)	23.2 (20.4 - 28.0)	0.602 [ε]
Waist : Hip ratio		*< 0.90*	0.86 (0.81 - 0.90)	0.87 (0.83 - 0.93)	0.86 (0.81 - 0.89)	***0.022*** [β]
Systolic BP	mmHg	*120 - 140*	110 (103 - 120)	109 (99 - 119)	111 (104 - 121)	0.198 [β]
Diastolic BP	mmHg	*80 - 90*	72 (66 - 79)	70 (66 - 77)	73 (66 - 80)	0.175 [β]
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	156 (113 - 196)	157 (105 - 217)	156 (114 - 196)	0.455 [β]
White cell count	$x10^9/L$	*4.0 - 11.0*	5.4 (4.1 - 6.5)	5.1 (3.9 - 6.0)	5.4 (4.1 - 6.8)	0.374 [ε]
C-reactive protein	mg/L	*< 5.0*	2.9 (0.9 - 6.6)	3.8 (1.3 - 9.7)	2.7 (0.9 - 5.9)	0.251 [β]
Haemoglobin	g/dL	*11.6 - 15.6*	11.7 (10.3 - 12.6)	11.6 (9.3 - 12.4)	11.7 (10.6 - 12.6)	0.142 [β]
MCV	fL	*80.0 - 100.0*	92.4 (88.1 - 96.1)	93.3 (89.0 - 96.9)	92.1 (87.9 - 95.9)	0.138 [β]
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 43)	37 (33 - 41)	39 (35 - 43)	0.096 [β]
ALT	IU/L	*10 - 41*	19 (15 - 26)	23 (18 - 28)	18 (14 - 26)	0.063 [ε]
Creatinine	µmol/L	*53 - 115*	64 (56 - 73)	65 (57 - 73)	64 (55 - 73)	0.855 [ε]
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.5 - 4.9)	4.7 (4.4 - 4.9)	0.785 [ε]
Fasting insulin	µU/mL	*0.2 - 9.4*	5.3 (2.5 - 8.6)	4.3 (2.5 - 7.6)	5.5 (2.5 - 8.9)	0.200 [β]
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.2)	3.9 (3.0 - 4.3)	3.7 (3.2 - 4.2)	0.323 [β]
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.7 - 1.2)	1.0 (0.8 - 1.4)	0.8 (0.7 - 1.1)	***0.026*** [β]
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.75 - 1.12)	0.89 (0.74 - 1.13)	0.95 (0.75 - 1.12)	0.464 [β]
LDL	mmol/L	*1.0 - 3.0*	2.3 (1.9 - 2.8)	2.2 (1.8 - 2.7)	2.3 (1.9 - 2.9)	0.103 [β]
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	1.8 (1.2 - 2.6)	2.5 (1.7 - 3.3)	0.067 [β]
Pyridoxine						
PLP	nmol/L	*> 25.0*	23.4 (16.2 - 38.4)	20.7 (16.2 - 53.7)	23.8 (15.7 - 36.8)	0.474 [β]
4-PA	nmol/L	*unknown*	17.3 (11.7 - 22.8)	20.7 (12.1 - 28.6)	16.7 (11.6 - 22.4)	0.169 [ε]

[β] Student's *t*-test

[ε] Wilcoxon rank-sum test

Table 4.6: Baseline characteristics in individuals with symptomatic DSP vs those without DSP (grouped data)

Variable	No. (%)			p-value
	Total (N=174)	SDSP (N=31)	No DSP (N=143)	
Female sex	123 (71%)	21 (68%)	102 (71%)	0.691 §
Age > 40 years	38 (22%)	12 (40%)	26 (18%)	***0.009*** §
Previous/Current TB	65 (37%)	18 (58%)	47 (33%)	***0.009*** §
Time of TB				***0.039*** ¶
Currently	29 (17%)	10 (32%)	19 (13%)	
< 1 year ago	12 (7%)	3 (10%)	9 (6%)	
1 year ago	5 (3%)	2 (6%)	3 (2%)	
2 years ago	5 (3%)	1 (3%)	4 (3%)	
> 2 years ago	14 (8%)	2 (6%)	12 (8%)	
Vit Bco supplement	170 (98%)	31 (100%)	139 (97%)	1.000 ¶
Vit B6 supplement	15 (9%)	8 (26%)	7 (5%)	***0.001*** ¶
WHO clinical stage				0.340 ¶
Stage 1	54 (32%)	8 (27%)	46 (33%)	
Stage 2	54 (32%)	7 (23%)	47 (34%)	
Stage 3	55 (32%)	13 (43%)	42 (30%)	
Stage 4	7 (4%)	2 (7%)	5 (4%)	
CD4 T-cell count				0.831 §
< 100 cells/mm ^{3}	28 (16%)	4 (13%)	24 (17%)	
100 - 200 cells/mm ^{3}	102 (60%)	18 (60%)	84 (60%)	
> 200 cells/mm ^{3}	40 (24%)	8 (27%)	32 (23%)	
Metabolic syndrome	21 (12%)	3 (10%)	18 (13%)	0.652 §
Body Mass Index				0.878 §
< 20 kg/m ^{2}	35 (21%)	7 (24%)	28 (20%)	
20 - 25 kg/m ^{2}	72 (43%)	11 (38%)	61 (44%)	
25 - 30 kg/m ^{2}	35 (21%)	7 (24%)	28 (20%)	
> 30 kg/m ^{2}	27 (16%)	4 (14%)	23 (16%)	
Alcohol last year	50 (29%)	6 (20%)	44 (31%)	0.213 §
IHDS score < 10	27 (21%)	4 (27%)	23 (20%)	0.549 §

¶ Fisher's exact test

§ χ^2 test

Univariate odds ratios and prevalence ratios (with 95% CIs) were calculated to determine univariate risk factors associated with symptomatic DSP and are shown in Table G-2 (Appendix G). In the stepwise backward multivariate logistic regression analysis, independent predictors of symptomatic DSP are reflected in Table 4.7. Variables included in

the initial model include: age, sex, waist-to-hip ratio, systolic and diastolic BP, previous/current TB, alcohol exposure, hs-CRP, haemoglobin, MCV, fasting insulin, LDL cholesterol and triglycerides, lactate, ALT, and 4-PA. Again, vitamin B6 supplementation was not included due to the possibility of covariance with previous or current TB therapy. Also, the wide confidence intervals make interpretation of the model difficult and introduce a degree of uncertainty in associations found. Further, other associations cannot be ruled out.

Table 4.7: Multivariate analysis of baseline symptomatic DSP risk factors – SDSP vs no DSP

Model	Odds Ratio	Odds Ratio 95% confidence interval	Variable p-value	Pseudo R^2	Model p-value
SDSP vs No DSP				0.38	***<0.001***
*Age **	1.14	1.05 - 1.23	***<0.001***		
ALT > 19 IU / L	12.99	2.40 - 70.31	***<0.001***		
Previous/Current TB	8.02	1.84 - 34.94	***0.010***		
C-reactive protein > 5 mg/L	5.07	1.21 - 21.27	***0.030***		

* Per one year increase

Further statistical analysis comparing individuals with symptomatic DSP to those without symptomatic DSP were also performed. Such analysis revealed similar results regarding the risk factors in multivariate analysis and is summarized in Appendix H

4.1.7 Cognitive screen at baseline

IHDS scores were performed randomly on 137 individuals at baseline. The median total score was 11.0 (IQR 10.5-12.0). Thirty-three individuals (24%) had an IHDS score of less than the accepted normal of 10 (Table 4.1). Further analysis of the total IHDS score across DSP groups is shown in Table I-1 in Appendix I. Individuals with DSP at baseline had a significantly lower total IHDS score compared to their counterparts without DSP (mean 10.4 vs 11.0; p=0.026). A poorer performance in all three subcategories of the IHDS score was observed in individuals with DSP, although only the psychomotor speed score reached significance (mean score 3.1 vs 3.8; p=0.035).

Although total IHDS score was associated with DSP, individuals with symptomatic DSP at baseline did not have significantly lower total IHDS score compared to individuals without DSP (p=0.55).

University of Cape Town

4.1.8 Comparison between asymptomatic and symptomatic DSP at baseline

To determine whether any potential risk factors could distinguish between individuals with symptomatic DSP (n=31) and those with asymptomatic DSP (n=10), a baseline comparison was performed between these groups. The results are shown in Table 4.8 for continuous variables and Table 4.9 for categorical variables.

Although fasting triglyceride levels were associated with symptomatic DSP at baseline, other lipid parameters associated with asymptomatic DSP at baseline; individuals with symptomatic DSP had significantly lower total cholesterol levels (p=0.010), mostly due to lower levels of HDL compared to those with asymptomatic DSP (p=0.023). Further, values of the 75th percentile for HDL are lower than reported normal range. Lower haemoglobin levels were observed in individuals with symptomatic DSP at baseline (p=0.032) but not when compared to those without DSP.

The IHDS scores were significantly lower in individuals with asymptomatic DSP compared to those with symptomatic DSP. The detailed analysis of total IHDS score across DSP groups is shown in Appendix I. Sixty per cent (n=6) of the individuals with asymptomatic DSP had an IHDS score less than 10 compared to 27% (n=4) with symptomatic DSP (p=0.020). Investigating the components of the IHDS, individuals with asymptomatic DSP performed significantly worse in the alternating hand sequence test adapted from the Luria sequencing test (psychomotor speed) (p=0.003) compared to the other two modalities where scores were similar between those individuals with symptomatic DSP and those with asymptomatic DSP (motor speed, p=0.31 and memory recall, p=0.21).

Taking into account the relatively small number of this group (n=41), the relatively weak correlations and risk inherent with multiple testing, these results should be interpreted with caution. Due to the limited sample number for this analysis, a multivariate regression model with more than 3 independent variables would have less than the recommended cut-off of 10 events per predictor to ensure valid results in a regression model, and was therefore not performed.

University of Cape Town

Table 4.8: Baseline characteristics in individuals with symptomatic DSP vs those with asymptomatic DSP (continuous data)

Variable	Unit	Normal range	Median (IQR)			p-value
			Total DSP (N=41)	SDSP (N=31)	ADSP (N=10)	
Clinical						
Age	years		31 (26 - 37)	37 (29 - 43)	39 (30 - 51)	0.531 β
Weight	kg		61 (55 - 71)	60 (53 - 71)	63 (59 - 70)	0.909 β
Height	metre		1.61 (1.56 - 1.66)	1.62 (1.55 - 1.68)	1.65 (1.55 - 1.71)	0.646 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.2 (20.4 - 28)	23.2 (20.8 - 25.9)	22.0 (20.2 - 27.3)	0.797 ε
Waist : Hip ratio		*< 0.90*	0.86 (0.81 - 0.89)	0.87 (0.83 - 0.93)	0.85 (0.80 - 0.92)	0.133 β
Systolic BP	mmHg	*120 - 140*	111 (104 - 121)	109 (99 - 119)	111 (101 - 129)	0.287 β
Diastolic BP	mmHg	*80 - 90*	73 (67 - 80)	70 (66 - 77)	74 (66 - 84)	0.310 ε
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	156 (114 - 196)	157 (105 - 217)	174 (117 - 193)	0.873 β
White cell count	$x10^9/L$	*4.0 - 11.0*	5.4 (4.1 - 6.8)	5.1 (3.9 - 6.0)	5.2 (4.1 - 5.9)	0.721 β
C-reactive protein	mg/L	*< 5.0*	2.7 (0.9 - 5.9)	3.8 (1.3 - 9.7)	2.6 (1.0 - 18.8)	0.984 β
Haemoglobin	g/dL	*11.6 - 15.6*	11.7 (10.6 - 12.6)	11.6 (9.3 - 12.4)	12.8 (11.5 - 14.0)	***0.032*** β
MCV	fL	*80.0 - 100.0*	92.1 (87.9 - 95.9)	93.3 (89 - 96.9)	92.3 (90.8 - 96.9)	0.862 β
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 43)	37 (33 - 41)	40 (39 - 43)	0.135 β
ALT	IU/L	*10 - 41*	18 (14 - 26)	23 (18 - 28)	24 (15 - 33)	0.652 β
Creatinine	µmol/L	*53 - 115*	64 (55 - 73)	65 (57 - 73)	69 (51 - 83)	0.570 β
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.5 - 4.9)	4.7 (4.4 - 5.4)	0.315 β
Fasting insulin	µU/mL	*0.2 - 9.4*	5.5 (2.5 - 8.9)	4.3 (2.5 - 7.6)	4.6 (1.7 - 7.2)	0.993 β
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.2)	3.9 (3.0 - 4.3)	4.9 (4.2 - 5.3)	***0.010*** β
Triglycerides	mmol/L	*0.5 - 2.0*	0.8 (0.7 - 1.1)	1.0 (0.8 - 1.4)	0.7 (0.6 - 1.5)	0.553 β
HDL	mmol/L	*1.15 - 1.68*	0.95 (0.75 - 1.12)	0.89 (0.74 - 1.13)	1.38 (0.88 - 1.74)	***0.023*** β
LDL	mmol/L	*1.0 - 3.0*	2.3 (1.9 - 2.9)	2.2 (1.8 - 2.7)	3.0 (2.6 - 3.2)	0.073 β
Lactate	mmol/L	*< 2.0*	2.5 (1.7 - 3.3)	1.8 (1.2 - 2.6)	2.4 (1.1 - 3.8)	0.499 β
Pyridoxine						
PLP	nmol/L	*> 25.0*	23.8 (15.7 - 36.8)	20.7 (16.2 - 53.7)	27.5 (22.9 - 32.0)	0.931 β
4-PA	nmol/L	*unknown*	16.7 (11.6 - 22.4)	20.7 (12.1 - 28.6)	19.0 (15.2 - 30.8)	0.734 ε

β Student's *t*-test

ε Wilcoxon rank-sum test

Table 4.9: Baseline characteristics in individuals with symptomatic DSP vs those with asymptomatic DSP (grouped data)

Variable	No. (%)			p-value
	Total DSP (N=41)	SDSP (N=31)	ADSP (N=10)	
Female sex	27 (66%)	21 (68%)	6 (60%)	0.712 ¶
Age > 40 years	16 (40%)	12 (40%)	4 (40%)	1.000 ¶
Previous/Current TB	22 (54%)	18 (58%)	4 (40%)	0.319 §
Time of TB				0.478 ¶
Currently	11 (27%)	10 (32%)	1 (10%)	
< 1 year ago	4 (10%)	3 (10%)	1 (10%)	
1 year ago	2 (5%)	2 (6%)	0 (0%)	
2 years ago	1 (2%)	1 (3%)	0 (0%)	
> 2 years ago	4 (10%)	2 (6%)	2 (20%)	
Vit Bco supplement	40 (98%)	31 (100%)	9 (90%)	0.244 ¶
Vit B6 supplement	8 (20%)	8 (26%)	0 (0%)	0.164 ¶
WHO clinical stage				0.692 ¶
Stage 1	11 (28%)	8 (27%)	3 (30%)	
Stage 2	11 (28%)	7 (23%)	4 (40%)	
Stage 3	16 (40%)	13 (43%)	3 (30%)	
Stage 4	2 (5%)	2 (7%)	0 (0%)	
CD4 T-cell count				0.775 ¶
< 100 cells/mm^3	6 (15%)	4 (13%)	2 (20%)	
100 - 200 cells/mm^3	24 (60%)	18 (60%)	6 (60%)	
> 200 cells/mm^3	10 (25%)	8 (27%)	2 (20%)	
Metabolic syndrome	4 (10%)	3 (10%)	1 (10%)	1.000 ¶
Body Mass Index				1.000 ¶
< 20 kg/m^2	9 (23%)	7 (24%)	2 (20%)	
20 - 25 kg/m^2	16 (41%)	11 (38%)	5 (50%)	
25 - 30 kg/m^2	9 (23%)	7 (24%)	2 (20%)	
> 30 kg/m^2	5 (13%)	4 (14%)	1 (10%)	
Alcohol last year	8 (20%)	6 (20%)	2 (20%)	1.000 ¶
IHDS score < 10	10 (45%)	4 (27%)	6 (86%)	***0.020*** ¶

¶ Fisher's exact test

§ χ^2 test

4.2 Follow-up results at week 24

4.2.1 Retention and attrition

Among the 184 individuals who were enrolled at baseline, 164 completed 12 weeks follow-up, and 144 completed 24 weeks follow-up (attrition rate of 21% at 24 weeks). The reasons for not completing follow-up are summarized in Figure 4.1. All follow-up data are described for the cohort of 144 individuals. Only individuals who were symptom-free at the baseline assessment had 2-week (n=111) and 4-week (n=114) follow-up assessments.

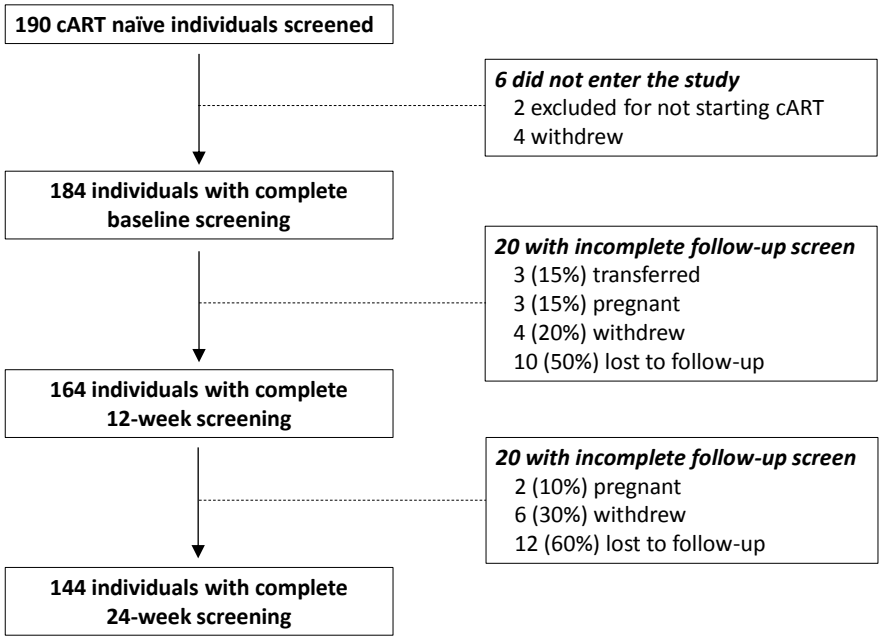


Figure 4.1: Flow chart of participant retention and attrition

4.2.2 Comparison between excluded individuals and the 24-week study cohort

A comparison of baseline characteristics was performed between those individuals who were excluded from the study and those who remained in the study until 24 weeks. This analysis was performed to establish any significant differences in the patient profile or risk factors investigated between these groups. Excluded individuals did not differ from the main study cohort. There were no statistically significant differences in terms of any of the potential risk factors investigated, including sex (p=0.25), age (p=0.44), WHO HIV stage (p=0.76), CD4 T-cell count (p=0.86) or any factors that associated with DSP at baseline [previous/current TB

(p=0.27), triglycerides (p=0.76), hs-CRP (p=0.15)]. The results are summarized in Table J-1 and Table J-2 in Appendix J.

4.2.3 Initiation of cART

Of the 144 individuals followed up for 24 weeks, 82 (57%) began cART with a stavudine-containing regimen, 16 (11%) with zidovudine, and 46 (32%) with tenofovir. Over the 24-week period, 9 individuals had within-class changes in cART therapy; five individuals changed from nevirapine (NVP) to efavirenz (EFV), two from stavudine to tenofovir, one from stavudine to zidovudine and one from efavirenz to nevirapine. One individual changed from nevirapine to zidovudine. No study participants underwent a class change from a non-NRTI-based to a protease inhibitor-based regimen.

All changes in cART were either secondary to commencing TB therapy or non-specific side effects, such as drug intolerance (excluding pain) or drug-induced skin rash. Two individuals who developed ATN during the 24-week follow-up period had a change in cART regimen from stavudine to tenofovir due to neuropathic symptoms. One of them experienced improvement in neurological symptoms after switching to tenofovir, the other had a change in regimen at week 24 and the symptoms had resolved at a 1-year follow-up. The seven final different drug combinations based on longest exposure time, on which further analysis was performed, are listed in Table 4.10.

Table 4.10: Combination ART regimens at 24 weeks

Drug combination	N	%
Stavudine / Lamivudine / Nevirapine	49	34
Stavudine / Lamivudine / Efavirenz	32	22
Tenofovir / Lamivudine / Nevirapine	23	16
Tenofovir / Lamivudine / Efavirenz	23	16
Zidovudine / Lamivudine / Nevirapine	11	8
Zidovudine / Lamivudine / Efavirenz	5	3
Zidovudine / Lamivudine / Tenofovir	1	1

Individuals with symptomatic DSP at baseline were more likely to have started on a stavudine-based regimen than those without symptomatic DSP. Seventy per cent of individuals (n=21) with symptomatic DSP at baseline were started on stavudine, compared to 58% (n=87) of those without symptomatic DSP (p=0.22).

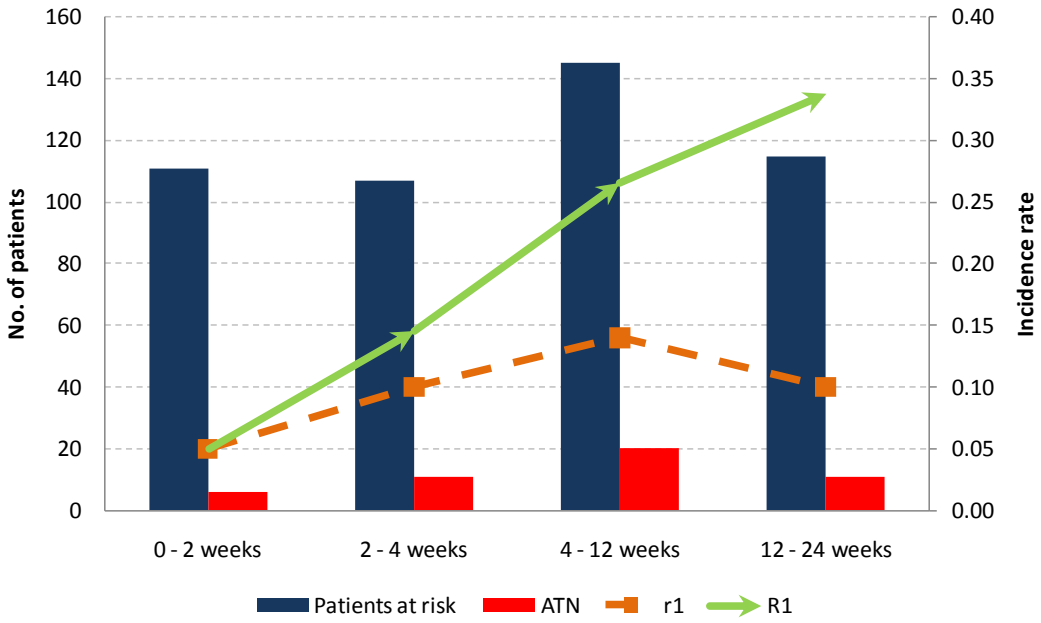
University of Cape Town

At week 24, plasma HIV viral load was used as a measure of virological control, and CD4 T-cell count as a measure of immunologic status. The cART treated cohort displayed viral control (i.e. viral load <200 copies/mL) in 68% of individuals seen at 24 weeks (n=87). CD4 T-cell count was significantly higher after 24 weeks of cART with a median of 272 cells/mm^3 (IQR 204-368, p=0.007) (Table 4.21, page 121).

4.2.4 Incidence of ATN and new neuropathic symptoms at 24 weeks

The primary outcome measure for the longitudinal study was a diagnosis of ATN, defined as the development of any new neuropathic symptoms (with or without neuropathic signs) or worsening in existing symptomatic DSP, after cART initiation. The incidence rates of ATN are shown in Figure 4.2, calculated as the ratio of the number of individuals who had development of ATN to the number of individuals at risk during the specific time interval (see section 2.8.2). The peak incidence of ATN was seen between week 4 and 12 (14%). The overall estimated crude ATN incidence rate for the total population after 24 weeks was 34%. Eight individuals who developed ATN did not complete the study period and were therefore excluded from the rest of the analysis.

University of Cape Town



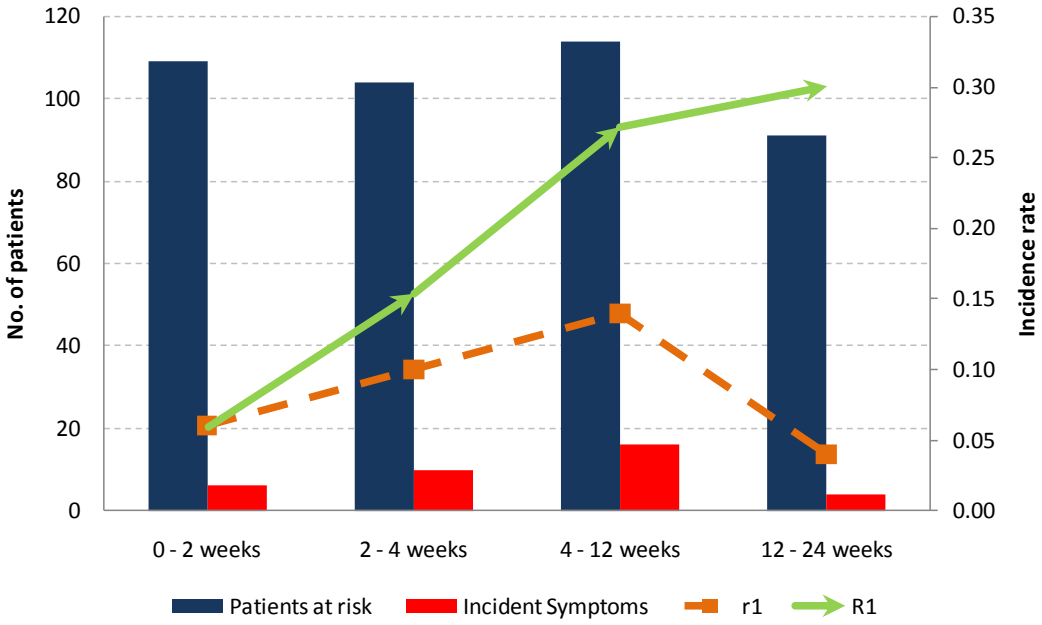
Interval (weeks)	Patients at risk	ATN	r_1	R_1
0 – 2	111	6	0.05	0.05
2 – 4	107	11	0.10	0.15
4 – 12	145	20	0.14	0.26
12 – 24	115	11	0.10	0.34

r_1 is calculated as the ratio of the number of patients who had development of ATN to the number of patients at risk during the interval
R_1 is the cumulative rate of development of ATN by end of the interval
Patients at risk are calculated as individuals at the specific study interval without ATN

Figure 4.2: Incidence rates for ATN

University of Cape

The development of new neuropathic symptoms (with or without signs) was also calculated, as it was the outcome measure used in the cytokine analysis (see Chapter 10). The greatest incidence in neuropathic symptoms was seen between week 4 and week 12 (14%) (Figure 4.3). The overall estimated crude incidence rate for the total population after 24 weeks was 30%.



Interval (weeks)	Patients at risk	Incident symptoms	r_1	R_1
0 – 2	109	6	0.06	0.06
2 – 4	104	10	0.10	0.15
4 – 12	114	16	0.14	0.27
12 – 24	91	4	0.04	0.30

r_1 is calculated as the ratio of the number of patients who had incident symptoms to the number of patients at risk during the interval
R_1 is the cumulative rate of incident symptoms by end of the interval
Patients at risk are calculated as individuals at the specific study interval who had not yet developed symptoms

Figure 4.3: Incidence rates for neuropathic symptoms

University of Cape Town

Overall incidence rates of DSP (as defined in section 2.6.1) for each time interval between study assessments were determined to compare incidence rates with other studies. Results are shown in Figure K-1 in Appendix K. The incidence rates of asymptomatic DSP are shown in Figure K-3 in Appendix K. In contrast to the incidence of ATN and new neuropathic symptoms, the incidence of asymptomatic DSP increased steadily over time with the highest incidence rate seen between week 12 and 24 (6%). The overall estimated crude asymptomatic DSP incidence rate for the total population after 24 weeks was 14%.

4.2.5 Characteristics of incident neuropathic symptoms and signs

Of all individuals with incident symptoms, 89% presented with positive sensory symptoms (pain and/or paraesthesiae). Paraesthesiae was marginally more frequent (69%) than numbness (64%). Pain was the presenting neuropathic symptom in 53% of individuals (Table 4.11).

Table 4.11: Incident neuropathic symptoms

Neuropathic Symptom	No. (%) Incident Symptoms (N=36)
Pain	19 (53%)
Paraesthesiae	25 (69%)
Numbness	23 (64%)
Positive Sensory*	32 (89%)

* Pain and/or paraesthesiae

The mean symptom severity score for incident symptoms did not differ between the different time points at which symptoms developed and ranged from 3.2 to 6.8, expressed as the mean VAS score (Table 4.12).

Table 4.12: Mean symptom severity for incident neuropathic symptoms at each specific visit

Visit	Mean symptom severity score	Standard deviation	p-value
Week 2*	5.7	3.6	
Week 4	3.2	2.8	0.146 β
Week 12	5.0	2.8	0.646 β
Week 24	6.8	2.8	0.623 β

* Reference category

β Student's *t*-test

Report of distal pain or paraesthesiae was strongly associated with objective findings of DSP. Twenty-eight individuals (78%) who developed new neuropathic symptoms during the 24-week follow-up period, and did not have DSP at baseline, had the presence of at least one

University of Cape Town

neuropathic sign occurring together with neuropathic symptoms. Of these 28 individuals, the most frequent neuropathic signs were altered pinprick sensibility and abnormal deep tendon reflexes, both occurring in 50% of individuals (Table 4.13). Vibration sensibility was altered in only 18% of individuals. None of the individuals with incident neuropathic symptoms developed distal muscle weakness after commencing cART.

Table 4.13: Frequency of neuropathic signs for individuals with incident SDSP

Neuropathic sign	No. (%)
Altered vibration sensibility	5 (18%)
Altered pin sensibility	14 (50%)
Altered proprioception	2 (7%)
Absent/reduced deep tendon reflexes	14 (50%)

4.2.6 Transition in DSP Status from baseline to 24 weeks

Transition within the tetratomic DSP states (asymptomatic DSP, symptomatic DSP, symptoms only and no DSP) is illustrated in a transition matrix (Table 4.14). It should be noted that only individuals who were symptom-free at the baseline assessment had 2- and 4-week follow-up assessments (as outlined in section 2.5). Individuals who did not attend a specific follow-up visit are included in Table 4.14 as the 'missing' group. For the purposes of this analysis, data will be described for 4-, 12-, and 24-week follow-up visits, and expressed as a percentage of individuals seen at the specific visit.

To summarize Table 4.14, of those with no DSP at baseline, the number of individuals with incident symptomatic DSP doubled from baseline to 12 weeks, but decreased after 24 weeks, with only a few individuals experiencing new neuropathic symptoms in isolation (five at week 12 and two at week 24). Further, the number of individuals with asymptomatic DSP steadily increased over the 24-week period.

Of 10 with asymptomatic DSP at baseline, nine were followed up to 24 weeks. Five had improvement in neurological signs and reverted to not having DSP, the other four remained stable. Of 31 individuals with symptomatic DSP at baseline examination, 24 were followed to 48 weeks. Three individuals had complete resolution in neuropathic symptoms at week 12, and therefore classified as asymptomatic DSP. Only one of these three continued to have

University of Cape Town

asymptomatic DSP at 24 weeks. By week 24, four individuals who had symptomatic DSP at baseline improved to having no DSP. The rest remained stable.

Five of seven individuals who had symptoms in isolation at baseline were followed up until 24 weeks; one progressed to symptomatic DSP while the symptoms in four individuals completely resolved. Therefore, although limited by a small sample size, these analyses suggest that neither asymptomatic DSP nor the presence of neuropathic symptoms in isolation are risk factors for developing symptomatic DSP after initiating cART within the first 24 weeks of cART ($p=0.36$ and $p=0.19$, respectively).

The transition between the DSP states at baseline and that at week 24 was further analysed to examine the transitions in-between these two examinations. For example, of 61 individuals with DSP at baseline who completed every examination visit, 84% remained DSP-free. The remaining 16% had brief transitions to one or more of the DSP states in-between the baseline and week 24 examinations. The transition rates for selected DSP states for interim visits are shown in Table L-1 in Appendix L (with individuals grouped according to their baseline and 24-week DSP states).

University of Cape Town

Table 4.14: Transition between four DSP states (No DSP, ADSP, SDSP and symptoms in isolation) across specific time points since baseline (left) and since the prior visit (right)

Transition matrices since Baseline

		Baseline				
		No DSP	ADSP	SDSP	Symptoms	Total
	Baseline count	**136**	**10**	**31**	**7**	
Week 4	**No DSP**	86 (76%)	3 (3%)	2 (2%)	0 (0%)	**91 (81%)**
	ADSP	4 (4%)	5 (4%)	0 (0%)	0 (0%)	**9 (8%)**
	SDSP	7 (6%)	0 (0%)	0 (0%)	1 (1%)	**8 (7%)**
	Symptoms	5 (4%)	0 (0%)	0 (0%)	0 (0%)	**5 (4%)**
	Total	**102 (90%)**	**8 (7%)**	**2 (2%)**	**1 (1%)**	**113 (100%)**
	Missing	*34*	*2*	*29*	*6*	*71*

		Baseline				
		No DSP	ADSP	SDSP	Symptoms	Total
Week 12	**No DSP**	90 (55%)	6 (4%)	4 (2%)	2 (1%)	**102 (63%)**
	ADSP	8 (5%)	3 (2%)	3 (2%)	0 (0%)	**14 (9%)**
	SDSP	19 (12%)	0 (0%)	19 (12%)	1 (1%)	**39 (24%)**
	Symptoms	5 (3%)	0 (0%)	0 (0%)	3 (2%)	**8 (5%)**
	Total	**122 (75%)**	**9 (6%)**	**26 (16%)**	**6 (4%)**	**163 (100%)**
	Missing	*16*	*1*	*5*	*1*	*23*

		Baseline				
		No DSP	ADSP	SDSP	Symptoms	Total
Week 24	**No DSP**	76 (53%)	5 (3%)	4 (3%)	4 (3%)	**89 (62%)**
	ADSP	18 (13%)	4 (3%)	1 (1%)	0 (0%)	**23 (16%)**
	SDSP	10 (7%)	0 (0%)	19 (13%)	1 (1%)	**30 (21%)**
	Symptoms	2 (1%)	0 (0%)	0 (0%)	0 (0%)	**2 (1%)**
	Total	**106 (74%)**	**9 (6%)**	**24 (17%)**	**5 (3%)**	**144 (100%)**
	Missing	*30*	*1*	*7*	*2*	*40*

Transition matrices since prior visit

		Baseline				
		No DSP	ADSP	SDSP	Symptoms	Total
	Baseline count	**136**	**10**	**31**	**7**	
Week 4	**No DSP**	86 (76%)	3 (3%)	2 (2%)	0 (0%)	**91 (81%)**
	ADSP	4 (4%)	5 (4%)	0 (0%)	0 (0%)	**9 (8%)**
	SDSP	7 (6%)	0 (0%)	0 (0%)	1 (1%)	**8 (7%)**
	Symptoms	5 (4%)	0 (0%)	0 (0%)	0 (0%)	**5 (4%)**
	Total	**102 (90%)**	**8 (7%)**	**2 (2%)**	**1 (1%)**	**113 (100%)**
	Missing	*34*	*2*	*29*	*6*	*71*

		Week 4				
		No DSP	ADSP	SDSP	Symptoms	Total
Week 12	**No DSP**	65 (61%)	5 (5%)	1 (1%)	3 (3%)	**74 (70%)**
	ADSP	10 (9%)	2 (2%)	0 (0%)	0 (0%)	**12 (11%)**
	SDSP	6 (6%)	2 (2%)	6 (6%)	1 (1%)	**15 (14%)**
	Symptoms	4 (4%)	0 (0%)	1 (1%)	0 (0%)	**5 (5%)**
	Total	**85 (80%)**	**9 (8%)**	**8 (8%)**	**4 (4%)**	**106 (100%)**
	Missing	*6*	*0*	*0*	*1*	*7*

		Week 12				
		No DSP	ADSP	SDSP	Symptoms	Total
Week 24	**No DSP**	72 (51%)	4 (3%)	7 (5%)	3 (2%)	**86 (61%)**
	ADSP	11 (8%)	7 (5%)	5 (4%)	0 (0%)	**23 (16%)**
	SDSP	5 (4%)	3 (2%)	21 (15%)	1 (1%)	**30 (21%)**
	Symptoms	0 (0%)	0 (0%)	0 (0%)	2 (1%)	**2 (1%)**
	Total	**88 (62%)**	**14 (10%)**	**33 (23%)**	**6 (4%)**	**141 (100%)**
	Missing	*12*	*0*	*6*	*2*	*20*

4.2.7 Trends in neuropathic symptoms and signs over time

The progression of symptoms and signs over the 24 weeks after cART initiation was investigated (Figure 4.4). Overall, the proportion of individuals experiencing neuropathic symptoms peaked at week 12 and then decreased again by week 24 (Figure 4.4 A). This observation was driven by the increase in incident symptoms (Figure 4.4 C); in those with symptomatic DSP at baseline (Figure 4.4 B), symptoms generally improved over the 24-week period, more notably in the first 12 weeks. By week 12, 29% of individuals had experienced complete resolution of symptoms.

Paraesthesiae remained the most frequent reported incident neuropathic symptom up to 12 weeks after cART (46%) (Figure 4.4 C). Burning or aching pain remained least frequently reported until week 12, at which time it became more frequent (peaking just below paraesthesiae at week 12). The symptom profile in those with incident symptoms irrespective of signs (Figure 4.4 C) did not differ compared to the group of individuals with incident symptomatic DSP (Figure 4.4 D).

University of Cape Town

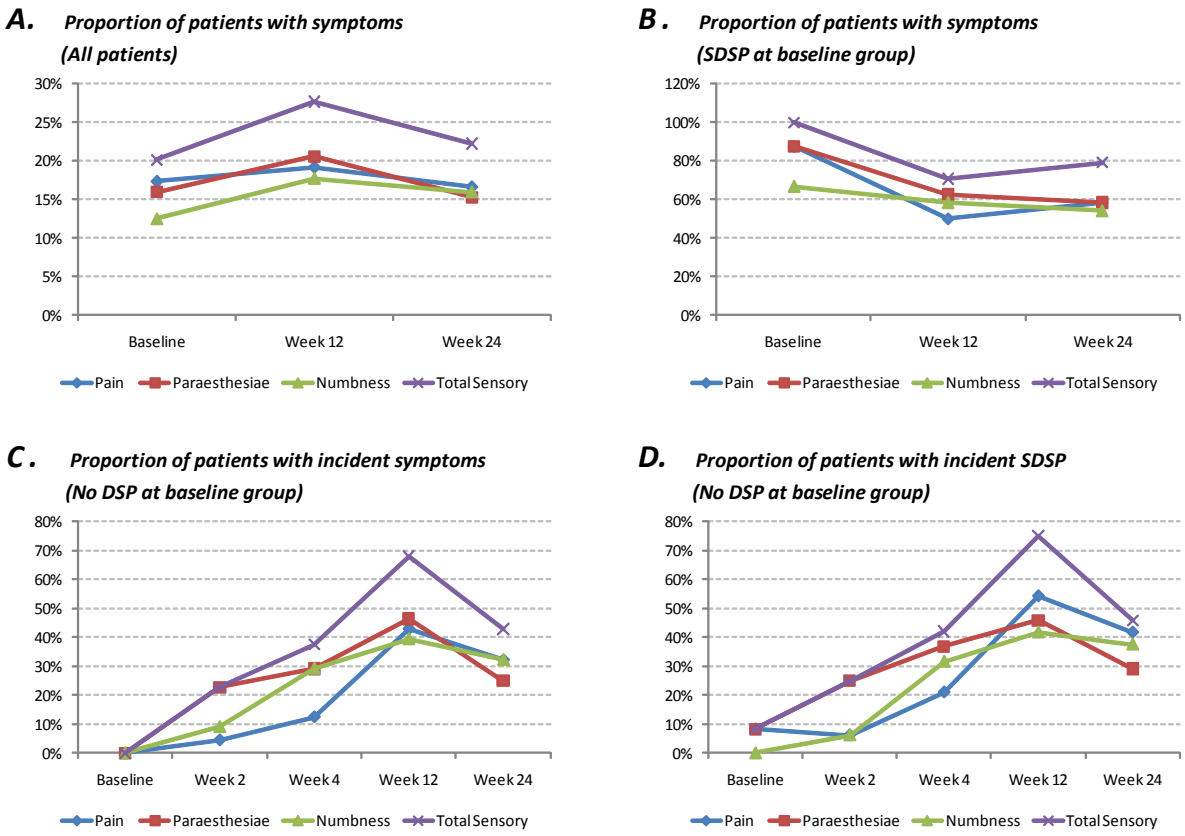


Figure 4.4: Trends in proportions of neuropathic symptoms over time for (A) all individuals, (B) individuals with SDSP at baseline, (C) individuals with incident symptoms and (D) individuals with incident SDSP

A repeated measures analysis was performed to assess change in symptom severity for individuals with symptomatic DSP at baseline, within 24 weeks after cART initiation (Table 4.15). Data are presented as a regression coefficient (with 95% CI), representing the rate of change in neuropathic symptoms as a function of time on cART. The mean VAS score decreased significantly over the 24-week follow-up period from 5.8 at baseline to 4.4 at week 24 (p=0.003). The greatest decrease in symptoms severity was observed within the first 12 weeks after initiation of cART (coefficient -1.80; p<0.001).

Table 4.15: Neuropathic symptom severity score over time for individuals with SDSP at baseline

Visit	Mean symptom severity score	Time effect coefficient[a]	Confidence Interval (95%)	Time effect p-value
Baseline	5.8			
Week 12	3.9	-1.80	(-2.69 ; -0.91)	***<0.001***
Week 24	4.4	-1.39	(-2.30 ; -0.48)	***0.003***

[a] Time effect is the effect of cART on mean symptom severity for the entire cohort

Figure 4.5 summarizes the frequency of neuropathic signs experienced over the follow-up period grouped according to DSP status (all individuals, symptomatic DSP, incident symptoms and incident symptomatic DSP). Both small and large fibre sensory modalities were affected. The upward trend over time in the proportion of individuals with neuropathic signs is similar to the upward trend seen in proportions of individuals experiencing neuropathic symptoms. However, overall there is not a distinct peak in the proportion of individuals with neuropathic signs at week 12 (Figure 4.5 A). Instead, there appears to be a steady increase in the proportion of individuals with neuropathic signs (including small and large fibre signs) over the 24-week follow-up period. Abnormal pinprick sensation remained the most frequent neuropathic sign overall during the 24 week follow-up period (Figure 4.5 A) and was the most frequent neuropathic sign in those individuals with incident symptoms and incident symptomatic DSP (Figure 4.5 C and D).

The overall upward trend in neuropathic signs appears to be influenced by the increased frequency of new neuropathic signs (Figure 4.5 D and E). There was no distinct trend in signs over time among those with symptomatic DSP at baseline (Figure 4.5 B), and signs generally improved in those with asymptomatic DSP at baseline (Figure 4.5 F). Abnormal pinprick sensation appeared to show the fastest recovery within the first 12 weeks after cART in those individuals with signs at baseline (Figure 4.5 B and F).

University of Cape Town

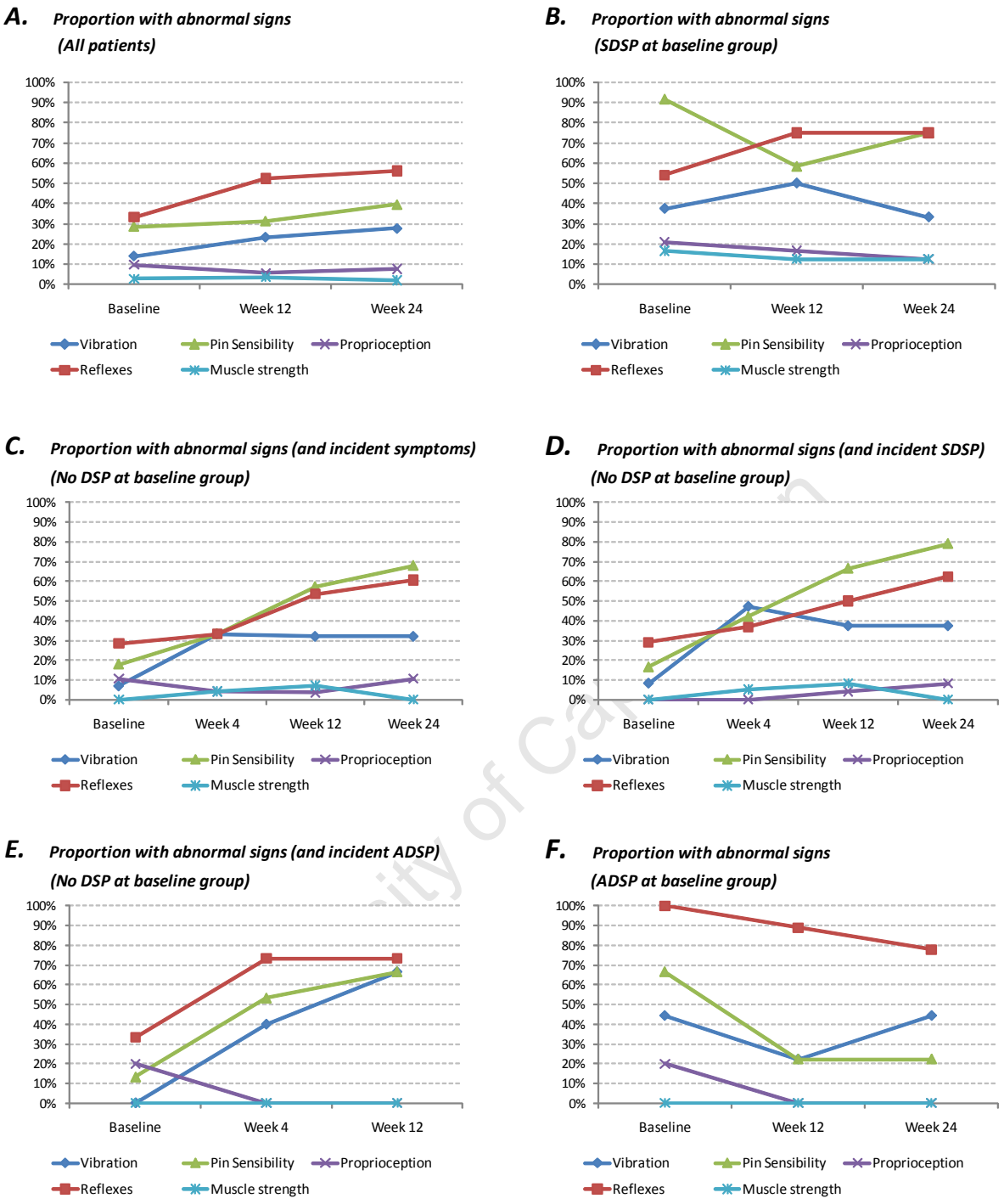


Figure 4.5: Trends in proportions of neuropathic signs over time for (A) all individuals, (B) individuals with SDSP at baseline, (C) individuals with incident symptoms, (D) individuals with incident SDSP, (E) individuals with incident ADSP and (F) individuals with ADSP at baseline

4.2.8 A longitudinal analysis of the relationship between neuropathic symptoms and signs

A longitudinal analysis was performed to investigate the relationship between neuropathic symptoms and signs, and whether individuals who experience worsening in symptoms also have worsening in signs. Twenty-four individuals with symptomatic DSP and 24 individuals with incident symptoms completed 24 weeks of follow-up.

4.2.8.1 Symptomatic DSP at Baseline

The correlations between changes in neuropathic symptoms and signs for individuals with symptomatic DSP at baseline are depicted in Table 4.16. The categories of improving, worsening and unchanged symptoms or signs were defined according to the criteria outlined in section 2.6. By week 12, just over half (n=13) improved in neuropathic symptoms whereas 11 worsened or remained unchanged (four worsened, seven unchanged). Within the first 12 weeks of cART, the improvement in symptoms correlated significantly with improvement in signs (p=0.005). None of those whose symptoms worsened showed and improvement in signs; in 50% the signs worsened and in the rest it remained unchanged. However, the correlation between symptoms and signs was not evident between 12 and 24 weeks (p=0.62). Small sample size precluded multivariate analyses to determine the effect of other variables, such as CD4 T-cell count or cART regimen on worsening or improving of symptoms and signs.

University of Cape Town

Table 4.16: Comparison between changes in neuropathic symptoms and changes in neuropathic signs for individuals with symptomatic DSP at baseline

Change in symptoms	Change in signs		p-value
	Improved	Worsened/ Unchanged	
Week 12 vs Baseline			
Symptoms Improved	9	4	***0.005*** ¶
Symptoms Worsened/Unchanged	1	10	
Week 24 vs Week 12			
Symptoms Improved	3	2	0.615 ¶
Symptoms Worsened/Unchanged	7	12	

¶ Fisher's exact test

4.2.8.2 Incident neuropathic symptoms

The correlation between changes in neuropathic symptoms versus changes in neuropathic signs for individuals with incident neuropathic symptoms after cART, over the 24-week follow-up period are summarised in a transition matrix in Table 4.17.

Table 4.17: Comparison between changes in neuropathic symptoms and changes in neuropathic signs for the group of individuals with incident neuropathic symptoms at any point within the 24-week period

Change in symptoms	Change in signs			
	Improved	Unchanged	Worsened	New signs
Week 2 vs Baseline				
Symptoms Improved	-	-	-	-
Symptoms Unchanged	5	12	-	-
Symptoms Worsened	-	-	-	-
New symptoms	6	-	-	-
Week 4 vs Week 2				
Symptoms Improved	-	-	3	-
Symptoms Unchanged	-	5	3	3
Symptoms Worsened	-	-	1	-
New symptoms	-	4	3	3
Week 12 vs Week 4				
Symptoms Improved	-	1	2	1
Symptoms Unchanged	1	2	4	2
Symptoms Worsened	-	2	1	-
New symptoms	-	8	6	2
Week 24 vs Week 12				
Symptoms Improved	4	6	5	-
Symptoms Unchanged	1	4	1	-
Symptoms Worsened	1	2	-	-
New symptoms	-	1	2	1

University of Cape Town

The three most important findings are:

a) Six individuals who experienced new symptoms, also had at least one new neuropathic sign. Eleven individuals with new symptoms had worsening of existing neuropathic signs.
b) Of all individuals who had experienced improvement in neuropathic symptoms within the first 12 weeks after cART, none had an improvement in signs.

c) After 12 weeks of study participation, general improvement in symptoms was not accompanied by improvement in signs. Whilst the proportion of individuals with neuropathic symptoms decrease after 12 weeks on cART, the proportion with neuropathic signs continues to increase, albeit at a lower rate than the first 12 weeks following cART.
d) By week 24, symptoms had resolved in 22, whereas the remainder had persistent or worsening symptoms.

From this data it appears that neuropathic signs may persist even after neuropathic symptoms have improved or resolved.

4.2.9 Comparison between symptoms at baseline and newly developed symptoms

To determine whether neuropathic symptoms that existed prior to cART initiation differ from symptoms that developed early after starting cART, a comparison was performed between individuals who had symptomatic DSP at baseline and individuals who developed neuropathic symptoms after cART (Table 4.18).

Table 4.18: Comparison of neuropathic symptoms – SDSP at baseline vs Incident Symptoms

Neuropathic Symptom	No. (%)		p-value
	SDSP at baseline (N=31)	Incident Symptoms (N=36)	
Pain	26 (84%)	19 (53%)	***0.005*** §
Paraesthesiae	26 (84%)	25 (69%)	0.163 ¶
Numbness	19 (61%)	23 (64%)	0.941 §
Positive Sensory*	30 (97%)	32 (89%)	0.209 ¶

* Pain and/or paraesthesiae

¶ Fisher's exact test

§ χ^2 test

While burning, aching pain and paraesthesiae were more frequent amongst individuals with symptomatic DSP pre-cART, paraesthesiae and numbness were more frequent amongst individuals with incident neuropathic symptoms. These differences were significant for the proportions of individuals with neuropathic pain (84% vs 53%, p=0.005). This may suggest

University of Cape Town

that paraesthesiae and numbness are earlier symptoms of painful neuropathy and that neuropathic pain is not the predominant feature of early ATN.

A comparison of neuropathic signs between individuals who had symptomatic DSP at baseline visit and individuals with incident symptoms with signs (i.e. incident symptomatic DSP) after cART initiation is presented in Table 4.19. The only difference in signs between the two groups was the significantly greater proportion of abnormal pinprick sensation in the group with symptomatic DSP at baseline (81% vs 50%, p=0.004), although it was also the most frequent sign together with abnormal deep tendon reflexes in individuals with incident symptomatic DSP.

Table 4.19: Comparison of neuropathic signs – SDSP at baseline vs Incident SDSP

Neuropathic Sign	No. (%)		p-value
	SDSP at baseline (N=31)	Incident SDSP (N=28)	
Altered vibration sensibility	11 (35%)	5 (18%)	0.146 ¶
Altered pin sensibility	25 (81%)	14 (50%)	***0.004*** §
Altered proprioception	5 (16%)	2 (7%)	0.425 ¶
Absent/reduced deep tendon reflexes	18 (58%)	14 (50%)	0.309 §

¶ Fisher's exact test

§ χ^2 test

University of Cape Town

4.2.10 Risk factors for the development of ATN

4.2.10.1 Cross-sectional analysis at 24 weeks

A comparison of baseline and 24-week factors between individuals who developed ATN and those without ATN are summarized in Table 4.20 and Table 4.21 for continuous data, and Table 4.22 for categorical data.

Table 4.20: Baseline characteristics for individuals with and without ATN (continuous data)

Baseline variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=144)	ATN (N=40)	No ATN (N=104)	
Clinical						
Age	years		33 (27 - 39)	35 (28 - 43)	32 (26 - 39)	0.165 β
Weight	kg		62 (55 - 71)	61 (54 - 70.5)	63 (56 - 71)	0.842 β
Height	metre		1.61 (1.56 - 1.68)	1.62 (1.58 - 1.67)	1.61 (1.56 - 1.69)	0.852 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.4 (20.3 - 28.0)	23.1 (20.1 - 28.5)	23.5 (20.3 - 27.8)	0.784 ε
Waist : Hip ratio		*< 0.90*	0.86 (0.82 - 0.91)	0.87 (0.82 - 0.91)	0.86 (0.82 - 0.90)	0.488 β
Systolic BP	mmHg	*120 - 140*	111 (103 - 122)	109 (100 - 118)	111 (104 - 122)	0.542 β
Diastolic BP	mmHg	*80 - 90*	72 (66 - 79)	71 (65 - 78)	73 (66 - 80)	0.453 ε
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	159 (121 - 193)	157 (116 - 212)	160 (123 - 191)	0.515 β
White cell count	$x10^9/L$	*4.0 - 11.0*	5.1 (4.0 - 6.5)	5.0 (4.0 - 6.5)	5.1 (4.0 - 6.5)	0.867 ε
C-reactive protein	mg/L	*< 5.0*	3.0 (1.0 - 7.1)	2.8 (0.9 - 6.4)	3.2 (1.0 - 7.5)	0.708 β
Haemoglobin	g/dL	*11.6 - 15.6*	11.8 (10.5 - 12.8)	11.8 (10.6 - 12.6)	11.8 (10.4 - 12.8)	0.862 β
MCV	fL	*80.0 - 100.0*	92.4 (88.5 - 96.0)	92.7 (88.6 - 97.0)	92.2 (88.5 - 95.8)	0.862 β
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 42)	39 (35 - 43)	39 (35 - 42)	0.992 β
ALT	IU/L	*10 - 41*	19 (14 - 27)	18 (13 - 24)	21 (15 - 28)	0.056 β
Creatinine	µmol/L	*53 - 115*	64 (56 - 73)	64 (57 - 74)	64 (55 - 73)	0.665 ε
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.4 - 5.0)	4.6 (4.4 - 4.9)	0.459 ε
Fasting insulin	µU/mL	*0.2 - 9.4*	5.2 (2.5 - 8.6)	4.7 (2.0 - 8.6)	5.5 (2.7 - 8.6)	0.313 β
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.2)	3.5 (3.0 - 4.2)	3.7 (3.3 - 4.3)	0.372 β
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.6 - 1.2)	0.8 (0.6 - 1.3)	0.9 (0.7 - 1.2)	0.449 β
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.75 - 1.12)	0.93 (0.75 - 1.08)	0.97 (0.74 - 1.13)	0.251 β
LDL	mmol/L	*1.0 - 3.0*	2.3 (2.0 - 2.9)	2.3 (1.9 - 2.8)	2.4 (2.0 - 2.9)	0.561 β
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	2.4 (1.4 - 3.3)	2.3 (1.7 - 3.0)	0.861 β
Pyridoxine						
PLP	nmol/L	*> 25.0*	24.3 (17.1 - 38.4)	23.3 (18.2 - 46.8)	24.7 (16.6 - 36.6)	0.493 β
4-PA	nmol/L	*unknown*	17.6 (11.8 - 24.5)	17.1 (11.7 - 22.8)	17.9 (12.0 - 25.5)	0.805 ε

β Student's *t*-test

ε Wilcoxon rank-sum test

Table 4.21: Week 24 characteristics for individuals with and without ATN (continuous data)

Week 24 variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=144)	ATN (N=40)	No ATN (N=104)	
Clinical						
Weight	kg		66 (57 - 75)	66 (55 - 74)	66 (58 - 75)	0.725 β
Height	metre		1.61 (1.57 - 1.68)	1.62 (1.57 - 1.66)	1.61 (1.57 - 1.69)	0.947 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	24.4 (21.0 - 29.2)	24.7 (20.8 - 29.1)	24.3 (21.5 - 29.3)	0.574 ε
Waist : Hip ratio		*< 0.90*	0.85 (0.80 - 0.89)	0.83 (0.79 - 0.91)	0.85 (0.80 - 0.89)	0.801 ε
Systolic BP	mmHg	*120 - 140*	115 (109 - 124)	117 (109 - 125)	115 (107 - 122)	0.932 β
Diastolic BP	mmHg	*80 - 90*	74 (69 - 80)	75 (71 - 80)	73 (68 - 80)	0.557 β
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	272 (204 - 368)	344 (223 - 457)	260 (186 - 337)	***0.007*** β
C-reactive protein*	mg/L	*< 5.0*	4.5 (1.3 - 9.7)	3.8 (1.5 - 8.3)	4.8 (1.0 - 10.7)	0.795 ε
Virological						
HIV viral load	copies/mL		20 (20 - 56)	20 (20 - 55)	20 (20 - 57)	0.875 ε
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.9 (4.7 - 5.2)	4.9 (4.7 - 5.2)	4.9 (4.7 - 5.2)	0.971 ε
Fasting insulin	µU/mL	*0.2 - 9.4*	5.5 (2.5 - 8.5)	4.9 (2.8 - 7.9)	5.8 (2.5 - 8.7)	0.384 ε
Total cholesterol	mmol/L	*3.1 - 5.2*	4.4 (3.9 - 5.1)	4.2 (3.7 - 5.1)	4.5 (3.9 - 5.1)	0.306 β
Triglycerides	mmol/L	*0.5 - 2.0*	0.8 (0.7 - 1.1)	0.8 (0.7 - 1.1)	0.8 (0.7 - 1.0)	0.402 ε
HDL	mmol/L	*1.15 - 1.68*	1.41 (1.12 - 1.64)	1.37 (1.05 - 1.56)	1.45 (1.20 - 1.82)	***0.027*** β
LDL	mmol/L	*1.0 - 3.0*	2.5 (2.0 - 2.9)	2.6 (2.1 - 2.9)	2.5 (2.0 - 2.9)	0.962 β
Pyridoxine						
PLP*	nmol/L	*> 25.0*	20.9 (13.3 - 34.7)	26.7 (13.1 - 35.1)	20.1 (13.4 - 33.5)	0.984 β
4-PA*	nmol/L	*unknown*	19.2 (12.5 - 27.7)	18.6 (13.7 - 23.3)	19.2 (12.2 - 30.3)	0.829 ε

* Week 12 blood samples

β Student's *t*-test

ε Wilcoxon rank-sum test

Table 4.22: Baseline and 24-week characteristics for individuals with and without ATN (grouped data)

Variable	No. (%)			p-value
	Total (N=144)	ATN (N=40)	No ATN (N=104)	
Female sex	97 (67%)	27 (68%)	70 (67%)	0.982 §
Age > 40 years	35 (24%)	13 (33%)	22 (21%)	0.155 §
Previous/Current TB	57 (40%)	13 (33%)	44 (42%)	0.281 §
Time of TB				***0.045*** ¶
Currently	11 (8%)	0 (0%)	11 (11%)	
< 1 year ago	26 (18%)	5 (13%)	21 (20%)	
1 year ago	4 (3%)	3 (8%)	1 (1%)	
2 years ago	3 (2%)	1 (3%)	2 (2%)	
> 2 years ago	12 (8%)	4 (10%)	8 (8%)	
Vit Bco supplement	143 (99%)	40 (100%)	103 (99%)	1.000 ¶
Vit B6 supplement	6 (4%)	2 (5%)	4 (4%)	0.875 ¶
WHO clinical stage				0.317 ¶
Stage 1	47 (33%)	11 (28%)	36 (35%)	
Stage 2	43 (30%)	16 (40%)	27 (26%)	
Stage 3	48 (34%)	11 (28%)	37 (36%)	
Stage 4	5 (4%)	2 (5%)	3 (3%)	
Baseline variables				
CD4 T-cell count				0.402 §
< 100 cells/mm ^{3}	22 (15%)	6 (15%)	16 (16%)	
100 - 200 cells/mm ^{3}	88 (62%)	22 (55%)	66 (65%)	
> 200 cells/mm ^{3}	32 (23%)	12 (30%)	20 (20%)	
Metabolic syndrome	17 (12%)	6 (15%)	11 (11%)	0.565 ¶
Body Mass Index				0.847 §
< 20 kg/m ^{2}	31 (22%)	10 (25%)	21 (21%)	
20 - 25 kg/m ^{2}	57 (40%)	14 (35%)	43 (42%)	
25 - 30 kg/m ^{2}	29 (20%)	8 (20%)	21 (21%)	
> 30 kg/m ^{2}	25 (18%)	8 (20%)	17 (17%)	
Alcohol last year	42 (30%)	11 (28%)	31 (30%)	0.734 §
IHDS score < 10	14 (10%)	7 (18%)	7 (7%)	0.062 ¶
Week 24 variables				
CD4 T-cell count				0.418 ¶
< 100 cells/mm ^{3}	5 (4%)	0 (0%)	5 (5%)	
100 - 200 cells/mm ^{3}	27 (21%)	7 (18%)	20 (22%)	
> 200 cells/mm ^{3}	98 (75%)	31 (82%)	67 (73%)	
HIV viral load				0.731 §
< 200 copies/mL	87 (68%)	25 (66%)	62 (69%)	
> 200 copies/mL	41 (32%)	13 (34%)	28 (31%)	
Antiretroviral drugs				
Stavudine	81 (56%)	24 (60%)	57 (55%)	0.574 §
Zidovudine	17 (12%)	3 (8%)	14 (13%)	0.399 ¶
Lamivudine	144 (100%)	40 (100%)	104 (100%)	N/A
Efavirenz	60 (42%)	15 (38%)	45 (43%)	0.529 §
Nevirapine	83 (58%)	25 (63%)	58 (56%)	0.464 §
Tenofovir	47 (33%)	13 (33%)	34 (33%)	0.982 §
Drug combination				0.614 ¶
3TC / NVP / TDF	23 (16%)	7 (18%)	16 (15%)	
3TC / EFV / TDF	23 (16%)	6 (15%)	17 (16%)	
AZT / 3TC / TDF	1 (1%)	0 (0%)	1 (1%)	
AZT / 3TC / NVP	11 (8%)	1 (3%)	10 (10%)	
AZT / 3TC / EFV	5 (3%)	2 (5%)	3 (3%)	
D4T / 3TC / NVP	49 (34%)	17 (43%)	32 (31%)	
D4T / 3TC / EFV	32 (22%)	7 (18%)	25 (24%)	

¶ Fisher's exact test

§ χ^2 test

The development of ATN was not associated with any of the baseline variables. Risk factors that were associated with symptomatic DSP at baseline in the univariate analysis, viz. age, waist-to-hip ratio, triglycerides and previous/current TB, were not significant predictors of the development of ATN (p=0.17; p=0.49; p=0.45 and p=0.28, respectively). Clinical features including sex (p=0.98), weight (p=0.84), height (p=0.85), BMI (p=0.78), systolic BP (p=0.54), and diastolic BP (p=0.45) were not predictors of the development of ATN. Also, there was no association with baseline HIV severity as measured by WHO HIV stage (p=0.32), baseline CD4 T-cell count (p=0.52) or hs-CRP (p=0.71).

Baseline fasting lipids and metabolic factors did not associate with the development of ATN including fasting total cholesterol (p=0.37), HDL (p=0.25), glucose (p=0.46), lactate (p=0.86), and the metabolic syndrome (p=0.57).

Baseline haematological factors including white cell count (p=0.87), haemoglobin (p=0.86) or MCV (p=0.86) were not significant predictors of ATN. Baseline biochemical factors including albumin (p=0.99) and creatinine (p=0.67) did not associate with the development of ATN. There was a trend towards lower ALT levels in individuals who developed ATN compared to those who did not (18.0 vs 21.0 IU/L, p=0.06). However, the interquartile range for ALT was still within normal range for both groups. Again, no difference was detected in alcohol exposure between the two groups in general (p=0.73). ATN was not associated with vitamin B6 indicators at baseline [PLP (p=0.49) and 4-PA (p=0.81)].

Of the 24-week factors, the development of ATN was associated with:

a) Higher CD4 T-cell count at week 24 (p=0.007). Individuals who developed ATN had a median CD4 T-cell count of 344 cells/mm^3 (IQR 223-457), compared to 260 cells/mm^3 (IQR 186-337) for the group who did not develop ATN. Although individuals with ATN showed greater CD4 T-cell reconstitution after 24 weeks compared with the ATN-free group, HIV viral loads at 24 weeks did not differ between the two groups (p=0.88).
b) Lower HDL levels at week 24 (p=0.03). Median HDL cholesterol levels for individuals who developed ATN was 1.37 mmol/L (IQR 1.05-1.56) compared to 1.45 mmol/L (IQR 1.20-1.82) for individuals who did not develop ATN. The HDL cholesterol levels for both groups are still considered within the normal range.

University of Cape Town

Apart from lower HDL levels at 24 weeks on cART, none of the other metabolic factors investigated at 24 weeks showed any associations with the development of ATN.

There was no significant difference in initial NRTI backbone between individuals who developed ATN compared to those who remained without ATN (p=0.61). Notably, only 30% of individuals who received stavudine developed ATN. Specifically, of individuals with ATN, 24 (60%) received stavudine, while in those who did not develop ATN, 57 (55%) received stavudine (p=0.57) (Table 4.22).

The presence of asymptomatic DSP at baseline was not associated with the development of ATN (p=0.21). Of note, on retrospective recall, only one of the 10 individuals with asymptomatic DSP at baseline reported having neuropathic symptoms in the past. These symptoms resolved spontaneously.

Univariate hazard ratios (with 95% CIs) were calculated to determine longitudinal risk factors associated with ATN within 24 weeks (Table M-7 in Appendix M). Individuals who were lost during follow-up were censored after the last known visit. Baseline factors significantly associated with ATN in the primary analysis (parametric, non-parametric and proportional) were examined by individual Cox regression. All baseline variables were explored for an association with ATN, but only variables showing significance level of $p<0.25$ in the primary analysis were included in in the multivariate analysis. In the multivariate Cox regression analysis (Table 4.23), only ALT >19 IU/L was associated with a decreased risk of developing ATN (p=0.034).

University of Cape Town

Table 4.23: Multivariate Cox regression analysis of baseline risk factors for ATN

Clinical variable	Hazard Ratio in multivariate analysis	Hazard Ratio 95% Confidence Interval	p-value
Female sex	1.10	0.47 - 2.59	0.822
Age > 40 years	2.13	0.82 - 5.55	0.120
Previous/Current TB	2.22	0.70 - 7.00	0.175
WHO clinical stage			
*Stage 1**	1.00		
Stage 2	1.75	0.73 - 4.18	0.207
Stage 3	0.32	0.09 - 1.20	0.091
Stage 4	0.60	0.07 - 5.39	0.652
IHDS score < 10	0.48	0.17 - 1.33	0.158
Alcohol last year	0.88	0.40 - 1.94	0.743
ALT > 19 IU/L	0.40	0.17 - 0.93	***0.034***
HDL < 1.0 mmol/L	1.84	0.86 - 3.94	0.117

* reference category

As discussed in section 2.6.1, a caveat in our definition of ATN is that worsening of neuropathic symptoms may be part of the natural history of DSP; the addition of cART as an additional neurotoxic insult does not necessarily imply that the worsening was due to cART. Furthermore, those individuals with symptomatic DSP at baseline formed part of the ATN-free comparative group. Therefore, to exclude this confounding effect, an analysis was performed looking at individuals who developed neuropathic symptoms, comparing them to individuals who never had neuropathic symptoms and the results are summarized in Appendix M. Individuals with incident symptoms showed a trend towards greater CD4 T-cell counts at 24 weeks (p=0.11, Table M-2 in Appendix M). Development of incident neuropathic symptoms was associated with lower ALT levels at baseline (p=0.003) and lower HDL levels at week 24 (p=0.038) (Table M-8 in Appendix M). In the multivariate Cox regression analysis, an ALT >19 IU/L was associated with a decreased risk of developing ATN (p=0.026) (Table 4.24).

University of Cape Town

Table 4.24: Multivariate Cox regression analysis of baseline risk factors for incident symptoms

Clinical variable	Hazard Ratio in multivariate analysis	Hazard Ratio 95% Confidence Interval	p-value
Female sex	1.09	0.47 - 2.55	0.840
Age > 40 years	1.92	0.74 - 4.98	0.176
Previous/Current TB	2.62	0.81 - 8.49	0.109
WHO clinical stage			
*Stage 1**	1.00		
Stage 2	1.84	0.78 - 4.35	0.163
Stage 3	0.30	0.08 - 1.14	0.077
Stage 4	0.48	0.05 - 4.47	0.521
IHDS score < 10	0.52	0.19 - 1.44	0.211
Alcohol last year	0.98	0.44 - 2.18	0.964
ALT > 19 IU/L	0.37	0.16 - 0.89	***0.026***
HDL < 1.0 mmol/L	1.85	0.87 - 3.97	0.112

* reference category

4.2.10.2 Longitudinal patterns of clinical and laboratory variables over 24 weeks in risk of developing ATN

In addition to an analysis of baseline factors, we performed repeated measures analysis to assess longitudinal patterns over the 24-week follow-up period. To determine the effect of cART initiation on risk factors investigated, as well as the difference in change in factors between individuals who developed ATN and those who remained without ATN, a cross-sectional time series analysis was performed (random effects model). Data is presented as a regression coefficient (with 95% CI), representing the rate of change in the variable (relative to baseline) between the two groups. Time on cART was used as within-individual factor and group (ATN vs no ATN) as the between-individual factor Table 4.25).

University of Cape Town

Table 4.25: Random effects model for the longitudinal risk factor analysis for ATN

Variable	Time effect[a]				Group effect[b]				
	Median	Coefficient	Confidence Interval (95%)	p-value	ATN Median	No ATN Median	Coefficient	Confidence Interval (95%)	p-value
CD4 T-cell count									
Baseline	159				157	160			0.515 β
Week 24	272	140.59	(121.06 ; 160.13)	***<0.001***	344	260	64.07	(22.40 ; 105.75)	***0.003***
Fasting glucose									
Baseline	4.7				4.7	4.6			0.459 ε
Week 12	4.9	0.24	(0.15 ; 0.34)	***<0.001***	4.9	4.8	0.10	(-0.11 ; 0.31)	0.339
Week 24	4.9	0.25	(0.16 ; 0.34)	***<0.001***	4.9	4.9	-0.07	(-0.28 ; 0.14)	0.510
Fasting insulin									
Baseline	5.2				4.7	5.5			0.313 β
Week 12	5.3	0.46	(-0.48 ; 1.40)	0.336	5.7	5.3	1.23	(-0.85 ; 3.32)	0.245
Week 24	5.5	0.53	(-0.41 ; 1.46)	0.271	4.9	5.8	-0.18	(-2.28 ; 1.92)	0.869
Total cholesterol									
Baseline	3.7				3.5	3.7			0.372 β
Week 12	4.2	0.53	(0.41 ; 0.64)	***<0.001***	4	4.2	-0.09	(-0.34 ; 0.17)	0.505
Week 24	4.4	0.68	(0.57 ; 0.80)	***<0.001***	4.2	4.5	-0.02	(-0.28 ; 0.24)	0.908
Triglycerides									
Baseline	0.9				0.8	0.9			0.449 β
Week 12	0.8	0.01	(-0.12 ; 0.15)	0.862	0.8	0.8	0.21	(-0.09 ; 0.51)	0.170
Week 24	0.8	-0.04	(-0.17 ; 0.10)	0.581	0.8	0.8	0.08	(-0.22 ; 0.38)	0.599
HDL									
Baseline	0.94				0.93	0.97			0.251 β
Week 12	1.3	0.36	(0.30 ; 0.42)	***<0.001***	1.19	1.33	-0.05	(-0.19 ; 0.08)	0.447
Week 24	1.41	0.51	(0.45 ; 0.57)	***<0.001***	1.37	1.45	-0.12	(-0.25 ; 0.02)	0.096
LDL									
Baseline	2.3				2.3	2.4			0.561 β
Week 12	2.4	0.13	(0.03 ; 0.23)	0.011	2.4	2.4	-0.07	(-0.29 ; 0.14)	0.506
Week 24	2.5	0.16	(0.06 ; 0.25)	0.002	2.6	2.5	0.11	(-0.11 ; 0.33)	0.336
Systolic BP									
Baseline	111				109	111			0.542 β
Week 12	115	3.72	(1.61 ; 5.84)	0.001	118	115	5.70	(1.10 ; 10.30)	***0.015***
Week 24	115	3.49	(1.39 ; 5.58)	0.001	117	115	1.68	(-2.96 ; 6.31)	0.479
Diastolic BP									
Baseline	72				71	73			0.453 ε
Week 12	73	1.17	(-0.39 ; 2.74)	0.143	73	73	1.92	(-1.52 ; 5.36)	0.274
Week 24	74	1.37	(-0.19 ; 2.93)	0.084	75	73	2.48	(-0.98 ; 5.95)	0.160
Weight									
Baseline	62				61	63			0.842 β
Week 12	64	1.70	(1.05 ; 2.36)	***<0.001***	62	65	0.47	(-0.97 ; 1.91)	0.520
Week 24	66	3.03	(2.38 ; 3.67)	***<0.001***	66	66	0.70	(-0.74 ; 2.14)	0.339
Body Mass Index									
Baseline	23.4				23.1	23.5			0.784 ε
Week 12	24.5	0.78	(0.48 ; 1.07)	***<0.001***	25	24.1	0.24	(-0.40 ; 0.89)	0.458
Week 24	24.4	1.15	(0.86 ; 1.44)	***<0.001***	24.7	24.3	0.10	(-0.55 ; 0.75)	0.763
Waist : Hip Ratio									
Baseline	0.86				0.87	0.86			0.488 β
Week 12	0.85	-0.01	(-0.03 ; 0.02)	0.670	0.83	0.85	-0.01	(-0.07 ; 0.04)	0.668
Week 24	0.85	0.00	(-0.02 ; 0.02)	0.996	0.83	0.85	-0.03	(-0.08 ; 0.03)	0.312
C-reactive protein									
Baseline	2.9				2.8	3.2			0.708 β
Week 12	4.4	5.36	(-0.13 ; 10.85)	0.056	3.8	4.8	1.47	(-11.02 ; 13.97)	0.817
PLP									
Baseline	24.3				23.3	24.7			0.493 β
Week 12	20.9	-2.01	(-6.88 ; 2.85)	0.417	26.7	20.1	-5.01	(-15.82 ; 5.80)	0.363
4PA									
Baseline	17.6				17.1	17.9			0.805 ε
Week 12	19.2	3.85	(-0.71 ; 8.40)	0.098	18.6	19.2	4.07	(-6.07 ; 14.20)	0.432

[a] Time effect is the effect of cART on mean concentrations for the entire cohort

[b] Group effect is the difference in mean concentrations between groups compared to baseline

β Student's *t*-test

ε Wilcoxon rank-sum test

Overall, CD4 T-cell count recovered significantly after 24 weeks on cART. The initiation of cART also had a significant effect on nutritional and metabolic factors. Metabolic parameters, including total cholesterol, HDL, LDL and fasting glucose rose significantly after cART treatment with the greatest increase occurring by week 24. Similarly clinical factors including, weight ($p<0.001$), BMI ($p<0.001$) and systolic BP ($p<0.001$) were significantly higher at 24 weeks compared to baseline concentrations. Diastolic BP, waist-to-hip ratio, fasting insulin, triglycerides, PLP and 4-PA remained unchanged during the course of follow-up.

A greater increase in CD4 T-cell count at 24 weeks was associated with development of ATN ($p=0.003$). Individuals who developed ATN had a greater increase in systolic BP at week 12 compared to those without ATN. Despite the general increase in most measures after cART initiation, there was no significant difference in magnitude of response between the individuals who developed ATN compared to those who did not develop ATN ("group effect") and included the following:

a) Clinical factors: Weight, BMI, waist-to-hip ratio and diastolic BP
b) Metabolic factors: Triglycerides, HDL, LDL, total cholesterol, glucose and insulin
c) Inflammatory markers: hs-CRP
d) Vitamin B6 indicators: PLP and 4-PA

The random effects model for individuals with incident symptoms compared to individuals without new symptoms is shown in Table N-1 in Appendix N. No significant associations with any risk factors were found.

University of Cape Town

4.2.11 Comparison between individuals with symptomatic DSP at baseline and individuals who developed symptomatic DSP

An analysis was performed comparing the group with symptomatic DSP at baseline to the group developing symptomatic DSP during 24 weeks follow-up to examine any significant differences between the two groups (refer to Table 4.26, Table 4.27 and Table 4.28). Only individuals who completed 24-week follow-up were included in this analysis.

Table 4.26: Baseline characteristics for individuals with SDSP at baseline vs those with incident SDSP (continuous data)

Baseline variable	Unit	Normal range	Median (IQR)		p-value	
			SDSP at baseline (N=24)	Incident SDSP (N=24)		
Clinical						
Age	years		38 (29 - 44)	33 (26 - 41)	0.090	β
Weight	kg		61 (55 - 73)	61 (55 - 66)	0.712	β
Height	metre		1.63 (1.56 - 1.68)	1.62 (1.58 - 1.66)	0.841	β
Body Mass Index	kg/m^2	*20.0 - 25.0*	24.1 (20.9 - 26.0)	21.8 (19.9 - 26.1)	0.793	β
Waist : Hip ratio		*< 0.90*	0.88 (0.85 - 0.93)	0.84 (0.79 - 0.90)	***0.034***	β
Systolic BP	mmHg	*120 - 140*	111 (100 - 121)	109 (100 - 117)	0.944	β
Diastolic BP	mmHg	*80 - 90*	70 (67 - 78)	71 (65 - 74)	0.626	β
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	167 (126 - 223)	141 (118 - 201)	0.496	ε
White cell count	$x10^9/L$	*4.0 - 11.0*	4.7 (3.8 - 6.0)	5.7 (4.8 - 7.1)	0.256	β
C-reactive protein	mg/L	*< 5.0*	3.8 (2.2 - 11.9)	2.9 (0.9 - 7.1)	0.601	β
Haemoglobin	g/dL	*11.6 - 15.6*	11.9 (9.6 - 12.8)	11.9 (10.7 - 13.1)	0.422	β
MCV	fL	*80.0 - 100.0*	93.3 (89.3 - 95.6)	92.3 (87.9 - 97.0)	0.646	β
Biochemical						
Albumin	g/L	*38 - 54*	38 (34 - 42)	41 (35 - 44)	0.210	β
ALT	IU/L	*10 - 41*	24 (18 - 29)	17 (13 - 20)	***0.009***	β
Creatinine	µmol/L	*53 - 115*	66 (58 - 74)	64 (58 - 76)	0.517	β
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.6 - 4.9)	4.7 (4.4 - 5.2)	0.622	β
Fasting insulin	µU/mL	*0.2 - 9.4*	4.6 (2.7 - 9.0)	5.6 (3.6 - 9.3)	0.429	β
Total cholesterol	mmol/L	*3.1 - 5.2*	4.0 (3.1 - 4.3)	3.7 (3.2 - 4.2)	0.988	β
Triglycerides	mmol/L	*0.5 - 2.0*	1.0 (0.8 - 1.4)	0.7 (0.6 - 1.0)	***0.009***	β
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.79 - 1.16)	0.90 (0.71 - 1.07)	0.309	β
LDL	mmol/L	*1.0 - 3.0*	2.5 (1.8 - 2.8)	2.6 (2.0 - 3.0)	0.189	β
Lactate	mmol/L	*< 2.0*	2.1 (1.2 - 2.6)	2.4 (1.7 - 3.3)	0.302	β
Pyridoxine						
PLP	nmol/L	*> 25.0*	20.7 (16.4 - 53.7)	21.4 (18.2 - 32.3)	0.807	β
4-PA	nmol/L	*unknown*	20.2 (12.0 - 26.2)	16.8 (11.7 - 21.6)	0.567	β

β Student's *t*-test

ε Wilcoxon rank-sum test

University of Cape Town

Table 4.27: Week 24 characteristics of individuals with SDSP at baseline vs those with incident SDSP (continuous data)

Week 24 variable	Unit	Median (IQR)		p-value	
		SDSP at baseline (N=24)	Incident SDSP (N=24)		
Clinical					
Weight	kg	66 (58 - 74)	66 (55 - 69)	0.496	β
Height	metre	1.61 (1.57 - 1.69)	1.62 (1.58 - 1.66)	0.900	β
Body Mass Index	kg/m^2	24.3 (21.6 - 27.1)	24.7 (20.1 - 26.7)	0.548	β
Waist : Hip ratio		0.85 (0.83 - 0.92)	0.83 (0.78 - 0.88)	0.084	ε
Systolic BP	mmHg	115 (109 - 130)	117 (109 - 124)	0.574	β
Diastolic BP	mmHg	73 (68 - 83)	75 (70 - 78)	0.687	β
Haematological					
CD4 T-cell count	$cells/mm^3$	260 (255 - 395)	344 (212 - 457)	0.848	β
Viral Load	copies/ml	20 (20 - 34)	20 (20 - 769)	0.102	ε
C-reactive protein*	mg/L	4.8 (1.3 - 11.4)	3.8 (1.7 - 16.5)	0.272	ε
Metabolic					
Fasting glucose	mmol/L	4.9 (4.8 - 5.2)	4.9 (4.6 - 5.3)	0.556	β
Fasting insulin	µU/mL	5.8 (2.9 - 8.3)	4.9 (2.9 - 7.4)	0.460	ε
Total cholesterol	mmol/L	4.5 (4.0 - 5.1)	4.2 (3.8 - 5.2)	0.877	β
Triglycerides	mmol/L	0.8 (0.7 - 1.0)	0.8 (0.7 - 1.3)	0.890	β
HDL	mmol/L	1.45 (1.11 - 1.65)	1.37 (1.06 - 1.51)	0.115	β
LDL	mmol/L	2.5 (2.1 - 2.8)	2.6 (2.1 - 3.3)	0.394	β
Pyridoxine					
PLP*	nmol/L	20.1 (11.9 - 33.1)	26.7 (10.4 - 33.2)	0.536	β
4-PA*	nmol/L	19.2 (12.6 - 27.0)	18.6 (12.4 - 23.1)	0.560	ε

* Week 12 blood samples

β Student's *t*-test

ε Wilcoxon rank-sum test

Table 4.28: Baseline and 24-week characteristics for individuals with SDSP at baseline vs those with incident SDSP (grouped data)

Variable	No. (%)		p-value	
	SDSP at baseline (N=24)	Incident SDSP (N=24)		
Female sex	15 (63%)	16 (67%)	1.000	¶
Age > 40 years	11 (46%)	6 (25%)	0.227	¶
Previous/Current TB	14 (58%)	7 (29%)	***0.042***	§
Time of TB			***0.015***	¶
Currently	7 (29%)	0 (0%)		
< 1 year ago	3 (13%)	4 (17%)		
1 year ago	2 (8%)	0 (0%)		
2 years ago	1 (4%)	1 (4%)		
> 2 years ago	1 (4%)	2 (8%)		
Vit Bco supplement	24 (100%)	24 (100%)		
Vit B6 supplement	6 (25%)	1 (4%)	***0.048***	¶
WHO clinical stage			***0.038***	¶
Stage 1	8 (33%)	6 (25%)		
Stage 2	4 (17%)	12 (50%)		
Stage 3	11 (46%)	4 (17%)		
Stage 4	1 (4%)	2 (8%)		
Baseline variables				
CD4 T-cell count			0.901	¶
< 100 cells/mm ^{3}	2 (8%)	2 (8%)		
100 - 200 cells/mm ^{3}	14 (58%)	16 (67%)		
> 200 cells/mm ^{3}	8 (33%)	6 (25%)		
Metabolic syndrome	1 (4%)	2 (8%)	1.000	¶
Body Mass Index			0.749	¶
< 20 kg/m ^{2}	5 (21%)	7 (29%)		
20 - 25 kg/m ^{2}	9 (38%)	11 (46%)		
25 - 30 kg/m ^{2}	6 (25%)	4 (17%)		
> 30 kg/m ^{2}	4 (17%)	2 (8%)		
Alcohol last year	6 (25%)	6 (25%)	1.000	¶
IHDS score < 10	3 (30%)	3 (13%)	0.328	¶
24 week variables				
CD4 T-cell count			0.666	¶
100 - 200 cells/mm ^{3}	4 (17%)	2 (10%)		
> 200 cells/mm ^{3}	19 (83%)	19 (90%)		
Viral Load < 200 copies/ml	16 (70%)	11 (52%)	0.354	¶

¶ Fisher's exact test

§ χ^2 test

There were a few differences at baseline. Fifty per cent of individuals with symptomatic DSP at baseline were HIV stage 3 or 4 compared to only 25% of individuals with incident symptomatic DSP (p=0.038). In those with incident symptomatic DSP, waist-to-hip ratios were lower (p=0.034), although height and weight were similar. The ALT and fasting triglyceride levels were lower in those with incident symptomatic DSP (p=0.009 and p=0.009, respectively). Interestingly, there was no difference in CD4 T-cell count, hs-CRP or fasting glucose levels between the two groups. After 24 weeks, all these longitudinal parameters were similar, although follow-up ALT levels were not performed as standard of care. Again, there was no difference in CD4 T-cell count or hs-CRP level (see further discussion in Chapter 10). However, grouping specific variables of interest showed that symptomatic DSP at baseline was significantly associated with previous or current TB therapy (p=0.042). Taking into account the relatively small number of this analysis (n=48), these results should be interpreted with caution.

University of Cape Town

Chapter 5 Clinical Discussion

University of Cape Town

5.1 The frequency of HIV-DSP at baseline and influence of the clinical tool used

HIV-DSP was present in 22% of a well-characterized cohort of individuals prior to commencing cART by using the case definitions of 1) at least two neuropathic signs (asymptomatic DSP) or 2) one or more neuropathic signs together with one or more neuropathic symptoms (symptomatic DSP). Previously, the 'two signs' definition was considered too stringent with loss of sensitivity (Simpson et al., 2006). However, it was decided *a priori* that this was necessary to enable accurate assessment for risk factors in this group, as asymptomatic DSP was purely a clinical diagnosis.

Other prospective studies in which the prevalence of HIV-DSP was reported prior to cART initiation are summarized in Table 5.1. Prevalence estimates vary substantially depending on the criteria employed to define DSP. In order to compare DSP prevalence from our study with those listed in Table 5.1, the respective definitions from these studies were applied to our data. By only using the BPNS, and applying a case definition of at least one sign (abnormal reflexes or vibration sensation), the prevalence of DSP in this cohort was 42% at baseline. In comparison, a North American study found the prevalence of DSP to be considerably lower (23%) using the same definition (Evans et al., 2011).

The inclusion of pin sensibility, which reflects small unmyelinated nerve fibre function, increased the sensitivity of the screening exam, given that vibration sensibility and tendon reflexes reflect large myelinated fibre function. The prevalence increased from 42% to 59% when abnormal pinprick sensibility from the mTNS was included as a neuropathic sign in defining DSP. Using the same definition as used herein, another African study also reported a high DSP prevalence of 43% prior to initiating cART, twofold more than observed in HIV-infected populations from the developed world (Sacktor et al., 2009).

University of Cape Town

Table 5.1: Comparative frequencies of DSP prior to cART initiation

Neuropathy definition	Frequency at baseline				
	Study cohort 2012	Forna 2007	Sacktor 2009	Mehta 2011	Evans 2011
DSP					
Current study definition [a]	22%				
≥ 1 sign, BPNS [b]	42%				23%
≥ 1 sign [c]	59%		43%		
Symptomatic DSP					
Current study definition [d]	17%				
Symptoms and ≥ 1 sign, BPNS [b]	12%			11%	4%
Other					
Symptoms or ≥ 1 sign [c]	63%	13%			

[a] Either (i) ≥ 2 signs or (ii) symptoms and ≥ 1 sign

[b] BPNS refers to assessment of deep tendon reflexes and vibration sensibility only

[c] Assessment of signs: pin sensibility, deep tendon reflexes and vibration sensibility

[d] Symptoms and ≥ 1 sign

University of Cape Town

The overall frequency of symptomatic DSP at baseline (pre-cART) in this cohort was 17%. Using only the BPNS (i.e. without pinprick sensibility), the frequency of symptomatic DSP was 12%. This is comparable to another African study, which reported a frequency of 11% (Mehta et al., 2011), but threefold higher than the frequency of 4% from a large North American cohort (Evans et al., 2011). Forna et al. also reported longitudinal data from an African cohort, but used a less stringent case definition of ≥1 neuropathic symptom or sign (Forna et al., 2007). Using this definition, our frequency was considerably higher than that described in their cohort. However, their study investigated safety and tolerability of NRTI therapy and not primarily the frequency of DSP. Also, study participants were evaluated by field officers and not clinicians. Previous work has demonstrated that the BPNS tool did not perform well in the hands of nurses trained in the administration of this tool when compared with clinicians or neurologists (Cettomai et al., 2010, Simpson et al., 2006).

Overall, after correcting for varying diagnostic criteria, our frequency figures for both DSP and symptomatic DSP are generally higher than those from developed countries. The increased frequency may reflect a higher prevalence of comorbidities, notably TB infection, that contribute to DSP in our study population. More advanced HIV disease as demonstrated by lower median CD4 T-cell counts in our study compared to that of Evans et al., may also affect DSP prevalence [158 vs 206 cells/mm^3 (Evans et al., 2011)]. Differences in execution

of neurological assessment and interpretation of signs may have contributed to the discrepant results. Sample sizes influence credibility of the frequency estimates, and ranged from 150 individuals (Mehta et al., 2011) to over a thousand (Evans et al., 2011).

5.2 Pain is a common clinical characteristics of HIV-DSP

HIV-DSP was characterized by a high frequency of positive sensory symptoms (73% with pain and/or paraesthesiae). Pain was the most prevalent symptom at baseline, affecting nearly two thirds of all individuals with DSP. Of those, 77% experienced their pain as at least moderately severe (VAS ≥4/10). This was similar to previous findings in a comparable population from this region (Maritz et al., 2010) Although numbness has previously been reported to be the most common symptom of HIV-DSP, it was the least frequent symptom in our cohort affecting only 46% of those with DSP at baseline (Harrison and Smith, 2011).

Decreased pinprick sensation was the most common neuropathic sign (76%), followed by abnormal ankle reflexes (68%). Distal mild toe extensor weakness occurred in only 15% and never as an isolated sign. The relatively high frequency of decreased pinprick sensation may be an important consideration when using the BPNS as a screening tool since this modality is not included in the BPNS. This could decrease the sensitivity of detecting DSP (see Table 5.1). In nearly a third (29%) of individuals with symptomatic DSP at baseline, decreased pinprick sensation was the only neuropathic sign and these individuals would therefore not have been included in the diagnosis of symptomatic DSP, had only the BPNS been used. Furthermore, the absence of investigating pinprick sensation would have excluded six of 10 individuals with asymptomatic DSP at baseline. These findings suggest that the use of the mTNS would be the preferred clinical tool for determining DSP status as the BPNS lacks sensitivity in the assessment of HIV-DSP.

Eliciting neuropathic symptoms may be clinically important in resource-limited settings where clinicians or special investigations are not always readily available. We found that the distal symptoms of pain, paraesthesiae or numbness was strongly associated with objective findings of HIV-DSP, with only 4% of individuals presenting with symptoms in isolation (Table 5.1), consistent with the findings of another group (Robinson-Papp et al., 2010). Therefore, symptom-based screening for DSP with a tool such as the single-question

University of Cape Town

neuropathy screen (SQNS) may prove useful in busy primary health care settings as suggested by Cettomai et al. (Cettomai et al., 2010).

5.3 Risk factors for DSP prior to cART initiation

Possible risk factors for HIV-DSP were investigated. This study supports the view that HIV-DSP is a multifactorial process and that insults to the peripheral nervous system are cumulative. Furthermore, risk factors found with this study are theoretically related to an increased susceptibility to mitochondrial damage (see section 1.8.3).

Age: Relatively few risk factors were independently associated with the presence of either DSP or symptomatic DSP, prior to initiating cART. Advancing age, which has proven to be an important risk factor for HIV-associated DSP in several studies (Tagliati et al., 1999, Morgello et al., 2004, Maritz et al., 2010, Evans et al., 2011, Lichtenstein et al., 2005) was associated with both DSP and symptomatic DSP at baseline. While ageing has an effect on the integrity of mtDNA resulting in oxidative stress (see section 1.8.3.2), it also influences the peripheral nervous system in other ways. Morphologic studies have reported loss of myelinated and unmyelinated nerve fibres in elderly individuals (Jacobs and Love, 1985, Bouche et al., 1993). Axonal atrophy occurs due to a reduction in the expression and axonal transport of cytoskeletal proteins in peripheral nerves (Stromska and Ochs, 1982). Delayed nerve regeneration after injury due to changes in neuronal, axonal, Schwann cell and macrophage responses, has also been described (Komiyama and Suzuki, 1992). Changes in innate and adaptive immune function associated with ageing may augment HIV immune dysfunction and also increase susceptibility to opportunistic infections or malignancies (Giunta et al., 2008, Robinson-Papp et al., 2012). Furthermore, due to age-associated comorbidities such as liver disease, vascular disease, renal disease or cumulative toxicities of substance abuse, ageing HIV-infected individuals have an increased risk of other toxicities and drug-drug interactions (Goulet, Fultz et al. 2007).

High-sensitivity CRP: Increased hs-CRP levels were independently associated with both DSP and symptomatic DSP at the baseline visit. This has not been previously reported and likely reflects a higher set point of HIV-induced systemic inflammation in these individuals. The higher hs-CRP levels in individuals with DSP suggest a greater degree of immune dysregulation with increased ROS production, possibly contributing to peripheral nerve

University of Cape Town

damage. As discussed in section 1.7.2, HIV immune dysregulation may indirectly contribute to peripheral nerve injury and dysfunction by promoting macrophage infiltration in peripheral nerves and the dorsal root ganglia with release of cytokines and ROS production. The time of DSP onset was not available for the individuals with pre-existing DSP at baseline. The left censored data meant that conclusions about possible causality could not be made. However, the association with hs-CRP levels strengthens the study hypothesis of underlying immune dysregulation contributing to DSP.

***Triglycerides**:* Fasting metabolic factors such as glucose and lipids were assessed at the point of cART initiation. Several associations emerged; both DSP and symptomatic DSP were associated with higher fasting triglyceride levels at baseline. However, 75th percentile values of both groups were still within normal range unlike a previous cohort from the developed world (Ances et al., 2009). An association with elevated triglyceride levels was reported by Ances et al. in which elevated fasting triglyceride levels (>1.70 mmol/L) conferred a 30% greater risk of having HIV-DSP, albeit in a mixed group of cART-naïve and cART exposed individuals (Ances et al., 2009). Similarly, Banerjee et al. showed that elevated but non-fasting triglyceride levels independently increased the risk for HIV-DSP (Banerjee et al., 2011). The association with higher triglyceride levels in our study, albeit still within normal range, suggests that triglycerides may be a surrogate for another unmeasured factor that serves as the causal link to DSP, such as mitochondrial dysfunction.

Several metabolic pathways are affected by mitochondrial respiratory chain dysfunction. Firstly, when ATP can no longer be synthesized efficiently through oxidative phosphorylation there is increased dependence on glycolysis. Secondly, the block of NADH utilization in the respiratory chain increases the intracellular NADH/NAD+ ratio. This alteration of the redox status promotes the conversion of pyruvate to lactate, inhibiting key enzymes involved in the mitochondrial β-oxidation of fatty acids, and consequently resulting in the accumulation of triglycerides (Fromenty and Pessayre, 1995). Individuals with elevated triglyceride levels may also be at higher risk of mitochondrial dysfunction through its effect on mitochondrial energy metabolism and membrane permeability (see section 1.8.3.4). Furthermore, in an animal model, plasma triglyceride levels positively correlated with small dense LDL (Griffin et al., 1994), a component of LDL vulnerable to oxidation (Isomaa et al., 2001). Mice fed high-fat diets had increased triglyceride-containing oxidized small dense LDL and showed evidence of systemic and nerve oxidative stress as well as altered nerve

University of Cape Town

conduction velocities and sensory function (Vincent et al., 2009). Small dense LDL particles were not measured in our study.

While triglyceride levels may be a marker of mitochondrial dysfunction, another possibility is that it may be a surrogate for early dyslipidaemia. Triglyceride levels correlated significantly with waist-to-hip ratio in our subjects, which in turn has been shown to be associated with dyslipidaemia (Jeong et al., 2005). Dyslipidaemia, a component of the metabolic syndrome, has been associated with a systemic low-grade inflammatory state and increased production of cytokines in several studies (Tamakoshi et al., 2003, Ridker et al., 2003, Han et al., 2002). It is worth noting that waist-to-hip ratio also significantly associated with DSP in a univariate analysis. Although other metabolic factors and markers of macronutritional status such as weight and BMI were not associated with DSP, waist-to-hip ratio rather than BMI has been suggested to be a more reliable indicator of early dyslipidaemia (Jeong et al., 2005). Therefore, the association between DSP and waist-to-hip ratio may be linked to early dyslipidaemia.

There remains some controversy regarding the accuracy of fasting triglycerides as an independent predictor of metabolic risk, because adjustment for other covariates in particular HDL, markedly decreases both the magnitude and significance of observed epidemiologic effects (Sarwar et al., 2007). However, in a multivariate model correcting for HDL and LDL, the raised triglyceride levels remained independently associated with DSP. Statin use has also been shown to increase risk for HIV-DSP, potentially mediating an association between triglycerides and DSP (Evans et al., 2012). However, none of the individuals in our cohort received statin drugs during the study period.

HIV/TB co-infection*:* Although we found <u>current</u> TB infection and/or its therapy to be independently associated with DSP and symptomatic DSP at baseline, a <u>history</u> of TB infection was not associated. This is in contrast to a previous cross-sectional study from a South African population, although it should be noted that their association was in cART-exposed individuals only (Maritz et al., 2010). Both the inflammation associated with TB infection as well as the metabolites of INH therapy may produce ROS, leading to mitochondrial damage (see section 1.9.1). The mitochondrial toxicity of stavudine together with the mitochondrial damage associated with TB and INH therapy may cumulatively lead to redox imbalance with subsequent oxidative stress and nerve damage. None of the individuals in our cohort were exposed to stavudine prior to the baseline assessment.

University of Cape Town

The frequency of previous TB infections in the study population was 22%. This is considerably lower than that reported previously from other HIV-infected cohorts from this region (47% to 52%) and could have limited the power to evaluate the impact of previous TB on HIV-associated DSP (Lawn et al., 2006, Maritz et al., 2010). The lower proportion of individuals with a history of previous TB may be attributed to a change in the government-sponsored programme with earlier initiation of cART at higher CD4 T-cell counts (350 vs 200 cells/mm^3) (Badri et al., 2002).

While it is possible that oxidative stress as a result of TB infection may lead to the manifestation of subclinical HIV-DSP (section 1.9.1.1), INH-associated painful neuropathy is a well known entity and is related to a deficiency in biologically active vitamin B6 (Van der Watt et al., 2011) and may have represented our association found with current TB treatment. Vitamin B6 and its association with DSP and *NAT2* will be discussed in further detail in Chapter 9.

Alanine aminotransferase (ALT)*:* The measurement of ALT was a routine part of the cART initiation workup performed by the clinic at baseline. The association between DSP and higher, albeit normal ALT levels, was therefore an unexpected finding. ALT levels above the normal limit were present in only 10% of individuals at baseline. The cause of this association, as well as its clinical significance, is unclear and this makes the interpretation difficult, particularly as liver function tests have high intra-individual variability (Kovari et al., 2010). Hepatitis C co-infection is known to affect ALT levels and cause peripheral neuropathy, and although concerns of possible synergistic effects of co-infection on the peripheral nervous system have been raised (Brew, 2003, Cornblath and Hoke, 2006, Estanislao et al., 2005), hepatitis C co-infection has not been shown to be a risk factor for HIV-DSP (Cherry et al., 2010, Evans et al., 2011). The background prevalence of hepatitis C infection is low in South Africa, although it varies between different population groups: in our community-based setting (Cape Town), the prevalence ranges from below 0.1% in blood donors to 1.6% amongst HIV-infected Caucasian men who have sex with men (Tucker et al., 1997, Amin et al., 2004). Our study cohort was predominantly female, as generally found in South African HIV-infected cohorts, and hepatitis C/HIV co-infection is expected to be rare.

Asymptomatic hepatic steatosis is common in HIV-infected individuals with metabolic abnormalities (Ingiliz et al., 2009). It has been shown that ALT, as a surrogate marker for hepatic steatosis, is relatively higher in individuals with increased BMI, central obesity,

University of Cape Town

lipodystrophy, dyslipidaemia, insulin resistance, or with alcohol consumption (Oh et al., 2006). Insulin resistance, increased pro-inflammatory cytokine production, oxidative stress and mitochondrial dysfunction leading to hepatocyte damage have all been posed as important pathophysiological mechanisms for raised ALT levels in hepatic steatosis (Schindhelm et al., 2006).

In our population, ALT correlated significantly with waist-to-hip ratio ($p<0.001$ $\rho=0.26$) and fasting triglycerides ($p=0.004$, $\rho=0.22$), but was not associated with raised BMI, impaired glucose metabolism, hs-CRP or alcohol consumption. Alcohol abuse is a known cause of neuropathy, but has not been shown to increase the risk for HIV-DSP in this cohort. However, it is possible that the lack of association between alcohol use, DSP and/or ALT may be due to the insensitivity of the measurement tool used to assess alcohol abuse. For example, an overly inclusive definition of alcohol use can potentially underestimate its impact on ALT or DSP.

Although an ALT level >19 IU/L was a strong predictor of DSP at baseline according to the multivariate model, the overall effect of other variables on DSP may have been underestimated due to the presence of mediating factors. For example, there was a significant correlation between ALT, fasting triglycerides and waist-to-hip ratio, but the latter two variables showed no independent association with symptomatic DSP after adjustment in a multivariate model. It would be incorrect to interpret that these variables are not associated with symptomatic DSP. Due to the observational nature of this study as well as the potential interactions between variables, the best multivariate model is not necessarily the most accurate description of what is influencing risk for symptomatic DSP. Furthermore, the dichotomization of ALT levels may have led to an inevitable loss of information and power, increasing the probability of false positive results. An ALT level of 19 IU/L was chosen to dichotomize the cohort as only eight individuals had ALT levels above normal limits. These results should therefore be interpreted with caution.

Stage of HIV disease: Data from pre-cART era cohorts indicate that DSP is more frequent in individuals with advanced immunosuppression and high viral load (Tagliati et al., 1999, Simpson et al., 2002, Childs et al., 1999). In this study, DSP with or without symptoms did not associate with lower baseline CD4 T-cell count or greater WHO stage of disease. Entry criteria limited enrolment to individuals about to commence cART with a CD4 T-cell count <350 cells/mm^3 and only 17% had counts <100 cells/mm^3. This may have selected a group of

University of Cape Town

individuals at lower risk for having DSP, possibly blunting the influence of CD4 T-cell count on the observed frequency of DSP at baseline.

Due to left censoring of data and risk factors at the baseline visit, it was not possible to account for all possible neurological insults. It is possible that confounding conditions not dependent on immunologic or virologic status, such as micronutrient deficiencies, previous infections, other medication or substance use may be important in the pathogenesis of DSP.

5.4 A comparison between symptomatic and asymptomatic HIV-DSP at baseline (pre-cART)

Many reports have tried to address the issue of whether asymptomatic DSP is a different entity from symptomatic DSP. Although only 10 individuals had asymptomatic DSP at baseline, they were more likely to have abnormal tendon reflexes compared to those with symptomatic DSP (100% vs 58%) and less likely to have reduced pin sensibility (60% vs 81%). Individuals with asymptomatic DSP at baseline performed significantly poorer in the IHDS score, particularly in the psychomotor speed performance compared to the other domains.

Individuals with symptomatic DSP at baseline had significantly lower fasting total cholesterol compared to those with asymptomatic DSP. The lower total cholesterol in symptomatic individuals was mostly driven by low HDL cholesterol, with the 75th percentile below normal range in this group. *In vitro* data have shown that HDL has anti-inflammatory properties and decreases oxidation (Hessler et al., 1979), possibly contributing to the redox imbalance leading to oxidative stress and ultimately to the development of painful DSP.

Although haemoglobin levels were significantly higher in individuals with asymptomatic compared to symptomatic DSP, a low haemoglobin level was not a predictor of symptomatic DSP (when compared to individuals without DSP). It remains possible though that anaemia may be a marker for other risk factors such as micronutrient deficiencies unmeasured in this study. Other risk factors for symptomatic DSP such as increasing age, previous or current TB and raised hs-CRP were equally distributed amongst participants with symptomatic and asymptomatic DSP.

University of Cape Town

5.5 The incidence rate of ATN peaked within 12 weeks after starting cART

Incidence estimates of symptomatic neuropathy after starting cART vary substantially depending on the population studied, the time period under investigation and the definition employed to determine neuropathy status. Using a definition of ATN, which included the development of new symptoms or worsening of symptomatic DSP at baseline, the cumulative incidence rate in this community clinic-based population was 34% after 24 weeks.

The peak period of developing neuropathic symptoms was between 2-4 weeks (10%) and 4-12 weeks (16%), with a decrease in incidence after 12 weeks. This is similar to a report from Europe/Australia showing a peak incidence within 90 days after commencing cART (Arenas-Pinto et al., 2008), However, in those who did not develop symptoms, but only accumulated signs, there was a steady increase over the 24-week follow-up period with the highest incidence of 6% evidently between 12 and 24 weeks. These findings suggest, to some degree, a separate pathogenetic mechanism for asymptomatic DSP. Further follow-up after this period will be useful in determining whether the incidence rate plateaus or continues to rise.

Other studies have not reported neuropathy incidence rates for the first 12 weeks after cART initiation, and as the incidence rate with this study was not constant over different periods of time, incidence density rates (per person time incidence rate) would be superficially inflated when expressed over longer time intervals. For this reason, cumulative incidence rates of other studies commencing cART, were compared to ours, applying the respective definitions of the comparative studies.

The cumulative incidence rate of 30% for new neuropathic symptoms within 6 months was similar to 38% reported by a Ugandan study (Sacktor et al., 2009). Using only the BPNS, the cumulative incidence of 21% was higher than that from a Kenyan study of 11% within one year after starting cART (Mehta et al., 2011). The incidences of both African studies were higher than that of the DELTA trial from Europe and Australia, reporting a cumulative incidence of 6% for neuropathic symptoms within three years after commencing cART (Arenas-Pinto et al., 2008).

University of Cape Town

Table 5.2: Reported cumulative incidence rates of ATN

Neuropathy incidence definition	Cumulative incidence rate				
	Study cohort 2012 (24 weeks)	**Forna 2007** (3 years)	**Arenas-Pinto 2008** (3 years)	**Sacktor 2009** (24 weeks)	**Mehta 2011** (1 year)
ATN					
Current study definition [a]	34%				
DSP					
Current study definition [b]	37%				
≥ 1 sign, BPNS [c]	63%				
≥ 1 sign [d]	76%			31%	
SDSP					
Current study definition [e]	22%				
Symptoms	30%		6%	38%	
Symptoms and ≥ 1 sign, BPNS [c]	21%				11%
Symptoms or ≥ 1 sign [d]	78%	36%			

[a] Incident symptoms or worsening of SDSP

[b] Either (i) ≥ 2 signs or (ii) symptoms and ≥ 1 sign

[c] BPNS refers to assessment of deep tendon reflexes and vibration sensibility only

[d] Assessment of signs: pin sensibility, deep tendon reflexes and vibration sensibility

[e] Symptoms and ≥ 1 sign

University of Cape Town

The different studies cannot be compared directly as the periods of observation as well as the observation points differ. However, after correcting for varying diagnostic criteria, the cumulative incidence for symptomatic neuropathy in this study appears generally higher. This observation may be as a result of detecting the peak incidence of symptomatic neuropathy by assessing individuals between short time intervals within 12 weeks after commencing cART. We directly estimated the incidence of symptomatic neuropathy over a specific exposure period for individuals at risk. It is possible that the comparative studies did not take into account substantial changes in incidence that may have occurred over a short time period early on in cART exposure or only calculated incidence proportions for the population initially at risk. Furthermore, differences in the execution and interpretation of neuropathic signs by non-clinicians have been documented (Simpson et al., 2006). In the study by Forna et al., study participants were evaluated by trained field officers and again, this may have contributed to the discrepant results. The increased incidence in this cohort might reflect a higher prevalence of comorbidities, notably TB infection, which could contribute to the pathogenesis of symptomatic DSP. The prevalence of previous TB in our study population was eight times higher than that documented in the Kenyan population (Mehta et al., 2011).

5.6 The progression of HIV-DSP after starting cART

More than half of the cohort was initiated on stavudine-containing cART regimen before the government-sponsored programme was changed and stavudine replaced with tenofovir. Although a preliminary study has suggested that cART may improve thermal and heat pain thresholds in HIV-infected individuals with pre-existing symptoms (Martin et al., 2000), the clinical effect of cART initiation on existing symptomatic or asymptomatic DSP has only been documented in an anecdotal report (Pialoux et al., 1997) and a small prospective study of three patients (Markus and Brew, 1998). We found that within the first 12 weeks of cART more than half of individuals (54%) with symptomatic DSP at baseline experienced improvement of neuropathic symptoms, of whom half had complete resolution of symptoms. However, one third experienced worsening of symptoms usually after 12 weeks of observation. A few individuals (n=7) reported symptoms without any neuropathic signs at baseline. Prospective evaluation of these individuals revealed that only two went on to develop neuropathic signs. Therefore, it is unlikely that this phenotype represents a transitional state of developing clinically evident neuropathy.

An important consideration in the treatment of HIV infection is whether the presence of asymptomatic DSP at baseline increases the risk to developing symptomatic ATN on cART, as it may preclude the use of potentially neurotoxic drugs in resource constrained settings. Based on our prospective clinical evaluations, asymptomatic DSP at baseline did not predict progression to symptomatic ATN. Of the 10 individuals with asymptomatic DSP at baseline, none developed ATN and half had complete resolution in neuropathic signs by 24 weeks on cART. However, there were few individuals with asymptomatic DSP at baseline and the sample size might have been too small to delineate the relationships between asymptomatic DSP and the progression to symptomatic DSP, or to accurately assess risk factors associated with asymptomatic DSP. In addition, whether progressive improvement in baseline DSP, be it symptomatic or asymptomatic DSP, occurred because of improvement in general condition or immunological status, a specific cART regimen, or because it simply reflected the progression of DSP, remains unclear as multivariate analyses were precluded due to small sample size.

University of Cape Town

Overall, the positive effect of cART on the cognitive performance screening tool was evident at 24 weeks; 10% had mild cognitive impairment as defined by an IHDS score <10, compared to 24% at baseline. The results are comparable to that from Sacktor et al., showing similar frequencies (13% at 24 weeks vs 33% at baseline) (Sacktor et al., 2009).

To summarize, over the first 24 weeks on cART, there was improvement in asymptomatic DSP as well as in cognitive performance. Those with painful symptoms at onset frequently improved on cART. However, approximately one third of individuals with no symptoms pre-cART developed new symptoms, usually within the first 12 weeks.

5.7 Clinical evolution of symptoms and signs over the first 24 weeks of cART

The most frequent incident neuropathic symptom after cART initiation was paraesthesiae, followed by numbness, and least frequently burning aching neuropathic pain. The latter was the most frequent symptom in the pre-cART group. This contrasts with previous work reporting that pain is an early feature and more intense, compared to HIV-DSP (pre-cART), although these cohorts had either more advanced HIV disease or longer duration of cART exposure (Berger et al., 1993, Moyle and Sadler, 1998, Maritz et al., 2010).

This study shows that neuropathic pain in individuals who had existing symptomatic DSP pre-cART appeared to be more severe compared with new positive sensory symptoms that developed after cART. It is likely that pre-cART symptoms may have been present for a longer period. Further follow-up would determine whether symptoms worsen over time in the ATN cohort.

The distribution of neuropathic signs did not differ significantly between the individuals who had symptomatic DSP at baseline, compared to those with ATN. Although more individuals with symptomatic DSP at baseline had abnormal pinprick sensation compared to individuals with ATN, it remained the most frequent neuropathic sign in both groups. This again emphasizes the poor sensitivity of the BPNS when used alone.

Several observations can be made from studying the natural progression of neuropathic signs at several time points over the first 24 weeks of starting cART. Firstly, within the first 12

University of Cape Town

weeks after cART, improvement in signs correlated significantly with improvement in symptoms in individuals with symptomatic DSP pre-cART. This correlation did not continue after 12 weeks. Secondly, in contrast to the peak incidence of symptomatic ATN seen between 4 and 12 weeks, the incidence of asymptomatic DSP continued to increase after each study visit until 24 weeks. Therefore, neuropathic signs appear to take longer to resolve and may persist even after the resolution of symptoms. Neuropathic signs also develop later than symptoms after cART initiation.

5.8 Risk factors for the development of ATN

After 24 weeks on cART, those developing ATN showed a significantly greater CD4 T-cell count recovery compared to those without ATN. This concurs with our hypothesis that an immune-mediated inflammatory response may be associated with nerve damage contributing to the development of ATN. The impact of cART-initiation on immune dysregulation or reconstitution, and how it may correlate with the development of ATN within the first 12 weeks of starting cART was investigated further and is discussed in Chapter 10.

Although advancing age, previous or current TB, increased baseline triglyceride levels, hs-CRP and waist-to-hip ratio showed an association with the presence of DSP at baseline, none of these factors correlated with development of ATN within 24 weeks of cART initiation. It is possible that the study period of 24 weeks may have been too short for a sizeable effect of cART on some covariates. Fasting triglyceride and HDL levels as well as the waist-to-hip ratios, all covariates that associated with symptomatic DSP at baseline, showed no significant change over the follow-up period after the addition of cART. These risk factors can therefore not be excluded as contributing to cumulative nerve injury over time and contributing to the development of ATN, although there was no effect noted within the first 24 weeks of cART. Also, a 24-week follow-up period is not sufficient to expose age-related associations. Increasingly, accelerated senescence is thought to be an important complication of HIV infection (Effros et al., 2008). As NRTIs inhibit the function of polymerase-γ, it is possible that these drugs contribute to accelerated ageing through mtDNA damage. An increasingly ageing cART-treated population may also accumulate metabolic risk factors (Letendre et al., 2009) leading to further mitochondrial damage. Also, due to age-associated comorbidities, ageing HIV-infected individuals have an increased risk of other toxicities and drug

University of Cape Town

interactions (Goulet et al., 2007).

The national government-sponsored cART protocol changed during the study period (section 1.9.2). Prior to May 2010, the first-line cART regimen NRTI backbone was either stavudine or zidovudine; in May 2010 the national guidelines replaced stavudine with tenofovir as the preferred first-line NRTI backbone agent. The effect was that 56% of our cohort received stavudine during the 24-week follow-up period. This offered the opportunity to assess, albeit unparalleled, the early effects of stavudine versus no stavudine on neuropathy status within the first 24 weeks. The use of d-drug cART was not associated with the development of ATN, with 30% of individuals on stavudine developing ATN compared to 27% on non-d-drug cART. Although some cART studies indicated that d-drugs might not only cause DSP, but exacerbate existing DSP (Moore et al., 2000, Berger et al., 1993), other trials have suggested that d-drug cART did not add further risk to the occurrence of HIV-associated DSP (Simpson et al., 2002, Schifitto et al., 2005, Morgello et al., 2004, Lichtenstein et al., 2005).

In some studies, the use of d-drug containing cART was found to be protective for DSP after the first year of therapy (Lichtenstein et al., 2005, Hung et al., 2008, Ellis et al., 2010). In a study by Lichtenstein et al., although the use of d-drugs was associated with incident DSP in the first year of their use, increasing duration of d-drug exposure was associated with a decreased incidence of DSP (Lichtenstein et al., 2005). These observations are important for resource-limited settings where more affordable drugs may be life saving. However, the long-term effects of d-drugs on mtDNA remain unknown.

Alternatively, the seemingly neuroprotective effect of d-drug NRTI previously described could be due to survival bias: individuals with early development of neurotoxic neuropathy may be more likely to discontinue d-drugs and be "weeded out" from subsequent cohort studies, thereby rendering paradoxical results (Simpson et al., 2010, Simpson et al., 2006). During the first 24 weeks after cART, this was not the case in our cohort. With this prospective analysis, albeit in a relatively small cohort, we found that only two individuals had changed in cART therapy from stavudine to tenofovir due to the development of neuropathic symptoms. It is possible that this low number of regimen changes is due to the under-recognition of DSP by clinicians in busy clinic settings, where examination time is limited.

University of Cape Town

Some have hypothesized that the risk-to-benefit ratio of NRTIs in terms of virological control versus neuropathic symptoms differs between cohorts based on the age of individuals, stage of disease, the level of immunosuppression or malnutrition, therefore giving neuroprotection to some individuals (as a result of recovery of immune and neurological function or better virological control). However, in others there is increasing risk of developing ATN due to the additional insult as part of the cumulative damage to mitochondria. This then manifests as a worsening in neuropathic symptoms in for example those individuals with pre-existing HIV-DSP.

It should be noted that data on adherence to antiviral regimens were retrieved from clinic folder reviews. Therefore, it is possible that individuals taking these drugs differed in terms of dose exposure and consequently in their risk for developing ATN. With compliance based on viral control, 68% of the overall cohort had an undetectable viral load after 24 weeks on cART. Seventy nine per cent had a viral load <400 copies/mL, which is higher than that observed in other resource-poor settings, as well as reports from developed countries (Ivers et al., 2005, Staszewski et al., 2001, Staszewski et al., 1999, Walmsley et al., 2002). Although HIV viral load was not associated with the development of ATN at 24 weeks, pre-cART levels were not available. It remains possible that an association with the viral set point and damage to the peripheral nervous system may have occurred at an earlier period associated with a higher viral load.

Other factors were also investigated but showed no association with the development of ATN. Similar to a North American study, we did not find impaired glucose metabolism to be associated with ATN (Evans et al., 2011). While they found an association with history of diabetes, these individuals were excluded from our analysis to minimise the possible confounding effect of diabetes. By including these individuals, the North American study however demonstrated that non-insulin diabetic therapy reduced the odds of converting from asymptomatic to symptomatic DSP (Evans et al., 2012). They concluded that apart from better glucose control, the prevention of oxidative stress neuronal injury may be another possible mechanism that explains the association. In dyslipidaemic individuals, metformin decreased serum levels of IL-6 and TNF-α, probably due to a reduction in the activity of nuclear factor kappa B (Gomez-Garcia et al., 2007, Li et al., 2005). Interestingly, in individuals with hepatic steatosis, metformin led to normalization of raised ALT levels, and a 50% decrease in liver fat, inflammation and necrosis as demonstrated on liver biopsy

University of Cape Town

(Bugianesi et al., 2005). However, we did not find an association between impaired glucose metabolism and ALT levels.

Although one prospective cohort found that women were almost 10 times more likely than men to develop symptomatic DSP in the first year of cART (Mehta et al., 2011), we found no gender differences in the distribution of ATN within the first 24 weeks of cART. The relationship between taller stature and HIV-associated DSP remains unclear. Height was not found to be associated with either HIV-DSP or ATN in this cohort, nor from a previous report from this population (Maritz et al., 2010). In contrast, increased height has been described as a risk factor for DSP in cART exposed individuals from Australia, South-East Asia and North America (Cherry et al., 2009, Evans et al., 2011), although it did not predict the transformation of asymptomatic to symptomatic DSP after long-term cART (Evans et al., 2012). Nevertheless, gender bias may have confounded the association between height and DSP as our population consisted mostly of females (70%) with a median height of 1.61 m (IQR 1.56-1.67). The narrow height range and the relatively low median height of our cohort may have limited the power to detect differences in height between those with DSP and those without.

Although cART had a significant effect on macronutritional status over the 24-week study period, with significant increases in body weight and BMI, we found no association between these and the development of ATN. Reports from the pre-cART era found nutritional indices such as decreased albumin, anaemia and weight loss to be associated with DSP in AIDS patients (Tagliati et al., 1999, So et al., 1988, Snider et al., 1983). However, the impact of nutritional status, including micronutrients on the development of ATN in the cART era has not been studied prospectively.

To summarize, these results illustrate the pathogenetic complexity of HIV-associated DSP in its broader sense in which not only HIV infection, but also its treatment, is a major contributor. HIV-DSP and ATN may therefore possibly be part of a continuum, being the result of various cumulative insults of which d-drugs are an important but not exclusive pathological entity.

University of Cape Town

5.9 Study limitations

Our study has several limitations. A major limitation in any study of DSP is the absence of a gold standard for diagnosing DSP especially in epidemiological field studies such as ours. Different research groups also use different tools and definitions. Our diagnosis was based on clinical measures, albeit using two different validated tools, without electrophysiological or histological confirmation of the diagnosis.

The comparator group, no-DSP, was defined as individuals not meeting the criteria for DSP and may therefore have included individuals with isolated neuropathic symptoms or one sign. The decision to define the main outcome as DSP versus no DSP and symptomatic DSP versus no DSP was based on the low proportion of individuals who were entirely free of any neuropathic symptoms and/or signs at baseline (only 40%). This is a finding not unique to this study. Wadley et al. documented a prevalence of 25% for individuals with isolated neuropathic symptoms or signs (Wadley et al., 2011).

The definition of worsening symptomatic DSP has not been uniformly defined. A change in the TNS score has been utilized in some studies (Simpson et al., 2006, Cavaletti et al., 2007), but the exact point-change associating with a clinically meaningful difference is not known. Further, worsening of one modality may be balanced out by an 'improvement' in another. As the primary objective of this study was to focus on painful neuropathy (section 1.10), it was decided *a priori* to define worsening symptomatic DSP as worsening in neuropathic symptoms. This definition was extrapolated from previous work where a 2-point improvement in pain (on an 11-point VAS) was validated against a standard patient global impression of change score (Farrar et al., 2001). Worsening in pain was not assessed and this definition may therefore not have construct validity. Furthermore, the population at risk for incident symptoms and those at risk for worsening symptoms (which would be individuals with symptomatic DSP at baseline) are mutually exclusive and with our definition of ATN, individuals with symptomatic DSP will be in both comparative groups. It was therefore decided to also perform longitudinal analyses on individuals only developing new neuropathic symptoms compared to individuals who remained symptom-free (Appendix M).

The study was undertaken at a local community clinic in Cape Town. Ascertainment bias through exclusion criteria such as known diabetes or individuals on TB therapy for less than one month hinders the extrapolation of these figures to the overall HIV population. There is a potential for selection bias as the analyses were restricted to individuals willing and able to

University of Cape Town

return for follow-up visits. Also, the results from a relatively small cohort may not be as robust as those from larger multicentre studies. Due to the small sample size, some effect estimates, although not significant, cannot rule out potential associations.

Individuals with symptomatic HIV-DSP at baseline were not examined at 2 and 4 weeks. It would be informative to investigate whether those who improved on cART at 12 weeks, experienced a transient period of worsening in symptoms prior to improvement, possibly due to the initial 'insult' of NRTI therapy. Interestingly, all three individuals who improved at 24 weeks initially worsened in symptoms at 12 weeks after cART initiation.

5.10 Study strengths

Both the BPNS and the mTNS were used to assess neuropathy status as each had its strength; the former allowed quantitative assessment of symptoms and the latter a more qualitative measure of neuropathy severity with the focus on small-fibre functioning, and thus increasing the specificity for the diagnosis of HIV-associated DSP. Individuals were assessed within days prior to commencing cART and 2-, 4-, 12- and 24-weeks thereafter. The incidence and evolution of ATN has not previously been studied within the first 4 weeks of commencing cART. These clinical outcomes can now be evaluated against biomarkers that we have hypothesized are important as contributing to the pathogenesis of ATN – the following chapters will discuss these further.

As the study included individuals with HIV-DSP at baseline, this allowed for a cross-sectional analysis of a 'clean' cART-naïve cohort as well as the effect of cART on progression of DSP. In contrast to cross-sectional analyses in which the temporal sequence of exposure and outcome is often not ascertained, the longitudinal observational design of this study allowed for a more precise determination of predictors. Loss to follow-up was random and occurred as a result of many factors (see section 4.2.1). More than half of individuals lost to follow-up defaulted cART at the local clinic and were therefore excluded (n=22). However, 78% of individuals completed follow-up assessments, and therefore provided unbiased estimates in the follow-up analyses.

An additional strength was having only two clinicians performing the clinical examination. Methodological issues, such as the assessment of ankle reflexes, have affected inter-rater

University of Cape Town

reliability in some studies where nurses were used (Simpson et al., 2006). In this study, the clinical assessments were performed by two clinicians trained and supervised by a neurologist and stringent criteria of ≥2 signs were used for the definition of asymptomatic DSP. Finally, due to the observational nature of the study, observed associations may not be causal. Furthermore, the author had direct involvement with all aspects of the study, which allowed consistency, strengthened internal validity and the reliability of study findings.

University of Cape Town

Chapter 6 NAT2 Results

University of Cape Town

6.1 Objectives

With the *NAT2* substudy we firstly investigated the variability of the *NAT2* gene in a sub-Saharan African Xhosa population. Secondly, the relationship between *NAT2* INH acetylation status and vitamin B6 levels was investigated; the hypothesis being that slow acetylation-phenotype would have an increased requirement for vitamin B6 and thus be at increased risk of deficiency, and potentially developing HIV-associated DSP.

6.2 NAT2 genotyping

NAT2 genotyping was performed on 165 consecutively selected individuals at baseline. Two of these individuals were excluded from the analyses related to DSP, as they did not commence cART within two weeks of the baseline (pre-cART) assessment.

Genomic DNA concentrations were satisfactory for further analysis and varied between 45 ng/µL and 231 ng/µL. PCR amplification of genomic DNA resulted in the amplification of an 895 bp fragment containing *NAT2*. As discussed in section 3.1.3.7, for *NAT2* polymorphism identification using RFLP, the wild-type sequences (*NAT2*4*) were discriminated from homozygous and heterozygous variations based on the pattern of restriction enzyme digestion of the amplified PCR products.

The variation in a single nucleotide at a particular position abolished specific restriction enzyme sites (*Msp*I for 191 G>A and 434 A>C, *Fok*I for 282 C>T, *Kpn*I for 481 C>T, *Taq*I for 590 G>A, *Dde*I for 803 A>G, *Dra*III for 845 A>C and *Bam*HI for 857 G>A) so that alleles were defined based on the availability of a specific endonuclease recognition sequence. Unique RFLP patterns were observed after restriction digestion of PCR products (see section 3.1.3.2). These distinct patterns were used to determine the presence or absence of certain SNPs. Figure 6.1 and Figure 6.2 represent images obtained from the PCR digestions after gel electrophoresis. Due to the lack of a known restriction enzyme site near base 341, the 341 T>C substitution was detected by using allele-specific PCR described in section 3.1.3.7. We identified seven variant SNPs that have all been previously reported (Doll et al., 1995).

University of Cape Town

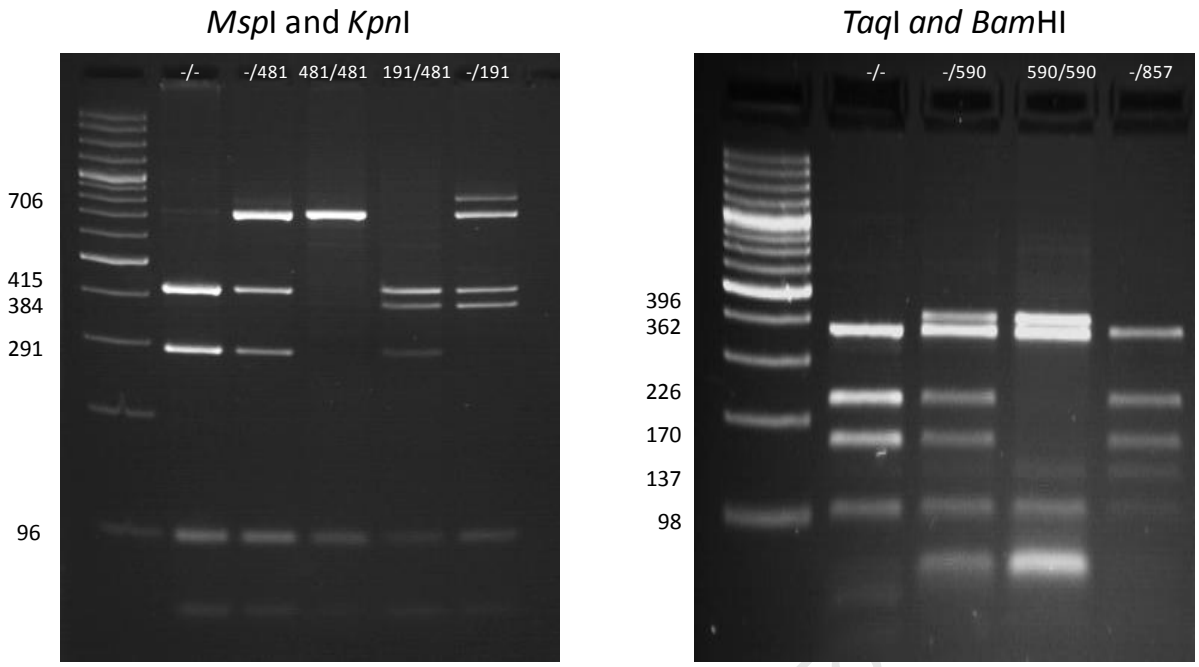


Figure 6.1: *NAT2* RFLP digests - *Msp*I & *Kpn*I (left) and *Taq*I & *Bam*HI (right)

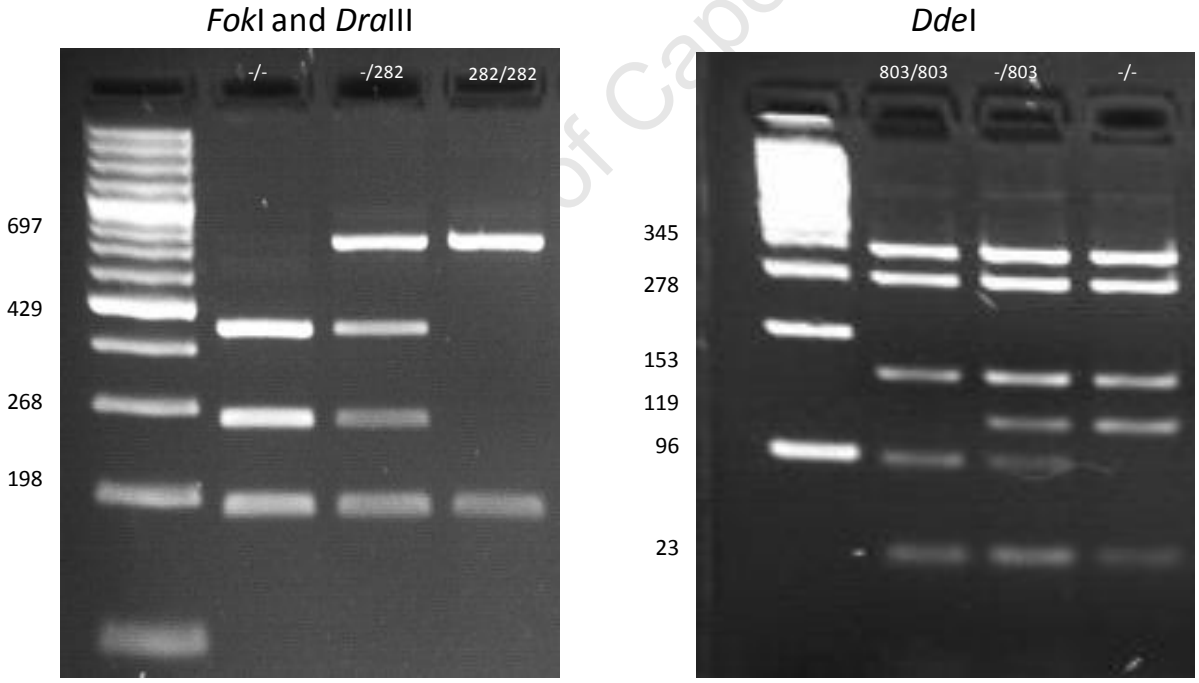


Figure 6.2: *NAT2* RFLP digests - *Fok*I & *Dra*III (left) and *Dde*I (right)

6.2.1 Allelic frequencies

The seven identified SNPs were polymorphic and in Hardy-Weinberg equilibrium ($p<0.05$). The genotypic and allelic frequencies are presented in Table 6.1. *NAT2*12* was the most common allele (51%) while *NAT2*7* was the least common (<1%). *NAT2*17* (434 A>C) and

*NAT2*18* (845 A>C) were not present in this cohort.

Table 6.1: *NAT2* allelic frequencies

***NAT2* allele**[a]	**SNP**	**Genotype frequency**	**Allele frequency**
		n (%)	**n (%)**
NAT2*5	**341 T>C**		83 (25%)
	TT	87 (53%)	
	TC	69 (42%)	
	CC	7 (4%)	
NAT2*6	**590 G>A**		83 (25%)
	GG	86 (53%)	
	GA	71 (44%)	
	AA	6 (4%)	
NAT2*7	**857 G>A**		1 (0%)
	GG	162 (99%)	
	GA	1 (1%)	
	AA	0 (0%)	
NAT2*11	**481 C>T**		74 (23%)
	CC	96 (59%)	
	CT	60 (37%)	
	TT	7 (4%)	
NAT2*12	**803 A>G**		166 (51%)
	AA	37 (23%)	
	AG	86 (53%)	
	GG	40 (25%)	
NAT2*13	**282 C>T**		121 (37%)
	CC	60 (37%)	
	CT	85 (52%)	
	TT	18 (11%)	
NAT2*14	**191 G>A**		19 (6%)
	GG	144 (88%)	
	GA	19 (12%)	
	AA	0 (0%)	

[a] Standard *NAT2* allele nomenclature

Although the frequencies of 341 T>C and 481 C>T were similar (25% vs 23%), the occurrence of 481 C>T was not as strongly linked to the occurrence of 341 C>T, as previously described in Caucasian populations (Sabbagh and Darlu, 2005). Of 83 alleles with

341 T>C substitutions, only 65 (78%) were also shown to have 481 C>T whereas the remaining 18 alleles (22%) showed the wild-type sequence for nucleotide 481, namely 481 C. This finding reflects a significant level of decay of linkage disequilibrium between these two SNPs and has also been observed in other African populations (Sabbagh et al., 2008).

6.2.2 Novel and uncommon *NAT2* polymorphisms

To confirm polymorphisms detected by RFLP and to identify any novel polymorphisms not detected by RFLP, the *NAT2* gene of 44 randomly selected individuals from the study population was subsequently sequenced. The bidirectional sequencing method is described in section 3.1.3.4. A novel 589 C>T polymorphism was found in one individual (allelic frequency 0.01) as well as three uncommon polymorphisms. Two of these uncommon polymorphisms, 403 C>G (allelic frequency 0.01) and 766 A>G (allelic frequency 0.01), were previously described in a central African population (Patin et al., 2006) and one, 345 C>T, was previously described in a North African population (allelic frequency 0.01) (Sabbagh et al., 2008).

The 589 C>T variant results in the arginine amino acid changing to a TGA stop codon. The 403 C>G polymorphism results in an amino acid change from cysteine to glycine and 766 A>G in a change from lysine to glutamic acid. The 345 C>T polymorphism is synonymous and does not change the amino acid sequence. The impact of these variants on NAT2 function will be discussed in section 6.3.1.

6.2.3 Distribution of *NAT2* SNPs and haplotypes

Sixteen distinct haplotypes were inferred by the PHASE programme (the results of which are shown in Table 6.2). The most frequent haplotypes were *NAT2*12A* (22%) and *NAT2*6A* (22%). Wild type *NAT2*4* was present in 6% of haplotypes. A unique haplotype, not compatible with standard *NAT2* nomenclature include the haplotype GCCTAAG with base changes 341 T>C, 481 C>T and 590 G>A (frequency 0.9%).

University of Cape Town

Table 6.2: PHASE-predicted *NAT2* haplotype frequencies

Haplotype[a]	*NAT2*[b]	Frequency
GCTCGAG	*4	0.061
GCCTGAG	*5A	0.015
GCCTGGG	*5B	0.164
GCCCGGG	*5C	0.064
GCCCGAG	*5D	0.003
GTTCAAG	*6A	0.215
GGTCAAG	*6B	0.027
GCTCAGG	*6F	0.003
GTTCGAA	*7B	0.003
GCTTGAG	*11A	0.018
GCTCGGG	*12A	0.221
GTTCGGG	*12B	0.030
GCTTGGG	*12C	0.024
GTTCGAG	*13	0.085
ACTCGAG	*14A	0.021
ATTCGAG	*14B	0.036
GCCTAAG	?	0.009

[a] Haplotype with seven positions: 191, 282, 341, 481, 590, 803 and 857

[b] Standard *NAT2* allele nomenclature

University of Cape Town

The synonymous base changes 481 C>T, 803 A>G and 282 C>T, encoding for *NAT2*11*, *NAT2*12* and *NAT2*13* respectively, do not result in functional change of the NAT2 enzyme and has previously been classified as *NAT2*4* (Bakayev et al., 2004, Dandara et al., 2003). We therefore grouped the abovementioned three alleles together with the ancestral reference genotype, *NAT2*4*. Using this classification, *NAT2*4* was the most common genotype (44%) while *NAT2*7* was the least common (0.3%). *NAT2*5* and *NAT2*6* were both present in 24.5%, as illustrated in Table 6.3.

Table 6.3: PHASE-predicted *NAT2* haplotype frequencies (alternative classification)

Haplotype[a]	*NAT2*[b]	Frequency
GCTCGAG	*4	0.439
GCCTGAG	*5A	0.015
GCCTGGG	*5B	0.164
GCCCGGG	*5C	0.064
GCCCGAG	*5D	0.003
GTTCAAG	*6A	0.215
GGTCAAG	*6B	0.027
GCTCAGG	*6F	0.003
GTTCGAA	*7B	0.003
ACTCGAG	*14A	0.021
ATTCGAG	*14B	0.036
GCCTAAG	?	0.009

[a] Haplotype with seven positions: 191, 282, 341, 481, 590, 803 and 857

[b] Standard *NAT2* allele nomenclature

A large number of different diplotype patterns of similar frequencies occurred in our cohort. Table 6.4 shows different combinations of the seven SNPs, which defined 49 distinct diplotype combinations. Diplotype frequencies ranged from 1% to 11%. Twenty-two diplotypes (45%) occurred with a frequency greater than 2%. PHASE v2.1.1 was used to generate genotypes for each diplotype configuration. The most frequent genotype was *NAT2*6A/12A*.

Computational genotypes from the PHASE programme were compared to actual genotypes obtained by means of allele-specific haplotype mapping and sequencing (Agundez et al., 2008). In most cases, genotype reconstruction fully corresponded to actual genotypes (Table 6.4). However, in some cases, the most probable predicted genotypes did not correspond to the actual genotype described by Agundez et al. (Agundez et al., 2008), viz. diplotypes number 5, 18, 42 and 44 noted in Table 6.4. This discrepancy may be as a result of differences in the two populations compared (African vs Spanish).

University of Cape Town

Table 6.4: *NAT2* diplotypes and PHASE reconstructed genotypes

No.	Diplotype							Frequency	Study results	Reference results (Agundez et al.)	
	191	282	341	481	590	803	857		PHASE reconstruction (probability)	Actual genotypes	PHASE reconstruction (probability)
1	GG	CC	TT	CC	GG	AG	GG	2%	*4/*12A (1.00)	*4/*12A	*4/*12A (1.00)
2	GG	CC	TT	CC	GG	GG	GG	2%	*12A/*12A (1.00)		
3	GG	CC	TT	CC	GA	AG	GG	1%	*6B/*12A (0.98)	*6B/*12A	*6B/*12A (1.00)
4	GG	CC	TT	CC	AA	AA	GG	1%	*6B/*6B (1.00)		
5	GG	CC	TT	CT	GG	AG	GG	1%	*11A/*12A (0.67)	*4/*12C	*4/*12C (1.00)
6	GG	CC	TT	CT	GG	GG	GG	1%	*12A/*12C (1.00)		
7	GG	CC	TC	CC	GG	AG	GG	1%	*4/*5C (0.75)	*4/*5C	*4/*5C (0.97) *5D/*12A (0.03)
8	GG	CC	TC	CC	GG	GG	GG	5%	*5C/*12A (1.00)		
9	GG	CC	TC	CT	GG	AA	GG	2%	*4/*5A (0.88)	*4/*5A	*4/*5A (1.00)
10	GG	CC	TC	CT	GG	AG	GG	2%	*4/*5B (0.63)	*4/*5B	*4/*5B (1.00)
11	GG	CC	TC	CT	GG	GG	GG	6%	*5B/*12A (0.95)	*5B/*12A	*5B/*12A (1.00)
12	GG	CC	TC	CT	GA	AG	GG	1%	*5B/*6B (0.69)	*5B/*6B	*5B/*6B (1.00)
13	GG	CC	TC	CT	GA	GG	GG	1%	*5B/*6F (0.55)		
14	GG	CC	TC	CT	AA	AA	GG	1%	*6B/? (0.95)[ς]		
15	GG	CC	TC	TT	GG	GG	GG	2%	*5B/*12C (1.00)	*5B/*12C	*5B/*12C (1.00)
16	GG	CC	TC	TT	GA	AG	GG	1%	*12C/? (0.61)[ς]		
17	GG	CC	CC	CT	GG	GG	GG	2%	*5B/*5C (1.00)	*5B/*5C	*5B/*5C (1.00)
18	GG	CC	CC	CT	GA	AG	GG	1%	*5C/? (0.67)[ς]	*5B/*5E	*5B/*5E (0.97)
19	GG	CC	CC	TT	GG	AG	GG	1%	*5A/*5B (1.00)	*5A/*5B	*5A/*5B (1.00)
20	GG	CC	CC	TT	GG	GG	GG	1%	*5B/*5B (1.00)	*5B/*5B	*5B/*5B (1.00)
21	GG	CT	TT	CC	GG	AA	GG	1%	*4/*13 (1.00)		
22	GG	CT	TT	CC	GG	AG	GG	6%	*12A/*13 (0.87)	*12A/*13 (n = 1) *4/*12B (n = 1)	*12A/*13 (0.70) *4/*12B (0.30)
23	GG	CT	TT	CC	GG	AG	GA	1%	*7B/*12A (0.44)	*7B/*12A	*7B/*12A (1.00)
24	GG	CT	TT	CC	GG	GG	GG	1%	*12A/*12B (1.00)		
25	GG	CT	TT	CC	GA	AA	GG	4%	*4/*6A (0.79)	*4/*6A	*4/*6A (1.00)
26	GG	CT	TT	CC	GA	AG	GG	11%	*6A/*12A (0.96)	*6A/*12A	*6A/*12A (0.96) *4/*6C (0.04)
27	GG	CT	TT	CC	AA	AA	GG	1%	*6A/*6B (1.00)	*6A/*6B	*6A/*6B (1.00)
28	GG	CT	TT	CT	GG	AA	GG	1%	*11A/*13 (0.98)		
29	GG	CT	TT	CT	GA	AA	GG	2%	*6A/*11A (0.93)		
30	GG	CT	TT	CT	GA	AG	GG	1%	*6A/*12C (0.95)	*6A/*12C	*6A/*12C (1.00)
31	GG	CT	TC	CC	GG	AG	GG	1%	*5C/*13 (0.94)		
32	GG	CT	TC	CC	GA	AG	GG	2%	*5C/*6A (0.99)	*5C/*6A	*5C/*6A (1.00)
33	GG	CT	TC	CT	GG	AG	GG	3%	*5B/*13 (0.93)	*5B/*13	*5B/*13 (0.98) *4/*5G (0.02)
34	GG	CT	TC	CT	GG	GG	GG	2%	*5B/*12B (0.97)		
35	GG	CT	TC	CT	GA	AA	GG	1%	*5A/*6A (0.80)	*5A/*6A	*5A/*6A (1.00)
36	GG	CT	TC	CT	GA	AG	GG	10%	*5B/*6A (0.98)	*5B/*6A	*5B/*6A (1.00)
37	GG	TT	TT	CC	GG	AA	GG	1%	*13/*13 (1.00)		
38	GG	TT	TT	CC	GA	AA	GG	4%	*6A/*13 (1.00)	*6A/*13	*6A/*13 (1.00)
39	GG	TT	TT	CC	GA	AG	GG	2%	*6A/*12B (0.97)		
40	GG	TT	TT	CC	AA	AA	GG	1%	*6A/*6A (1.00)	*6A/*6A	*6A/*6A (1.00)
41	GA	CC	TT	CC	GG	AA	GG	1%	*4/*14A (1.00)	*4/*14A	*4/*14A (1.00)
42	GA	CC	TT	CC	GG	AG	GG	3%	*12A/*14A (0.98)	*4/*14E	*12A/*14A (0.97) *4/*14E (0.03)
43	GA	CT	TT	CC	GG	AA	GG	1%	*4/*14B (0.52)		
44	GA	CT	TT	CC	GG	AG	GG	1%	*12A/*14B (0.86)	*4/*14G	*4/*14G (1.00)
45	GA	CT	TT	CC	GA	AA	GG	1%	*6A/*14A (0.76)	*6A/*14A (n = 3) *4/*14D (n = 1)	*6A/*14A (0.60) *4/*14D (0.40)
46	GA	CT	TC	CC	GG	AA	GG	1%	*5D/*14B (0.94)		
47	GA	CT	TC	CC	GG	AG	GG	1%	*5C/*14B (0.98)		
48	GA	CT	TC	CT	GG	AG	GG	2%	*5B/*14B (0.98)		
49	GA	TT	TT	CC	GA	AA	GG	3%	*6A/*14B (0.99)		

[ς] The new allele (indicated as '?') contains mutations at positions 341, 481 and 590.

University of Cape Town

6.3 NAT2 phenotypes

From the *NAT2* genotypes we inferred the distribution of rapid, intermediate and slow acetylation phenotypes in our cohort. The distribution of NAT2 phenotypes were assessed using three pre-specified classifications:

A) **Classification 1**: Rapid acetylators compared to individuals with at least one slow allele.

B) **Classification 2**: Comparing rapid, slow and intermediate acetylators. Heterozygote individuals bearing a rapid and a slow haplotype were considered intermediate acetylators (Cascorbi et al., 1995).

C) **Classification 3**: Comparing rapid, slow, intermediate and ambiguous *NAT2* genotypes grouped as slow/intermediate. Slow/intermediate acetylation included individuals with two or more SNPs associated with slow acetylation occurring with a rapid allele. Assuming the slow SNPs occur on the same chromosome would make the individual an intermediate acetylator. If they occur on different chromosomes, the individual would be a slow acetylator.

of Cape Town

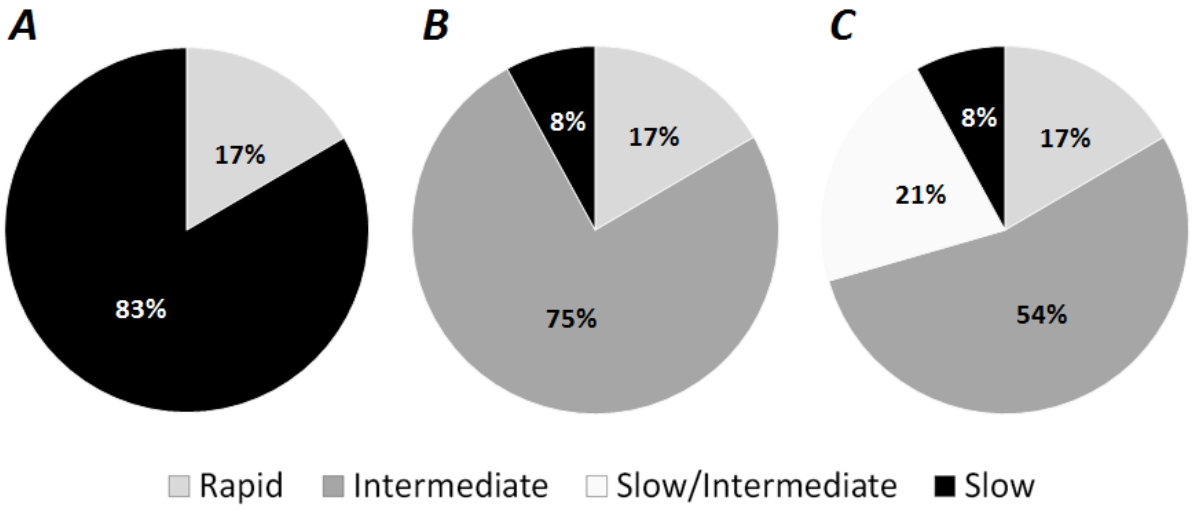


Figure 6.3: NAT2 acetylation phenotype distribution using three different classifications

Figure 6.3 illustrates the distribution of rapid, intermediate and slow acetylation phenotypes according to the three different classifications. One slow allele was present in 135 individuals (83%) (Figure 6.3 A), 17% were rapid acetylators, 8% slow and 54% were intermediate acetylators (Figure 6.3 B). In 35 individuals (21%) uncertainty remained in determining

NAT2 phenotype and was classified as slow/intermediate (Figure 6.3 C). Computational PHASE analysis predicted a slow phenotype in all 35 ambiguous genotypes.

6.3.1 Predicted functional effect of novel and rare polymorphisms on NAT2 activity

To determine the effects of the SNPs on NAT2 enzyme activity, we theoretically translated each *NAT2* haplotype into its corresponding amino acid sequences. The aim was to assess the unknown functional impact of two SNPs (345 C>T and 589 C>T). Two rare SNPs previously reported to have no effect on NAT2 activity (403 C>G and 766 A>G) were also investigated. We also used the web-based tools PolyPhen-2 and PANTHER to predict the effect of the variant on protein activity by measuring the evolutionary constraint acting on the site where it appeared. In order to test the reliability of PolyPhen-2 and PANTHER, the SNPs resulting in amino acid change where the impact on NAT2 activity is well known, were included as controls. The SNPs 191 G>A, 341 T>C, 590 G>A, 857 G>A are known to alter NAT2 activity while 803 A>G does not alter NAT2 activity. Our analysis predicted that 403 C>G, 345 C>T and 766 A>G do not alter NAT2 functional activity, while 589 C>T resulting in a stop codon, likely does.

6.4 The association between NAT2 and HIV-DSP

6.4.1 *NAT2* and DSP at baseline

The allele distribution between the group with symptomatic DSP at baseline and the group without DSP at baseline are presented in Table 6.5. Data were analysed using three pre-specified genetic models (described in section 3.1.3.6). None of the SNPs linked to slow acetylation showed a significant association with symptomatic DSP at baseline for any of the three genetic models, with relatively equal distributions of these SNPs between both groups.

The allele distribution between the individuals with DSP at baseline and those without were also investigated and are presented in Table O-5 in Appendix O. DSP was not associated with any of the alleles investigated using any of the three models. Although a greater proportion of individuals with DSP at baseline had the 341 T>C SNP compared to those without DSP (57% vs 43%), the difference was not statistically significant (p=0.08). However, adjusting for the

University of Cape Town

effect of a history of previous TB therapy, the association was significantly strengthened (p=0.032), suggesting a possible augmenting interaction between *NAT2* and previous TB therapy. The DSP odds ratios for allelic groups after correcting for previous TB therapy are summarized in Table P-1 in Appendix P.

Table 6.5: Allele distribution between SDSP and No DSP shown in three genetic models

General Genetic Model

SNP	SDSP n (%)	No DSP n (%)	Total n (%)	p-value
191 G>A				0.371 ¶
GG	28 (93%)	107 (86%)	135 (88%)	
GA	2 (7%)	17 (14%)	19 (12%)	
AA	0 (0%)	0 (0%)	0 (0%)	
282 C>T				0.918 ¶
CC	11 (37%)	46 (37%)	57 (37%)	
CT	15 (50%)	64 (52%)	79 (51%)	
TT	4 (13%)	14 (11%)	18 (12%)	
341 T>C				0.313 ¶
TT	13 (43%)	71 (57%)	84 (55%)	
TC	16 (53%)	47 (38%)	63 (41%)	
CC	1 (3%)	6 (5%)	7 (5%)	
481 C>T				1.000 ¶
CC	18 (60%)	74 (60%)	92 (60%)	
CT	11 (37%)	44 (35%)	55 (36%)	
TT	1 (3%)	6 (5%)	7 (5%)	
590 G>A				0.812 ¶
GG	14 (47%)	67 (54%)	81 (53%)	
GA	15 (50%)	52 (42%)	67 (44%)	
AA	1 (3%)	5 (4%)	6 (4%)	
803 A>G				0.607 §
AA	9 (30%)	27 (22%)	36 (23%)	
AG	15 (50%)	66 (53%)	81 (53%)	
GG	6 (20%)	31 (25%)	37 (24%)	
857 G>A				1.000 ¶
GG	30 (100%)	123 (99%)	153 (99%)	
GA	0 (0%)	1 (1%)	1 (1%)	
AA	0 (0%)	0 (0%)	0 (0%)	

Dominant Genetic Model

SNP	SDSP n (%)	No DSP n (%)	Total n (%)	p-value
191 G>A				0.371 ¶
GG	28 (93%)	107 (86%)	135 (88%)	
GA+AA	2 (7%)	17 (14%)	19 (12%)	
282 C>T				0.965 §
GG	11 (37%)	46 (37%)	57 (37%)	
GA+AA	19 (63%)	78 (63%)	97 (63%)	
341 T>C				0.169 §
GG	13 (43%)	71 (57%)	84 (55%)	
GA+AA	17 (57%)	53 (43%)	70 (45%)	
481 C>T				0.974 §
GG	18 (60%)	74 (60%)	92 (60%)	
GA+AA	12 (40%)	50 (40%)	62 (40%)	
590 G>A				0.468 §
GG	14 (47%)	67 (54%)	81 (53%)	
GA+AA	16 (53%)	57 (46%)	73 (47%)	
803 A>G				0.339 §
GG	9 (30%)	27 (22%)	36 (23%)	
GA+AA	21 (70%)	97 (78%)	118 (77%)	
857 G>A				1.000 ¶
GG	30 (100%)	123 (99%)	153 (99%)	
GA+AA	0 (0%)	1 (1%)	1 (1%)	

Additive Genetic Model

SNP	SDSP n (%)	No DSP n (%)	Total n (%)	p-value
191 G>A				0.548 ¶
G	58 (97%)	231 (93%)	289 (94%)	
A	2 (3%)	17 (7%)	19 (6%)	
282 C>T				0.859 §
G	37 (62%)	156 (63%)	193 (63%)	
A	23 (38%)	92 (37%)	115 (37%)	
341 T>C				0.319 §
G	42 (70%)	189 (76%)	231 (75%)	
A	18 (30%)	59 (24%)	77 (25%)	
481 C>T				0.879 §
G	47 (78%)	192 (77%)	239 (78%)	
A	13 (22%)	56 (23%)	69 (22%)	
590 G>A				0.596 §
G	43 (72%)	186 (75%)	229 (74%)	
A	17 (28%)	62 (25%)	79 (26%)	
803 A>G				0.358 §
G	33 (55%)	120 (48%)	153 (50%)	
A	27 (45%)	128 (52%)	155 (50%)	
857 G>A				1.000 ¶
G	60 (100%)	247 (100%)	307 (100%)	
A	0 (0%)	1 (0%)	1 (0%)	

¶ Fisher's exact test

§ χ^2 test

The distribution of slow, intermediate and rapid acetylators between the individuals with symptomatic DSP at baseline and those without DSP is presented in Table 6.6. There was no significant difference in distribution of NAT2 acetylation phenotypes between the symptomatic DSP group and those individuals without DSP.

Table 6.6: NAT2 acetylation phenotype distribution between baseline SDSP and No DSP

Variable	No. (%)			p-value
	Total (N=163)	SDSP (N=30)	No DSP (N=124)	
Acetylation Classification 1				0.943 §
Rapid	25 (16%)	5 (17%)	20 (16%)	
Slow	129 (84%)	25 (83%)	104 (84%)	
Acetylation Classification 2				1.000 ¶
Rapid	25 (16%)	5 (17%)	20 (16%)	
Intermediate	116 (75%)	23 (77%)	93 (75%)	
Slow	13 (8%)	2 (7%)	11 (9%)	
Acetylation Classification 3				0.552 ¶
Rapid	25 (16%)	5 (17%)	20 (16%)	
Intermediate	84 (55%)	14 (47%)	70 (56%)	
Slow/Intermediate	32 (21%)	9 (30%)	23 (19%)	
Slow	13 (8%)	2 (7%)	11 (9%)	

¶ Fisher's exact test

§ χ^2 test

University of Cape Town

The distribution of slow, intermediate and rapid acetylators between individuals with DSP and those without DSP are presented in Table O-1 in Appendix O. No significant difference in distribution in the NAT2 acetylation phenotype was found.

6.4.2 *NAT2* and ATN

No single SNP significantly associated with the development of ATN within 12 weeks of cART using the dominant, recessive and additive models. The allele distribution between the ATN and the ATN-free group are presented in Table O-7 in Appendix O. The distribution of slow, intermediate and rapid acetylator phenotypes between the ATN and the ATN-free groups are presented in Table 6.7. The data demonstrated a tendency of ATN to be associated with slow acetylator phenotype (although not statistically significant) with 91% of the ATN cohort having at least one slow allele compared to 81% without ATN (p = 0.17). In the Cox

regression analysis, there was a trend for individuals with at least one slow allele to develop ATN (OR 3.13; 95% CI 0.75-13.11; p=0.12).

Table 6.7: NAT2 acetylation phenotype distribution between individuals with and without ATN

Variable	No. (%)			p-value
	Total (N=157)	ATN (N=33)	No ATN (N=124)	
Acetylation Classification 1				0.165 §
Rapid	27 (17%)	3 (9%)	24 (19%)	
Slow	130 (83%)	30 (91%)	100 (81%)	
Acetylation Classification 2				0.188 ¶
Rapid	27 (17%)	3 (9%)	24 (19%)	
Intermediate	117 (75%)	29 (88%)	88 (71%)	
Slow	13 (8%)	1 (3%)	12 (10%)	
Acetylation Classification 3				0.312 ¶
Rapid	27 (17%)	3 (9%)	24 (19%)	
Intermediate	84 (54%)	21 (64%)	63 (51%)	
Slow/Intermediate	33 (21%)	8 (24%)	25 (20%)	
Slow	13 (8%)	1 (3%)	12 (10%)	

¶ Fisher's exact test

§ χ^2 test

The proportion of individuals developing new neuropathic symptoms was significantly associated with the presence of at least one slow allele (Table 6.8). In the Cox regression analysis, there was only a trend for individuals with at least one slow allele to develop new neuropathic symptoms (OR 3.22; 95% CI 0.77-13.49; p=0.11) and the trend remained after adjusting for a history of previous INH TB therapy (p=0.069, Table P-7 in Appendix P).

University of Cape Town

Table 6.8: NAT2 acetylation phenotype distribution between individuals with and without Incident Symptoms

Variable	No. (%)			p-value
	Total (N=132)	Incident Symptoms* (N=28)	No Symptoms* (N=104)	
Acetylation Classification 1				***0.036*** [§]
Rapid	22 (17%)	1 (4%)	21 (20%)	
Slow	110 (83%)	27 (96%)	83 (80%)	
Acetylation Classification 2				***0.041*** [¶]
Rapid	22 (17%)	1 (4%)	21 (20%)	
Intermediate	99 (75%)	26 (93%)	73 (70%)	
Slow	11 (8%)	1 (4%)	10 (10%)	
Acetylation Classification 3				0.086 [¶]
Rapid	22 (17%)	1 (4%)	21 (20%)	
Intermediate	73 (55%)	20 (71%)	53 (51%)	
Slow/Intermediate	26 (20%)	6 (21%)	20 (19%)	
Slow	11 (8%)	1 (4%)	10 (10%)	

[¶] Fisher's exact test

[§] χ^2 test

[*] Patients with SDSP at baseline were excluded

Chapter 7 NAT2 Discussion

University of Cape Town

The study demonstrated the diversity of the *NAT2* gene in a sub-Saharan African Xhosa population by genotyping 330 chromosomes. Individual *NAT2* SNP frequencies in this indigenous African population were similar to those reported elsewhere from the Western Cape and other African populations (Adams et al., 2003, Parkin et al., 1997, Sabbagh et al., 2008). However, some differences were also observed. Comparative allele frequencies of the seven common *NAT2* SNPs in African populations are shown in Figure 7.1. (Sabbagh et al., 2008).

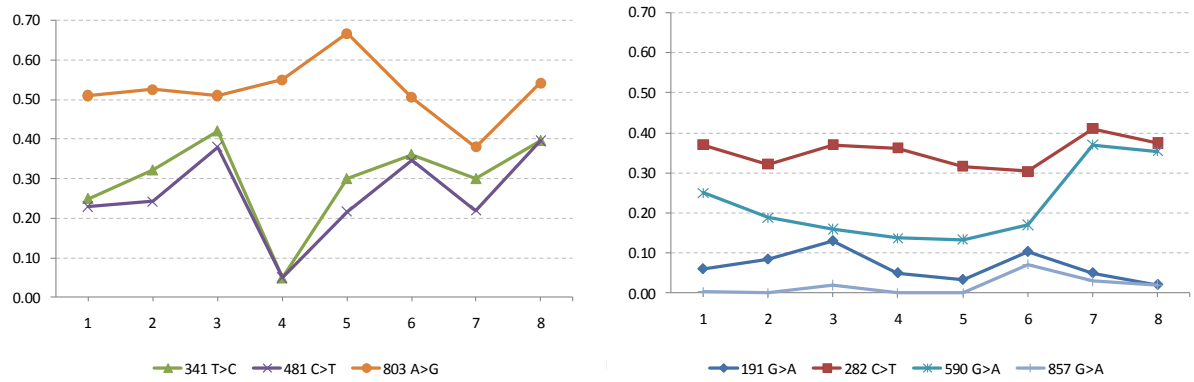


Figure 7.1: SNP frequencies in 8 different African populations. (1) Study population (Xhosa); (2) Tswana; (3) Ateke Bantus; (4) Bakola Pygmies; (5) Baka Pygmies; (6) Mandenka; (7) Dogons and (8) Somali (Functional SNPs 341 T>C, 191 G>A, 590 G>A) (Adapted from Sabbagh et al., 2008)

The base change 803 A>G, which defines the *NAT2*12* allelic group, is the one that occurred with the highest frequency in our population (51%), consistent with other African populations. The two most frequent allelic groups associated with a slow acetylation phenotype were *NAT2*5* (341 T>C) and *NAT2*6* (590 G>A), both occurring with a frequency of 25%. Intraracial variability has been reported for 341 T>C with frequencies in other African populations ranging from 5% to 42%. Compared to our population, 590 G>A has previously been found at lower frequencies among Tswana, Tanzanian, Venda and Zimbabwean populations (Dandara et al., 2003), although higher in Northern African (e.g. Somali) populations. The *NAT2*14* allelic group (191 G>A) has functional implications, and is regarded as an ancestral African-specific group, rarely occurring in Caucasian and Asian populations. Although 191 G>A occurred at a frequency of 6%, it is similar to other African populations (3-9%) (Dandara et al., 2003, Loktionov et al., 2002, Sabbagh et al., 2008). The

functional 857 G>A variant is primarily found in Asia and Central America and as expected, had a very low frequency in our population (<1%). In addition, we found a novel 589 C>T polymorphism in one individual and three uncommon polymorphisms previously described in African populations, viz. 403 C>G, 766 A>G and 345 C>T (Sabbagh et al., 2008, Patin et al., 2006).

A marked degree of linkage disequilibrium exists in Caucasian populations between SNP positions 341 and 481, where 341 T>C rarely occurs without 481 C>T (Lin et al., 1993). In our cohort, similar to that reported for other African populations (Bakayev et al., 2004, Sabbagh et al., 2008), the linkage disequilibrium was less pronounced: of 83 alleles with 341 T>C substitutions, only 78% were also shown to have 481 C>T. In addition, we found a high occurrence of nucleotide changes 803 A>G and 282 C>T in isolation (defining alleles *NAT2*12A* and *NAT2*13* respectively). In contrast, these nucleotide changes are almost always tightly linked to other polymorphisms in European populations (Cascorbi and Roots, 1999).

A common problem in *NAT2* genotyping is that the allelic effect of variations in *cis* or *trans* is not apparent and may influence the phenotype. Rather than indicating actual genotypes, the results of most genotyping methods indicate the predicted sum of two haplotypes (diplotypes), and many such diplotypes can represent different allelic combinations with different functional consequences (Sabbagh et al., 2008, Agundez et al., 2008). Forty-nine different diplotypes with similar frequencies occurred in our study population (Table 6.4). Ambiguous genotypes, where two or more enzyme-inactivating SNPs may be located either in *cis* or *trans* were demonstrated in 35 individuals (21%). These individuals may therefore be intermediate acetylators (*cis*) or slow acetylators (*trans*). To overcome this ambiguity, PHASE computational haplotype reconstruction was performed, which predicted a slow acetylation phenotype in all 35 individuals with ambiguous genotypes.

To date, no comparisons between actual haplotypes and reconstructed haplotypes have been performed in a study group large enough to include rare variant alleles in African individuals. Actual *NAT2* genotypes cannot be fully determined by haplotype prediction techniques. In Caucasian populations the ambiguity of the haplotype reconstruction with PHASE v2.1.1 ranged from 0.1% to 1.2% in previous studies (Golka et al., 2008, Agundez et al., 2008). However, an error rate of approximately 3% has been reported in an African population (Sabbagh and Darlu, 2005). This may be due to a greater frequency of multiple heterozygotes

University of Cape Town

with ambiguous multilocus genotypes, creating difficulty in the statistical inference of haplotypes. For this reason, in addition to performing PHASE reconstruction, we compared the PHASE predicted genotypes to a reference set which converted diplotypes to actual genotypes based on molecular haplotype mapping (Agundez et al., 2008). In our dataset, 6% of the PHASE predicted genotypes did not correspond to the actual genotype described by Agundez et al. (Agundez et al., 2008). It is possible that this discrepancy reflects the difference between the two populations in the comparison (African vs Spanish). In addition, over half of the diplotypes from our cohort occurred with a frequency <2%, and as demonstrated by Stephens et al., lower-frequency variants are less easily estimated statistically, leading to possible PHASE reconstruction error (Stephens et al., 2001). Consequently, due to the possibility that rare haplotypes may be important for disease risk or for predicting drug response, molecular haplotyping may be necessary to unambiguously determine linkage phase in African populations (Sabbagh and Darlu, 2005).

To determine the distribution of NAT2 acetylation phenotypes in this population, we inferred the distribution of rapid, intermediate and slow acetylation phenotypes from the *NAT2* genotypes as described in section 3.1.3.7. Consistent with previous African populations, the distribution of NAT2 phenotypes in this cohort was skewed toward the slow phenotype, with 83% having at least one slow allele. In most European populations, the proportions of slow and rapid acetylators are equal, whereas in Alaskan Inuits the majority are rapid acetylators (95%) and Saudi Arabians are generally slow acetylators (95%) (Matar et al., 2004).

NAT2 determines acetylation and influences the risk for INH-associated neuropathy. Furthermore, symptomatic DSP is independently associated with previous TB and thus possibly with INH exposure. We therefore investigated whether *NAT2* genotype and the deduced acetylation phenotype was a risk factor for HIV-associated symptomatic DSP. Overall, none of the SNPs associated with symptomatic DSP at baseline and neither did the NAT2 acetylation phenotypes. At 24 weeks, although none of the SNPs associated with the development of ATN, there was a trend for slow acetylation to predict the development of ATN. Furthermore, the development of new neuropathic symptoms over 24 weeks was significantly associated with the slow acetylation phenotype (Table 6.8).

Despite the association found between slow acetylation and new neuropathic symptoms, previous or current TB therapy was not associated with the development of new neuropathic symptoms after cART initiation (see section 4.2.10). The association between slow

University of Cape Town

acetylation and incident symptoms after cART, regardless of a history of previous TB, suggests the possibility that slow acetylation may be an independent risk factor for the development of neuropathic symptoms on cART. This relationship may be the result of slow acetylation increasing vulnerability to mechanisms such as oxidative stress that may be attributed to TB/HIV co-infection, cART initiation, or concomitant cotrimoxazole treatment (see later).

It is important to note that the slow acetylation phenotype is not homogeneous and is dependent upon an individual's specific *NAT2* haplotype or allelic profile. With 49 different *NAT2* diplotypes observed in this cohort, a wide variation in NAT2 enzyme activity between different individuals is to be expected. With this relatively small sample size and wide confidence intervals, important associations between acetylation phenotype and HIV-associated DSP may not be accurately represented.

This study, as with most other *NAT2* studies, used phenotypes inferred from genotyping results and thus the validity of the derived conclusions may be compromised by *NAT2* genotype-phenotype discrepancies (Walraven et al., 2008). In healthy individuals, there is a high correlation between *NAT2* genotype and phenotype activity. Previously, discrepancies between genotype and phenotype occurred in only 7% where rapid genotypes showed slow acetylation phenotypes (Cascorbi et al., 1995). Similar low rates of discordance are reported by other studies, some of which employed test substrates other than caffeine (Mrozikiewicz et al., 1994, Graf et al., 1992, Blum et al., 1991). However, in the context of chronic inflammatory disease, the relationship between genotype and phenotype is not clear. For example, acetylation phenotype/genotype discordance has been reported in 17% of HIV-infected individuals (O'Neil et al., 2000) and 35% in patients with AIDS (Wolkenstein et al., 2000). In both these studies, enzyme activity was quantified using caffeine as probe.

The reasons for the discordance between measured and predicted phenotype may be due to the method used to determine NAT2 phenotype. For example, the genotype/phenotype discordance rate is higher when caffeine rather than dapsone is used as a probe in HIV-positive patients (O'Neil et al., 2000). The determination of acetylation phenotype using dapsone is dependent upon determination of only a primary metabolite, monoacetyl dapsone (Zuidema et al., 1986). However, when using caffeine as a probe, the substrate for the NAT2 enzyme is not caffeine itself but rather a secondary metabolite (5-acetylamino-6-formylamino-3-methyluracil) which is formed by the hepatic cytochrome P450 1A2

University of Cape Town

(CYP1A2) enzyme system (Grant et al., 1984). There is considerable variation in CYP1A2 metabolic activity due to genetic factors, environmental factors, and drug-drug interactions. The NAT2 acetylation phenotype determination using caffeine as probe is thus potentially highly variable.

The discrepancy between phenotype and genotype may also be due to undetected rare nucleotide changes in *NAT2* when using conventional PCR-RFLP genotyping methods (Kaufmann et al., 1996, O'Neil et al., 2000, O'Neil et al., 1997). By sequencing only a third of our cohort, we found a novel non-synonymous 589 C>T variant with a predicted effect on protein structure and functional activity. Three rare variants, only previously described in African populations (Patin et al., 2006), were also found although these are unlikely to translate into any functional effect. Our results suggest that DNA sequencing for the entire intron-exon organization of the *NAT2* gene may provide more detailed information about genetic diversity that potentially affect NAT2 activity in this population.

Apart from these difficulties in accurately determining *NAT2* acetylation phenotype, other factors such as environmental, genetic or epigenetic factors may influence an individual's capacity to acetylate a substrate. Concomitant medication is potentially one such candidate in HIV-infected populations exposed to polypharmacy, although there are no known inhibitors or inducers of the NAT2 enzyme. Furthermore, gene-gene interactions may have confounded our results. McKeown-Eyssen et al. associated *NAT2* and *CYP2D6* gene-gene interaction with multiple chemical sensitivities (McKeown-Eyssen et al., 2004). Their results suggest that individuals with the rapid-metabolizing forms of both enzymes were 18 times more likely to have chemical hypersensitivity to drugs such as sulfamethoxazole than individuals with normal metabolizing forms of these enzymes. Gene-gene interactions between CYP2D6 and another P450 enzyme, CYP3A4, have also been found to influence the metabolism of commonly used pharmaceutical agents (Le Corre et al., 2004). Co-administration of NAT2 substrates such as sulfamethoxazole, a component of cotrimoxazole, may have affected acetylation phenotype. Sulfamethoxazole is metabolized by the NAT2 and cytochrome P450 enzymes (Alfirevic et al., 2003). Given that most of our cohort (97%) received cotrimoxazole during the study period, this may have influenced our results. Slow acetylation would be expected to result in shunting of this drug to an alternative oxidative pathway, resulting in toxic metabolites, hydroxyl amine and nitroso compounds (Alfirevic et al., 2003). These toxic metabolites are normally detoxified by ROS scavengers such as glutathione and other

University of Cape Town

antioxidants (Cribb and Spielberg, 1992). As discussed in section 1.8.3.6, HIV-infected individuals have low concentrations of glutathione (Buhl et al., 1989) leading to the accumulation of toxic metabolites that may bind to macromolecules, causing protein damage and cellular injury.

Malnutrition may also affect *NAT2* acetylation. HIV-associated wasting is characterized by a hypermetabolic state that includes disruption of carbohydrate and lipid metabolism (Hellerstein et al., 1990). This disruption could lead to diminished pools of NAT2 cofactors, such as acetyl coenzyme A, with a concomitant loss of NAT2 activity.

Finally, as acetylation occurs in the liver and the acetylation products are excreted by the kidneys, both hepatic and renal dysfunction influence acetylation phenotype. Hepatic disease has emerged as the most common non-AIDS–related cause of death among HIV-infected patients, accounting for 14%–18% of all deaths (Price and Thio, 2010, Palella et al., 2006). Furthermore, renal impairment, based on a single creatinine clearance measurement, was very common among African HIV-infected adults with clinically non-advanced HIV disease: 19% had moderate and 2% severe renal impairment (Struik et al., 2011).

To conclude, this research adds to the knowledge base for *NAT2* genetic variation and acetylation phenotypes in this South African population. A considerable prevalence of genotype-deduced slow acetylators was demonstrated. Although we were only able to show a significant association between a NAT2 acetylation phenotype and the development of new neuropathic symptoms, further studies investigating the interaction between cART and/or sulfamethoxazole and anti-TB therapy in the development of HIV-associated DSP is warranted. The growing practice of relying solely on genotype determination to assess the potential role of metabolic differences as a contributor to disease or drug-induced toxicity may need to be reconsidered, at least for NAT2 substrates in HIV/TB co-infection. To this end, acetylation of INH as a substrate in HIV/TB co-infected individuals and their NAT2 acetylation phenotype should be correlated, and may be helpful to determine vitamin B6 requirements (see Chapter 8).

University of Cape Town

Chapter 8 Vitamin B6 Results

University of Cape Town

8.1 Demographic Characteristics

The nested cohort for the vitamin B6 study consisted of 159 consecutively enrolled individuals who had neurological data available at baseline and at week 12. The individuals were genotyped for *NAT2* and also had vitamin B6 concentrations measured. These results are summarized in Table 8.1 and Table 8.2. The demographic and clinical features for this nested cohort were similar to the total longitudinal cohort described in Chapter 4 (see Table 4.1 and Table 4.2, page 89).

Table 8.1: Vitamin B6 cohort baseline characteristics (continuous data)

Variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=159)	SDSP (N=31)	No SDSP (N=128)	
Clinical						
Age	years		33 (26 - 39)	37 (29 - 43)	32 (26 - 38)	***0.019*** β
Weight	kg		61 (55 - 71)	60 (53 - 68)	62 (55 - 71)	0.555 ε
Height	metre		1.61 (1.56 - 1.67)	1.62 (1.55 - 1.68)	1.61 (1.56 - 1.67)	0.698 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.2 (20.3 - 28.0)	23.6 (20.8 - 25.9)	23.2 (20.3 - 28.0)	0.691 ε
Waist : Hip ratio		*< 0.90*	0.86 (0.81 - 0.90)	0.87 (0.83 - 0.92)	0.86 (0.81 - 0.90)	0.066 β
Systolic BP	mmHg	*120 - 140*	111 (103 - 122)	109 (98 - 119)	111 (104 - 122)	0.067 β
Diastolic BP	mmHg	*80 - 90*	71 (66 - 79)	70 (66 - 77)	72 (66 - 80)	0.170 β
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	156 (113 - 193)	167 (121 - 217)	151 (112 - 191)	0.271 ε
White cell count	$x10^9/L$	*4.0 - 11.0*	5.4 (4.1 - 6.5)	4.7 (3.9 - 5.9)	5.4 (4.3 - 6.7)	0.310 ε
C-reactive protein	mg/L	*< 5.0*	2.9 (0.9 - 6.6)	3.8 (1.4 - 11.9)	2.6 (0.9 - 6.3)	0.169 β
Haemoglobin	g/dL	*11.6 - 15.6*	11.7 (10.3 - 12.8)	11.8 (9.4 - 12.6)	11.7 (10.3 - 12.8)	0.406 β
MCV	fL	*80.0 - 100.0*	92.1 (88.0 - 96.1)	93.5 (89.8 - 96.9)	91.7 (87.9 - 96.1)	0.134 β
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 43)	38 (34 - 42)	40 (35 - 43)	0.114 β
ALT	IU/L	*10 - 41*	20 (15 - 27)	24 (18 - 29)	19 (14 - 26)	0.171 β
Creatinine	µmol/L	*53 - 115*	64 (55 - 74)	66 (57 - 73)	64 (55 - 74)	0.700 ε
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.5 - 4.9)	4.6 (4.4 - 4.9)	0.813 ε
Fasting insulin	µU/mL	*0.2 - 9.4*	5.1 (2.5 - 8.6)	4.3 (2.5 - 7.6)	5.4 (2.5 - 8.6)	0.286 β
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.3)	3.9 (3.1 - 4.3)	3.7 (3.2 - 4.3)	0.532 β
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.7 - 1.3)	1.0 (0.8 - 1.4)	0.8 (0.6 - 1.2)	***0.045*** β
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.74 - 1.13)	0.89 (0.77 - 1.13)	0.94 (0.73 - 1.13)	0.592 β
LDL	mmol/L	*1.0 - 3.0*	2.4 (1.9 - 2.9)	2.3 (1.8 - 2.7)	2.4 (1.9 - 2.9)	0.185 β
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	2.0 (1.2 - 2.6)	2.4 (1.7 - 3.1)	0.277 β
Pyridoxine						
PLP	nmol/L	*> 25.0*	23.8 (16.5 - 38.4)	20.7 (16.2 - 53.7)	24.1 (17.4 - 36.8)	0.512 β
4-PA	nmol/L	*unknown*	17.4 (11.9 - 23.3)	20.7 (12.1 - 28.6)	17.0 (11.7 - 22.8)	0.212 ε

β Student's *t*-test

ε Wilcoxon rank-sum test

University of Cape Town

Table 8.2: Vitamin B6 cohort baseline characteristics (grouped data)

Variable	No. (%)			p-value
	Total (N=159)	SDSP (N=31)	No SDSP (N=128)	
Female sex	110 (69%)	17 (65%)	93 (70%)	0.647 §
Age > 40 years	39 (25%)	11 (42%)	28 (21%)	***0.023*** §
Previous/Current TB	59 (37%)	16 (62%)	43 (32%)	***0.005*** §
Time of TB				***0.020*** ¶
Currently	24 (15%)	8 (31%)	16 (12%)	
< 1 year ago	11 (7%)	3 (12%)	8 (6%)	
1 year ago	5 (3%)	2 (8%)	3 (2%)	
2 years ago	4 (3%)	1 (4%)	3 (2%)	
> 2 years ago	15 (9%)	2 (8%)	13 (10%)	
Vit Bco supplement	155 (97%)	26 (100%)	129 (97%)	1.000 ¶
Vit B6 supplement	12 (8%)	7 (27%)	5 (4%)	***0.001*** ¶
WHO clinical stage				0.285 ¶
Stage 1	51 (33%)	8 (31%)	43 (33%)	
Stage 2	49 (31%)	5 (19%)	44 (34%)	
Stage 3	50 (32%)	12 (46%)	38 (29%)	
Stage 4	6 (4%)	1 (4%)	5 (4%)	
CD4 T-cell count				0.264 §
< 100 cells/mm ^{3}	26 (17%)	2 (8%)	24 (18%)	
100 - 200 cells/mm ^{3}	96 (62%)	16 (62%)	80 (62%)	
> 200 cells/mm ^{3}	34 (22%)	8 (31%)	26 (20%)	
Body Mass Index				0.939 §
< 20 kg/m ^{2}	34 (22%)	6 (23%)	28 (22%)	
20 - 25 kg/m ^{2}	65 (42%)	10 (38%)	55 (42%)	
25 - 30 kg/m ^{2}	30 (19%)	6 (23%)	24 (18%)	
> 30 kg/m ^{2}	27 (17%)	4 (15%)	23 (18%)	
Alcohol last year	44 (28%)	6 (23%)	38 (29%)	0.524 §
IHDS score < 10	29 (25%)	3 (30%)	26 (25%)	0.716 §
Neuropathy status				
DSP	35 (22%)			
Symptomatic DSP	26 (16%)			
Asymptomatic DSP	9 (6%)			
Symptoms only	6 (4%)			

¶ Fisher's exact test

§ χ^2 test

University of Cape Town

The majority of the nested cohort was female (69%) with ages ranging between 19 and 63 years [median age of 33 (IQR 26-39)]. Overall, 59 individuals (37%) had a history of TB therapy (current or previous INH exposure). Of these, 35 completed TB treatment before study entry and 24 started before study entry and continued treatment during the study period. Eight individuals developed TB infection after the baseline assessment (six started treatment between 4 and 12 weeks, and two after 12 weeks).

Nearly two thirds (n=100, 64%) were classified as HIV WHO stage 1 or 2, with only six individuals (4%) in stage 4. The baseline median CD4 T-cell count was 156 cells/mm^3 (IQR 113-193) with 79% having counts <200 cells/mm^3 and 17% <100 cells/mm^3.

The BMI ranged from 16.5 to 39.5 kg/m^2, with a median of 23.2 kg/m^2. Median fasting lipid values were within normal ranges. Serum albumin, done on 145 of the 159 individuals (91%), did not correlate with BMI at baseline (ρ=0.12, p=0.14). Serum albumin <38 g/L was found in 65 (45%) and of these, 34% were eutrophic and 34% underweight. Of the 55% with normal serum albumin levels, 49% were eutrophic and 13% were underweight. Thirty-seven individuals (23%) had a haemoglobin level <10 g/dL.

Nearly all individuals (97%) were prescribed two tablets of vitamin B complex supplements at the time of enrolment[2]. Twelve individuals (8%) were prescribed additional 25 mg/day vitamin B6 supplementation.

During the follow-up period, 10 of 24 individuals (42%) on TB therapy received additional vitamin B6 supplementation, i.e. 25 mg/day (six of whom had symptomatic DSP at baseline). Two individuals completed TB therapy less than six months prior to enrolment, and were still prescribed additional vitamin B6 supplementation at the time of the baseline assessment.

In this nested cohort, the distribution of DSP status was comparable to that of the total cohort and was categorised as follows:

- No DSP, 124 (78%) – individuals not meeting criteria of symptomatic DSP or asymptomatic DSP including six individuals (4%) with neuropathic symptoms in isolation.
- Asymptomatic DSP, 9 (6%)
- Symptomatic DSP, 26 (16%)

[2] Pyridoxine doses in vitamin B complex preparations may vary according to the manufacturer, but this currently equates to 2-4 mg/day in South African government-sponsored programmes (Rossiter et al., 2012).

University of Cape Town

8.2 Pyridoxine concentrations in a South African community-based HIV-infected cohort before and after cART

8.2.1 Plasma PLP and 4-PA concentrations at baseline (before cART)

At the baseline assessment, PLP concentrations ranged from 5.8 to 128.0 nmol/L with a median of 23.8 nmol/L. Over half of the cohort (53%) was vitamin B6 deficient at baseline (PLP <25 nmol/L). Figure 8.1 illustrates the positively skewed distribution of baseline plasma PLP and 4-PA concentrations overall. Baseline plasma 4-PA concentrations ranged from 4.9 to 197.0 nmol/L with a median of 17.4 nmol/L, not indicative of any ongoing or recent vitamin B6 supplementation (Bates et al., 1999, Bor et al., 2003).

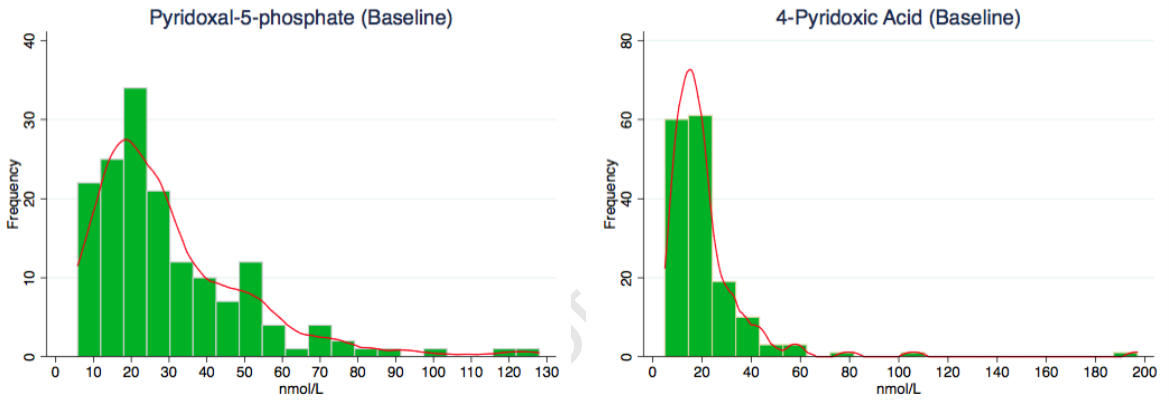


Figure 8.1: Baseline concentrations of PLP (left) and 4-PA (right)

8.2.2 Factors influencing plasma PLP concentrations at baseline

To investigate the relationship between PLP and NAT2 acetylation phenotype, as well as previously identified factors associated with symptomatic DSP, immunity and metabolic status, Spearman correlation coefficients were calculated (Table 8.3). There was a significant albeit moderate positive correlation between PLP and 4-PA (ρ=0.57; p<0.001) as well as between PLP and albumin levels (ρ=0.29; p<0.001) and a weak correlation between PLP and haemoglobin levels at baseline (ρ=0.19; p=0.018). Therefore, poor micronutritional status reflected by low albumin and haemoglobin levels correlated with suboptimal PLP levels. There was an inverse correlation between PLP and hs-CRP, i.e. higher hs-CRP levels correlated with lower PLP concentrations (ρ=-0.25; p=0.002). Of the risk factors associated with symptomatic DSP at baseline (see section 4.1.6.2), PLP levels showed a significant although weak correlation with ALT (ρ=0.17; p=0.036). The correlation coefficients between

PLP and other variables investigated appear in Table 8.3. Baseline PLP concentration did not correlate with any other clinical factors related to macronutritional status, including weight, BMI and waist-to-hip ratio, nor with any of the metabolic factors, such as total cholesterol, HDL, LDL, triglycerides or fasting glucose.

Table 8.3: Spearman correlations between baseline PLP levels and baseline factors possibly related to nutritional status or symptomatic DSP

Variable	Correlation coefficient	p-value
Clinical		
Age	0.06	0.428
Weight	0.08	0.339
Height	0.07	0.376
Body Mass Index	0.06	0.440
Waist : Hip ratio	0.14	0.081
Systolic BP	0.05	0.544
Diastolic BP	-0.10	0.219
Haematological		
CD4 T-cell count	0.01	0.941
White cell count	-0.02	0.826
C-reactive protein	-0.25	***0.002***
Haemoglobin	0.19	***0.018***
MCV	0.04	0.620
Biochemical		
Albumin	0.29	***<0.001***
ALT	0.17	***0.036***
Creatinine	0.00	0.994
Metabolic		
Fasting glucose	0.01	0.860
Fasting insulin	0.08	0.345
Total cholesterol	0.11	0.194
Triglycerides	0.04	0.656
HDL	0.14	0.083
LDL	0.06	0.448
Lactate	0.04	0.646
Pyridoxine		
4-PA	0.57	***<0.001***

University of Cape Town

To determine whether there is a significant difference in PLP concentrations between the group variables of interest, PLP median values between groups were compared (Table 8.4). Although no significant correlations were detected, there was a trend for men to have higher median PLP levels compared to women (29.0 vs 22.8 nmol/L, p=0.10). As a measure of HIV severity, individuals were grouped using two arbitrary CD4 T-cell count cut-offs, viz. 100 and 200 cells/mm^3. In both these groups, no significant relationship was found between lower CD4 T-cell count and low PLP concentrations; those with a CD4 T-cell count <100 cells/mm^3 had a median PLP of 24.8 nmol/L compared to the level of 23.8 nmol/L in the group with a higher CD4 T-cell count (p=0.23). Vitamin B6 supplementation by prescription, either as vitamin B complex or as 25 mg vitamin B6 had no significant effect on PLP concentrations overall (p=0.52).

The effect of prior TB therapy on PLP concentrations was investigated. Table 8.4 shows that there was no difference in median PLP concentrations between the 35 individuals who were previously exposed to INH therapy, compared to INH-naïve individuals (p=0.99). Twenty-four individuals started TB therapy before study entry and continued on INH during the study period. While there was a trend towards higher PLP concentrations in these individuals compared to INH-naïve individuals, the differences observed were modest (27.4 vs 23.1 nmol/L, p=0.15).

To establish whether individuals with a slow NAT2 acetylation phenotype had lower vitamin B6 concentrations compared to those with a rapid acetylation phenotype, plasma PLP and 4-PA concentrations were compared between different NAT2 acetylation phenotypes deduced from *NAT2* genotypes (Table 8.4). PLP concentrations were not significantly different between rapid, intermediate and slow acetylators (p=0.69), with median PLP concentrations of 23.8, 23.4 and 26.7 nmol/L respectively. Comparing individuals homozygous for rapid acetylator genotype to individuals bearing at least one slow allele (acetylation phenotype 1, Table 8.4), no significant association with PLP or 4-PA concentrations was shown (p=0.41).

University of Cape Town

Table 8.4: Comparison of PLP medians between different categories possibly related to nutritional status or symptomatic DSP

Variable	Median (IQR)	p-value
Sex		0.102 β
Female	22.8 (16.2 - 34.5)	
Male	29.0 (19.7 - 46.8)	
Age groups		0.901 β
> 40 years	24.4 (16.6 - 46.8)	
< 40 years	23.8 (16.5 - 38.4)	
Previous/Current TB		0.421 β
Yes	23.8 (14.7 - 49.2)	
No	23.9 (17.5 - 36.6)	
Current TB		0.148 β
Yes	27.4 (17.1 - 60.1)	
No	23.1 (16.5 - 36.8)	
Previous TB		0.988 β
Yes	22.4 (13.9 - 40.8)	
No	23.9 (17.5 - 36.6)	
Vit B6 supplement		0.520 β
Yes	28.4 (15.6 - 54.9)	
No	23.7 (16.6 - 36.8)	
Acetylation-phenotype 1		0.410 β
Rapid	23.8 (12.8 - 34.0)	
Slow	23.7 (17.4 - 38.5)	
Acetylation-phenotype 2		0.689 ϕ
Rapid	23.8 (12.8 - 34.0)	
Intermediate	23.4 (17.5 - 39.4)	
Slow	26.7 (13.0 - 32.3)	
WHO Stage		0.798 β
Stage 1 or 2	24.8 (18.0 - 36.6)	
Stage 3 or 4	22.5 (13.3 - 48.0)	
CD4 T-cell count		0.227 β
< 100 cells/mm^3	24.8 (14.7 - 33.6)	
> 100 cells/mm^3	23.8 (17.4 - 43.7)	
Alcohol last year		0.434 β
Yes	23.1 (17.3 - 33.8)	
No	24.8 (16.6 - 43.4)	
IHDS score		0.240 β
< 10	27.8 (22.9 - 36.8)	
≥ 10	23.4 (14.5 - 36.8)	

β Student's *t*-test

ϕ Analysis of Variance

Not all individuals on INH therapy were prescribed 25 mg/day additional vitamin B6 supplementation and this may have had an effect on PLP concentrations. To understand the possible effect of the additional vitamin B6 supplementation (25mg/day) on PLP and 4-PA concentrations among individuals on INH therapy during the study period, the group with 25mg/day vitamin B6 supplementation was compared to those without (Table 8.5). In both these groups all individuals were prescribed Vitamin B complex. PLP and 4-PA concentrations were not significantly different between individuals who were prescribed 25 mg/day of vitamin B6 supplementation and those who were not (p=0.22 and p=0.42, respectively).

Table 8.5: PLP and 4-PA levels for individuals currently on TB treatment grouped by vitamin B6 supplementation (25 mg/day)

Pyridoxine	Unit	Normal range	Median (IQR)		p-value
			Additional vitamin B6 supplement* (N=10)	No additional vitamin B6 supplement* (N=14)	
PLP	nmol/L	*> 25.0*	23.6 (12.5 - 38.2)	28.1 (18.0 - 76.5)	0.218 β
4-PA	nmol/L	*unknown*	22.4 (15.7 - 33.2)	33.0 (18.2 - 46.2)	0.418 β

* All patients received vitamin Bco containing up to 4mg vitamin B6; Additional vitamin B6 refers to 25 mg/day

β Student's *t*-test

Univariate odds ratios and prevalence ratios (with a 95% CI) were calculated to determine factors associated with vitamin B6 deficiency (PLP <25 nmol/L). All variables were explored for an association, but only variables showing a significance level of p<0.25 in the primary analysis were included in the multivariate analysis and are shown in Table Q-15 in Appendix Q. A multivariate logistic regression analysis identified factors independently associated with vitamin B6 deficiency at baseline. The results are summarized in Table 8.6. Higher levels of fasting triglycerides (>0.9 mmol/L) and ALT (>19 IU/L) remained significantly associated with PLP concentrations <25 nmol/L while higher albumin levels (>38 g/L) was associated with PLP concentration ≥25 nmol/L. Pyridoxic acid (4-PA) was not included into the model due to covariance.

University of Cape Town

Table 8.6: Multivariate analysis of risk factors for PLP deficiency at baseline

Model	Odds Ratio	Odds Ratio 95% Confidence Interval	Variable p-value	Pseudo R^2	Model p-value
PLP > 25 nmol/L vs PLP < 25 nmol/L				0.15	***<0.001***
Albumin > 38 g/L	3.86	1.59 - 9.35	***0.003***		
Triglycerides > 0.9 mmol/L	0.39	0.17 - 0.92	***0.031***		
ALT > 19 IU/L	0.34	0.15 - 0.78	***0.011***		

Grouping PLP and 4-PA levels into quartiles did not provide any additional insights into the relationship between vitamin B6 levels and the various aforementioned factors (See Table Q-3 to Table Q-14 in Appendix Q).

8.2.3 Low plasma pyridoxine concentrations as a risk factor for HIV-DSP at baseline

The association of vitamin B6 levels with DSP status was investigated. At baseline, individuals with DSP were compared to those without. None of the DSP types (DSP, symptomatic DSP or asymptomatic DSP) were associated with lower baseline measures of circulating plasma vitamin B6 concentrations (PLP and 4-PA) (Table 8.7).

University of Cape Town

Table 8.7: Baseline and week 12 vitamin B6 levels categorized by baseline DSP status

Baseline DSP status	N	Baseline		Week 12		Group effect [λ]
		Baseline PLP (nmol/L)		12-week PLP (nmol/L)		Δ PLP
		Median (IQR)	p-value	Median (IQR)	p-value	p-value
DSP	35	25.5 (16.5 - 53.7)	0.392 [ε]	20.0 (11.2 - 35.1)	0.562 [ε]	0.108 [λ]
No DSP	124	23.8 (15.7 - 36.8)		21.2 (14.2 - 33.4)		
SDSP	26	20.7 (16.2 - 53.7)	0.474 [ε]	19.2 (11.2 - 31.1)	0.597 [ε]	0.166 [λ]
No DSP	124	23.8 (15.7 - 36.8)		21.2 (14.2 - 33.4)		
ADSP	9	27.5 (22.9 - 32.0)	0.563 [ε]	29.2 (11.6 - 37.6)	0.766 [ε]	0.515 [λ]
No DSP	124	23.8 (15.7 - 36.8)		21.2 (14.2 - 33.4)		
		Baseline 4-PA (nmol/L)		12-week 4-PA (nmol/L)		Δ 4-PA
		Median (IQR)	p-value	Median (IQR)	p-value	p-value
DSP	35	20.5 (12.1 - 28.6)	0.089 [ε]	20.3 (12.2 - 28.2)	0.983 [ε]	0.810 [λ]
No DSP	124	16.7 (11.6 - 22.4)		19.1 (12.5 - 26.9)		
SDSP	26	20.7 (12.1 - 28.6)	0.169 [ε]	18.9 (12.2 - 25.7)	0.782 [ε]	0.660 [λ]
No DSP	124	16.7 (11.6 - 22.4)		19.1 (12.5 - 26.9)		
ADSP	9	19.0 (15.2 - 30.8)	0.235 [ε]	21.4 (16.4 - 31.0)	0.585 [ε]	0.785 [λ]
No DSP	124	16.7 (11.6 - 22.4)		19.1 (12.5 - 26.9)		

[ε] Wilcoxon rank-sum test for difference in median vitamin B6 level between DSP groups at a specific time point

[λ] Random effects model for the difference in mean vitamin B6 level between DSP groups at week 12 compared to baseline

University of Cape Town

Comparison of proportions of individuals with DSP, symptomatic DSP or asymptomatic DSP at baseline across the vitamin B6 quartiles and halves (PLP and 4-PA) were assessed. No significant differences were found between the different groups and these results are summarized in Table Q-16 – Table Q-19 in Appendix Q.

To assess the impact of previous TB therapy on current vitamin B6 status, we stratified the cohort by TB history and investigated the association of low vitamin B6 concentrations with symptomatic DSP in only those individuals with a history of previous or current TB therapy (n=59). Symptomatic DSP at baseline was significantly associated with PLP deficiency (p=0.029) (Table 8.8).

Table 8.8: Vitamin B6 levels in individuals with previous TB: comparing SDSP to No DSP

Plasma pyridoxine level	No. (%)			Odds Ratio	Odds Ratio 95% Confidence Interval	p-value
	Total (N=59)	SDSP (N=16)	No DSP (N=43)			
PLP						
Below 25 nmol/L	30 (51%)	12 (75%)	18 (42%)	4.17	(1.15 ; 15.04)	***0.029***
Above 25 nmol/L	29 (49%)	4 (25%)	25 (58%)			
4-PA						
Below median (17.4 nmol/L)	28 (48%)	8 (50%)	20 (47%)	1.15	(0.36 ; 3.63)	0.812
Above median (17.4 nmol/L)	31 (53%)	8 (50%)	23 (54%)			

The median plasma PLP concentrations in symptomatic DSP and DSP-free individuals with different NAT2 genotypes are summarized in Table Q-1 in Appendix Q. There is no indication that the plasma PLP values were lower amongst the symptomatic slow acetylators on enrolment.

8.2.4 Plasma PLP and 4-PA concentrations after 12 weeks on cART

Figure 8.2 illustrates the positively skewed distribution of PLP and 4-PA concentrations after 12 weeks of cART when 55% of individuals had vitamin B6 deficiency (PLP <25 nmol/L). The median plasma PLP level was 21.0 nmol/L and 4-PA had a median of 19.2 nmol/L.

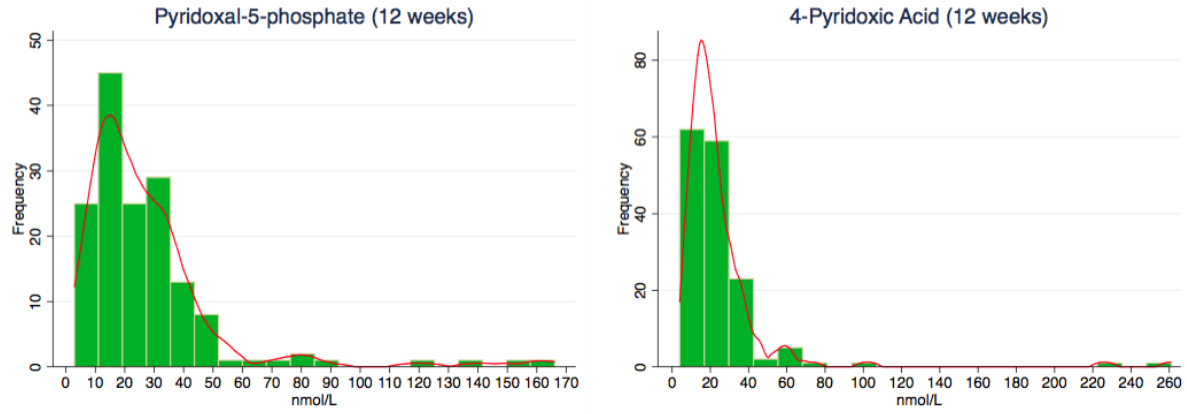


Figure 8.2: Week 12 concentrations of PLP (left) and 4-PA (right)

8.2.5 Changes in vitamin B6 status between different DSP groups after 12 weeks of cART

For the cohort overall, plasma PLP concentrations after 12 weeks did not change significantly from baseline concentrations (23.8 to 21.0 nmol/L; p=0.27). An insignificant increase in

median 4-PA concentration was observed during the first 12 weeks on cART (17.4 to 19.2 nmol/L; p=0.11).

Table 8.7 (page 186) illustrates the median plasma PLP and 4-PA concentrations at baseline and 12 weeks after cART grouped according to baseline DSP status. Compared to baseline, median PLP concentrations at week 12 were lower in all comparative groups except for the nine individuals who had asymptomatic DSP, in whom the median PLP concentration was higher. However, a random effects model showed no significant differences in the mean vitamin B6 levels in any DSP groups between baseline and week 12 (Table 8.7).

8.2.6 Factors influencing plasma PLP concentrations after 12 weeks of cART

To determine whether any factors had an effect on PLP levels over the 12-week period, or any factors predispose an individual to a greater decrease in PLP after commencing cART, the longitudinal relationship between PLP and the various factors were investigated (Table 8.9). Factors of interest included those associated with symptomatic DSP such as stavudine, nutritional factors, immune-related factors and *NAT2* acetylation phenotype.

At week 12, 59% were receiving cART with a stavudine-containing cART regimen, 28% with tenofovir, and 12% with zidovudine. Plasma PLP differed significantly according to the use of individual antiretroviral agents. The PLP levels at week 12 were significantly higher in the group of individuals on stavudine (d4T) compared to the other individuals (Table 8.9). The regression coefficient showed that the average increase in PLP over 12 weeks is 9.96 nmol/L higher in the group of individuals prescribed stavudine compared to the rest (p=0.032). To investigate this further, an association between stavudine use and 25 mg/day vitamin B6 supplementation was explored but none was found (p=1.00).

The baseline BMI <20 kg/m^2 was associated with lower PLP concentrations after 12 weeks (p=0.043). There was a trend towards lower PLP concentrations in individuals with an IHDS score <10, which may be a marker of advancing HIV disease. However, this was not supported by associations with CD4 T-cell count and WHO HIV stage. None of the factors that showed a correlation with PLP at baseline affected PLP concentrations over 12 weeks on cART. These included 4-PA, hs-CRP, albumin and ALT. Prescriptions of vitamin B6 supplementation (25 mg/day) were also not associated with PLP levels after 12 weeks of cART (p=0.24). Factors associated with symptomatic DSP at baseline did not affect PLP levels over the 12-week period such as age, waist-to-hip ratio and previous/current TB. All

University of Cape Town

other factors investigated are summarized in Table 8.9.

Table 8.9: Effect of baseline factors on 12-week PLP level compared to baseline PLP level

Variable	Coefficient*	Confidence Interval (95%)	p-value
Clinical			
Female sex	-0.27	(-10.05 ; 9.52)	0.957
Age > 40 years	-2.81	(-13.35 ; 7.73)	0.601
WHO clinical stage 3 or 4	-2.95	(-12.27 ; 6.36)	0.534
IHDS score < 10	-10.71	(-21.80 ; 0.37)	0.058
Previous / Current TB	-2.28	(-11.58 ; 7.01)	0.631
Current TB	0.47	(-12.93 ; 13.88)	0.945
Previous TB	-3.31	(-12.65 ; 6.04)	0.488
Stavudine Exposure	9.96	(0.83 ; 19.09)	***0.032***
Vitamin B6 supplementation (25mg)	9.41	(-6.14 ; 24.96)	0.236
Alcohol last year	6.89	(-3.27 ; 17.06)	0.184
Weight > 61 kg	4.47	(-4.66 ; 13.60)	0.337
Height > 1.61 metre	-3.18	(-12.36 ; 6.00)	0.497
Body Mass Index < 20 kg/m^2	-11.17	(-0.33 ; -22.00)	***0.043***
Body Mass Index > 25 kg/m^2	2.36	(-7.12 ; 11.84)	0.625
Waist : Hip ratio > 0.9	-7.35	(-18.23 ; 3.54)	0.186
Haematological			
CD4 T-cell count < 200 cells/mm^3	1.28	(-10.17 ; 12.72)	0.827
CD4 T-cell count < 100 cells/mm^3	11.33	(-0.94 ; 23.60)	0.070
White cell count > 5.4 x10^9/L	3.96	(-5.41 ; 13.33)	0.407
C-reactive protein > 5 mg/L	1.01	(-8.75 ; 10.77)	0.840
Haemoglobin > 11.7 g/dL	-0.35	(-9.53 ; 8.82)	0.940
MCV > 92 fL	-1.29	(-10.52 ; 7.95)	0.785
Biochemical			
Albumin < 37 g/L	2.52	(-7.22 ; 12.27)	0.612
ALT > 19 IU/L	2.39	(-6.82 ; 11.60)	0.611
Creatinine > 64 µmol/L	-1.63	(-10.79 ; 7.53)	0.727
Metabolic			
Fasting glucose > 5.6 mmol/L	0.29	(-20.42 ; 20.99)	0.978
Fasting insulin > 5.1 µU/mL	-2.75	(-12.07 ; 6.58)	0.564
Total cholesterol > 3.7 mmol/L	2.75	(-6.60 ; 12.10)	0.564
Triglycerides > 0.9 mmol/L	-4.21	(-13.55 ; 5.12)	0.376
HDL < 1.41 mmol/L	-6.03	(-15.92 ; 3.86)	0.232
LDL > 2.4 mmol/L	-6.32	(-15.60 ; 2.96)	0.182
Lactate > 2 mmol/L	-1.97	(-11.79 ; 7.86)	0.695
NAT2 Acetylation			
Slow acetylation	5.80	(-13.38 ; 24.99)	0.553
Intermediate acetylation	-1.85	(-14.04 ; 10.34)	0.767

* Difference in mean 12-week PLP level between groups compared to baseline

8.2.7 Low plasma vitamin B6 concentrations as a risk factor for ATN

The development of ATN, incident symptomatic DSP or incident neuropathic symptoms were not associated with lower measures of circulating plasma pyridoxine concentrations at baseline (PLP and 4-PA) (Table 8.10). At 12 weeks, despite the decrease in PLP levels after cART initiation overall, there was no significant difference in magnitude of response between the individuals who developed ATN compared to those who did not (p=0.41) or between individuals with incident symptoms and those without (p=0.53).

Both groups showed slight increases in 4-PA concentration over time, although this increase was not statistically significant and the mean increase was not significantly different between the ATN group and their ATN-free counterparts (p=0.68) or between individuals with incident symptoms and those without (p=1.00).

Table 8.10: Baseline and week 12 vitamin B6 concentrations categorized by week 12 DSP status

Week 12 DSP status	N	Baseline		Week 12		Group effect [λ]
		Baseline PLP (nmol/L)		12-week PLP (nmol/L)		Δ PLP
		Median (IQR)	p-value	Median (IQR)	p-value	p-value
ATN	33	24.4 (18.0 - 36.8)	0.930 [ε]	23.7 (13.1 - 32.3)	0.850 [ε]	0.407 [λ]
No ATN	122	23.8 (16.2 - 38.5)		20.1 (13.4 - 34.2)		
Incident SDSP	20	20.1 (18.1 - 34.6)	0.626 [ε]	24.1 (15.5 - 32.8)	0.918 [ε]	0.610 [λ]
No SDSP	109	24.4 (16.7 - 36.8)		21.0 (13.4 - 34.2)		
Incident symptoms	28	21.4 (16.4 - 34.7)	0.486 [ε]	24.1 (13.0 - 31.1)	0.901 [ε]	0.525 [λ]
No symptoms	101	24.4 (17.4 - 38.4)		20.2 (14.2 - 34.5)		
		Baseline 4-PA (nmol/L)		12-week 4-PA (nmol/L)		Δ 4-PA
		Median (IQR)	p-value	Median (IQR)	p-value	p-value
ATN	33	16.6 (11.7 - 22.7)	0.579 [ε]	17.0 (12.5 - 22.1)	0.474 [ε]	0.676 [λ]
No ATN	122	17.9 (12.1 - 24.2)		19.3 (12.5 - 28.8)		
Incident SDSP	20	16.1 (12.1 - 20.6)	0.580 [ε]	18.7 (12.4 - 23.2)	0.569 [ε]	0.899 [λ]
No SDSP	109	17.5 (12.0 - 24.8)		19.1 (12.6 - 28.8)		
Incident symptoms	28	16.8 (12.1 - 22.4)	0.793 [ε]	18.7 (13.0 - 23.2)	0.805 [ε]	0.998 [λ]
No symptoms	101	17.5 (12.0 - 24.2)		19.1 (12.5 - 28.8)		

[ε] Wilcoxon rank-sum test for difference in median vitamin B6 level between DSP groups at a specific time point

[λ] Random effects model for the difference in mean vitamin B6 level between DSP groups at week 12 compared to baseline

University of Cape Town

Two groups were formed using a 50 nmol/L cut-off level to test it as a possible critical level for the presence HIV-associated DSP. The level was chosen by visual inspection of the follow-up data upon noticing a higher frequency of ATN among those with PLP <50 nmol/L at baseline. This level was also consistent with PLP levels from healthy cohorts ranging between 40 and 60 nmol/L (Bailey et al., 1999, Shen et al., 2010, Ye et al., 2010). The proportions of individuals in these groups for various DSP categories are shown in Table 8.11 and Table 8.12.

Table 8.11: Distribution of DSP status per PLP group below and above 50 nmol/L

Baseline DSP status	Baseline PLP < 50 nmol/L (N = 135)	Baseline PLP > 50 nmol/L (N = 24)	p-value
DSP	25 (19%)	10 (42%)	***0.012*** §
SDSP	18 (13%)	8 (33%)	***0.011*** §
ADSP	7 (5%)	2 (8%)	0.626 ¶

§ χ^2 test

¶ Fisher's exact test

In the group of individuals with baseline PLP levels >50 nmol/L, the frequency of symptomatic DSP at baseline was higher than for the group with lower PLP levels (p=0.011). When correcting for vitamin B6 supplementation, this association was no longer evident (OR 0.37; 95% CI 0.13-1.05; p=0.06). Therefore, individuals with symptomatic DSP may have higher PLP concentrations at baseline due to additional vitamin B6 supplementation and this may be the result of the clinician's response to symptoms (Table 8.2). However, these results should be interpreted with caution due to the relatively small number of people in the group with PLP >50nmol/L.

Although not statistically significant, lower baseline PLP levels were seen in those who developed ATN. By comparing individuals with incident symptoms to individuals without, and excluding those with symptomatic DSP at baseline, there is a trend towards lower baseline PLP levels in those with incident symptoms after commencing cART (p=0.096) (Table 8.12).

University of Cape Town

Table 8.12: Distribution of 12-week DSP status per baseline PLP group below and above 50 nmol/L

Week 12 DSP status	Baseline PLP < 50 nmol/L (N = 131)	Baseline PLP > 50 nmol/L (N = 24)	p-value
ATN	30 (23%)	3 (13%)	0.253 §
Incident symptoms	28 (21%)	1 (4%)	0.096 §
Incident SDSP	19 (15%)	1 (4%)	0.274 §

§ χ^2 test

ATN was significantly associated with a 12-week PLP level <50 nmol/L (p=0.032) (Table 8.13). Excluding individuals with symptomatic DSP at baseline from the analysis (to remove the effect of the association found between symptomatic DSP and PLP >50nmol/L) showed that the development of new neuropathic symptoms was significantly associated with a PLP <50 nmol/L (p=0.043). These results may not be credible due to the relatively small number of people in the group with 12-week PLP >50nmol/L.

Table 8.13: Distribution of 12-week DSP status per week 12 PLP group below and above 50 nmol/L

Week 12 DSP status	Week 12 PLP < 50 nmol/L	Week 12 PLP > 50 nmol/L	p-value
	(n = 142)	(n = 15)	
ATN	34 (24%)	0 (0%)	***0.032*** §
	(n = 118)	(n = 13)	
Incident symptoms	29 (25%)	0 (0%)	***0.043*** §
Incident SDSP	20 (17%)	0 (0%)	0.107 §

¶ Fisher's exact test

§ χ^2 test

Taken together, vitamin B6 levels were low overall and appear to co-segregate with other markers of micronutritional deficiency such as low haemoglobin and albumin. At 12 weeks there was a tendency for PLP levels to decrease further. Those individuals with higher PLP levels were few but did not develop neuropathic symptoms after cART initiation.

University of Cape Town

Chapter 9 Vitamin B6 Discussion

University of Cape Town

This study demonstrates the high prevalence of suboptimal circulating vitamin B6 in a South African HIV-infected cohort. Vitamin B6 deficiency was present in 53% of this cohort prior to commencing cART with a median PLP concentration of 24 nmol/L. Plasma vitamin B6 levels remained low despite vitamin B complex supplementation which forms part of the government's HIV standard of care treatment programme. A previous South African study, albeit in a group of HIV/TB co-infected children prior to initiating TB therapy, found that 50% of vitamin B6 levels were suboptimal, with a median PLP concentration of 34 nmol/L (Cilliers et al., 2010).

A summary of studies investigating vitamin B6 deficiency is included in Table 9.1. In North American HIV-infected cohorts deficient vitamin B6 levels were reported in 11% to 34% of cART naïve individuals with WHO Stage I HIV infection (Baum et al., 1991, Tang et al., 1997a) and in 53% of individuals with more advanced HIV infection (Beach et al., 1992). However, others did not find higher prevalence of vitamin B6 deficiency in HIV-infected individuals compared with the general population (Skurnick et al., 1996, Look et al., 2001). For example, in a small study of 17 individuals on protease inhibitors, a modest increase in vitamin B6 concentrations was shown three months after cART initiation (48.2 to 63.5 nmol/L), despite not receiving vitamin supplementation (Look et al., 2001). These levels are nearly 2-fold higher than those shown in our cohort. In the same study, mean PLP concentrations were similar to HIV-negative individuals.

The reported differences in vitamin B6 levels may be attributed to a number of confounders including age of study participants, stage of HIV disease, comorbid conditions, or the method used to determine vitamin B6 levels. Compared to the static (direct) method used in our study (performed by Dr M Rybak, CDC, Atlanta, GA, USA), the functional (indirect) method used in earlier reports has been shown to be less accurate (Krishnamurthy et al., 1967, Lumeng et al., 1974, Beach et al., 1992).

University of Cape Town

Table 9.1: Summary of studies performed investigating vitamin B6 deficiency in HIV-infected cohorts and in general populations

Group	Study population	Cohort size	PLP deficiency definition (nmol/L)	% deficient	Mean PLP (nmol/L)
HIV-infected cohorts					
Baum et al. 1991	HIV-infected men who have sex with men (WHO Stage I)(20-50 years), USA	112	Not specified	34%[†]	Not specified
Beach et al. 1992	HIV-infected men who have sex with men, USA	100	Not specified	53%[†]	Not specified
Skurnick et al. 1996	HIV-infected adults, USA	64	179.0[‡]	2%	389.0
	HIV negative adults, USA	33	179.0[‡]	None	317.0
Tang et al. 1997	HIV-infected men who have sex with men (WHO Stage I), USA	310	88.0[¶]	11%	223.0
Look et al. 2001	HIV-infected individuals starting protease inhibitor cART regimen (25-65 years), Germany	17	Not specified[¶ §]	None	48.1
	HIV-infected individuals 100 days after cART initiation (25-65 years), Germany	17	Not specified[¶ §]	None	63.5[a]
	Healthy control individuals (24-82 years), Germany	42	Not specified[¶ §]	None	58.3
Cilliers et al. 2010	HIV-infected children <13 years on TB treatment, South Africa	19	24.3*	52%	33.6
	HIV negative children <13 years on TB treatment, South Africa	33	24.3*	18%	45.6
General cohorts					
Bailey et al. 1999	Volunteers from local authority schools, local health centres & general practitioners, UK	51 (adolescent 13-14 yr)	34.4[¶]	50%	36.4 (males), 43.5 (females)
		131 (adult 20-64 yr)	34.4[¶]	38%	39.2 (males), 40.0 (females)
		68 (elderly 68-73 yr)	34.4[¶]	48%	34.8 (males), 35.3 (females)
		44 (old >73 yr)	34.4[¶]	27%	57.8 (males), 49.0 (females)
Friso et al. 2001	Population-based Framingham Heart Study cohort, CRP<6 mg/L, USA	834	Not specified[¶]	Not specified	55.8
	Population-based Framingham Heart Study cohort, CRP>6 mg/L, USA	57	Not specified[¶]	Not specified	36.5
Friso et al. 2004	Individuals with severe multivessel Coronary Artery Disease, USA	475	36.3 (median)[¶]	50%	33.9
	Individuals free from Coronary Artery Disease, USA	267	36.3 (median)[¶]	50%	31.2
Rybak et al. 2004	Nonrepresentative population subset, USA	303	Not specified[¶]	Not specified	42.4[b]
Visser et al. 2004	Ambulatory patients on TB treatment, South Africa	19	24.3*	89%	16.0[c]
Woolf et al. 2008	Population-based females with/without rheumatoid arhtritis (55-82 years), USA	18 (Healthy)	20.0[¶]	15%	46.0
		33 (Rheumatoid)	20.0[¶]	56%	20.0
Matarese et al. 2009	Intestinal transplant patients, USA	29	13.4[¶]	1%	13.4
Shen et al. 2010	Population-based Puerto Rican adults, USA	1205	Not specified*	Not specified	42.4[d]
Ye et al. 2010	Population-based Puerto Rican adults (45-75 years), USA	1236	30.0*	29%	44.3

[†] Erythrocyte transaminase assay, activity coefficient >1.85 for deficiency
[‡] Microbioogical assay
[¶] High performance liquid chromatography
[§] Cystathionine level used as surrogate
* Tyrosine decarboxylase test

[a] 100 days after cART initiation
[b] Geometric mean
[c] Graphic interpretation - median
[d] Median

Differences in the amount of vitamin B6 supplementation, which is not always well documented, may have affected the results. Vitamin B complex supplementation containing up to 4 mg vitamin B6, was virtually universal within our cohort, as per standard of care. However, the pattern of plasma vitamin B6 levels did not reflect ongoing or recent supplementation as neither of the two static measurements of vitamin B6 significantly improved within the 12 weeks for the overall cohort. It was previously reported that after a 30-day period of daily vitamin B6 supplementation (40 mg/day), PLP levels increased more than tenfold and 4-PA levels by fiftyfold in a cohort with a median baseline PLP below 25 nmol/L (Bor et al., 2003). In addition, in individuals on INH TB therapy but without HIV infection, a dose of 6 mg/day of vitamin B6 increased the mean B6 concentration more than threefold after 12 weeks (31 to 161 nmol/L) (Krishnamurthy et al., 1967). As mentioned above, one group from the developed world found that cART initiation was associated with an increase in vitamin B6 levels without supplementation. From our study it appears that daily vitamin B complex supplement (containing up to 4 mg vitamin B6) is not sufficient to maintain adequate vitamin B6 levels for HIV-infected individuals, with or without concomitant TB prior to commencing cART. This level of supplementation also seems insufficient to maintain adequate vitamin B6 levels after cART initiation. However, data on prescription of vitamin B6 supplementation were retrieved from clinic folder reviews and it is possible that individuals differed in terms of compliance and therefore dose exposure. This may have affected the plasma levels measured.

The study data also imply that health facilities are not prescribing vitamin B6 at dosages of 25 mg/day to individuals on TB therapy, who are at higher risk of developing DSP. Of those on INH TB therapy at the time of the study, only 42% were prescribed 25 mg vitamin B6 daily. The underuse of vitamin B6 supplementation may be attributed to the variability in the recommendations regarding vitamin B6 supplementation in the context of HIV/TB co-infection (Van der Watt et al., 2011). South African guidelines for example, do not recommend additional vitamin B6 supplementation above standard of care vitamin B complex for individuals on TB therapy without evidence of risk factors such as alcohol abuse, pregnancy, diabetes or epilepsy (South African Department of Health, 2004). In contrast to international guidelines, HIV is not mentioned as a high risk group (WHO, 2009, South African Department of Health, 2004). Other possible reasons why additional vitamin B6 supplementation remains underprescribed include underestimation of the risk for and clinical impact of DSP, and supply-chain shortages.

University of Cape Town

Given that a history of TB therapy was associated with increased risk for DSP in previous African cohorts (Maritz et al., 2010, Forna et al., 2007) and that INH-associated painful neuropathy is preventable with adequate vitamin B6 supplementation (Biehl and Sklavem, 1953), another study objective was to investigate the association between vitamin B6 deficiency and existing DSP (including symptomatic DSP) or the development of ATN after cART initiation.

For the cohort overall, we found no association between vitamin B6 deficiency (defined as PLP <25 nmol/L) and DSP, with or without symptoms, pre-cART. It is possible that an association between vitamin B6 deficiency and DSP was concealed by the positively skewed distribution of vitamin B6 concentrations, which limited the power to detect any differences in concentrations between groups. Another possible confounder was additional vitamin B6 supplementation, as it was shown that the prescription of 25 mg/day vitamin B6 supplementation was associated with symptomatic DSP at baseline as well as significantly higher 4-PA concentrations (Table Q-6 in Appendix Q). This may be the result of clinic staff prescribing additional vitamin B6 in response to the recognition of neuropathic symptoms. Exact vitamin intake is unknown as data on supplementation were retrieved from clinic folder reviews. However, the higher 4-PA levels suggest recent intake in those individuals prescribed additional vitamin B6.

By investigating the association between DSP and vitamin B6 levels only in individuals with a history of TB therapy, PLP levels were significantly lower in those with symptomatic DSP at baseline (Table 8.8). This is in line with other findings suggesting that vitamin B6 deficiency contributes to the risk of symptomatic DSP in INH-exposed HIV-infected individuals; a randomized control trial showed that multivitamin supplementation which included 25 mg pyridoxine daily reduced the risk for symptomatic DSP in HIV/TB co-infected individuals (Villamor et al., 2008).

After 12 weeks of cART, although not associated with vitamin B6 deficiency at baseline or 12 weeks, ATN was significantly associated with a 12-week PLP level <50 nmol/L (p=0.032). Excluding individuals with symptomatic DSP at baseline from the analysis (to remove the effect of the association found between symptomatic DSP and PLP ≥50 nmol/L), the development of new neuropathic symptoms remained significantly associated with a PLP level <50 nmol/L (p=0.043) compared to individuals who never had any neuropathic symptoms. These findings suggest that circulating PLP levels of ≥50 nmol/L is possibly

University of Cape Town

beneficial in reducing the risk of developing neuropathic symptoms after cART. The cut-off level of 50 nmol/L was chosen by visual inspection of the data and was also guided by PLP levels from healthy cohorts ranging between 40 and 60 nmol/L (Bailey et al., 1999, Shen et al., 2010, Ye et al., 2010). Due to the subjective cut-off level, the association found may be specific to this cohort. The small number of individuals with PLP levels ≥50 nmol/L compared to those with PLP <50 nmol/L may also have influenced the results.

The variation in the literature regarding the laboratory definition of vitamin B6 deficiency contributed to the difficulties in interpreting the data. There is no consensus on the biochemical cut-off point that defines 'deficiency' and the lower end of PLP reference ranges may vary from 20 nmol/L to 30 nmol/L (Leklem, 1990, Lui et al., 1985) (Table 9.1). At the other extreme, another study defined vitamin B6 deficiency as plasma PLP <88 nmol/L (Tang et al., 1997a). Furthermore, dichotomization of a continuous variable leads to an inevitable loss of information and power and may increase the probability of false positive results. We based our definition of 25 nmol/L on the South African definition for PLP deficiency (Visser et al., 2004, Cilliers et al., 2010). However, irrespective of the definition of vitamin B6 deficiency, HPLC measured plasma PLP levels from North American cohorts are substantially higher than this African population.

Another reason why a clear association between vitamin B6 deficiency and HIV-associated DSP could not be shown, may be that the mechanism for nerve injury in HIV-infected individuals is multifactorial and not entirely related to nutrition (see section 1.7). One such mechanism, which may contribute to vitamin B6 deficiency, is inflammation. Previous studies have shown that low plasma PLP concentrations are characteristic of individuals with inflammatory conditions such as rheumatoid arthritis or inflammatory bowel disease (Chiang et al., 2005a, Roubenoff et al., 1995, Saibeni et al., 2003). Furthermore, inverse relationships have been reported between vitamin B6 levels and markers of inflammation in the general population (Kelly et al., 2004, Gori et al., 2006, Friso et al., 2001) and animal models (Chiang et al., 2005b). We examined the association of hs-CRP and serum albumin with PLP. In this cohort, plasma PLP levels inversely associated with albumin and hs-CRP levels but the association with hs-CRP was no longer evident after correcting for serum albumin in a multivariate model. Serum albumin is a major confounder of plasma PLP concentration and in this ambulant group of individuals, nearly half had hypoalbuminaemia. The level of albumin may reflect nutritional status but may also be a negative acute-phase reactant. It is

University of Cape Town

therefore difficult to ascertain whether the relationship between PLP and albumin level is caused by an association between malnutrition and inflammation or an association between two markers of malnutrition. The observation that albumin levels are inversely related to hs-CRP and the relationship between them was partly attenuated by adjustment for PLP and 4-PA, is consistent with the possibility that both inflammation and malnutrition may be contributory to the association between PLP and albumin.

In HIV-infected individuals, other factors may contribute to vitamin B6 deficiency, thereby weakening the association between vitamin B6 and inflammation. These factors include insufficient dietary intake (as a result of anorexia, dysphagia, odynophagia), malabsorption and diarrhoea, malignancies, HIV-related enteropathy, impaired storage and altered metabolism due to hepatic or renal impairment (Keusch and Farthing, 1990, Tang and Smit, 1998). This malnutrition may lead to further immunosuppression, opportunistic infections and mucosal damage with failure of normal intestinal mucosal turnover and healing, all of which could exacerbate the vitamin B6 deficiency.

There was an inverse correlation between PLP and triglycerides but not with any other lipid markers. While vitamin B6 supplementation has been reported to be an effective strategy to ameliorate plasma triglycerides and total cholesterol levels, the biological mechanism remains unknown (de Gomez Dumm et al., 2003). However, as the interquartile ranges of triglyceride levels were within normal limits, it is likely that triglyceride levels are serving as a surrogate marker for another process related to vitamin B6 deficiency. For example, excessive alcohol usage is known to cause vitamin B6 deficiency and higher triglycerides. No such association was found in this study but this may theoretically be the result of an inadequate measure used to assess alcohol abuse. In addition, as discussed in Chapter 5, mitochondrial dysfunction leads to the accumulation of triglycerides. If the mitochondrial dysfunction is related to increased ROS as a result of inflammation (see section 1.8), vitamin B6 may be utilized at an increased rate and result in reduced concentrations in the plasma. Since the liver is known to play a central role in vitamin B6 metabolism, hepatic impairment may lead to PLP deficiency (Lumeng et al., 1974, Labadarios et al., 1977). Plasma PLP levels correlated inversely with serum ALT, although only 10% of ALT concentrations were raised. Therefore, the role of hepatic impairment is unlikely to have been a significant contributory factor to the low serum PLP levels in these individuals. As with triglycerides, it is likely that ALT concentrations could be reflecting other factors related to vitamin B6 deficiency.

University of Cape Town

This study was unable to demonstrate any correlation between vitamin B6 status and CD4 T-cell count or WHO disease stage, although all individuals had a CD4 T-cell count of less than ≤350 cells/mm^3, possibly blunting the influence of CD4 T-cell count on vitamin B6 levels. Among cART-naïve individuals, previous observational studies have shown low or deficient serum concentrations of several micronutrients, including thiamine, selenium, zinc, and vitamins A, -B3, -B6, -B12, -C, -D, and -E to be associated with either low CD4 T-cell counts or HIV disease progression (Visser et al., 2004, Tang et al., 1997b, Tang et al., 1993, Haug et al., 1998, Baum et al., 1995, Abrams et al., 1993).

The relationship between NAT2 acetylation phenotype and vitamin B6 levels were investigated, the hypothesis being that a slow acetylation phenotype would increase vitamin B6 requirements and thus the risk for deficiency. An association between low circulating vitamin B6 levels (plasma PLP and 4-PA) and slow NAT2 acetylation phenotype could not be demonstrated, even after adjusting for a history of previous TB therapy. Possible explanations for this finding could be the relatively small sample size with overrepresentation of slow acetylation phenotype in this cohort, or the positively skewed distribution of both PLP and 4-PA levels. Discordance between genotype-deduced phenotype and true phenotype cannot be ruled out and may have affected associations observed (as discussed in Chapter 7).

The question remains: What constitutes sufficient vitamin B6 supplementation? Unpublished research from our group in a cohort of hospitalized HIV/TB co-infected individuals suggest that 25 mg/day of observed vitamin B6 supplementation is probably sufficient to prevent or reverse vitamin B6 deficiency, and that administering more than 25 mg/day may be unnecessary (Centner, 2012). Mean PLP levels were four times higher than that of our cohort. However, as these individuals were non-fasting and plasma samples were obtained within a median of six hours after a pyridoxine dose, the observed elevated PLP levels could be explained by the pharmacokinetic peak after supplementation (Ubbink et al., 1987).

Saturation levels for ambulant HIV-infected populations remain undetermined. Others have demonstrated that 50 mg/day of vitamin B6 might be the saturation level for critically ill hospitalized patients and between 40 and 100 mg/day in healthy HIV-negative individuals, with patients being unable to efficiently utilize vitamin B6 doses higher than these levels (Cheng et al., 2006, Ubbink et al., 1987, Bor et al., 2003). A recent randomized controlled trial involving individuals initiating cART in Tanzania demonstrated that "high-dose" multivitamin supplementation (including 25 mg vitamin B6 daily) reduced the risk of

University of Cape Town

neuropathy compared with standard-dose supplementation (1.3 mg vitamin B6 daily) (Isanaka et al., 2012). However, they found that overall "high-dose" multivitamin supplementation (including thiamine, riboflavin, niacin, folic acid, vitamin B12, -C and -E) was associated with increased ALT levels. The exact mechanism of action for high-dose multivitamins to cause ALT elevation in the context of cART remains unknown although increased mortality after cART initiation has been observed among patients with low BMI in several analyses from sub-Saharan Africa (Johannessen et al., 2008, Stringer et al., 2006, Zachariah et al., 2006). Overly aggressive multivitamin regimens coinciding with cART initiation may propagate metabolic disturbances and contribute to poor clinical outcomes among patients who are severely malnourished. Compared to our cohort, individuals from the Tanzanian study had more advanced HIV infection with CD4 T-cell counts <100 cells/mm^3 in 40% and 7% with a BMI <16 kg/m^2. For these reasons, it is difficult to extrapolate their ALT findings to our population.

Based on the above findings and our study results, 4 mg vitamin B6 daily is not sufficient to correct vitamin B6 deficiency in an HIV-infected population about to commence cART who does not have evidence of overt malnutrition, a median CD4 T-cell count of 156 cells/mm^3 and a high prevalence of TB co-infection. Furthermore, in those prescribed 25 mg/day, it did not significantly affect PLP concentrations. Although poor compliance may have affected our findings, we recommend that higher dosage of vitamin B6 supplementation (at least greater than 4 mg/day) should be considered standard of care in all HIV-infected patients, irrespective of TB therapy.

In summary, the current longitudinal observational study is novel in the investigation of fasting plasma PLP and 4-PA concentrations in an African HIV-infected population about to commence cART, and evaluating the effect of the first 12 weeks of cART on vitamin B6 status. We have shown a high prevalence of suboptimal circulating plasma PLP levels in this African cohort. Half of the population was vitamin B6 deficient at baseline and after 12 weeks on cART, despite vitamin B complex supplementation. The low vitamin B6 status correlated with low albumin levels and could be a reflection of a general state of micronutrient deficiency. The results from our study support previous findings in which the protective effect of 25 mg vitamin B6 supplementation on peripheral neuropathy was demonstrated, not only in individuals with INH treated TB infection but also in individuals commencing cART (Villamor et al., 2008, Isanaka et al., 2012). Our HIV population has a

University of Cape Town

high TB co-infection rate and both these infections may impact on nutrition and consequently vitamin B6 status. A concerted effort to educate health professionals and patients regarding optimal nutrition is necessary. This study suggests that adequate vitamin B6 replacement is an area that requires attention and that guidelines should be altered particularly in areas where HIV/TB co-infection is common. We suggest that public health care policies should implement the recommendations of the developed world regarding vitamin B6 supplementation (10-25 mg) (JTC, 1998, CDC, 2003, WHO, 2009, Chan and Iseman, 2002), prior to commencing cART in all individuals.

University of Cape Town

Chapter 10 Cytokine Results

University of Cape Town

10.1 Objective

We investigated the association between incident painful neuropathy and the concentrations of markers previously shown to be associated with painful neuropathies and/or immune reconstitution within 12 weeks after cART-initiation. These included hs-CRP, CD4 T-cell count, specific cytokines TNF-α, IL-2, IL-4, IL-6 and their soluble receptors.

10.2 Cohort characteristics

As part of the prospective cohort followed up for 24 weeks (n=144), 32 individuals (27%) developed neuropathic symptoms within the first 12 weeks (see section 4.2.4). Two individuals had detectable viral loads at week 24, and as the substudy was examining inflammation, these individuals were excluded; the remaining 30 comprised the incident symptom-group.

A nested-control group was selected and consisted of 30 individuals who remained symptom-free for 24 weeks. The groups were paired using risk factors previously described in the literature such as age (difference ≤3 years), gender, previous isoniazid exposure, baseline CD4 T-cell count (difference ≤25 cells/mm^3) and dNRTI containing regimen (Lichtenstein et al., 2005, Maritz et al., 2010, Morgello et al., 2004) (Table 10.1 and Table 10.2). Of note, factors such as height, weight, white cell count and hs-CRP, which were not paired, showed similar distributions between the two groups. Even though median CD4 T-cell counts were similar, the groups were stratified for counts <100 cells/mm^3, 100-200 and >200 cell/mm^3. Again, the distribution between cases and controls remained similar (p=0.86). Baseline HIV viral loads were unavailable.

University of Cape Town

Table 10.1: Demographic characteristics for the cytokine cohort (continuous data)

Variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=60)	Incident symptoms (N=30)	No symptoms (control) (N=30)	
Clinical						
Age	years		33 (26 - 37)	32 (26 - 35)	34 (26 - 41)	0.215 β
Weight	kg		58.5 (53.0 - 68.0)	58.0 (52.5 - 68.5)	59.5 (54.0 - 64.0)	0.866 β
Height	metre		1.61 (1.55 - 1.64)	1.58 (1.55 - 1.64)	1.62 (1.57 - 1.65)	0.235 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	22.6 (19.8 - 28.0)	22.7 (20.1 - 28.0)	22.6 (19.8 - 25.3)	0.598 β
Waist : Hip ratio		*< 0.90*	0.85 (0.81 - 0.88)	0.86 (0.82 - 0.90)	0.85 (0.81 - 0.87)	0.516 β
Systolic BP	mmHg	*120 - 140*	110 (103 - 123)	110 (106 - 123)	110 (101 - 123)	0.925 β
Diastolic BP	mmHg	*80 - 90*	73 (65 - 80)	73 (65 - 81)	73 (69 - 80)	0.797 β
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	141 (110 - 189)	145 (109 - 181)	138 (110 - 194)	0.753 β
White cell count	$x10^9/L$	*4.0 - 11.0*	5.7 (4.3 - 7.1)	5.7 (3.9 - 6.4)	5.7 (4.4 - 7.9)	0.270 β
C-reactive protein	mg/L	*< 5.0*	2.7 (0.9 - 6.7)	2.6 (0.9 - 5.4)	2.9 (0.9 - 6.7)	0.693 β
Haemoglobin	g/dL	*11.6 - 15.6*	11.7 (10.1 - 13.1)	12.3 (10.5 - 13.3)	11.4 (9.7 - 12.6)	0.118 β
MCV	fL	*80.0 - 100.0*	91.4 (87.6 - 95.6)	90.9 (87.5 - 94.5)	91.5 (87.6 - 97.0)	0.599 β
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 43)	40 (36 - 43)	39 (35 - 43)	0.426 β
ALT	IU/L	*10 - 41*	17 (14 - 25)	18 (15 - 28)	16 (13 - 20)	0.070 β
Creatinine	µmol/L	*53 - 115*	65 (57 - 73)	66 (56 - 72)	64 (57 - 74)	0.782 β
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.8)	4.6 (4.4 - 5.0)	4.7 (4.4 - 4.8)	0.633 ε
Fasting insulin	µU/mL	*0.2 - 9.4*	5.0 (3.0 - 7.9)	5.2 (3.8 - 8.9)	4.9 (2.2 - 7.3)	0.343 β
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.0 - 4.3)	3.9 (3.2 - 4.4)	3.5 (3.0 - 4.2)	0.298 β
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.0)	0.8 (0.6 - 1.2)	0.736 β
HDL	mmol/L	*1.15 - 1.68*	0.88 (0.71 - 1.09)	0.92 (0.73 - 1.14)	0.86 (0.67 - 1.04)	0.354 β
LDL	mmol/L	*1.0 - 3.0*	2.3 (1.9 - 3.0)	2.4 (1.9 - 3.1)	2.3 (1.8 - 2.8)	0.180 β
Lactate	mmol/L	*< 2.0*	2.5 (1.7 - 3.3)	2.5 (1.8 - 3.3)	2.4 (1.7 - 3.3)	0.597 β
Pyridoxine						
PLP	nmol/L	*> 25.0*	23.2 (14.7 - 36.8)	24.4 (16.7 - 40.3)	20.2 (14.7 - 34.5)	0.642 β
4-PA	nmol/L	*unknown*	16.3 (11.3 - 22.1)	15.8 (11.2 - 21.1)	16.6 (11.7 - 22.1)	0.616 β

β Student's *t*-test

ε Wilcoxon rank-sum test

Table 10.2: Demographic characteristics for the cytokine cohort (grouped data)

Variable	No. (%)			p-value
	Total (N=60)	Incident symptoms (N=30)	No symptoms (control) (N=30)	
Female sex	44 (73%)	22 (73%)	22 (73%)	1.000 ¶
Age > 40 years	12 (20%)	9 (30%)	3 (10%)	0.104 ¶
Previous/Current TB	14 (23%)	7 (23%)	7 (23%)	1.000 ¶
Time of TB				1.000 ¶
Currently	0 (0%)	0 (0%)	0 (0%)	
< 1 year ago	6 (10%)	3 (10%)	3 (10%)	
1 year ago	0 (0%)	0 (0%)	0 (0%)	
2 years ago	1 (2%)	0 (0%)	1 (3%)	
> 2 years ago	7 (12%)	4 (13%)	3 (10%)	
Vit Bco supplement	58 (97%)	29 (97%)	29 (97%)	1.000 ¶
Vit B6 supplement	0 (0%)	0 (0%)	0 (0%)	1.000 ¶
WHO clinical stage				0.172 ¶
Stage 1	18 (30%)	6 (20%)	12 (40%)	
Stage 2	28 (47%)	18 (60%)	10 (33%)	
Stage 3	12 (20%)	5 (17%)	7 (23%)	
Stage 4	2 (3%)	1 (3%)	1 (3%)	
CD4 T-cell count				0.864 ¶
< 100 cells/mm ^{3}	11 (18%)	5 (17%)	6 (20%)	
100 - 200 cells/mm ^{3}	39 (65%)	19 (63%)	20 (67%)	
> 200 cells/mm ^{3}	10 (17%)	6 (20%)	4 (13%)	
Body Mass Index	16 (28%)	9 (30%)	7 (25%)	0.981 ¶
< 20 kg/m ^{2}	16 (28%)	9 (30%)	7 (25%)	
20 - 25 kg/m ^{2}	23 (40%)	12 (40%)	11 (39%)	
25 - 30 kg/m ^{2}	11 (19%)	5 (17%)	6 (21%)	
> 30 kg/m ^{2}	8 (14%)	4 (13%)	4 (14%)	
Alcohol last year	17 (29%)	8 (27%)	9 (32%)	0.775 ¶
IHDS score < 10	5 (11%)	3 (12%)	2 (9%)	1.000 ¶

¶ Fisher's exact test

University of Cape Town

Nineteen individuals (33%) had elevated hs-CRP levels >5mg/L (IQR 5.2-161 mg/L) of which 11 were >10 mg/L. One had an hs-CRP >100 mg/L for which no obvious reason was found on baseline examination or careful folder review; there was no record of any acute infection or inflammatory condition in the clinic folder, and the white cell count was 9.98 x10^9/L. Only one individual had an elevated white cell count (16.5x10^9/L), but without cause; the hs-CRP was 1.8 mg/L.

Of those who developed neuropathic symptoms, the peak incidence was between weeks 4 and 12 (14%). The overall estimated crude incidence rate after 24 weeks was 30% (see Figure 4.3, section 4.2.4). In this substudy, the most frequent symptom was paraesthesiae (25/30), followed by numbness (21/30) and pain (15/30). At initial presentation median pain/paraesthesiae severity by VAS was 4 (IQR 1-6). By week 24, neuropathic symptoms had

resolved in 18, whereas the remainder had persistent or worsening symptoms. Of the 30 with incident symptoms, 15 (50%) developed ≥1 new/worsening neuropathic sign(s).

10.3 Cytokine analysis

10.3.1 Baseline cytokine concentrations

The baseline cytokine concentrations at the pre-cART visit are tabulated in Table 10.3. Median concentrations of IL-4, IL-12(p70) and IL-13 were below the minimum detectable level at baseline. Levels of IL-4 were undetectable in 97% of individuals.

Table 10.3: Baseline cytokine concentrations, minimum detectable concentrations and the percentage of undetectable levels for each cytokine in the overall cytokine cohort.

Cytokine	Baseline median (IQR) (pg/mL)	Minimum detectable concentration (pg/mL)	No. undetected (%)
Interleukin-1β	0.12 (0.01 ; 0.36)	0.06	17 (29%)
Interleukin-2	0.99 (0.14 ; 2.38)	0.16	16 (27%)
Interleukin-4	0.12 (0.12 ; 0.12)	0.13	58 (97%)
Interleukin-5	0.56 (0.32 ; 1.11)	0.01	1 (2%)
Interleukin-6	2.84 (1.92 ; 5.11)	0.10	1 (2%)
Interleukin-7	2.06 (0.67 ; 3.68)	0.12	4 (7%)
Interleukin-8	3.77 (2.46 ; 5.25)	0.11	0 (0%)
Interleukin-10	14.91 (7.03 ; 29.35)	0.15	0 (0%)
Interleukin-12	0.01 (0.01 ; 1.02)	0.11	37 (62%)
Interleukin-13	0.47 (0.47 ; 7.26)	0.48	34 (57%)
IFN-γ	1.20 (0.01 ; 3.23)	0.29	22 (37%)
GM-CSF	0.73 (0.17 ; 1.51)	0.46	23 (38%)
TNF-α	6.64 (4.53 ; 10.98)	0.05	0 (0%)

University of Cape Town

10.3.2 Trends in cytokine concentrations over the 12 week follow-up

The initiation of cART significantly affected both pro- and anti-inflammatory cytokine concentrations, irrespective of the development of neuropathic symptoms (see heat map Figure 10.1 and Figure R-1 to Figure R-4 in Appendix R). Almost all the cytokines measured, whether pro- or anti-inflammatory, demonstrated significantly higher concentrations at week 2 and -4 returning to baseline levels by week 12; these included IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p(70), IL-13, IFN-γ, GM-CSF and TNF-α [Table 10.4 and Table R-1 in Appendix R (Time effect)]. The exception was IL-8 with persistently lower levels from week

2 onwards compared to those at baseline. Irrespective of symptom-status, TNF-α increased from pre-cART levels, peaked between weeks 2 and -4, but then decreased at week 12 to a lower level compared with baseline (p=0.008). At week 12, IL-6 remained higher than baseline levels (p=0.033).

Table 10.4: Cross-sectional time series analysis for selected cytokines: IL-1β, IL-2, IL-4, IL-6, IL-10 and TNF-α

Cytokine	Time effect[a]				Group effect[b]				
	Median (pg/mL)	Coefficient	Confidence interval (95%)	p-value	Incident symptoms Median (pg/mL)	No symptoms Median (pg/mL)	Coefficient	Confidence interval (95%)	p-value
Interleukin-1β									
Baseline	0.12				0.11	0.16			0.608 [ε]
Week 2	1.71	1.52	(1.24 ; 1.79)	***<0.001***	1.20	2.23	-0.57	(-1.10 ; -0.03)	***0.038***
Week 4	1.31	1.15	(0.87 ; 1.43)	***<0.001***	1.11	1.65	-0.19	(-0.73 ; 0.35)	0.486
Week 12	0.26	0.26	(0.00 ; 0.53)	0.054	0.29	0.24	0.18	(-0.34 ; 0.70)	0.497
Interleukin-2									
Baseline	0.99				1.01	0.95			0.796 [ε]
Week 2	10.73	10.99	(8.97 ; 13.00)	***<0.001***	8.54	14.44	-3.94	(-7.90 ; 0.01)	0.051
Week 4	9.90	9.01	(6.98 ; 11.05)	***<0.001***	7.94	10.29	-0.99	(-4.99 ; 3.02)	0.629
Week 12	1.69	1.92	(-0.03 ; 3.87)	0.054	1.80	1.34	0.54	(-3.29 ; 4.37)	0.782
Interleukin-4									
Baseline	0.12				0.12	0.12			0.147 [ε]
Week 2	1.21	4.11	(1.11 ; 7.11)	***0.007***	0.12	2.79	-0.69	(-6.64 ; 5.26)	0.820
Week 4	0.12	2.22	(-0.81 ; 5.25)	0.151	0.13	0.12	-1.37	(-7.38 ; 4.65)	0.656
Week 12	0.12	1.60	(-1.31 ; 4.51)	0.282	0.12	0.12	2.74	(-3.05 ; 8.53)	0.354
Interleukin-6									
Baseline	2.84				2.84	2.97			0.693 [ε]
Week 2	8.71	8.18	(5.37 ; 10.99)	***<0.001***	6.79	9.15	1.39	(-4.18 ; 6.97)	0.624
Week 4	8.71	6.76	(3.91 ; 9.61)	***<0.001***	7.62	8.79	0.35	(-5.29 ; 6.00)	0.903
Week 12	4.09	2.95	(0.24 ; 5.66)	***0.033***	3.37	4.21	4.53	(-0.84 ; 9.91)	0.098
Interleukin-10									
Baseline	14.91				14.31	16.25			0.720 [ε]
Week 2	36.28	25.52	(9.47 ; 41.57)	***0.002***	36.68	35.68	12.36	(-19.56 ; 44.27)	0.448
Week 4	31.82	19.53	(3.27 ; 35.80)	***0.019***	30.94	33.99	-10.80	(-43.13 ; 21.54)	0.513
Week 12	10.68	7.93	(-7.53 ; 23.39)	0.315	13.99	9.89	-3.99	(-34.71 ; 26.74)	0.799
TNF-α									
Baseline	6.64				7.36	6.53			0.820 [ε]
Week 2	14.19	6.55	(4.59 ; 8.50)	***<0.001***	12.47	15.98	-1.22	(-5.09 ; 2.66)	0.539
Week 4	14.52	6.79	(4.81 ; 8.77)	***<0.001***	13.94	14.76	-1.90	(-5.82 ; 2.02)	0.343
Week 12	5.52	-2.56	(-4.44 ; -0.67)	***0.008***	5.71	5.20	1.50	(-2.24 ; 5.24)	0.432

[a] Time effect is the effect of cART on mean cytokine concentration for the entire cohort

[b] Group effect is the difference in mean cytokine concentration between groups compared to baseline

[ε] Wilcoxon rank-sum test

University of Cape Town

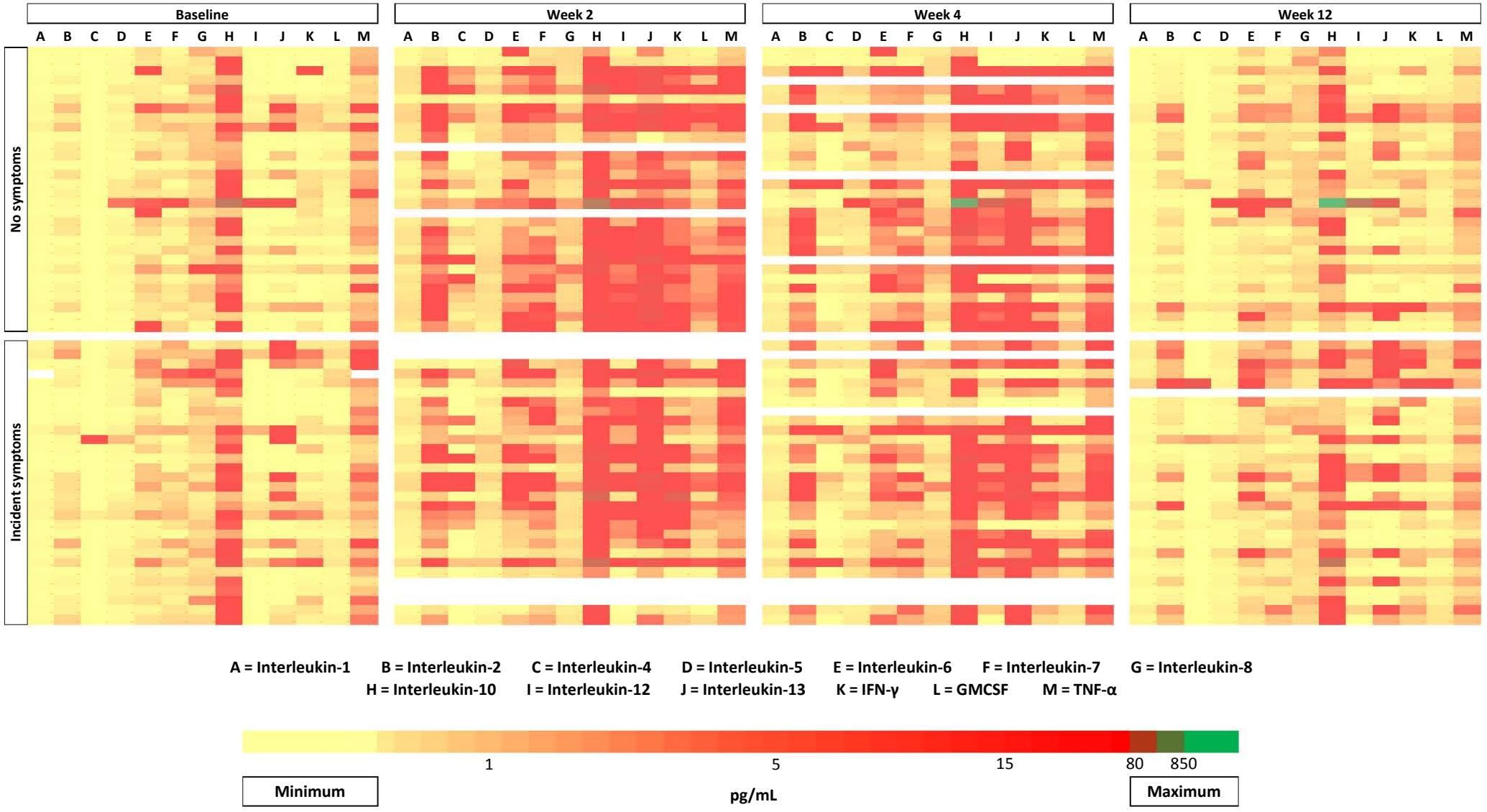


Figure 10.1: Heat map of cytokine concentrations for all individuals at four different time points

The pro-inflammatory Th1 cytokines showed the greatest increases from baseline concentrations; IL-12p(70) (≈700-fold), IL-2 (≈300-fold) and IFN-γ (≈200-fold). Although TNF-α concentrations only increased 3-fold, these were at higher baseline levels. Compared with baseline, the Th2 cytokine levels also responded to cART initiation; IL-13 increased 59-fold and IL-4 36-fold at 2 weeks. IL-5 and IL-10 increased by comparatively modest amounts (4- to 5-fold).

10.3.3 Within-individual stability across time

To assess the impact of random variability and fluctuations within an individual's cytokine peak measurements over time, rank correlation analysis was performed. Through this analysis, we gained insight into the predictability of the trend observed in concentration for a particular cytokine. For the interval of 12 weeks, cytokine levels showed within-individual stability and predictability by correlating at two different time points for 11 cytokines, and at three time points for three of these (IL-5, IL-10 and IFN-γ) (Table 10.5).

Table 10.5: Spearman rank correlation coefficients between baseline and 3 follow-up visits

Cytokine	Week 2	Week 4	Week 12
Interleukin-1β	0.22	0.52**	0.22
Interleukin-2	0.15	0.44*	0.29
Interleukin-4	0.22	0.14	0.29
Interleukin-5	0.40*	0.69**	0.56**
Interleukin-6	0.50**	0.57**	0.19
Interleukin-7	0.28	0.41*	0.41*
Interleukin-8	0.26	0.14	0.19
Interleukin-10	0.49**	0.71**	0.54**
Interleukin-12	0.30	0.52**	0.28
Interleukin-13	0.20	0.43*	0.35
IFN-γ	0.43*	0.68**	0.39*
GM-CSF	0.23	0.50**	0.47**
TNF-α	0.33	0.63**	0.47**

* $p < 0.05$
** $p < 0.01$

10.3.4 Comparison of cytokines between cases and controls

To determine whether an elevated cytokine concentration at baseline is a predictor of development of neuropathic symptoms over the 24 weeks of follow-up, a cross-sectional comparison of cytokine levels was performed. The baseline pre-cART cytokine concentrations between the symptomatic and symptom-free groups were similar (Table 10.4).

University of Cape Town

To determine the effect of cART initiation on cytokine production, as well as the difference in cytokine production between individuals with incident neuropathic symptoms and those who remained symptom-free, a cross-sectional time series analysis was performed (Table 10.4). Despite the general increase in cytokine concentrations, there was a tendency for lower peaks in the symptomatic- compared to the symptom-free group. The marginally lower cytokine production in the symptomatic group compared to the individuals who remained symptom-free was only statistically significant for IL-1β and IL-13 at week 2 (p=0.038 and p=0.029, respectively), and a trend towards significance for IL-2 and GM-CSF (p=0.051 and p=0.072, respectively).

10.4 Soluble receptors and IL-1 receptor antagonists

Accumulating evidence indicates that biologic effects and regulation of cytokines *in vivo* may be modulated not only by cytokine secretion or the activity of one cytokine by other cytokines, but also by their respective soluble receptors and receptor antagonists. As the half-lives of soluble cytokine receptors are longer than the respective cytokines they may be more informative of chronic cytokine activity (Arend et al., 1998, Chikanza et al., 1993). Therefore, we investigated the association of plasma concentrations of cytokine receptors sIL-1RI, sIL-1RII, sIL-2Rα, sIL-4R, sIL-6R, sTNFRI, sTNFRII, and IL-1RA and the development of neuropathic symptoms (Table 10.6).

University of Cape Town

Table 10.6: Cross-sectional time series analysis for soluble receptors over 12 weeks after starting cART

Cytokine	Time effect[a]				Group effect[b]				
	Median (pg/mL)	Coefficient	Confidence interval (95%)	p-value	Incident symptoms Median (pg/mL)	No symptoms Median (pg/mL)	Coefficient	Confidence interval (95%)	p-value
sIL1RI									
Baseline	29.6				33.3	25.5			0.159 [ε]
Week 2	34.0	4.7	(-0.4 ; 9.8)	0.073	36.9	33.6	-0.7	(-10.8 ; 9.4)	0.893
Week 4	37.6	4.0	(-1.2 ; 9.1)	0.132	37.5	38.7	-6.2	(-16.4 ; 4.1)	0.237
Week 12	28.2	1.4	(-3.6 ; 6.3)	0.585	34.5	25.2	1.3	(-8.5 ; 11.1)	0.789
sIL1RII									
Baseline	4571.9				4160.6	4922.8			0.413 [ε]
Week 2	4885.7	-94.4	(-1249.1 ; 1060.4)	0.873	4744.4	4905.8	-294.9	(-2600.0 ; 2010.3)	0.802
Week 4	5116.7	781.2	(-388.7 ; 1951.1)	0.191	5115.7	5751.6	-626.6	(-2960.5 ; 1707.3)	0.599
Week 12	5806.5	941.3	(-178.8 ; 2061.5)	0.100	5604.6	6152.1	433.5	(-1802.0 ; 2669.0)	0.704
sIL2rα									
Baseline	1102.3				1068.2	1210.3			0.554 [ε]
Week 2	1049.2	-238.1	(-647.9 ; 171.7)	0.255	1188.4	866.1	870.5	(63.8 ; 1677.1)	***0.034***
Week 4	881.2	-573.8	(-988.7 ; -158.9)	***0.007***	745.5	1032.1	695.8	(-121.3 ; 1512.8)	0.095
Week 12	733.3	-840.6	(-1236.0 ; -445.2)	***<0.001***	647.1	823.8	396.5	(-381.9 ; 1174.9)	0.318
sIL6R									
Baseline	9379.3				9379.3	9545.9			0.575 [ε]
Week 2	9480.6	-210.6	(-1457.6 ; 1036.4)	0.741	10038.4	9183.6	2064.7	(-387.2 ; 4516.6)	0.099
Week 4	11538.0	170.5	(-1093.0 ; 1434.1)	0.791	11248.9	11940.5	702.1	(-1781.4 ; 3185.6)	0.580
Week 12	8123.9	-1366.0	(-2568.2 ; -163.9)	***0.026***	8250.4	7518.7	2598.0	(234.6 ; 4961.5)	***0.031***
sTNFRI									
Baseline	536.2				551.4	489.4			0.693 [ε]
Week 2	560.9	13.4	(-69.8 ; 96.6)	0.752	647.5	516.4	81.9	(-83.9 ; 247.7)	0.333
Week 4	606.9	77.2	(-7.1 ; 161.5)	0.072	646.0	571.0	4.6	(-163.5 ; 172.7)	0.957
Week 12	455.1	-50.7	(-130.9 ; 29.5)	0.215	532.9	415.2	53.3	(-106.5 ; 213.1)	0.513
sTNFRII									
Baseline	5441.7				5441.7	5582.4			0.638 [ε]
Week 2	5104.3	-979.0	(-1695.3 ; -262.7)	***0.007***	5680.6	4236.9	1517.7	(116.3 ; 2919.2)	***0.034***
Week 4	4757.1	-1343.6	(-2069.2 ; -618.0)	***<0.001***	4307.4	5766.0	-352.0	(-1771.9 ; 1068.0)	0.627
Week 12	4564.9	-1421.8	(-2112.7 ; -730.9)	***<0.001***	4485.4	4579.4	-92.1	(-1443.5 ; 1259.3)	0.894
IL1ra									
Baseline	4.4				10.2	1.9			***0.030*** [ε]
Week 2	3.4	2.5	(-13.5 ; 18.5)	0.760	11.3	0.4	5.5	(-26.2 ; 37.1)	0.734
Week 4	5.4	0.4	(-15.7 ; 16.4)	0.965	5.7	2.1	-7.3	(-39.1 ; 24.6)	0.654
Week 12	3.4	9.7	(-5.8 ; 25.2)	0.219	5.1	0.0	19.3	(-11.4 ; 50.0)	0.219

[a] Time effect is the effect of cART on mean cytokine concentration for the entire cohort

[b] Group effect is the difference in mean cytokine concentration between groups compared to baseline

[ε] Wilcoxon rank-sum test

University of Cape Town

In the group who developed symptoms compared to those remaining symptom-free, median IL-1RA concentrations were significantly higher pre-cART (10.2 vs 1.9 pg/mL, p=0.030), and remained higher throughout the study period. However, 26 of 60 individuals (six symptomatic and 20 symptom-free) had baseline IL-1RA concentrations lower than the detectable level.

After starting cART, the overall concentrations of sIL-2Rα and sTNFRII declined significantly from pre-cART/baseline levels to those at 12 weeks irrespective of symptom status ($p<0.001$). However, a repeated measures analysis for both sTNFRII and sIL-2Rα showed significant differences in the median levels between symptomatic and symptom-free groups at week 2 (sTNFRII, $p=0.034$; sIL-2Rα, $p=0.034$).

Soluble IL-6R levels showed an overall upward trend over the first four weeks before declining, although concentrations in the symptomatic group were still higher at week 12 ($p=0.031$). Soluble receptors IL-1RI, sIL-1RII and sTNFRI remained stable throughout the observation period without any differences between the two groups.

It has been suggested that the biological cytokine activity may depend on the balance between the cytokine concentration and that of its soluble receptor. Therefore, the cytokine:soluble receptor ratios may provide an estimate of plasma biological activity (Arend et al., 1998, Cox et al., 2006, Dodoo et al., 2002, Hagiwara et al., 1996). Concentrations of all the soluble receptors tested were considerably higher than their respective cytokines. There was no significant difference in either the TNF-α/sTNFRI or TNF-α/sTNFRII ratios between individuals who developed neuropathic symptoms compared to control individuals. The ratios between IL-1β and its receptors sIL-RI and sIL-RII, IL-6 and its receptor sIL-6R and IL-2 and sIL-2Rα were also investigated (Table R-2 in Appendix R). At week 2 after cART, individuals with incident symptoms compared with symptom-free individuals showed lower ratios of IL-1β/sIL-1RI ($p=0.004$), IL-1 β/IL-1RA ($p=0.006$) and IL-2/sIL-2Rα ($p=0.014$). At the same time, "pain-associated" cytokines of *a priori* interest showed higher ratios in the symptomatic-group for TNF-α/IL-4 ($p=0.022$) and a trend for IL-6/IL-4 ($p=0.054$) and IFN-γ/IL-4 ($p=0.061$). At week 12 the symptom-group still had a higher IFN-γ/IL-10 ratio ($p=0.044$).

10.4.1 Possible factors affecting cytokine concentrations

Increased cytokine production has been associated with low CD4 T-cell counts (Bebell et al., 2008, Stylianou et al., 1999). A comparison of cytokine concentrations stratified by CD4 T-cell count (below and above 100 cells/mm^3) is shown in Table 10.7. Apart from significantly higher IL-6 and IL-10 concentrations in individuals with a CD4 T-cell count <100 cells/mm^3 ($p=0.006$ and $p=0.013$ respectively), other cytokine concentrations did not differ significantly between the two groups.

University of Cape Town

Table 10.7: Comparison of baseline cytokine concentrations across CD4 T-cell groups

Cytokine	CD4 < 100 cells/mm^3 (n = 11)	CD4 > 100 cells/mm^3 (n = 49)	p-value	
Interleukin-1β	0.1 (0.0 - 0.3)	0.1 (0.0 - 0.4)	0.555	β
Interleukin-2	0.4 (0.0 - 1.7)	1.0 (0.1 - 2.5)	0.482	β
Interleukin-4	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.1)	0.499	ε
Interleukin-5	0.6 (0.3 - 1.6)	0.5 (0.3 - 1.0)	0.653	β
Interleukin-6	4.6 (2.6 - 14.0)	2.8 (1.6 - 4.5)	***0.006***	ε
Interleukin-7	2.2 (1.2 - 9.1)	2.0 (0.7 - 3.6)	0.338	β
Interleukin-8	3.8 (2.7 - 7.4)	3.8 (2.4 - 4.8)	0.897	β
Interleukin-10	27.4 (20.4 - 41.0)	13.1 (6.9 - 25.4)	***0.011***	β
Interleukin-12	0.0 (0.0 - 0.2)	0.0 (0.0 - 1.1)	0.301	ε
Interleukin-13	0.5 (0.5 - 10.3)	0.5 (0.5 - 7.2)	0.622	ε
IFN-γ	2.5 (1.0 - 4.9)	0.9 (0.0 - 2.8)	0.085	β
GMCSF	0.7 (0.1 - 1.3)	0.7 (0.2 - 1.5)	0.847	β
TNF-α	9.2 (6.0 - 11.4)	6.2 (4.3 - 10.9)	0.168	β

β Student's *t*-test

ε Wilcoxon rank-sum test

Stratifying individuals by dNRTI exposure, previous TB history or vitamin B6 deficiency, did not show an association with cytokine concentrations at any sampling point (see Table R-3 – Table R-6 in Appendix R). However, the small sample size may limit the power to detect differences between stratified groups.

10.4.1.1 Symptom duration and severity

Cytokine concentrations of individuals with transient neuropathic symptoms (18/30) were compared to those individuals whose symptoms persisted after week 12 (12/30). The IL-6 peak at week 2 was significantly higher for individuals with persistent symptoms compared to those with transient symptoms (p=0.023) (Table 10.8). To evaluate whether the pattern of cytokine release was related to severity of symptoms, individuals were stratified according to symptom severity grade. No statistical difference was found between individuals with a symptom grade ≥2 (representing VAS ≥6/10) compared to individuals with a symptom grading <2 (Table 10.8 and Table R-7 in Appendix R).

University of Cape Town

Table 10.8: Effect of symptom duration and severity on selected cytokines IL-1β, IL-2, IL-4, IL-6, IL-10 and TNF-α

Cytokine	Symptom duration group effect[a]			Symptom severity group effect[b]		
	Coefficient	Confidence interval (95%)	p-value	Coefficient	Confidence interval (95%)	p-value
Interleukin-1β						
Week 2	-0.39	(-1.31 ; 0.52)	0.402	-0.15	(-0.97 ; 0.68)	0.724
Week 4	0.02	(-0.91 ; 0.94)	0.975	0.24	(-0.58 ; 1.07)	0.565
Week 12	0.08	(-0.85 ; 1.01)	0.868	0.28	(-0.52 ; 1.09)	0.491
Interleukin-2						
Week 2	-2.89	(-9.48 ; 3.71)	0.391	-1.97	(-7.93 ; 4.00)	0.518
Week 4	1.05	(-5.60 ; 7.69)	0.758	2.67	(-3.29 ; 8.64)	0.380
Week 12	-1.51	(-8.23 ; 5.21)	0.661	0.44	(-5.35 ; 6.24)	0.880
Interleukin-4						
Week 2	-3.00	(-15.49 ; 9.50)	0.638	1.56	(-9.69 ; 12.80)	0.786
Week 4	-2.17	(-14.75 ; 10.42)	0.736	-0.90	(-12.15 ; 10.34)	0.875
Week 12	10.13	(-2.57 ; 22.83)	0.118	9.77	(-1.17 ; 20.72)	0.080
Interleukin-6						
Week 2	-11.50	(-21.43 ; -1.56)	***0.023***	7.92	(-1.37 ; 17.21)	0.095
Week 4	-3.66	(-13.69 ; 6.37)	0.474	6.61	(-2.68 ; 15.89)	0.163
Week 12	-7.90	(-18.05 ; 2.25)	0.127	5.75	(-3.20 ; 14.70)	0.208
Interleukin-10						
Week 2	-6.48	(-42.28 ; 29.32)	0.723	-1.79	(-34.73 ; 31.15)	0.915
Week 4	-9.07	(-45.18 ; 27.05)	0.623	-7.71	(-40.65 ; 25.23)	0.646
Week 12	24.18	(-12.35 ; 60.71)	0.195	-22.73	(-54.54 ; 9.07)	0.161
TNF-α						
Week 2	-3.02	(-8.81 ; 2.78)	0.307	-4.89	(-10.22 ; 0.44)	0.072
Week 4	-1.18	(-7.02 ; 4.67)	0.693	-1.22	(-6.55 ; 4.10)	0.652
Week 12	-1.00	(-6.91 ; 4.91)	0.741	-2.04	(-7.19 ; 3.10)	0.436

[a] Difference in mean cytokine concentration between resolved symptoms and unresolved symptoms compared to baseline

[b] Difference in mean cytokine concentration between grade ≥ 2 severity and grade 1 severity compared to baseline

University of Cape Town

10.5 CD4 T-cell reconstitution and hs-CRP analysis

After 24 weeks individuals with incident symptoms showed greater CD4 T-cell reconstitution compared with the symptom-free group (335 vs 237 cells/mm^3, p=0.008). Overall, and compared to pre-cART, hs-CRP levels peaked at 2 weeks (p=0.001) although without significant differences between symptom and symptom-free individuals (Table 10.9).

Table 10.9: CD4 T-cell count and hs-CRP before cART initiation and longitudinally over 12 weeks categorized by Incident symptoms

Variable	Time effect[a]		Group effect[b]		
	Median (IQR) (pg/mL)	p-value	Incident symptoms Median (IQR) (pg/mL)	No symptoms Median (IQR) (pg/mL)	p-value
CD4 T-cell count					
Baseline	141 (110 ; 189)		145 (109 ; 181)	138 (110 ; 194)	0.920 [ε]
24-week	272 (204 ; 368)	***<0.001***	335 (210 ; 403)	237 (185 ; 260)	***0.008***
C-reactive protein					
Baseline	2.7 (0.9 ; 6.7)		2.9 (0.9 ; 6.7)	2.6 (0.9 ; 5.4)	0.693 [ε]
Week 2	6.7 (2.1 ; 18.5)	***0.001***	8.4 (3.4 ; 17.0)	5.3 (1.8 ; 19.3)	0.606
Week 4	6.5 (3.3 ; 26.5)	0.137	8.4 (3.5 ; 30.6)	4.2 (3.3 ; 15.0)	0.930
Week 12	4.0 (1.5 ; 7.2)	0.651	3.8 (1.5 ; 8.3)	4.2 (1.0 ; 6.7)	0.216

[ε] Wilcoxon rank-sum test

[a] Difference between follow-up visit and baseline

[b] Difference between groups compared to baseline

Chapter 11 Cytokine Discussion

University of Cape Town

We demonstrate a transient but significant burst in both pro- and anti-inflammatory plasma cytokine concentrations, as well as hs-CRP levels, within 2-4 weeks after starting cART in all individuals, irrespective of neuropathic symptom status. Twelve of the 13 cytokines analysed increased significantly within 4 weeks of starting cART; some such as IL-2, IL-12(p70) and IFN-γ, increased by more than 200-times relative to pre-cART/baseline concentrations before returning to these levels at 12 weeks. Although Padilla et al. measured inflammatory markers (IL-6 and hs-CRP) longitudinally starting four weeks after cART initiation/re-initiation, the 2-week sampling visit proved informative in our study (Padilla et al., 2011).

Comparing plasma cytokine concentrations in those developing neuropathic symptoms with those remaining symptom-free at 24 weeks, the symptomatic group demonstrated a trend towards lower peak cytokine concentrations between 2 and 4 weeks post-cART initiation. This was evident for both pro- and anti-inflammatory cytokines. However, only IL-1β and IL-13 concentrations at week 2 were significantly lower in the symptomatic-group.

Soluble cytokine receptors were studied as an indicator of a more chronic systemic inflammatory response (Arend et al., 1998, Chikanza et al., 1993). Interestingly, the IL-1RA concentrations were 5-times higher in the symptomatic group preceding cART initiation. Two weeks after starting cART, the sIL-2rα and sTNFRII showed higher peak concentrations in the symptomatic- compared with the symptom-free group. At week 24, the symptomatic-group showed greater reconstitution of CD4 T-cell count. In those with symptoms, higher IL-6 levels associated with symptoms lasting up to 24 weeks compared to the levels in those with transient symptoms. Importantly, two individuals with detectable viral loads at week 24 were excluded to minimize the confounding effect of uncontrolled HIV infection. Vitamin B6 concentrations did not correlate with the change in cytokine concentrations. However, other antioxidants such as vitamin E and selenium levels were not accounted for in this analysis and may have also affected cytokine production. Taken together these results suggest that within the first 12-24 weeks there is evidence to support enhanced subclinical inflammation and immune reconstitution in the systemic compartment in individuals developing neuropathic symptoms compared with those remaining symptom-free. Individuals susceptible to developing neuropathic symptoms possibly have insults driving chronic subclinical inflammation preceding the additional effect observed with the initiation of cART.

University of Cape Town

Previously, higher levels of systemic TNF-α- and IL-2-mRNA were reported in HIV-negative painful neuropathies when compared with painless neuropathies (Uceyler et al., 2007). Although in our study plasma IL-2 and TNF-α concentrations did not mirror the soluble receptor levels or segregate by symptom status, the systemic cytokine concentrations of both were substantially lower than the concentrations of their respective soluble receptors (sIL-2rα, sTNFRI and sTNFRII). A possible explanation is that IL-2 and TNF-α, secreted by Th1 cells, act locally in an autocrine or a paracrine manner and are neutralized systemically by their soluble receptors as an immune regulatory mechanism (Mosmann and Coffman, 1989). Furthermore, the longer plasma half-life of these soluble receptors and their rapid biological inactivation of systemic cytokines suggest that the elevated levels detected in the symptomatic group may indicate a relatively higher level of inflammation or injury, even prior to cART initiation (Barnes, 1998, Chikanza et al., 1993).

In order to further explore a profile of immune dysregulation we assessed the ratios between key cytokines previously implicated in neuropathic pain (Tyor et al., 1995, Uceyler et al., 2007). The group with symptoms had significantly higher ratios of TNF-α/IL-4 and IL-6/IL-4 2 weeks after cART initiation, and IFN-γ/IL-10 at 12 weeks. This suggests that in individuals developing neuropathic symptoms, there is an imbalance between pro- and anti-inflammatory cytokines in which Th1 subsets and their cytokine products including IL-6, IFN-γ and TNF-α, dominate over an immunoregulatory Th2 subset and their cytokines, IL-4 and IL-10. This imbalance may be due to either HIV-associated immune dysregulation, greater imbalance between ROS and antioxidants, or predisposing genetic factors inducing reduced or enhanced cytokine gene transcription upon any pathology.

Although peak cytokine concentrations in this cohort overall were lower than those associated with hospitalized TB-IRIS patients (Conesa-Botella et al., 2012, Tadokera et al., 2011), this study population comprised ambulatory individuals attending a community-based clinic. Parasitic and non-parasitic infections, as well as nutritional deficiencies could contribute to the degree of observed immune activation once the HIV-induced functional inhibition is reversed by cART. However, all individuals were well and by selection had achieved viral control by 24 weeks.

We suggest that the lower cytokine peak in the symptomatic group may be the consequence of systemic neutralization by the concomitant and significantly higher concentration of IL-1RA and soluble receptors. For example, IL-1RA is released from hepatocytes stimulated by

University of Cape Town

circulating inflammatory mediators, and also synthesized by locally activated monocytes to reduce the systemic responses to localized IL-1β production (Dinarello, 1996, Nicklin et al., 2000). High levels of circulating IL-1RA have been reported in many inflammatory settings including target-organ autoimmune diseases such as rheumatoid arthritis; in the latter, systemic and local synovial production of IL-1RA is thought to be inadequate to dampen the IL-1 mediated synovial damage (Arend et al., 1998). The finding of high plasma levels of IL-1RA prior to cART initiation in individuals who developed symptoms suggests that IL-1RA may be part of the host's homeostatic mechanism aimed at downregulating a pre-existing inflammatory challenge.

Importantly, the symptom-free group at baseline had a similar IL-1β/IL-1RA ratio to a previous and comparable cohort of asymptomatic African women with reasonably preserved CD4 T-cell counts not on cART (Thea et al., 1996). By comparison, our symptomatic group showed a markedly altered IL-1β/IL-1RA ratio at baseline/pre-cART due to the significantly higher IL-1RA levels (Table 10.6). We propose that this higher plasma IL-1RA level in the group predicted to develop symptoms reflects the cytokine imbalance at the dorsal root ganglion particularly when the blood-nerve barrier is impaired (Thea et al., 1996).

The dorsal root ganglion plays a pivotal role in the pathogenesis of HIV-associated neuropathy, which likely involves both indirect cytokine- and direct viral protein-mediated neurotoxicity (Jones et al., 2005). Neuronal toxicity in the dorsal root ganglion is associated with upregulated IL-1β and TNF-α expression (Acharjee et al., 2011, Jones et al., 2005). Subclinical levels of inflammation such as local IL-1β production, may prime proteinase-activated receptors $(PAR)_2$ on small dorsal root ganglion neurons and their afferent axons (Vergnolle et al., 2001). A "second hit" such as the cART-associated subclinical "cytokine burst" herein described, may facilitate further PAR_2 activation with augmentation of nociceptive signalling via central pathways (Alier et al., 2008, Vergnolle et al., 2001).

An alternative explanation for the lower cytokine peaks seen in individuals with symptoms may be that these individuals had already reached a threshold of mitochondrial damage preceding cART initiation. Mitochondrial damage leads to oxidative stress (Lewis et al., 2003) and this in turn impacts on several T-cell functions including lymphocyte responsiveness and cytokine release (Grisham, 2004). The impairment of T-lymphocyte function by oxidative stress has been shown to be relevant in several models and conditions such as rheumatoid arthritis and HIV infection (Cayota et al., 1994, Maurice et al., 1997,

University of Cape Town

Stefanova et al., 1996). Although it may be argued that oxidative stress and impairment in T-lymphocyte functioning may impact on cytokine release after cART initiation, the timing of increased levels of several soluble receptors that coincides with the rising incident peak of ATN and neuropathic symptoms, negates this.

As the principal downstream mediator of the acute-phase response, hs-CRP may account for the integrated effects of certain cytokines, including TNF-α and IL-6 (Liu et al., 2007). Similar to the trend shown in the cytokine concentrations, an overall increase in hs-CRP concentrations were observed between 2 and 4 weeks after starting cART. However, no significant differences were observed in hs-CRP concentrations during the 24-week follow period between individuals who developed neuropathic symptoms and individuals who remained symptom-free.

The substudy has several limitations. Firstly, plasma levels may not reliably reflect local activation due to the local perineural action of cytokines at low concentrations. Therefore, systemic cytokine levels might not accurately demonstrate the extent of neural inflammation in symptomatic individuals. A more definitive measure of local inflammatory reactions at nerve level would be nerve biopsy; however this is impractical for community-based studies at fortnightly intervals. Secondly, our conclusions are based on a small sample reducing our statistical power to detect weaker associations. Fluctuation in cytokine levels may have affected results although rank correlation analyses demonstrated good within-individual stability and predictability for the majority of cytokines. A random effects model was used to limit the effect of variability and fluctuations on the longitudinal analysis of cytokine levels. Exclusion criteria such as known diabetes or individuals on TB therapy limit the generalizability of our findings. In addition, although individuals with detectable viral loads at 24 weeks were excluded from this study, a degree of undetectable viral replication may have confounded the interpretation of the cytokine results. Methodologic limitations also need to be taken into account. The analysis was hindered in part by the low plasma levels of some of the examined cytokines, such that, for instance, IL-4 and IL-12 were not detectable in several samples even by using ultrasensitive Multiplex kits. Furthermore, measurement error may influence results, which is a limitation in any study.

Cytokine:receptor ratios for all groups were not normally distributed and showed a large variability with significant skewing. Ratios should therefore be confirmed in a larger study population. Also, due to the extreme complexity of the cytokine network, investigating ratios

University of Cape Town

of one pro-inflammatory cytokine to another anti-inflammatory cytokine may not be an accurate reflection of trends in cytokine responses.

Strengths of this study include the examination of individuals (by clinicians) a few days prior to starting cART, in addition to four intervening clinical and sampling visits spanning the first 24 weeks after cART initiation. The prospective design of our study allowed us to assess the variation in the inflammatory biomarkers over the early stages of exposure to cART. Variation in inflammatory markers may be as a result of natural antagonists in the plasma and their effect on cytokine levels may be better observed in longitudinal data compared to a cross-sectional analysis. We were also able to demonstrate significant differences in trends between soluble receptors and cytokine concentrations.

In conclusion, we present longitudinal plasma cytokine levels in a community-based, otherwise asymptomatic cohort starting cART. Overall, cART resulted in a significant increase in cytokine production from pre-treatment levels within the first 2-4 weeks after initiating therapy, possibly contributing to the general susceptibility to manifesting pathological IRIS in this window period. We show that individuals developing neuropathic symptoms within the following 12 weeks already have higher levels of IL-1RA pre-cART. We propose that this represents a homeostatic response to chronic higher levels of background inflammatory signals such as IL-1β. An additional "insult" such as initiating cART with the observed cytokine burst, altered and higher ratios of pain- associated cytokines (TNF-α/IL-4, IL-6/IL-4 and IFN-γ/IL-10), and concomitant increases in soluble cytokine receptors (sIL-2Rα and sTNFRII), on a background of HIV-associated immune dysregulation and/or susceptible genetic factors may give rise to neuropathic symptoms via complex direct and indirect signalling mechanisms.

University of Cape Town

Chapter 12 Conclusion, recommendations and future work

University of Cape Town

12.1 Conclusions

The purpose of this community-based longitudinal observational study was firstly to investigate the characteristics and prevalence of HIV-associated DSP, with and without symptoms, prior to the introduction of cART. Secondly, we sought to investigate the progression in clinical characteristics of HIV-associated DSP within the first 24 weeks of cART initiation.

This study provides novel data on the clinical evolution of HIV-associated DSP status within the first 24 weeks of starting cART to the extent that all individuals were examined days before cART initiation and followed up at four subsequent assessments. We found that HIV-DSP remains a clinically relevant problem in this population with 22% of individuals having HIV-DSP prior to commencing cART, of whom 77% were symptomatic. Interestingly, 54% of the symptomatic individuals experienced improvement of neuropathic symptoms within 12 weeks of starting cART. Half of the group with symptom improvement experienced complete resolution of symptoms on cART. In addition, 50% of those with asymptomatic DSP at the time of cART initiation (2 neuropathic signs by definition) showed complete resolution of both signs within 24 weeks; the neuropathic sign that resolved most frequently was distal pin loss. These results suggest that there are early beneficial effects associated with cART on the peripheral sensory pathways, either via virological control or immune recovery, which become evident within 12 weeks. The small sample size of this latter group precluded multivariate analyses. As a result, we were unable to investigate any differences in nutritional state, CD4 T-cell counts or viral loads at baseline in individuals who improve after cART.

An important research aim was to determine the incidence and characteristics of symptomatic ATN as a clinically relevant outcome in the first 24 weeks of starting cART. The incidence of painful ATN was 34%, occurring most frequently between 4 and 12 weeks, after which the incidence rate decreased. The development of neuropathic signs on the other hand, increased steadily over the 24-week period. Although the sample was small and further longitudinal observations are necessary to assess the long-term trend in this cohort, it does suggest either a separate pathogenesis of asymptomatic DSP compared to symptomatic DSP, or a distinct mechanism related to the development of early painful ATN. Continued follow-up of this well-characterized cohort will be useful in determining whether there remains a difference in incidence rates between symptomatic and asymptomatic DSP. It also appears, albeit based on

University of Cape Town

a small sample, that the presence of asymptomatic DSP pre-cART is not a risk factor to the development of ATN within the first 24 weeks.

The longitudinal association of candidate risk factors based on previous reports, notably age, d-drug history and previous TB infection with the development of ATN, were examined. Interestingly, there was no significant difference in NRTI backbone (d-drug vs no d-drug) between individuals who developed ATN compared to those who remained ATN-free. Although advancing age, relatively higher triglycerides (although still normal) and hs-CRP levels, as well as current INH TB therapy were all independent predictors of HIV-DSP at baseline/pre-cART, these factors did not associate with the development of ATN. This may be due to the short follow-up interval or due to the limitation of sample size.

As symptomatic ATN appears early after cART and the timing coincides with the manifestations of IRIS, we hypothesized that incident painful ATN may represent an immune reconstitution manifestation. As such, after 24 weeks on cART the development of ATN was significantly associated with a greater CD4 T-cell count recovery. We observed a transient but significant burst in both pro- and anti-inflammatory plasma cytokine concentrations, as well as hs-CRP levels, within 2-4 weeks after starting cART in all individuals, irrespective of neuropathic symptom status. However, the symptomatic group showed higher sIL-2rα and sTNFRII peak concentrations at week 2, and significantly higher pro-inflammatory/anti-inflammatory ratios for TNFα/IL-4 and IFNγ/IL-10 compared to the symptom-free group. Half of the symptomatic individuals only had transient symptoms despite remaining on the same cART regimen throughout the study period. Those individuals who had symptoms lasting up to 24 weeks had significantly higher IL-6 levels than those with transient symptoms. These results show that in individuals with early symptomatic ATN there is evidence of systemic dysregulation of cytokines previously implicated in painful neuropathies. Importantly, in the symptomatic group the IL-1RA concentrations, which may represent an acute phase protein response, were 5-times higher even before cART was initiated. Taken together, these results suggest that those who develop ATN already had a higher set point of the pro-inflammatory signalling cascade, albeit measured systemically, prior to initiating cART.

Based on associations between a history of TB infection and HIV-associated DSP previously described in African populations where there is a high co-infection rate, we investigated whether plasma vitamin B6 levels were compromised in individuals with a slow NAT2

University of Cape Town

acetylation phenotype thus predisposing them to HIV-associated DSP. In contrast to European populations, the overall distribution of NAT2 acetylation phenotypes in this African cohort was skewed toward the slow phenotype. The slow acetylation phenotype was overrepresented in individuals developing ATN within 12 weeks of starting cART. This association that was strengthened by taking into account a history of INH exposure, suggesting a possible augmenting effect between slow NAT2 acetylation phenotype and previous TB therapy on the development of ATN.

Overall, NAT2 acetylation phenotypes showed no association with vitamin B6 levels. However, the absence of a statistical association may be a result of the overall low vitamin B6 levels in this cohort; vitamin B6 deficiency was present in 53% of individuals at baseline. Plasma vitamin B6 levels remained low at week 12, despite "standard of care" vitamin B complex supplementation (containing ≤4mg pyridoxine). Although not statistically significant, there was a weak correlation between low plasma PLP and the development of ATN, suggesting a contributory role in the pathogenesis of ATN.

University of Cape Town

12.2 Proposed multifactorial model of HIV-associated DSP pathogenesis

Notwithstanding the possible differences in the incidence of symptomatic and asymptomatic DSP in relation to cART initiation, we propose that oxidative stress may represent a mechanistic pathway through which HIV-associated chronic immune dysregulation in association with low vitamin B6, slow NAT2 acetylation phenotype, previous opportunistic infections such as TB, dysglycaemia, dyslipidaemia, increasing age and NRTI-associated neurotoxicity and/or immune reconstitution, may contribute to HIV-associated DSP. These factors probably contribute to DSP largely in combination, although not in equal magnitude and may differ among individuals. A synergistic, dynamic multifactorial model for the development of DSP is outlined in Figure 12.1 where HIV-DSP and ATN represent a continuum of a similar pathological process over a period of time.

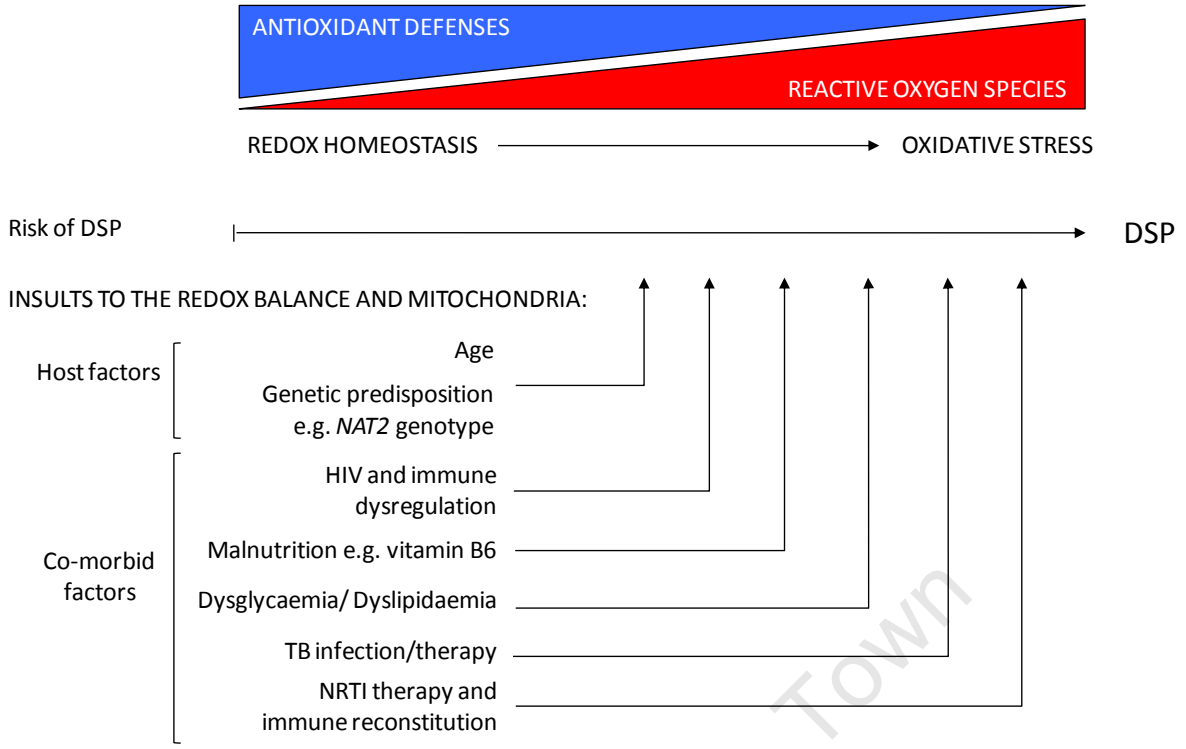


Figure 12.1: Cumulative effect of HIV-infection, vitamin deficiency, opportunistic infections and NRTIs on mtDNA damage eventually leading to disruption in redox homeostasis, impaired mitochondrial function with oxidative stress and mtDNA damage.

University of Cape Town

Multiple insults may affect the redox homeostasis by either directly increasing ROS or decreasing antioxidants, eventually contributing to oxidative stress and impairment of mitochondrial function. Once a critical threshold of mitochondrial dysfunction is surpassed, it would compromise cellular redox homeostasis, driving oxidative stress and further mtDNA damage. Antiretroviral therapy, particularly NRTI-containing regimens, in predisposed individuals such as those with increasing age, inadequately nourished individuals, advanced HIV disease, comorbid infections or genetic predisposition such as slow NAT2 acetylation or certain mitochondrial haplogroups, thus provide the context for multiple insults compromising the redox balance, eventually leading to peripheral nerve injury or dysfunction.

Depending on factors such as the extent of nerve dysfunction or injury, susceptibility of nerve fibres and whether the exposure to the insult is acute or chronic, this process may lead to pain via complex direct and indirect signalling mechanisms. The timing and duration of symptoms will depend on the individual's susceptibility to oxidative stress, which in turn may be influenced by age, general health, genetic background, antioxidant defence and nutrition,

comorbid conditions, metabolic disturbances, underlying subclinical nerve inflammation or nerve injury, inflammatory response related to HIV infection and/or cART initiation, T-cell function and nerve injury repair mechanisms. Although antiretroviral therapy is seen as an insult in our proposed model, these previously mentioned factors might all influence the potential neurotoxicity of NRTIs in a particular individual. For that reason, some individuals may benefit from NRTI therapy as a result of recovery of immune function and/or better virological control, thereby counterbalancing its potential neurotoxic effect.

Although the model outlined in Figure 12.1 attempts to unify different pathogenetic mechanisms involving oxidative stress as a central component in the development of HIV-associated DSP, some individuals develop a symptomatic neuropathy and others an asymptomatic neuropathy. The interplay between these two clinical manifestations is not clear. Of interest however, was the observation, albeit in a highly selected nested-case-control cohort, that individuals who developed neuropathic symptoms soon after cART initiation showed higher peak concentrations of sIL-2rα and sTNFRII and a significant imbalance between pro-and anti-inflammatory cytokines compared to a symptom-free control group. Furthermore, the symptomatic individuals showed a markedly altered IL-1β/IL-1RA ratio at baseline/pre-cART due to significantly higher IL-1RA levels. These results suggest an inflammatory process associated with cytokine imbalance at a higher set point even before cART initiation. Regions where the blood-nerve barrier may be less effective or impaired, such as the dorsal root ganglion and distal axons, may be particularly vulnerable to this altered cytokine milieu predisposing these individuals to the development of painful ATN.

The dorsal root ganglion plays a pivotal role in the pathogenesis of HIV-associated neuropathy, which likely involves both indirect cytokine- and direct viral protein-mediated neurotoxicity, as previously discussed. Neuronal toxicity in the dorsal root ganglion has been associated with upregulated IL-1β and TNF-α expression (Acharjee et al., 2011, Jones et al., 2005). HIV-associated immune dysregulation with subclinical levels of local IL-1β production may prime proteinase-activated receptor-2 (PAR_2) on small dorsal root ganglion neurons and their afferent axons (Vergnolle et al., 2001). The cytokine burst triggered by the introduction of cART may facilitate further PAR_2 activation with augmentation of nociceptive signalling via complex central pathways (Alier et al., 2008, Vergnolle et al., 2001). Theoretically, the increase in cytokine and ROS production may also change axonal properties and trigger the activation of multiple intracellular signalling pathways such as

University of Cape Town

mitogen activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB), both shown to play a major role in neural inflammatory pain (Kamata et al., 2005, Takada et al., 2003).

The observed cytokine burst on a background of HIV-associated immune dysregulation, underlying nerve inflammation and/or susceptible genetic and environmental factors may also provide the final push over a critical threshold of mitochondrial dysfunction, resulting in oxidative stress and pain. That is to say these individuals may have reached their threshold of mitochondrial damage prior to cART initiation. Given that oxidative stress may also result in impaired T-lymphocyte functioning, which may impact on cytokine release after cART initiation, the timing of increased levels of several soluble receptors in symptomatic individuals which coincides with the rising incident peak of ATN and neuropathic symptoms, contradicts this as a theory for painful ATN soon after cART initiation. Rather, surpassing the critical threshold of mitochondrial dysfunction with its effect on mtDNA may result in more latent consequences.

The study period of 24 weeks may have been too short for the effect of cART to be significant on some covariates and to determine the natural progression of HIV-associated DSP after cART initiation. We suggest further follow-up for a longer period to determine its natural progression and the effect of metabolic and biochemical factors, such as waist-to-hip ratio, triglycerides and ALT levels on the development of HIV-associated DSP. In addition, not all neurological or mitochondrial insults could be accounted for with this study. Due to left censoring of the baseline DSP group, it was not possible to accurately assess all possible risk factors or the progression of symptoms and signs prior to cART for the group of patients who already had DSP at baseline assessment. There is also uncertainty about the relative contribution of the virus itself and the initial associated inflammatory response related to seroconversion.

University of Cape Town

12.3 Clinical implications of the study findings

Neuropathic pain is distressing and disabling and DSP remains undertreated. For clinicians treating HIV-infected individuals, there is a need for greater sensitivity in diagnosis and closer monitoring of DSP in primary health care clinics. It is possible that early diagnosis with intervention may lead to better outcomes. It has been suggested that a screening tool such as the BPNS could be incorporated into routine assessments (Luma et al., 2012).

However, a tuning fork and reflex hammer are necessary and such equipment is not readily available in resource-limited clinic settings. Another caution is that agreement between ankle reflexes performed by non-clinician healthcare workers, such as nurses, and by clinicians has been shown to be poor (Cettomai et al., 2010, Simpson et al., 2006). Our results show that the most common neuropathic sign associated with DSP was impaired pin sensibility, suggesting this as the preferred examination modality, but it is not tested in the BPNS. Assessment of pin sensibility can be rapidly performed and for non-clinicians it may be easier to perform than eliciting reflexes since it requires only a sharp object. However, distal neuropathic symptoms were strongly associated with objective findings of DSP. Therefore, symptom-based screening for DSP with a tool such as the single-question neuropathy screen (SQNS) may prove useful in busy primary health care settings. It is important that individuals should be routinely questioned about their experience of pain.

This research expands our understanding of the pathophysiology of HIV-associated DSP. The results have implications not only on individuals who develop painful HIV-associated DSP and the early effects of commencing cART. The cART-associated cytokine burst is an important finding in all individuals commencing cART. It illustrates the susceptibility to pathology related to immune reconstitution and hypercytokinemia in all individuals commencing cART, which may result in oxidative stress and tissue damage as part of a multifactorial process in predisposed individuals. Whether the cytokine burst early after cART initiation may contribute to an increased susceptibility towards mitochondrial damage with possible long-term effects on mitochondrial function in an increasingly ageing HIV-treated population remains unknown.

Our nested case-control cohort focussing on the cytokine response early after cART initiation consisted of a relatively small sample size. Therefore, the possibility of using IL-1RA as a biomarker for the prediction of ATN development may need to be explored in a larger sample size. However, its use as a biomarker for the prediction of ATN development is unlikely due to the high cost of the assay and the often transient characteristics of early ATN symptoms.

Although we are as yet unable to predict what drives the higher inflammatory set point in individuals predicted to develop early incident painful neuropathy, preventative strategies should be directed at multiple levels to maintain adequate redox homeostasis. Careful attention to micronutritional status and concomitant drugs in all individuals prior to starting cART, may provide a simple measure to reduce cellular oxidative stress known to augment

University of Cape Town

inflammation, thereby preventing nerve injury. Micronutrient supplementation may be particularly critical in areas with high rates of HIV/TB co-infection, such as Southern Africa (Allard et al., 1998, Israel et al., 1992).

During the first weeks of starting cART, the production of free radicals may be greater than their normal clearance. The host's endogenous antioxidant system would play a major role to prevent or limit the deleterious effects of free radicals and maintain immune function, especially during the first 24 weeks in all individuals commencing cART (Jones et al., 2006, Tang et al., 2000). We demonstrated that vitamin B6 deficiency is frequent in this cohort, and although not classified as an antioxidant compound, vitamin B6 has been shown to have highly effective antioxidant properties (Matxain et al., 2006, Stocker et al., 2003). There is also evidence to suggest that vitamin B6 deficiency can accelerate mitochondrial decay and thus contribute to senescence and neurodegeneration (Ames, 2004, Atamna et al., 2002). In a recent randomized controlled trial, the use of high-dose vitamin supplementation which included 25 mg vitamin B6 daily, reduced the risk of neuropathy in individuals commencing cART (Isanaka et al., 2012). Apart from vitamin B6 deficiency, numerous other vitamin and/or mineral deficiencies that were not measured in our study may be present, likely due to increased utilization of antioxidant micronutrients because of increased oxidative stress. Vitamin B6 and other micronutrient deficiencies may also contribute to immunosuppression with reduced T-lymphocyte effector function (Baum et al., 1991, Rall and Meydani, 1993, Willis-Carr and St Pierre, 1978). Theoretically, this may result in a reduced capacity of lymphocytes to respond to relevant stimuli affecting repair mechanisms to local damage.

Optimizing the redox homeostasis and host repair mechanisms against the various insults described earlier by improving antioxidant status prior to initiating cART may prove to be the best measure to prevent the development of HIV-associated DSP. Vitamin supplementation should not be withheld in this population, and an emphasis should be placed on compliance. Vitamin B complex is widely administered in HIV infection, but was not sufficient to prevent vitamin B6 deficiency. An argument can therefore be made for instituting higher doses of vitamin B6 supplementation to all HIV-infected individuals. Public health care policies should implement the recommendations of the developed world regarding pyridoxine supplementation (10-25 mg per day). Our results also suggest that there is a need to commence vitamin supplementation prior to cART in all individuals which may then reduce the redox imbalance in those with a higher set point of subclinical inflammation at cART

University of Cape Town

commencement. Combination ART has led to a substantial reduction in TB incidence (Badri et al., 2002) and earlier cART initiation may therefore have a critical role in addressing the HIV/TB co-epidemic in South Africa and other African countries although this has cost implications in a high disease burden setting.

Plasma and serum samples are available for all participants. To explore the oxidative stress model further, markers of oxidative stress and mitochondrial dysfunction are avenues for investigation. However, the demonstration of mitochondrial dysfunction and oxidative stress is complex and relies on a combination of pathological, biochemical and molecular genetic studies.

Theoretically the presence of oxidative stress may be tested in one of three ways: (1) direct measurement of ROS; (2) downstream markers of oxidative damage to biomolecules/tissue; and (3) the detection of antioxidant levels. Most ROS are highly reactive and short lived and therefore difficult to measure directly. Markers to measure damaged proteins, lipids, DNA, RNA or other biomolecules are more stable and therefore provide a more reliable method. Increased systemic levels of lipid peroxidation products were shown in NRTI-treated individuals with symptomatic hyperlactatemia, lactic acidosis, or lipoatrophy (McComsey and Morrow, 2003), but not with DSP (Hulgan et al., 2006). It is possible that measuring the oxidative degradation of lipids may relate poorly to a disease process related to axonal injury. Furthermore, the oxidative stress related to nerve damage or dysfunction may be localized and therefore not of sufficient magnitude to alter systemic lipid or protein peroxidation profiles. Local measures of oxidative stress, or even inflammatory reactions at nerve level might be more informative, but challenging. Hahn et al. investigated markers of oxidative stress on post mortem sciatic nerve biopsies of AIDS patients, but failed to demonstrate increased levels of protein oxidation in those with clinical signs of neuropathy (Hahn et al., 2008). Performing sural nerve biopsies on cART treated individuals may prove to be challenging in resource-limited settings, and it would be unethical to perform biopsies on DSP-free individuals as a control group. Furthermore, this strategy would investigate the distal aspect of the sensory neural pathway and not the more proximal elements such as the dorsal root ganglion.

As the measurement of oxidative stress particularly in the context of the nervous system has been challenging, another approach might be to measure the “mopping-up defences” such as levels of antioxidant enzymes and other redox molecules which serve to counterbalance ROS

University of Cape Town

generated in cells. Assays are available to measure the activity of specific antioxidants, such as glutathione, catalase and superoxide dismutase but access to nervous tissue is again a limitation.

An additional avenue that might be explored would be to investigate markers of inflammatory pain such as guanosine triphosphate cyclohydrolase (GCH1). GCH1 is the rate-limiting enzyme for tetrahydrobiopterin (BH4) synthesis, an essential cofactor for catecholamine, serotonin and nitric oxide production (Tegeder et al., 2006). GCH1 was identified as a modulator of neuropathic inflammatory pain in an animal model, and a functional polymorphic haplotype of the *GCH1* gene showed an association with reduced levels of non-HIV neuropathic pain (Tegeder et al., 2006). Systemic levels of GCH1 may be interesting to explore for an association with painful neuropathy in HIV-infected individuals by studying our overall longitudinal cohort after further observation.

University of Cape Town

Appendices

Appendix A Ethical approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: nosi.tywabi@uct.ac.za

05 June 2008

REC REF: 221/2008

Dr J Heckmann
Department of Medicine
Division of Neurology

Dear Dr Heckmann

PROJECT TITLE: PREVALENCE OF HIV NEUROPATHY AND RISK FACTORS FOR ANTIRETROVIRAL TOXIC NEUROPATHY IN AN HIV POSITIVE COHORT OF BLACK SOUTH AFRICANS.

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above mentioned at a full committee meeting on the 30th May 2008.

The study is granted approval for one year until 30 May 2009.

The Committee determined that the research involves no greater than minimal risk and no additional risks were identified, therefore continuing review will be conducted using the expedited procedure, according to category 9 of OHRP guidance.

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

Please quote the REC. REF in all your correspondence.

Yours sincerely

Wesley Henley

pp **PROFESSOR M BLOCKMAN**
CHAIRPERSON, HSF HUMAN ETHICS

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-

lemjedi

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: sumayahariefdien@uct.ac.za

23 November 2009

REC REF: 221/2008

A/Prof J Heckmann
Neurology

Dear A/Prof Heckmann

PROJECT TITLE:- PREVALENCE OF HIV NEUROPATHY AND RISK FACTORS FOR ANTIRETROVIRAL TOXIC NEUROPATHY IN AN HIV POSITIVE COHORT OF BLACK SOUTH AFRICANS

Thank you for your letter to the Research Ethics Committee dated 18th November 2009.

It is a pleasure to inform you that the Ethics Committee has granted **approval** to perform two additional investigations:

1. NAT 2 genotyping on DNA
2. PYRIDOXINE plasma levels at baseline and three months.

We note that a sub-set of this sample will be interviewed regarding their experiences of participating in neuropsychological testing.

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

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

sAriefdien

Appendix B Neuropathy assessment form

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

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

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

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

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

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

Nn1 Score 1a	

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

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

Nn2 score 1b	

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

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

Nn3 Score 1c	

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

0 = Grade 0 1-3 = Grade 1
4- 6 = Grade 2 7-8 = Grade 3
9-10= Grade 4 **Total sensory severity GRADE** ____/4 nn4

1d. If a symptom was present in the past, but not now i.e "Currently Absent" Yes (1) N/A (0) nn5

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

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

1g. Do you think the ARV treatment helped your symptoms? Yes (1) No (0) Unknwn (2) nn8

1h. Did the sensory symptoms in your feet start or get worse within 2-4 months of starting ARV Rx?
Yes (1) No (0) Unknwn (2) nn9

1i. Did the sensory symptoms in your feet start or get worse within 2-4 months of changing the dose of ARV Rx?
Yes (1) No (0) Unknwn (2) nn10

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

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

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

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

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

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

	Right	Left		
Ankle Reflexes:	_____	_____	(3) use highest value____/4	nn12

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

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

Reduced TNS score: tick in the box :

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

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

Neuropathy classification according to TNS:

Symptomatic DSP= a ≥1 and b≥1 or c≥1 or d≥1 or significant touch-evoked pain yes/ no _____ n20

Asymptomatic DSP= TNS ≥3 yes/ no _____ nn21

Indeterminate DSP= a ≥1 only yes/ no _____ nn22

Punch Biopsy performed yes/ no _____ nn23

Examination shows another form of peripheral neuropathy (not symmetric distal sensory) yes/no_____ nn24
refer for further evaluation to neuromuscular HIV clinic at GSH (1st wed am of every month; 404-3209)

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

Appendix C Patient information sheet

PATIENT INFORMATION SHEET – The study of inflammation and the development of neuropathy in HIV patients in the first 3 months of ARV treatment (McHAART substudy)

You have already been told about the McHAART study. Painful feet may develop in some patients with HIV infection. It is thought that this may be the result of the virus having an effect on the nerves of the feet or that some people are very sensitive to some of the antiretroviral treatments (ARVs). We are trying to understand this problem better so that we can change the treatment in people who may be at risk of developing these problems. We are asking you to consider taking part in this study because you do not have painful feet. We are not expecting you to develop painful feet but we do know that some patients in your position may develop painful feet within the first three months of starting ARVs. We also want to see whether there is a change in the speed with which your brain allows you to do certain movements of the fingers and hands. We do not expect the speed of movement to change but we want to see whether these do change if you also perhaps develop symptoms in your feet. You may not directly benefit from this study but we are hoping that the information from this study will benefit people like you in the future.

You do not have to take part in the substudy- please feel free to ask questions or to say no. This study is part of the main McHAART study, but if you want to only take part in the main study, you may do so. In the main study Dr van der Watt will ask you about symptoms in your feet and examine the nerves in your legs (10 minutes). He will also do 3 brain function tests such as checking your memory by asking you to remember four words and test how fast you can perform certain movement of your fingers and hands (5 minutes).

For the substudy you will only need to fill in a sheet of questions about possible symptoms in your feet at 2 of your regular Crossroads clinic visits; the visit at 14 days after starting ARV's, and the visit after 1 month. At the one-month visit Dr van der Watt will again examine your feet briefly and ask you to do the movements of your fingers and hands to check how fast you can do it- both these will be repeated again at three months. At both these 2 visits (14 days and 1 month) Dr van der Watt will also take blood from you for special tests at the university laboratory to study the way your blood cells are reacting to the new ARV treatments; normally bloods will not be taken at the 2 week visit although blood

University of Cape Town

may be taken at the 1 month visit to monitor for side effects. We will only need 10 mL (about 2 teaspoons) of blood for these tests. To compensate you for your time (filling in the questions and for the blood sample) and help with your transport we will give you R50 for the two-week visit and R50 for the 1 month visit.

If you do want to take part in this substudy the following will happen;

- First visit – McHAART study visit at Groote Schuur Hospital. We will organise and pay for your transport to Groote Schuur Hospital and back; we will give you R150, as you will spend the morning with study related activities.
- Two weeks later at your regular clinic visit at Crossroads we will do a questionnaire to ask about your feet and take 10 mL (2 teaspoons) of blood (Dr Van der Watt).
- The one-month visit at Crossroads – we will do the same procedure as in the two-week visit. We will ask you to answer a few questions about your feet and we will take 10 mL (2 teaspoons) of blood but we will also spend 15 minutes examining your feet and movement in hands.
- The three-month visit will be the regular longer visit of the McHAART study at Groote Schuur Hospital where you will have an examination and blood tests. You will be given R150 for transport at this visit.

Trained people speaking both English and Xhosa will help with all these questions. Your participation in all these visits is very important as we wish to study how your body reacts to the ARVs at each visit. If you want to re-schedule a visit please contact Dr van der Watt at 084 954 3305

University of Cape Town

_____	_____	_____
Name	Signature	Date
_____	_____	_____
Study Doctor	Signature	Date

Appendix D International HIV Dementia Scale (IHDS)

INTERNATIONAL HIV DEMENTIA SCALE (IHDS)

Patient Number: _____ **Date of Visit (DD/MM/YYYY): ___/___/20__**

Visit: _____

Total International HIV Dementia Scale Score: This is the sum of the scores on items 1-3. The maximum possible score is 12 points. A patient with a score of ≤ 10 should be evaluated further for possible dementia.

Memory-Registration – *Give four words to recall (dog, hat, carrot, red) – 1 second to say each. Then ask the patient all four words after you have said them. Repeat words if the patient does not recall them all immediately. Tell the patient you will ask for recall of the words again a bit later.*

1. Motor Speed: Tap the first two fingers of the non-dominant hand as widely and as quickly as possible.

4 = 15 in 5 seconds

3 = 11-14 in 5 seconds

2 = 7-10 in 5 seconds

1 = 3-6 in 5 seconds

0 = 0-2 in 5 seconds

___/4 nn25

2. Psychomotor Speed: *Perform the following movements with the non-dominant hand as quickly as possible:*

1) Clench hand in fist on flat surface.

2) Put hand flat on surface with palm down.

3) Put hand perpendicular to flat surface on the side of the 5th digit. Demonstrate and have patient perform twice for practice.

4 = 4 sequences in 10 seconds

3 = 3 sequences in 10 seconds

2 = 2 sequences in 10 seconds

1 = 1 sequence in 10 seconds

0 = unable to perform

`

___/4 nn26

3. Memory-Recall: *Ask the patient to recall the four words. For words not recalled, prompt with a semantic clue as follows: animal (dog); piece of clothing (hat); vegetable (carrot); color (red).*

Give 1 point for each word spontaneously recalled.

Give 0.5 points for each correct answer after prompting

Maximum – 4 points.

___/4 nn27

TOTAL ___/12 nn28

Appendix E Clinic folder review form

LONGITUDINAL STUDY CLINIC FOLDER REVIEW

Study number: Clinic folder number:

Date of HIV diagnosis: _____ WHO Stage at commencement of cART: _____

Sex: Male / Female Date of Birth (dd/mm/yyyy): _____

cART REGIMEN

Date Started	Date Stopped	Drugs and dosages							Reason stopped		
		D4T	TDF	3TC	EFV	NVP	AZT	Other	Failure	Toxicity	Other
1											
2											
3											
4											

What toxicity? 1.) _____

2.) _____

MEDICATION AND DOSAGES

Vit BCO	Bactrim
Pyridoxine	Other

TB HISTORY

Previous TB	Yes	No	When
Current TB	Yes	No	When

BLOOD RESULTS

	B/L	6 mo			
CD4					
Nadir CD4					
Viral load					
ALT					
Creat					
WCC Hb MCV					
Plt					
CXR					
PPD					

OTHER MEDICATIONS

_____

_____

_____

_____

_____

_____

OTHER COMMENTS

_____

_____

Appendix F DNA extraction procedure

Step 1: Lyse

- Pipet 20 µL QIAGEN Protease (or Proteinase K) into the bottom of a 1.5 mL microcentrifuge tube.
- Add 200 µL buffy coat sample to the microcentrifuge tube.
- Add 200 µL Buffer AL to the sample. Mix by pulse-vortexing for 15 seconds.
- Incubate at 56°C for 10 min.

Step 2: Lyse

- Add 200 µL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 seconds.
- Apply mixture to the spin column (in a 2 mL collection tube) without wetting the rim, close the cap, and centrifuge at 6000 g for 1 min.
- Place the spin column in a clean 2 mL collection tube, and discard the tube containing the filtrate.

Step 3: Wash (Buffer AW1)

- Open the spin column and add 500 µL Buffer AW1 without wetting the rim.
- Close the cap and centrifuge at 6000 g for 1 min.
- Place the spin column in a clean 2 mL collection tube, and discard the collection tube containing the filtrate.

Step 4: Wash (Buffer AW2)

- Open the spin column and add 500 µL Buffer AW2 without wetting the rim.
- Close the cap and centrifuge at full speed (20 000 g for 3 min).

Step 5: Elute

- Place the spin column in a clean 1.5 mL microcentrifuge tube, and discard the collection tube containing the filtrate. Open the spin column and add 200 µL Buffer AE or distilled water.
- Incubate at room temperature for 30 min, and then centrifuge at 6000 g for 1 min.

University of Cape Town

Appendix G Odds ratios in univariate analysis

Table G-1: Odds ratios for baseline DSP risk factors – DSP compared to No DSP

Variable	Odds Ratio in univariate analysis	Odds Ratio 95% Confidence Interval	Prevalence Ratio in univariate analysis	Prevalence Ratio 95% Confidence Interval	p-value
Clinical					
Female sex	0.78	0.37 - 1.61	0.82	0.47 - 1.45	0.503
Age > 40 years	0.34	1.39 - 6.37	0.45	1.31 - 3.78	***0.006***
IHDS score < 10	3.33	1.28 - 8.67	2.01	0.71 - 5.64	***0.016***
Previous/Current TB	1.98	0.85 - 4.66	1.71	0.89 - 3.26	0.122
Current TB	2.39	1.03 - 5.56	1.88	1.07 - 3.33	***0.048***
Previous TB	1.98	0.85 - 4.66	1.71	0.89 - 3.26	0.122
Time of TB					0.227
*Never**	1.00		1.00		
Currently	2.93	1.20 - 7.13	2.22	1.19 - 4.14	
< 1 year ago	2.25	0.63 - 8.05	1.86	0.75 - 4.64	
1 year ago	3.37	0.53 - 21.54	2.42	0.77 - 7.64	
2 years ago	1.26	0.13 - 11.94	1.21	0.20 - 7.33	
> 2 years ago	1.68	0.49 - 5.78	1.51	0.59 - 3.88	
Vitamin B6 supplementation	4.83	1.63 - 14.33	2.79	1.58 - 4.93	***0.019***
Alcohol last year	0.55	0.23 - 1.28	0.62	0.30 - 1.24	0.149
Waist : Hip ratio > 0.9	2.20	1.01 - 4.77	1.80	1.03 - 3.14	***0.046***
Diastolic BP > 80 mmHg	0.62	0.13 - 2.91	0.68	0.18 - 2.49	0.542
Haematological					
C-reactive protein > 5 mg/L	1.58	0.71 - 3.52	1.42	0.77 - 2.61	0.264
MCV > 92 fL	1.86	0.84 - 4.13	1.64	0.86 - 3.15	0.125
Biochemical					
ALT > 19 IU/L	2.56	1.21 - 5.39	2.09	1.15 - 3.80	***0.014***
Metabolic					
Fasting insulin > 5.1 µU/mL	N/A		N/A		
Triglycerides > 0.9 mmol/L	1.69	0.80 - 3.59	1.52	0.84 - 2.76	0.169
Lactate > 2 mmol/L	0.60	0.27 - 1.34	0.67	0.36 - 1.25	0.210
Pyridoxine					
4-PA > 17.4 nmol/L	1.71	0.80 - 3.66	1.52	0.83 - 2.77	0.169

* Reference category

Table G-2: Odds ratios for baseline symptomatic DSP risk factors - SDSP compared to No DSP

Variable	Odds Ratio in univariate analysis	Odds Ratio 95% confidence interval	Prevalence Ratio in univariate analysis	Prevalence Ratio 95% confidence interval	p-value
Clinical					
Female sex	0.85	0.37 - 1.96	0.87	0.44 - 1.72	0.693
Age > 40 years	2.97	1.28 - 6.93	2.35	1.25 - 4.44	***0.014***
IHDS score < 10	1.45	0.42 - 4.99	1.39	0.48 - 4.02	0.560
Previous/Current TB	2.83	1.28 - 6.26	2.32	1.22 - 4.42	***0.010***
Current TB	3.11	1.27 - 7.60	2.38	1.26 - 4.51	***0.016***
Previous TB	2.11	0.79 - 5.60	1.86	0.84 - 4.13	0.143
Time of TB					0.097
*Never**	1.00		1.00		
Currently	3.89	1.49 - 10.15	2.89	1.41 - 5.91	
< 1 year ago	2.46	0.59 - 10.28	2.10	0.69 - 6.33	
1 year ago	4.92	0.75 - 32.28	3.35	1.02 - 11.01	
2 years ago	1.85	0.19 - 17.81	1.68	0.27 - 10.41	
> 2 years ago	1.23	0.25 - 6.13	1.20	0.30 - 4.76	
Vitamin B6 supplementation	7.13	2.34 - 21.72	3.86	2.08 - 7.16	***0.003***
Alcohol last year	0.55	0.21 - 1.43	0.60	0.26 - 1.38	0.199
Waist : Hip ratio > 0.9	2.40	1.02 - 5.66	2.01	1.04 - 3.88	***0.045***
Systolic BP > 120 mmHg	0.66	0.08 - 5.53	0.70	0.11 - 4.50	0.698
Diastolic BP > 80 mmHg	0.84	0.18 - 3.99	0.86	0.23 - 3.22	0.824
Haematological					
C-reactive protein > 5 mg/L	1.46	0.58 - 3.64	1.36	0.65 - 2.88	0.420
Haemoglobin < 11.7 g/dL	1.28	0.58 - 2.83	1.23	0.64 - 2.35	0.536
MCV > 92 fL	1.55	0.69 - 3.48	1.43	0.73 - 2.81	0.293
Biochemical					
Albumin < 37 g/L	0.34	0.14 - 0.85	0.41	0.19 - 0.89	***0.021***
ALT > 19 IU/L	2.43	1.05 - 5.60	2.09	1.03 - 4.22	***0.037***
Metabolic					
Fasting insulin > 5.1 µU/mL	N/A		N/A		
Triglycerides > 0.9 mmol/L	2.16	0.92 - 5.07	1.90	0.93 - 3.90	0.076
LDL > 2.4 mmol/L	0.78	0.34 - 1.78	0.81	0.40 - 1.62	0.551
Lactate > 2 mmol/L	0.48	0.20 - 1.16	0.54	0.26 - 1.12	0.101
Pyridoxine					
4-PA > 17.4 nmol/L	1.55	0.66 - 3.65	1.44	0.71 - 2.92	0.313

* Reference category

Table G-3: Odds ratios for baseline symptomatic DSP risk factors – SDSP vs No SDSP

Variable	Odds Ratio in univariate analysis	Odds Ratio 95% Confidence Interval	Prevalence Ratio in univariate analysis	Prevalence Ratio 95% Confidence Interval	p-value
Clinical					
Female sex	0.88	0.38 - 2.00	0.89	0.45 - 1.79	0.754
Age > 40 years	2.71	1.18 - 6.23	2.22	1.17 - 4.23	***0.022***
Previous/Current TB	2.77	1.26 - 6.09	2.31	1.21 - 4.41	***0.011***
Current TB	3.17	1.30 - 7.69	2.44	1.28 - 4.65	***0.014***
Previous TB	2.02	0.77 - 5.33	1.81	0.81 - 4.05	0.163
Time of TB					0.084
*Never**	1.00		1.00		
Currently	3.92	1.51 - 10.18	2.95	1.44 - 6.06	
< 1 year ago	2.35	0.57 - 9.67	2.04	0.67 - 6.24	
1 year ago	5.23	0.80 - 34.28	3.54	1.08 - 11.62	
2 years ago	1.96	0.20 - 18.91	1.77	0.28 - 10.99	
> 2 years ago	1.12	0.23 - 5.50	1.11	0.27 - 4.46	
Vitamin B6 supplementation	7.67	2.52 - 23.36	4.11	2.22 - 7.64	***0.002***
Alcohol last year	0.57	0.22 - 1.48	0.62	0.27 - 1.42	0.226
Waist : Hip ratio > 0.9	2.31	0.99 - 5.39	1.96	1.01 - 3.82	0.053
Systolic BP > 120 mmHg	0.54	0.07 - 4.43	0.59	0.09 - 3.88	0.566
Diastolic BP > 80 mmHg	0.90	0.19 - 4.30	0.92	0.25 - 3.43	0.898
Haematological					
C-reactive protein > 5 mg/L	1.38	0.56 - 3.43	1.31	0.62 - 2.79	0.482
Haemoglobin < 11.7 g/dL	1.34	0.61 - 2.95	1.28	0.66 - 2.46	0.461
MCV > 92 fL	1.58	0.70 - 3.54	1.47	0.74 - 2.89	0.267
Biochemical					
Albumin < 37 g/L	0.32	0.13 - 0.80	0.39	0.18 - 0.84	***0.014***
Metabolic					
Fasting insulin > 5.1 µU/mL	N/A		N/A		
Triglycerides > 0.9 mmol/L	2.19	0.94 - 5.12	1.94	0.94 - 3.98	0.070
LDL > 2.4 mmol/L	0.71	0.31 - 1.63	0.75	0.38 - 1.51	0.426
Lactate > 2 mmol/L	0.47	0.19 - 1.13	0.53	0.26 - 1.10	0.091
Pyridoxine					
4-PA > 17.4 nmol/L	1.47	0.63 - 3.44	1.38	0.68 - 2.82	0.374

* Reference category

Appendix H Baseline comparison - SDSP vs No SDSP group

Table H-1: Baseline characteristics in individuals with and without symptomatic DSP at baseline (continuous data)

Variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=184)	SDSP (N=31)	No SDSP (N=153)	
Clinical						
Age	years		33 (26 - 39)	37 (29 - 43)	32 (26 - 37)	***0.019*** β
Weight	kg		61 (54 - 71)	60 (53 - 71)	62 (55 - 71)	0.608 β
Height	metre		1.61 (1.56 - 1.67)	1.62 (1.55 - 1.70)	1.61 (1.56 - 1.66)	0.617 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.2 (20.3 - 27.7)	23.2 (20.2 - 26.1)	23.2 (20.4 - 28.0)	0.647 ε
Waist : Hip ratio		*< 0.90*	0.86 (0.81 - 0.90)	0.87 (0.83 - 0.93)	0.86 (0.81 - 0.89)	***0.019*** β
Systolic BP	mmHg	*120 - 140*	110 (103 - 122)	109 (100 - 122)	111 (104 - 121)	0.178 β
Diastolic BP	mmHg	*80 - 90*	72 (66 - 80)	70 (66 - 78)	73 (67 - 80)	0.113 ε
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	158 (114 - 196)	157 (105 - 199)	158 (114 - 196)	0.465 β
White cell count	$x10^9/L$	*4.0 - 11.0*	5.3 (4.1 - 6.5)	5.1 (3.9 - 6.0)	5.3 (4.1 - 6.8)	0.388 β
C-reactive protein	mg/L	*< 5.0*	2.8 (0.9 - 6.6)	3.8 (1.2 - 11.9)	2.6 (0.9 - 5.9)	0.285 β
Haemoglobin	g/dL	*11.6 - 15.6*	11.8 (10.4 - 12.6)	11.6 (9.6 - 12.9)	11.8 (10.6 - 12.6)	0.100 β
MCV	fL	*80.0 - 100.0*	92.4 (88.5 - 96.1)	93.3 (89.8 - 96.9)	92.1 (87.9 - 95.9)	0.165 β
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 43)	37 (34 - 43)	40 (35 - 43)	0.077 β
ALT	IU/L	*10 - 41*	19 (15 - 26)	23 (18 - 29)	19 (14 - 26)	0.262 β
Creatinine	µmol/L	*53 - 115*	64 (55 - 73)	65 (55 - 75)	64 (55 - 73)	0.732 β
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.5 - 4.9)	4.7 (4.4 - 4.9)	0.776 β
Fasting insulin	µU/mL	*0.2 - 9.4*	5.2 (2.5 - 8.6)	4.3 (1.8 - 7.6)	5.5 (2.5 - 8.9)	0.224 β
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.3)	3.9 (3.0 - 4.4)	3.7 (3.2 - 4.2)	0.263 β
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.7 - 1.2)	1.0 (0.7 - 1.4)	0.8 (0.7 - 1.1)	***0.034*** β
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.75 - 1.13)	0.89 (0.74 - 1.25)	0.97 (0.75 - 1.12)	0.344 β
LDL	mmol/L	*1.0 - 3.0*	2.3 (1.9 - 2.9)	2.2 (1.8 - 2.9)	2.4 (1.9 - 2.9)	0.080 β
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	1.8 (1.2 - 2.8)	2.5 (1.7 - 3.3)	0.072 β
Pyridoxine						
PLP	nmol/L	*> 25.0*	23.8 (16.5 - 38.4)	20.7 (16.5 - 53.7)	24.1 (15.7 - 36.8)	0.512 β
4-PA	nmol/L	*unknown*	17.4 (11.9 - 23.3)	20.7 (12.1 - 28.6)	17.0 (11.6 - 22.4)	0.212 ε

β Student's *t*-test

ε Wilcoxon rank-sum test

Table H-2: Baseline characteristics in individuals with and without symptomatic DSP at baseline (grouped data)

Variable	No. (%)			p-value
	Total (N=184)	SDSP (N=31)	No SDSP (N=153)	
Female sex	129 (70%)	21 (68%)	108 (71%)	0.752 §
Age > 40 years	42 (23%)	12 (40%)	30 (20%)	***0.016*** §
Previous/Current TB	69 (38%)	18 (58%)	51 (33%)	***0.009*** §
Time of TB				***0.033*** ¶
Currently	30 (16%)	10 (32%)	20 (13%)	
< 1 year ago	13 (7%)	3 (10%)	10 (7%)	
1 year ago	5 (3%)	2 (6%)	3 (2%)	
2 years ago	5 (3%)	1 (3%)	4 (3%)	
> 2 years ago	16 (9%)	2 (6%)	14 (9%)	
Vit Bco supplement	179 (97%)	31 (100%)	148 (97%)	0.591 ¶
Vit B6 supplement	15 (8%)	8 (26%)	7 (5%)	***<0.001*** ¶
WHO clinical stage				0.310 ¶
Stage 1	57 (32%)	8 (27%)	49 (33%)	
Stage 2	58 (32%)	7 (23%)	51 (34%)	
Stage 3	58 (32%)	13 (43%)	45 (30%)	
Stage 4	7 (4%)	2 (7%)	5 (3%)	
CD4 T-cell count				0.814 §
< 100 cells/mm ^{3}	30 (17%)	4 (13%)	26 (17%)	
100 - 200 cells/mm ^{3}	108 (60%)	18 (60%)	90 (60%)	
> 200 cells/mm ^{3}	42 (23%)	8 (27%)	34 (23%)	
Metabolic syndrome	22 (12%)	3 (10%)	19 (12%)	0.668 §
Body Mass Index				0.876 §
< 20 kg/m ^{2}	37 (21%)	7 (24%)	30 (20%)	
20 - 25 kg/m ^{2}	77 (43%)	11 (38%)	66 (44%)	
25 - 30 kg/m ^{2}	37 (21%)	7 (24%)	30 (20%)	
> 30 kg/m ^{2}	28 (16%)	4 (14%)	24 (16%)	
Alcohol last year	52 (29%)	6 (20%)	46 (31%)	0.239 §
IHDS score < 10	10 (7%)	1 (7%)	9 (7%)	1.000 ¶

¶ Fisher's exact test

§ χ^2 test

Table H-3: Multivariate analysis of baseline risk factors for Symptomatic DSP vs No symptomatic DSP

Model	Odds Ratio	Odds Ratio 95% confidence interval	Variable p-value	Pseudo R^2	Model p-value
SDSP vs No SDSP				0.28	***<0.001***
*Age **	1.11	1.03 - 1.19	***<0.001***		
Previous/Current TB	9.16	2.22 - 37.81	***<0.001***		
Triglycerides > 0.9 mmol/L	5.26	1.35 - 20.52	***0.020***		
Sex (Male)	3.91	1.00 - 15.24	***0.050***		

* Per one year increase

Appendix I IHDS score comparison across DSP groups

Table I-1: Baseline IHDS score comparison between different DSP groups

Variable	Mean ± SD			p-value	
	Total (N=137)	**SDSP (N=15)**	**No SDSP (N=122)**		
Motor Speed	3.7 ± 0.5	3.7 ± 0.5	3.8 ± 0.5	0.484	β
Psychomotor Speed	3.4 ± 0.8	3.5 ± 0.6	3.4 ± 0.8	0.580	β
Memory Recall	3.7 ± 0.6	3.8 ± 0.4	3.7 ± 0.6	0.864	β
IHDS Score	11.3 ± 1.1	10.8 ± 1.4	11.4 ± 1.0	0.851	β
	Total (N=137)	**DSP (N=22)**	**No DSP (N=115)**		
Motor Speed	3.7 ± 0.5	3.6 ± 0.5	3.8 ± 0.4	0.083	β
Psychomotor Speed	3.4 ± 0.8	3.1 ± 1.1	3.5 ± 0.7	***0.035***	β
Memory Recall	3.7 ± 0.6	3.7 ± 0.5	3.8 ± 0.6	0.623	β
IHDS Score	11.3 ± 1.1	10.4 ± 1.7	11.0 ± 1.1	***0.026***	β
	DSP (N=22)	**ADSP (N=7)**	**SDSP (N=15)**		
Motor Speed	3.6 ± 0.5	3.4 ± 0.5	3.7 ± 0.5	0.313	β
Psychomotor Speed	3.1 ± 1.1	2.1 ± 1.3	3.5 ± 0.6	***0.003***	β
Memory Recall	3.7 ± 0.5	3.5 ± 0.8	3.8 ± 0.4	0.298	β
IHDS Score	10.4 ± 1.7	9.1 ± 2.0	11.0 ± 1.1	***0.009***	β
	Total (N=130)	**SDSP (N=15)**	**No DSP (N=115)**		
Motor Speed	3.8 ± 0.4	3.7 ± 0.5	3.8 ± 0.4	0.383	β
Psychomotor Speed	3.5 ± 0.7	3.5 ± 0.6	3.5 ± 0.7	0.815	β
Memory Recall	3.8 ± 0.6	3.8 ± 0.4	3.8 ± 0.6	0.931	β
IHDS Score	11.0 ± 1.1	10.8 ± 1.4	11.5 ± 0.8	0.882	β
	Total (N=122)	**ADSP (N=7)**	**No DSP (N=115)**		
Motor Speed	3.8 ± 0.5	3.4 ± 0.5	3.8 ± 0.4	***0.049***	β
Psychomotor Speed	3.4 ± 0.8	2.1 ± 1.3	3.5 ± 0.7	***<0.001***	β
Memory Recall	3.7 ± 0.6	3.5 ± 0.8	3.8 ± 0.6	0.309	β
IHDS Score	10.9 ± 1.3	9.1 ± 2.0	11.5 ± 0.8	***<0.001***	β

β Student's *t*-test

Appendix JComparison between excluded individuals and those that completed the study

Table J-1: Baseline characteristics - Excluded individuals compared to those who completed 24-week follow-up (continuous data)

Variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=184)	Excluded (N=40)	Completed (N=144)	
Clinical						
Age	years		33 (26 - 39)	32 (28 - 37)	33 (26 - 39)	0.435 β
Weight	kg		61 (54 - 71)	59 (53 - 69)	62 (55 - 71)	0.380 β
Height	metre		1.61 (1.56 - 1.67)	1.60 (1.56 - 1.65)	1.61 (1.56 - 1.68)	0.850 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.2 (20.3 - 27.7)	22.5 (20.7 - 26.3)	23.4 (20.3 - 28.0)	0.521 ε
Waist : Hip ratio		*< 0.90*	0.86 (0.81 - 0.90)	0.83 (0.80 - 0.87)	0.86 (0.82 - 0.91)	0.195 β
Systolic BP	mmHg	*120 - 140*	110 (103 - 122)	110 (103 - 119)	111 (103 - 122)	0.865 β
Diastolic BP	mmHg	*80 - 90*	72 (66 - 80)	74 (67 - 79)	72 (66 - 79)	0.813 ε
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	158 (114 - 196)	152 (104 - 196)	158 (121 - 195)	0.862 β
White cell count	$x10^9/L$	*4.0 - 11.0*	5.3 (4.1 - 6.5)	5.5 (4.6 - 6.5)	5.2 (4.0 - 6.5)	0.863 β
C-reactive protein	mg/L	*< 5.0*	2.8 (0.9 - 6.6)	1.3 (0.7 - 5.6)	3.0 (1.0 - 7.1)	0.149 β
Haemoglobin	g/dL	*11.6 - 15.6*	11.8 (10.4 - 12.6)	11.8 (10.5 - 12.5)	11.8 (10.4 - 12.8)	0.666 β
MCV	fL	*80.0 - 100.0*	92.4 (88.5 - 96.1)	93.6 (87.7 - 96.5)	92.3 (88.5 - 95.8)	0.788 β
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 43)	42 (34 - 43)	39 (35 - 42)	0.503 β
ALT	IU/L	*10 - 41*	19 (15 - 26)	20 (15 - 25)	19 (15 - 27)	0.609 β
Creatinine	µmol/L	*53 - 115*	64 (55 - 73)	62 (51 - 75)	64 (56 - 73)	0.354 β
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.5 - 4.9)	4.7 (4.4 - 4.9)	0.724 β
Fasting insulin	µU/mL	*0.2 - 9.4*	5.2 (2.5 - 8.6)	5.3 (3.1 - 6.7)	5.2 (2.5 - 8.6)	0.666 β
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.3)	3.8 (3.2 - 4.3)	3.7 (3.2 - 4.2)	0.480 β
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.7 - 1.2)	0.8 (0.7 - 1.2)	0.9 (0.6 - 1.2)	0.758 β
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.75 - 1.13)	0.99 (0.77 - 1.20)	0.94 (0.75 - 1.12)	0.338 β
LDL	mmol/L	*1.0 - 3.0*	2.3 (1.9 - 2.9)	2.4 (1.8 - 2.9)	2.3 (2.0 - 2.9)	0.815 β
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	2.4 (1.7 - 3.4)	2.4 (1.7 - 3.1)	0.551 β
Pyridoxine						
PLP	nmol/L	*> 25.0*	23.8 (16.5 - 38.4)	22.9 (13.9 - 38.4)	24.3 (17.1 - 38.4)	0.659 β
4-PA	nmol/L	*unknown*	17.4 (11.9 - 23.3)	14.7 (12.7 - 19.2)	17.6 (11.8 - 24.5)	0.254 ε

β Student's *t*-test

ε Wilcoxon rank-sum test

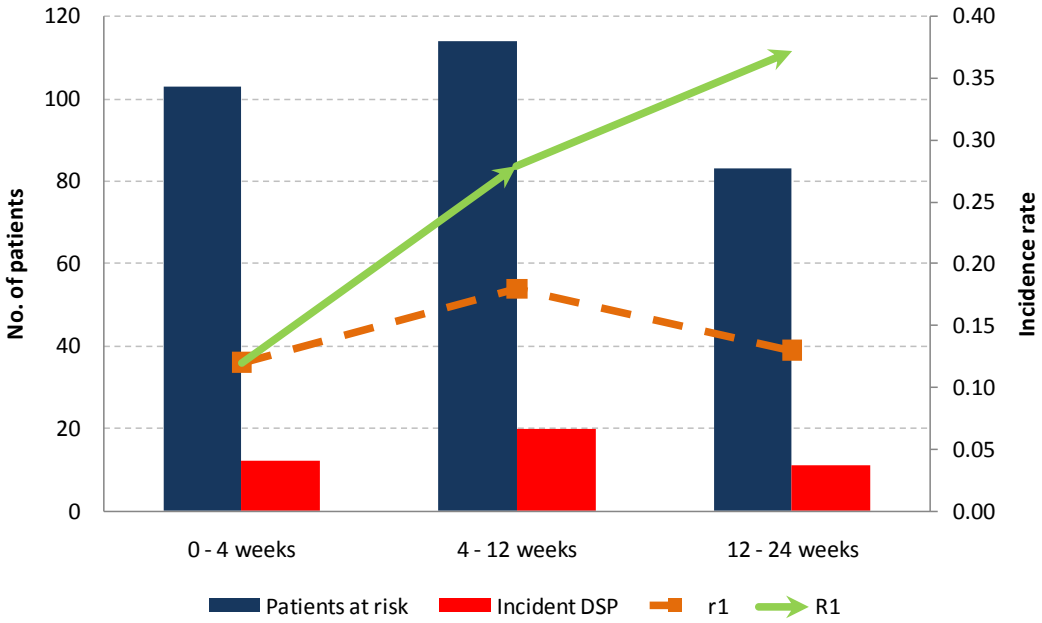
Table J-2: Baseline characteristics - Excluded individuals compared to those who completed 24-week follow-up (grouped data)

Variable	No. (%)			p-value
	Total (N=184)	Excluded (N=40)	Completed (N=144)	
Female sex	129 (70%)	31 (78%)	98 (68%)	0.248 §
Age > 40 years	42 (23%)	7 (18%)	35 (24%)	0.444 §
Previous/Current TB	69 (38%)	18 (45%)	51 (35%)	0.268 §
Time of TB				0.446 ¶
Currently	30 (16%)	9 (23%)	21 (15%)	
< 1 year ago	13 (7%)	2 (5%)	11 (8%)	
1 year ago	5 (3%)	2 (5%)	3 (2%)	
2 years ago	5 (3%)	2 (5%)	3 (2%)	
> 2 years ago	16 (9%)	3 (8%)	13 (9%)	
Vit Bco supplement	179 (97%)	39 (98%)	140 (97%)	1.000 ¶
Vit B6 supplement	15 (8%)	3 (8%)	12 (8%)	0.910 ¶
WHO clinical stage				0.761 ¶
Stage 1	57 (32%)	10 (27%)	47 (33%)	
Stage 2	58 (32%)	14 (38%)	44 (31%)	
Stage 3	58 (32%)	11 (30%)	47 (33%)	
Stage 4	7 (4%)	2 (5%)	5 (4%)	
CD4 T-cell count				0.394 §
< 100 cells/mm ^{3}	30 (17%)	9 (24%)	21 (15%)	
100 - 200 cells/mm ^{3}	108 (60%)	20 (53%)	88 (62%)	
> 200 cells/mm ^{3}	42 (23%)	9 (24%)	33 (23%)	
Body Mass Index				0.323 §
< 20 kg/m ^{2}	37 (21%)	6 (16%)	31 (22%)	
20 - 25 kg/m ^{2}	77 (43%)	20 (54%)	57 (40%)	
25 - 30 kg/m ^{2}	37 (21%)	8 (22%)	29 (20%)	
> 30 kg/m ^{2}	28 (16%)	3 (8%)	25 (18%)	
Alcohol last year	52 (29%)	10 (26%)	42 (30%)	0.694 §
IHDS score < 10	33 (24%)	9 (28%)	24 (23%)	0.542 §
Neuropathy status				
Symptomatic DSP	31 (17%)	7 (18%)	24 (17%)	0.901 §
Asymptomatic DSP	10 (5%)	1 (3%)	9 (6%)	0.370 ¶
Symptoms only	7 (4%)	2 (5%)	5 (3%)	0.693 ¶
DSP	41 (22%)	8 (20%)	33 (23%)	0.482 §

¶ Fisher's exact test

§ χ^2 test

Appendix K DSP incidence rates



Interval (weeks)	Patients at risk	Incident DSP	r_1	R_1
0 – 4	103	12	0.12	0.12
4 – 12	114	20	0.18	0.28
12 – 24	83	11	0.13	0.37

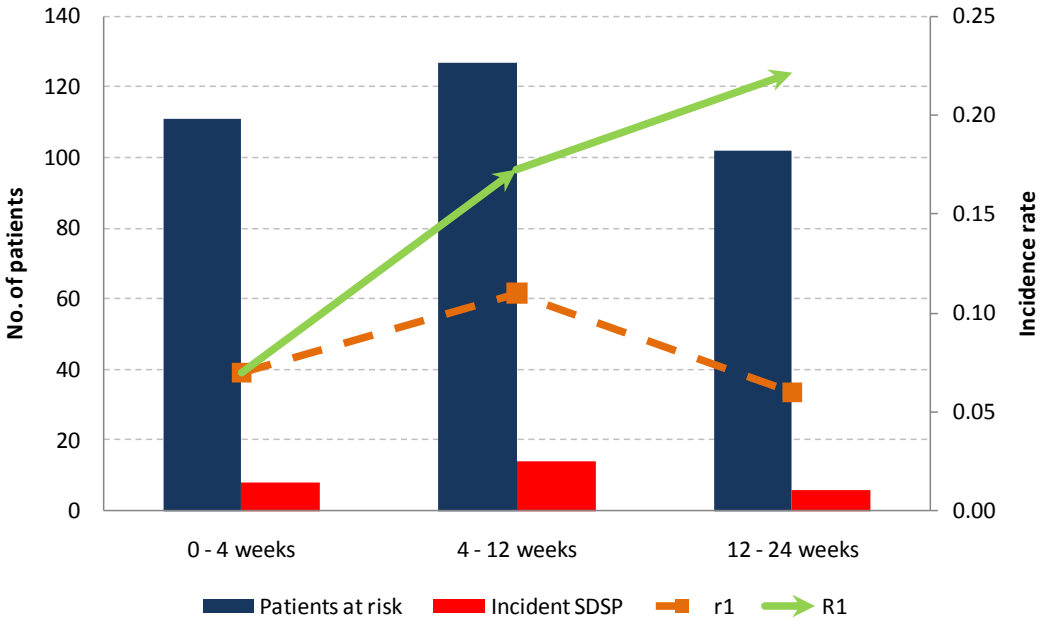
r_1 is calculated as the ratio of the number of patients who had development of DSP to the number of patients at risk during the interval

R_1 is the cumulative rate of development of DSP by end of interval

Patients at risk are calculated as individuals at the specific interval without DSP

Figure K-1: Incidence rates for DSP

University of Cap



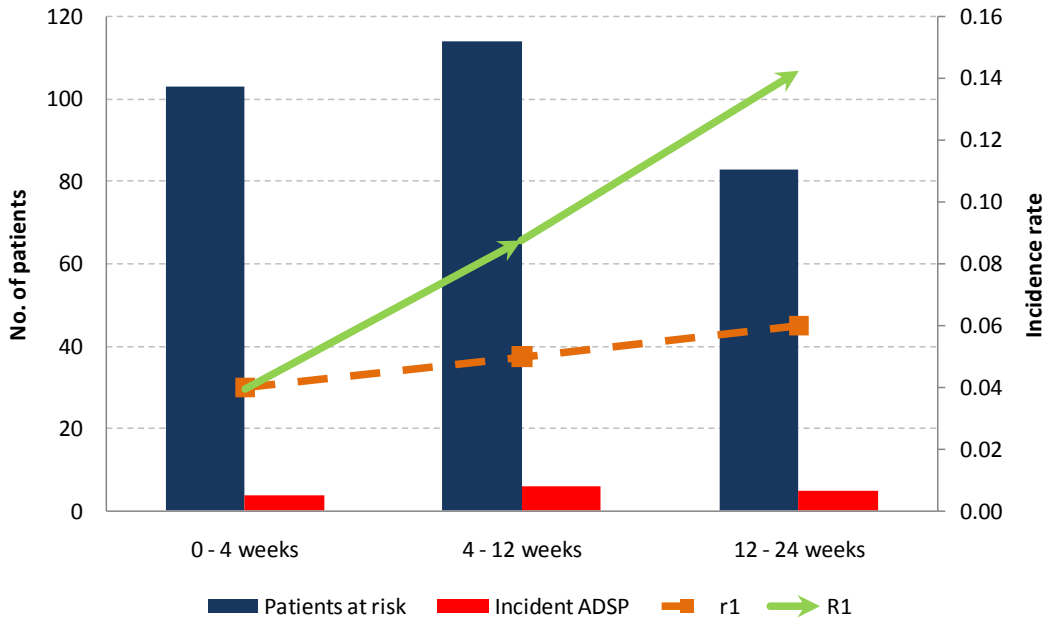
Interval (weeks)	Patients at risk	Incident SDSP	r_1	R_1
0 – 2	111	8	0.07	0.07
4 – 12	127	14	0.11	0.17
12 - 24	102	6	0.06	0.22

r_1 is calculated as the ratio of the number of patients who had development of SDSP to the number of patients at risk during the interval

R_1 is the cumulative rate of development of SDSP by end of the interval

Figure K-2: Incidence rates for symptomatic DSP

University of Cape Town



Interval (weeks)	Patients at risk	Incident ADSP	r_1	R_1
0 – 4	103	4	0.04	0.04
4 – 12	114	6	0.05	0.09
12 - 24	83	5	0.06	0.14

r_1 is calculated as the ratio of the number of patients who had development of ADSP to the number of patients at risk during the interval

R_1 is the cumulative rate of development of ADSP by end of the interval

Figure K-3: Incidence rates for asymptomatic DSP

University of Cape Town

Appendix LDSP movements for baseline to 24-week transitions

Table L-1: Transition from baseline to week 24 showing interim movements between DSP states

Overall transition from Baseline to Week 24

		Baseline				
		No DSP	ADSP	SDSP	Symptoms	Total
Week 24	No DSP	76 (53%)[a]	5 (3%)	4 (3%)	4 (3%)	**89 (62%)**
	ADSP	18 (13%)[b]	4 (3%)	1 (1%)	0 (0%)	**23 (16%)**
	SDSP	10 (7%)[c]	0 (0%)	19 (13%)	1 (1%)	**30 (21%)**
	Symptoms	2 (1%)	0 (0%)	0 (0%)	0 (0%)	**2 (1%)**
	Total	**106 (74%)**	**9 (6%)**	**24 (17%)**	**5 (3%)**	**144 (100%)**
Baseline count		**136**	**10**	**31**	**7**	

[a,b,c] Transition between interim visits (Week 4 to week 12) shown below

[a] DSP-free at Baseline and DSP-free at Week 24

		Week 4					
		No DSP	ADSP	SDSP	Symptoms	Missed	Total
Week 12	No DSP	51		1	2	10	**64**
	ADSP	2					**2**
	SDSP	2		1	1	2	**6**
	Symptoms	1					**1**
	Missed	2				1	**3**
	Total	**58**	**0**	**2**	**3**	**13**	**76**

[b] DSP-free at Baseline and ADSP at Week 24

		Week 4					
		No DSP	ADSP	SDSP	Symptoms	Missed	Total
Week 12	No DSP	4	2			3	**9**
	ADSP	3				1	**4**
	SDSP	2	1	2			**5**
	Symptoms						**0**
	Missed						**0**
	Total	**9**	**3**	**2**	**0**	**4**	**18**

[c] DSP-free at Baseline and SDSP at Week 24

		Week 4					
		No DSP	ADSP	SDSP	Symptoms	Missed	Total
Week 12	No DSP	3					**3**
	ADSP	2					**2**
	SDSP		1	2		2	**5**
	Symptoms						**0**
	Missed						**0**
	Total	**5**	**1**	**2**	**0**	**2**	**10**

Appendix M Neuropathy comparisons – Baseline & 24-week factors

Table M-1: Baseline characteristics in individuals with and without incident symptoms (continuous data)

Baseline variable	Unit	Normal range	Median (IQR) Total (N=120)	Incident Symptoms** (N=28)	No Symptoms** (N=92)	p-value	
Clinical							
Age	years		32 (26 - 38)	34 (26 - 40)	31 (27 - 38)	0.678	β
Weight	kg		62 (56 - 71)	61 (56 - 69)	63 (56 - 72)	0.623	β
Height	metre		1.61 (1.56 - 1.67)	1.61 (1.57 - 1.64)	1.61 (1.56 - 1.69)	0.373	β
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.3 (20.3 - 28.0)	23.6 (20.6 - 27.8)	23.3 (20.3 - 28.0)	0.995	ε
Waist : Hip ratio		*< 0.90*	0.86 (0.81 - 0.90)	0.85 (0.81 - 0.88)	0.86 (0.81 - 0.90)	0.662	β
Systolic BP	mmHg	*120 - 140*	111 (103 - 122)	109 (100 - 120)	111 (104 - 122)	0.473	β
Diastolic BP	mmHg	*80 - 90*	72 (66 - 81)	72 (67 - 78)	73 (66 - 81)	0.737	ε
Haematological							
CD4 T-cell count	$cells/mm^3$	*> 500*	158 (118 - 193)	138 (114 - 193)	163 (125 - 193)	0.630	β
White cell count	$x10^9/L$	*4.0 - 11.0*	5.1 (4.0 - 6.6)	5.4 (4.3 - 7.1)	5.1 (4.0 - 6.6)	0.445	ε
C-reactive protein	mg/L	*< 5.0*	2.7 (0.9 - 6.7)	2.9 (0.9 - 6.7)	2.6 (0.9 - 6.5)	0.664	β
Haemoglobin	g/dL	*11.6 - 15.6*	11.8 (10.6 - 12.8)	11.5 (9.7 - 12.8)	11.9 (10.7 - 12.8)	0.376	β
MCV	fL	*80.0 - 100.0*	92.1 (88.5 - 96.4)	91.6 (87.9 - 97.0)	92.2 (88.5 - 95.8)	0.762	β
Biochemical							
Albumin	g/L	*38 - 54*	39 (35 - 43)	39 (35 - 41)	39 (35 - 43)	0.627	β
ALT	IU/L	*10 - 41*	19 (14 - 26)	16 (12 - 20)	20 (15 - 28)	***0.003***	β
Creatinine	µmol/L	*53 - 115*	64 (56 - 73)	64 (58 - 77)	64 (55 - 73)	0.490	ε
Metabolic							
Fasting glucose	mmol/L	*4.1 - 5.6*	4.6 (4.4 - 4.9)	4.7 (4.4 - 4.8)	4.6 (4.4 - 4.9)	0.828	ε
Fasting insulin	µU/mL	*0.2 - 9.4*	5.4 (2.4 - 8.6)	4.8 (2.2 - 7.6)	5.5 (2.5 - 8.9)	0.419	β
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.2)	3.5 (3.0 - 4.2)	3.7 (3.3 - 4.2)	0.282	β
Triglycerides	mmol/L	*0.5 - 2.0*	0.8 (0.6 - 1.1)	0.8 (0.6 - 1.1)	0.9 (0.6 - 1.0)	0.361	β
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.74 - 1.11)	0.86 (0.71 - 1.04)	0.99 (0.74 - 1.13)	0.100	β
LDL	mmol/L	*1.0 - 3.0*	2.3 (2.0 - 2.9)	2.3 (1.9 - 2.9)	2.4 (2.0 - 2.9)	0.567	β
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	2.5 (1.7 - 3.3)	2.4 (1.7 - 3.0)	0.970	β
Pyridoxine							
PLP	nmol/L	*> 25.0*	24.4 (17.8 - 36.7)	21.4 (18.0 - 32.3)	26.8 (17.6 - 38.5)	0.444	β
4-PA	nmol/L	*unknown*	17.5 (11.6 - 24.5)	17.0 (11.5 - 22.1)	17.7 (12.0 - 27.3)	0.535	ε

β Student's *t*-test

ε Wilcoxon rank-sum test

** SDSP at baseline cohort excluded

Table M-2: Week 24 characteristics in individuals with and without incident symptoms (continuous data)

Week 24 variable	Unit	Normal range	Median (IQR) Total (N=120)	Incident Symptoms** (N=28)	No Symptoms** (N=92)	p-value	
Clinical							
Weight	kg		66 (57 - 75)	65 (56 - 70)	66 (57 - 75)	0.643	β
Height	metre		1.61 (1.57 - 1.67)	1.61 (1.56 - 1.64)	1.61 (1.57 - 1.69)	0.387	β
Body Mass Index	kg/m^2	*20.0 - 25.0*	24.4 (20.8 - 29.3)	25.0 (20.8 - 29.2)	24.3 (20.8 - 29.5)	0.887	ε
Waist : Hip ratio		*< 0.90*	0.84 (0.79 - 0.88)	0.83 (0.78 - 0.86)	0.85 (0.80 - 0.88)	0.473	ε
Systolic BP	mmHg	*120 - 140*	115 (107 - 123)	115 (109 - 124)	115 (107 - 122)	0.569	β
Diastolic BP	mmHg	*80 - 90*	73 (69 - 79)	74 (71 - 78)	73 (69 - 80)	0.786	β
Haematological							
CD4 T-cell count	$cells/mm^3$	*> 500*	261 (186 - 364)	298 (210 - 457)	260 (179 - 351)	0.110	β
C-reactive protein *	mg/L	*< 5.0*	4.6 (1.2 - 9.0)	4.6 (1.6 - 8.6)	4.7 (1.0 - 10.3)	0.714	ε
Virological							
HIV viral load	copies/mL		20 (20 - 36)	20 (20 - 44)	20 (20 - 36)	0.861	ε
Metabolic							
Fasting glucose	mmol/L	*4.1 - 5.6*	4.9 (4.7 - 5.2)	4.9 (4.6 - 5.2)	4.9 (4.7 - 5.2)	0.775	ε
Fasting insulin	µU/mL	*0.2 - 9.4*	5.6 (2.4 - 8.7)	4.7 (2.8 - 7.9)	5.9 (2.0 - 8.7)	0.519	ε
Total cholesterol	mmol/L	*3.1 - 5.2*	4.4 (3.9 - 5.0)	4.2 (3.7 - 5.2)	4.4 (3.9 - 4.9)	0.859	β
Triglycerides	mmol/L	*0.5 - 2.0*	0.8 (0.6 - 1.1)	0.9 (0.7 - 1.1)	0.8 (0.6 - 1.0)	0.532	ε
HDL	mmol/L	*1.15 - 1.68*	1.39 (1.12 - 1.63)	1.28 (0.99 - 1.55)	1.44 (1.21 - 1.72)	***0.038***	β
LDL	mmol/L	*1.0 - 3.0*	2.5 (2.0 - 3.0)	2.7 (2.2 - 3.3)	2.4 (2.0 - 2.9)	0.341	β
Pyridoxine							
PLP *	nmol/L	*> 25.0*	21.6 (13.7 - 34.7)	22.6 (12.9 - 32.3)	21.6 (14.7 - 35.6)	0.197	β
4-PA *	nmol/L	*unknown*	19.2 (12.5 - 28.0)	18.8 (15.1 - 23.3)	19.2 (12.2 - 30.3)	0.759	ε

* 12-week blood samples

** SDSP at baseline cohort excluded

β Student's *t*-test

ε Wilcoxon rank-sum test

University of Cape Town

Table M-3: Baseline and week 24 characteristics in individuals with and without incident symptoms (grouped data)

Variable	No. (%) Total (N=120)	Incident Symptoms** (N=28)	No Symptoms** (N=92)	p-value	
Baseline factors					
Female sex	82 (68%)	20 (71%)	62 (67%)	0.688	§
Age > 40 years	24 (20%)	7 (25%)	17 (18%)	0.450	§
Previous/Current TB	42 (35%)	7 (25%)	35 (38%)	0.205	§
Time of TB					
Currently	10 (8%)	0 (0%)	10 (11%)	0.155	¶
< 1 year ago	17 (14%)	2 (7%)	15 (16%)	0.155	¶
1 year ago	2 (2%)	1 (4%)	1 (1%)	0.155	¶
2 years ago	2 (2%)	0 (0%)	2 (2%)	0.155	¶
> 2 years ago	11 (9%)	4 (14%)	7 (8%)	0.155	¶
Vit Bco supplement	119 (99%)	28 (100%)	91 (99%)	1.000	¶
Vit B6 supplement	6 (5%)	1 (4%)	5 (5%)	1.000	¶
WHO clinical stage					
Stage 1	39 (33%)	8 (29%)	31 (34%)	0.114	¶
Stage 2	39 (33%)	14 (50%)	25 (27%)	0.114	¶
Stage 3	37 (31%)	5 (18%)	32 (35%)	0.114	¶
Stage 4	4 (3%)	1 (4%)	3 (3%)	0.114	¶
CD4 T-cell count					
< 100 cells/mm^3	20 (17%)	5 (18%)	15 (17%)	0.969	§
100 - 200 cells/mm^3	74 (63%)	17 (61%)	57 (63%)	0.969	§
> 200 cells/mm^3	24 (20%)	6 (21%)	18 (20%)	0.969	§
Metabolic syndrome	16 (13%)	5 (18%)	11 (12%)	0.525	¶
Body Mass Index					
< 20 kg/m^2	26 (22%)	6 (21%)	20 (22%)	0.838	§
20 - 25 kg/m^2	48 (41%)	11 (39%)	37 (41%)	0.838	§
25 - 30 kg/m^2	23 (19%)	7 (25%)	16 (18%)	0.838	§
> 30 kg/m^2	21 (18%)	4 (14%)	17 (19%)	0.838	§
Alcohol last year	36 (31%)	9 (32%)	27 (30%)	0.830	§
IHDS score < 10	14 (10%)	3 (11%)	11 (10%)	1.000	¶
24 week factors					
CD4 T-cell count				0.561	¶
< 100 cells/mm^3	5 (5%)	0 (0%)	5 (6%)		
100 - 200 cells/mm^3	25 (23%)	6 (22%)	19 (23%)		
> 200 cells/mm^3	79 (72%)	21 (78%)	58 (71%)		
HIV viral load					
< 200 copies/mL	76 (71%)	18 (67%)	58 (73%)		
> 200 copies/mL	31 (29%)	9 (33%)	22 (28%)		
Antiretroviral drugs					
Stavudine	67 (56%)	18 (64%)	49 (53%)	0.304	§
Zidovudine	14 (12%)	2 (7%)	12 (13%)	0.516	¶
Lamivudine	120 (100%)	28 (100%)	92 (100%)		
Efavirenz	44 (37%)	7 (25%)	37 (40%)	0.143	§
Nevirapine	76 (63%)	21 (75%)	55 (60%)	0.143	§
Tenofovir	39 (33%)	8 (29%)	31 (34%)	0.612	§
Drug combination				0.335	¶
3TC / NVP / TDF	20 (17%)	5 (18%)	15 (16%)		
3TC / EFV / TDF	19 (16%)	3 (11%)	16 (17%)		
AZT / 3TC / NVP	11 (9%)	1 (4%)	10 (11%)		
AZT / 3TC / EFV	3 (3%)	1 (4%)	2 (2%)		
D4T / 3TC / NVP	45 (38%)	15 (54%)	30 (33%)		
D4T / 3TC / EFV	22 (18%)	3 (11%)	19 (21%)		

** SDSP at baseline cohort excluded

¶ Fisher's exact test

§ χ^2 test

Table M-4: Baseline characteristics in individuals with and without HIV-SN (continuous data)

Baseline variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=144)	HIV-SN (N=48)	No HIV-SN (N=96)	
Clinical						
Age	years		33 (27 - 39)	35 (27 - 43)	32 (27 - 38)	0.099 [β]
Weight	kg		62 (55 - 71)	61 (55 - 68)	63 (56 - 72)	0.456 [β]
Height	metre		1.61 (1.56 - 1.68)	1.62 (1.58 - 1.68)	1.60 (1.56 - 1.68)	0.483 [β]
Body Mass Index	kg/m^2	*20.0 - 25.0*	23.4 (20.3 - 28.0)	23.1 (20.1 - 26.0)	23.6 (20.3 - 28.3)	0.324 [ε]
Waist : Hip ratio		*< 0.90*	0.86 (0.82 - 0.91)	0.87 (0.83 - 0.92)	0.86 (0.81 - 0.90)	0.284 [β]
Systolic BP	mmHg	*120 - 140*	111 (103 - 122)	109 (100 - 118)	111 (104 - 123)	0.118 [β]
Diastolic BP	mmHg	*80 - 90*	72 (66 - 79)	70 (66 - 76)	73 (66 - 83)	0.088 [ε]
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	159 (121 - 193)	159 (121 - 212)	160 (118 - 191)	0.508 [β]
White cell count	$x10^9/L$	*4.0 - 11.0*	5.1 (4.0 - 6.5)	5.4 (4.0 - 6.3)	5.0 (4.0 - 7.0)	0.865 [ε]
C-reactive protein	mg/L	*< 5.0*	3.0 (1.0 - 7.1)	3.7 (1.4 - 11.9)	2.6 (0.9 - 6.5)	0.125 [β]
Haemoglobin	g/dL	*11.6 - 15.6*	11.8 (10.5 - 12.8)	11.9 (10.2 - 12.9)	11.7 (10.5 - 12.6)	0.886 [β]
MCV	fL	*80.0 - 100.0*	92.4 (88.5 - 96.0)	93.3 (89.3 - 97.0)	92.1 (88.5 - 95.5)	0.385 [β]
Biochemical						
Albumin	g/L	*38 - 54*	39 (35 - 42)	39 (35 - 43)	39 (35 - 42)	0.800 [β]
ALT	IU/L	*10 - 41*	19 (14 - 27)	19 (14 - 26)	20 (14 - 28)	0.365 [β]
Creatinine	µmol/L	*53 - 115*	64 (56 - 73)	65 (58 - 74)	64 (55 - 73)	0.443 [ε]
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.7 (4.4 - 4.9)	4.7 (4.4 - 4.9)	4.6 (4.4 - 4.9)	0.192 [ε]
Fasting insulin	µU/mL	*0.2 - 9.4*	5.2 (2.5 - 8.6)	4.9 (3.1 - 9.3)	5.3 (2.0 - 8.4)	0.859 [β]
Total cholesterol	mmol/L	*3.1 - 5.2*	3.7 (3.2 - 4.2)	3.9 (3.2 - 4.3)	3.7 (3.2 - 4.2)	0.986 [β]
Triglycerides	mmol/L	*0.5 - 2.0*	0.9 (0.6 - 1.2)	0.9 (0.6 - 1.3)	0.9 (0.7 - 1.1)	0.815 [β]
HDL	mmol/L	*1.15 - 1.68*	0.94 (0.75 - 1.12)	0.93 (0.75 - 1.08)	0.97 (0.74 - 1.12)	0.284 [β]
LDL	mmol/L	*1.0 - 3.0*	2.3 (2.0 - 2.9)	2.5 (2.0 - 2.9)	2.3 (2.0 - 2.8)	0.765 [β]
Lactate	mmol/L	*< 2.0*	2.4 (1.7 - 3.1)	2.1 (1.4 - 3.3)	2.4 (1.7 - 3.0)	0.427 [β]
Pyridoxine						
PLP	nmol/L	*> 25.0*	24.3 (17.1 - 38.4)	20.7 (16.6 - 46.8)	26.4 (17.4 - 36.8)	0.903 [β]
4-PA	nmol/L	*unknown*	17.6 (11.8 - 24.5)	17.4 (11.9 - 22.7)	17.7 (11.3 - 25.5)	0.998 [ε]

[β] Student's *t*-test

[ε] Wilcoxon rank-sum test

Table M-5: Week 24 characteristics in individuals with and without HIV-SN (continuous data)

Week 24 variable	Unit	Normal range	Median (IQR)			p-value
			Total (N=144)	HIV-SN (N=48)	No HIV-SN (N=96)	
Clinical						
Weight	kg		66 (57 - 75)	66 (56 - 70)	66 (58 - 78)	0.341 β
Height	metre		1.61 (1.57 - 1.68)	1.63 (1.58 - 1.68)	1.61 (1.57 - 1.68)	0.446 β
Body Mass Index	kg/m^2	*20.0 - 25.0*	24.4 (21.0 - 29.2)	24.1 (20.8 - 26.9)	24.8 (21.5 - 30.1)	0.180 ε
Waist : Hip ratio		*< 0.90*	0.85 (0.80 - 0.89)	0.85 (0.82 - 0.91)	0.84 (0.79 - 0.88)	0.254 ε
Systolic BP	mmHg	*120 - 140*	115 (109 - 124)	115 (109 - 125)	115 (107 - 122)	0.771 β
Diastolic BP	mmHg	*80 - 90*	74 (69 - 80)	75 (69 - 80)	73 (69 - 80)	0.547 β
Haematological						
CD4 T-cell count	$cells/mm^3$	*> 500*	272 (204 - 368)	311 (236 - 400)	260 (175 - 351)	***0.018*** β
C-reactive protein *	mg/L	*< 5.0*	4.5 (1.3 - 9.7)	4.5 (1.6 - 11.4)	4.5 (1.0 - 9.0)	0.426 ε
Virological						
HIV viral load	copies/mL		20 (20 - 56)	20 (20 - 73)	20 (20 - 47)	0.353 ε
Metabolic						
Fasting glucose	mmol/L	*4.1 - 5.6*	4.9 (4.7 - 5.2)	5.0 (4.7 - 5.2)	4.9 (4.7 - 5.2)	0.325 ε
Fasting insulin	µU/mL	*0.2 - 9.4*	5.5 (2.5 - 8.5)	5.0 (2.9 - 8.0)	5.9 (2.2 - 8.7)	0.625 ε
Total cholesterol	mmol/L	*3.1 - 5.2*	4.4 (3.9 - 5.1)	4.4 (3.8 - 5.2)	4.4 (3.9 - 4.9)	0.731 β
Triglycerides	mmol/L	*0.5 - 2.0*	0.8 (0.7 - 1.1)	0.9 (0.7 - 1.1)	0.8 (0.6 - 1.0)	0.196 ε
HDL	mmol/L	*1.15 - 1.68*	1.41 (1.12 - 1.64)	1.38 (1.08 - 1.60)	1.44 (1.17 - 1.72)	0.457 β
LDL	mmol/L	*1.0 - 3.0*	2.5 (2.0 - 2.9)	2.5 (2.1 - 3.1)	2.4 (2.0 - 2.9)	0.421 β
Pyridoxine						
PLP *	nmol/L	*> 25.0*	20.9 (13.3 - 34.7)	19.2 (11.2 - 33.2)	22.4 (14.7 - 34.8)	0.210 β
4-PA *	nmol/L	*unknown*	19.2 (12.5 - 27.7)	17.2 (12.4 - 23.3)	19.5 (12.5 - 30.5)	0.349 ε

* 12-week blood samples

β Student's *t*-test

ε Wilcoxon rank-sum test

Table M-6: Baseline and 24-week characteristics in individuals with and without HIV-SN (grouped data)

Variable	No. (%)			p-value
	Total (N=144)	HIV-SN (N=48)	No HIV-SN (N=96)	
Baseline factors				
Female sex	98 (68%)	31 (65%)	67 (70%)	0.527 §
Age > 40 years	35 (24%)	17 (35%)	18 (19%)	***0.028*** §
Previous/Current TB	57 (40%)	21 (44%)	36 (38%)	0.470 §
Time of TB				0.058 ¶
Currently	11 (8%)	1 (2%)	10 (10%)	
< 1 year ago	26 (18%)	12 (25%)	14 (15%)	
1 year ago	4 (3%)	3 (6%)	1 (1%)	
2 years ago	3 (2%)	2 (4%)	1 (1%)	
> 2 years ago	13 (9%)	3 (6%)	10 (10%)	
Vit Bco supplement	143 (99%)	48 (100%)	95 (99%)	1.000 ¶
Vit B6 supplement	6 (4%)	2 (4%)	4 (4%)	1.000 ¶
WHO clinical stage				0.539 ¶
Stage 1	46 (32%)	14 (29%)	32 (34%)	
Stage 2	43 (30%)	16 (33%)	27 (28%)	
Stage 3	49 (34%)	15 (31%)	34 (36%)	
Stage 4	5 (4%)	3 (6%)	2 (2%)	
CD4 T-cell count				0.266 §
< 100 cells/mm^3	22 (15%)	5 (10%)	17 (18%)	
100 - 200 cells/mm^3	88 (62%)	29 (60%)	59 (63%)	
> 200 cells/mm^3	32 (23%)	14 (29%)	18 (19%)	
Metabolic syndrome	17 (12%)	3 (6%)	14 (15%)	0.178 ¶
Body Mass Index				0.693 §
< 20 kg/m^2	31 (22%)	12 (25%)	19 (20%)	
20 - 25 kg/m^2	57 (40%)	20 (42%)	37 (39%)	
25 - 30 kg/m^2	29 (20%)	10 (21%)	19 (20%)	
> 30 kg/m^2	25 (18%)	6 (13%)	19 (20%)	
Alcohol last year	42 (30%)	12 (25%)	30 (32%)	0.393 §
IHDS score < 10	14 (10%)	9 (20%)	5 (5%)	***0.013*** ¶
Week 24 factors				
CD4 T-cell count				0.088 ¶
< 100 cells/mm^3	5 (4%)	0 (0%)	5 (6%)	
100 - 200 cells/mm^3	27 (21%)	6 (14%)	21 (24%)	
> 200 cells/mm^3	98 (75%)	38 (86%)	60 (70%)	
HIV viral load < 200 copies/mL	87 (68%)	27 (61%)	60 (71%)	0.246 §
Antiretroviral drugs				
Stavudine	81 (56%)	29 (60%)	52 (54%)	0.476 §
Zidovudine	17 (12%)	4 (8%)	13 (14%)	0.424 ¶
Lamivudine	144 (100%)	48 (100%)	96 (100%)	
Efavirenz	60 (42%)	23 (48%)	37 (39%)	0.282 §
Nevirapine	83 (58%)	24 (50%)	59 (61%)	0.190 §
Tenofovir	47 (33%)	16 (33%)	31 (32%)	0.900 §
Drug combination				0.075 ¶
3TC / NVP / TDF	23 (16%)	8 (17%)	15 (16%)	
3TC / EFV / TDF	23 (16%)	7 (15%)	16 (17%)	
AZT / 3TC / TDF	1 (1%)	1 (2%)	0 (0%)	
AZT / 3TC / NVP	11 (8%)	0 (0%)	11 (11%)	
AZT / 3TC / EFV	5 (3%)	3 (6%)	2 (2%)	
D4T / 3TC / NVP	49 (34%)	16 (33%)	33 (34%)	
D4T / 3TC / EFV	32 (22%)	13 (27%)	19 (20%)	

¶ Fisher's exact test

§ χ^2 test

Table M-7: Univariate Cox regression analysis of baseline risk factors for ATN

Clinical variable	Hazard Ratio in univariate analysis	Hazard Ratio 95% Confidence Interval	p-value
Female sex	0.88	0.43 - 1.81	0.726
Age > 40 years	1.38	0.65 - 2.92	0.404
Previous/Current TB	0.71	0.34 - 1.47	0.357
WHO clinical stage			
*Stage 1**	1.00		
Stage 2	1.87	0.89 - 3.92	0.100
Stage 3	0.60	0.22 - 1.62	0.315
Stage 4	0.99	0.13 - 7.67	0.992
IHDS score < 10	0.50	0.19 - 1.29	0.153
Alcohol last year	0.82	0.40 - 1.69	0.584
ALT > 19 IU/L	0.51	0.25 - 1.04	0.062
HDL < 1.0 mmol/L	1.85	0.94 - 3.67	0.077

* reference category

Table M-8: Univariate Cox regression analysis of baseline risk factors for incident symptoms

Clinical variable	Hazard Ratio in univariate analysis	Hazard Ratio 95% Confidence Interval	p-value
Female sex	0.85	0.41 - 1.75	0.654
Age > 40 years	1.30	0.59 - 2.85	0.510
Previous/Current TB	0.59	0.28 - 1.21	0.149
WHO clinical stage			
*Stage 1**	1.00		
Stage 2	1.88	0.90 - 3.96	0.095
Stage 3	0.54	0.20 - 1.46	0.223
Stage 4	0.78	0.10 - 6.07	0.816
IHDS score < 10	0.51	0.20 - 1.31	0.160
Alcohol last year	0.92	0.44 - 1.89	0.814
ALT > 19 IU/L	0.51	0.25 - 1.04	0.063
HDL < 1.0 mmol/L	1.87	0.95 - 3.71	0.072

* reference category

University of Cape Town

Appendix N Longitudinal analysis of possible neuropathy risk factors (random effects model)

Table N-1: Longitudinal risk factor analysis for incident symptoms – random effects model

Variable	Group effect*				
	Incident Symptoms** Median	No symptoms Median	Coefficient	Confidence Interval (95%)	p-value
CD4 T-cell count					
Baseline	138	163			0.630 β
Week 24	298	260	43.12	(-4.65 ; 90.90)	0.077
Fasting glucose					
Baseline	4.7	4.6			0.828 ε
Week 12	4.9	4.8	0.01	(-0.22 ; 0.25)	0.933
Week 24	4.9	4.9	-0.06	(-0.30 ; 0.17)	0.603
Insulin					
Baseline	4.8	5.5			0.419 β
Week 12	5.0	5.3	0.52	(-1.83 ; 2.87)	0.663
Week 24	4.7	5.9	-0.07	(-2.45 ; 2.30)	0.951
Total cholesterol					
Baseline	3.5	3.7			0.282 β
Week 12	3.9	4.2	-0.02	(-0.31 ; 0.27)	0.883
Week 24	4.2	4.4	0.14	(-0.16 ; 0.43)	0.360
Triglycerides					
Baseline	0.8	0.9			0.361 β
Week 12	0.8	0.8	-0.15	(-0.49 ; 0.18)	0.377
Week 24	0.9	0.8	0.09	(-0.24 ; 0.43)	0.585
HDL					
Baseline	0.86	0.99			0.100 β
Week 12	1.19	1.33	-0.04	(-0.19 ; 0.12)	0.645
Week 24	1.28	1.44	-0.10	(-0.26 ; 0.05)	0.204
LDL					
Baseline	2.3	2.4			0.567 β
Week 12	2.4	2.4	0.09	(-0.16 ; 0.34)	0.474
Week 24	2.7	2.4	0.22	(-0.03 ; 0.46)	0.088
Systolic BP					
Baseline	109	111			0.473 β
Week 12	112	115	2.22	(-3.03 ; 7.47)	0.408
Week 24	115	115	-0.59	(-5.88 ; 4.71)	0.827
Diastolic BP					
Baseline	72	73			0.737 ε
Week 12	73	73	0.00	(-3.90 ; 3.91)	0.998
Week 24	74	73	0.51	(-3.43 ; 4.45)	0.800
Weight					
Baseline	61	63			0.623 β
Week 12	62	65	0.61	(-1.00 ; 2.23)	0.458
Week 24	65	66	0.79	(-0.84 ; 2.42)	0.343
Body Mass Index					
Baseline	23.6	23.3			0.995 ε
Week 12	25.0	24.4	0.54	(-0.19 ; 1.26)	0.147
Week 24	25.0	24.3	0.23	(-0.50 ; 0.96)	0.529
Waist : Hip Ratio					
Baseline	0.85	0.86			0.662 β
Week 12	0.83	0.85	-0.01	(-0.07 ; 0.06)	0.848
Week 24	0.83	0.85	-0.02	(-0.08 ; 0.04)	0.518
C-reactive protein					
Baseline	2.9	2.4			0.664 β
Week 12	2.9	2.4	2.12	(-11.85 ; 16.09)	0.766
PLP					
Baseline	21.4	26.8			0.444 β
Week 12	22.6	21.6	-3.61	(-15.97 ; 8.74)	0.566
4PA					
Baseline	17.0	17.7			0.535 ε
Week 12	18.8	19.2	-2.13	(-13.71 ; 9.45)	0.718

* Group effect is the difference in mean concentrations between groups compared to baseline

** SDSP at baseline cohort excluded

β Student's *t*-test

ε Wilcoxon rank-sum test

Table N-2: Longitudinal risk factor analysis for HIV-SN – random effects model

Variable	Group effect*				
	HIV-SN Median	No HIV-SN Median	Coefficient	Confidence Interval (95%)	p-value
CD4 T-cell count					
Baseline	159	160			0.508 β
Week 24	311	260	44.74	(4.16 ; 85.32)	***0.031***
Fasting glucose					
Baseline	4.7	4.6			0.192 ε
Week 12	5.0	4.8	0.08	(-0.11 ; 0.28)	0.407
Week 24	5.0	4.9	0.00	(-0.20 ; 0.20)	0.993
Insulin					
Baseline	4.9	5.3			0.859 β
Week 12	5.3	5.3	-0.20	(-2.18 ; 1.78)	0.842
Week 24	5.0	5.9	-0.14	(-2.14 ; 1.85)	0.890
Total cholesterol					
Baseline	3.9	3.7			0.986 β
Week 12	4.1	4.2	-0.05	(-0.29 ; 0.19)	0.686
Week 24	4.4	4.4	0.07	(-0.18 ; 0.32)	0.576
Triglycerides					
Baseline	0.9	0.9			0.815 β
Week 12	0.8	0.8	0.23	(-0.05 ; 0.51)	0.114
Week 24	0.9	0.8	0.08	(-0.21 ; 0.36)	0.602
HDL					
Baseline	0.93	0.97			0.284 β
Week 12	1.24	1.35	0.00	(-0.13 ; 0.12)	0.943
Week 24	1.38	1.44	0.00	(-0.13 ; 0.13)	0.969
LDL					
Baseline	2.5	2.3			0.765 β
Week 12	2.4	2.4	-0.09	(-0.30 ; 0.12)	0.405
Week 24	2.5	2.4	0.08	(-0.13 ; 0.29)	0.432
Systolic BP					
Baseline	109	111			0.118 β
Week 12	118	114	7.28	(2.92 ; 11.64)	***0.001***
Week 24	115	115	4.68	(0.33 ; 9.04)	***0.035***
Diastolic BP					
Baseline	70	73			0.088 ε
Week 12	72	73	2.94	(-0.31 ; 6.18)	0.076
Week 24	75	73	4.91	(1.66 ; 8.15)	***0.003***
Weight					
Baseline	61	63			0.456 β
Week 12	62	65	0.30	(-1.08 ; 1.68)	0.669
Week 24	66	66	0.36	(-1.01 ; 1.72)	0.610
Body Mass Index					
Baseline	23.1	23.6			0.324 ε
Week 12	23.6	24.5	0.10	(-0.52 ; 0.72)	0.748
Week 24	24.1	24.8	0.06	(-0.55 ; 0.67)	0.850
Waist : Hip Ratio					
Baseline	0.87	0.86			0.284 β
Week 12	0.86	0.85	-0.01	(-0.06 ; 0.05)	0.846
Week 24	0.85	0.84	0.04	(-0.01 ; 0.10)	0.093
C-reactive protein					
Baseline	3.7	2.6			0.125 β
Week 12	4.5	4.5	1.26	(-10.55 ; 13.08)	0.834
PLP					
Baseline	20.7	26.4			0.903 β
Week 12	19.2	22.4	-7.57	(-17.77 ; 2.63)	0.146
4PA					
Baseline	17.4	17.7			0.998 ε
Week 12	17.2	19.5	1.75	(-7.87 ; 11.38)	0.721

* Group effect is the difference in mean concentrations between groups compared to baseline

β Student's *t*-test

ε Wilcoxon rank-sum test

Appendix O NAT2 acetylation phenotype comparisons

Table O-1: NAT2 acetylation phenotype distribution between individuals with and without DSP

Variable	No. (%)			p-value
	Total (N=163)	DSP (N=39)	No DSP (N=124)	
Acetylation Classification 1				0.790 §
Rapid	27 (17%)	7 (18%)	20 (16%)	
Slow	136 (83%)	32 (82%)	104 (84%)	
Acetylation Classification 2				0.899 ¶
Rapid	27 (17%)	7 (18%)	20 (16%)	
Intermediate	123 (75%)	30 (77%)	93 (75%)	
Slow	13 (8%)	2 (5%)	11 (9%)	
Acetylation Classification 3				0.376 ¶
Rapid	27 (17%)	7 (18%)	20 (16%)	
Intermediate	88 (54%)	18 (46%)	70 (56%)	
Slow/Intermediate	35 (21%)	12 (31%)	23 (19%)	
Slow	13 (8%)	2 (5%)	11 (9%)	

¶ Fisher's exact test

§ χ^2 test

Table O-2: NAT2 acetylation phenotype distribution between ADSP and No DSP

Variable	No. (%)			p-value
	Total (N=163)	ADSP (N=9)	No DSP (N=154)	
Acetylation Classification 1				0.639 §
Rapid	27 (17%)	2 (22%)	25 (16%)	
Slow	136 (83%)	7 (78%)	129 (84%)	
Acetylation Classification 2				0.844 ¶
Rapid	27 (17%)	2 (22%)	25 (16%)	
Intermediate	123 (75%)	7 (78%)	116 (75%)	
Slow	13 (8%)	0 (0%)	13 (8%)	
Acetylation Classification 3				0.643 ¶
Rapid	27 (17%)	2 (22%)	25 (16%)	
Intermediate	88 (54%)	4 (44%)	84 (55%)	
Slow/Intermediate	35 (21%)	3 (33%)	32 (21%)	
Slow	13 (8%)	0 (0%)	13 (8%)	

¶ Fisher's exact test

§ χ^2 test

University of Cape Town

Table O-3: NAT2 acetylation phenotype distribution between individuals with and without SDSP

Variable	No. (%)			p-value
	Total (N=163)	SDSP (N=30)	No SDSP (N=133)	
Acetylation Classification 1				0.987 §
Rapid	27 (17%)	5 (17%)	22 (17%)	
Slow	136 (83%)	25 (83%)	111 (83%)	
Acetylation Classification 2				1.000 ¶
Rapid	27 (17%)	5 (17%)	22 (17%)	
Intermediate	123 (75%)	23 (77%)	100 (75%)	
Slow	13 (8%)	2 (6%)	11 (8%)	
Acetylation Classification 3				0.623 ¶
Rapid	27 (17%)	5 (17%)	22 (16%)	
Intermediate	88 (54%)	14 (46%)	74 (56%)	
Slow/Intermediate	35 (21%)	9 (30%)	26 (20%)	
Slow	13 (8%)	2 (7%)	11 (8%)	

¶ Fisher's exact test
§ χ^2 test

Table O-4: NAT2 acetylation phenotype distribution between individuals with and without HIV-SN

Variable	No. (%)			p-value
	Total (N=157)	HIV-SN (N=44)	No HIV-SN (N=113)	
Acetylation Classification 1				0.790 §
Rapid	27 (17%)	7 (16%)	20 (18%)	
Slow	130 (83%)	37 (84%)	93 (82%)	
Acetylation Classification 2				0.911 ¶
Rapid	27 (17%)	7 (16%)	20 (18%)	
Intermediate	117 (75%)	34 (77%)	83 (73%)	
Slow	13 (8%)	3 (7%)	10 (9%)	
Acetylation Classification 3				0.899 ¶
Rapid	27 (17%)	7 (16%)	20 (18%)	
Intermediate	84 (54%)	23 (52%)	61 (54%)	
Slow/Intermediate	33 (21%)	11 (25%)	22 (19%)	
Slow	13 (8%)	3 (7%)	10 (9%)	

¶ Fisher's exact test
§ χ^2 test

University of Cape Town

Table O-5: Allele distribution between individuals with and without DSP shown in 3 genetic models

General Genetic Model

SNP	DSP n (%)	No DSP n (%)	Total n (%)	p-value	
191 G>A				0.250	¶
GG	37 (95%)	107 (86%)	144 (88%)		
GA	2 (5%)	17 (14%)	19 (12%)		
AA	0 (0%)	0 (0%)	0 (0%)		
282 C>T				1.000	¶
CC	14 (36%)	46 (37%)	60 (37%)		
CT	21 (54%)	64 (52%)	85 (52%)		
TT	4 (10%)	14 (11%)	18 (11%)		
341 T>C				0.135	¶
TT	16 (41%)	71 (57%)	87 (53%)		
TC	22 (56%)	47 (38%)	69 (42%)		
CC	1 (3%)	6 (5%)	7 (4%)		
481 C>T				0.765	¶
CC	22 (56%)	74 (60%)	96 (59%)		
CT	16 (41%)	44 (35%)	60 (37%)		
TT	1 (3%)	6 (5%)	7 (4%)		
590 G>A				0.799	¶
GG	19 (49%)	67 (54%)	86 (53%)		
GA	19 (49%)	52 (42%)	71 (44%)		
AA	1 (3%)	5 (4%)	6 (4%)		
803 A>G				0.877	§
AA	10 (26%)	27 (22%)	37 (23%)		
AG	20 (51%)	66 (53%)	86 (53%)		
GG	9 (23%)	31 (25%)	40 (25%)		
857 G>A				1.000	¶
GG	39 (100%)	123 (99%)	162 (99%)		
GA	0 (0%)	1 (1%)	1 (1%)		
AA	0 (0%)	0 (0%)	0 (0%)		

Dominant Genetic Model

SNP	DSP n (%)	No DSP n (%)	Total n (%)	p-value	
191 G>A				0.250	¶
GG	37 (95%)	107 (86%)	144 (88%)		
GA+AA	2 (5%)	17 (14%)	19 (12%)		
282 C>T				0.892	§
GG	14 (36%)	46 (37%)	60 (37%)		
GA+AA	25 (64%)	78 (63%)	103 (63%)		
341 T>C				0.076	§
GG	16 (41%)	71 (57%)	87 (53%)		
GA+AA	23 (59%)	53 (43%)	76 (47%)		
481 C>T				0.718	§
GG	22 (56%)	74 (60%)	96 (59%)		
GA+AA	17 (44%)	50 (40%)	67 (41%)		
590 G>A				0.562	§
GG	19 (49%)	67 (54%)	86 (53%)		
GA+AA	20 (51%)	57 (46%)	77 (47%)		
803 A>G				0.615	§
GG	10 (26%)	27 (22%)	37 (23%)		
GA+AA	29 (74%)	97 (78%)	126 (77%)		
857 G>A				1.000	¶
GG	39 (100%)	123 (99%)	162 (99%)		
GA+AA	0 (0%)	1 (1%)	1 (1%)		

Additive Genetic Model

SNP	DSP n (%)	No DSP n (%)	Total n (%)	p-value	
191 G>A				0.265	¶
G	76 (97%)	231 (93%)	307 (94%)		
A	2 (3%)	17 (7%)	19 (6%)		
282 C>T				0.990	§
G	49 (63%)	156 (63%)	205 (63%)		
A	29 (37%)	92 (37%)	121 (37%)		
341 T>C				0.217	§
G	54 (69%)	189 (76%)	243 (75%)		
A	24 (31%)	59 (24%)	83 (25%)		
481 C>T				0.927	§
G	60 (77%)	192 (77%)	252 (77%)		
A	18 (23%)	56 (23%)	74 (23%)		
590 G>A				0.734	§
G	57 (73%)	186 (75%)	243 (75%)		
A	21 (27%)	62 (25%)	83 (25%)		
803 A>G				0.656	§
G	40 (51%)	120 (48%)	160 (49%)		
A	38 (49%)	128 (52%)	166 (51%)		
857 G>A				1.000	¶
G	78 (100%)	247 (100%)	325 (100%)		
A	0 (0%)	1 (0%)	1 (0%)		

¶ Fisher's exact test

§ χ^2 test

Table O-6: Allele distribution between individuals with and without SDSP shown in 3 genetic models

General Genetic Model					*Dominant Genetic Model*					*Additive Genetic Model*				
SNP	**SDSP** n (%)	**No SDSP** n (%)	**Total** n (%)	**p-value**	**SNP**	**SDSP** n (%)	**No SDSP** n (%)	**Total** n (%)	**p-value**	**SNP**	**SDSP** n (%)	**No SDSP** n (%)	**Total** n (%)	**p-value**
191 G>A				0.531 ¶	**191 G>A**				0.531 ¶	**191 G>A**				0.544 ¶
GG	28 (93%)	116 (87%)	144 (88%)		GG	28 (93%)	116 (87%)	144 (88%)		G	58 (97%)	249 (94%)	307 (94%)	
GA	2 (7%)	17 (13%)	19 (12%)		GA+AA	2 (7%)	17 (13%)	19 (12%)		A	2 (3%)	17 (6%)	19 (6%)	
AA	0 (0%)	0 (0%)	0 (0%)											
282 C>T				0.878 ¶	**282 C>T**				0.986 §	**282 C>T**				0.829 §
CC	11 (37%)	49 (37%)	60 (37%)		GG	11 (37%)	49 (37%)	60 (37%)		G	37 (62%)	168 (63%)	205 (63%)	
CT	15 (50%)	70 (53%)	85 (52%)		GA+AA	19 (63%)	84 (63%)	103 (63%)		A	23 (38%)	98 (37%)	121 (37%)	
TT	4 (13%)	14 (11%)	18 (11%)											
341 T>C				0.377 ¶	**341 T>C**				0.222 §	**341 T>C**				0.372 §
TT	13 (43%)	74 (56%)	87 (53%)		GG	13 (43%)	74 (56%)	87 (53%)		G	42 (70%)	201 (76%)	243 (75%)	
TC	16 (53%)	53 (40%)	69 (42%)		GA+AA	17 (57%)	59 (44%)	76 (47%)		A	18 (30%)	65 (24%)	83 (25%)	
CC	1 (3%)	6 (5%)	7 (4%)											
481 C>T				1.000 ¶	**481 C>T**				0.892 §	**481 C>T**				0.833 §
CC	18 (60%)	78 (59%)	96 (59%)		GG	18 (60%)	78 (59%)	96 (59%)		G	47 (78%)	205 (77%)	252 (77%)	
CT	11 (37%)	49 (37%)	60 (37%)		GA+AA	12 (40%)	55 (41%)	67 (41%)		A	13 (22%)	61 (23%)	74 (23%)	
TT	1 (3%)	6 (5%)	7 (4%)											
590 G>A				0.812 ¶	**590 G>A**				0.459 §	**590 G>A**				0.572 §
GG	14 (47%)	72 (54%)	86 (53%)		GG	14 (47%)	72 (54%)	86 (53%)		G	43 (72%)	200 (75%)	243 (75%)	
GA	15 (50%)	56 (42%)	71 (44%)		GA+AA	16 (53%)	61 (46%)	77 (47%)		A	17 (28%)	66 (25%)	83 (25%)	
AA	1 (3%)	5 (4%)	6 (4%)											
803 A>G				0.542 §	**803 A>G**				0.291 §	**803 A>G**				0.310 §
AA	9 (30%)	28 (21%)	37 (23%)		GG	9 (30%)	28 (21%)	37 (23%)		G	33 (55%)	127 (48%)	160 (49%)	
AG	15 (50%)	71 (53%)	86 (53%)		GA+AA	21 (70%)	105 (79%)	126 (77%)		A	27 (45%)	139 (52%)	166 (51%)	
GG	6 (20%)	34 (26%)	40 (25%)											
857 G>A				1.000 ¶	**857 G>A**				1.000 ¶	**857 G>A**				1.000 ¶
GG	30 (100%)	132 (99%)	162 (99%)		GG	30 (100%)	132 (99%)	162 (99%)		G	60 (100%)	265 (100%)	325 (100%)	
GA	0 (0%)	1 (1%)	1 (1%)		GA+AA	0 (0%)	1 (1%)	1 (1%)		A	0 (0%)	1 (0%)	1 (0%)	
AA	0 (0%)	0 (0%)	0 (0%)											

¶ Fisher's exact test

§ χ^2 test

Table O-7: Allele distribution between individuals with and without ATN shown in 3 genetic models

General Genetic Model

SNP	ATN n (%)	No ATN n (%)	Total n (%)	p-value	
191 G>A				0.553	¶
GG	28 (85%)	110 (89%)	138 (88%)		
GA	5 (15%)	14 (11%)	19 (12%)		
AA	0 (0%)	0 (0%)	0 (0%)		
282 C>T				0.782	¶
CC	14 (42%)	43 (35%)	57 (36%)		
CT	16 (48%)	66 (53%)	82 (52%)		
TT	3 (9%)	15 (12%)	18 (11%)		
341 T>C				0.202	¶
TT	14 (42%)	71 (57%)	85 (54%)		
TC	18 (55%)	47 (38%)	65 (41%)		
CC	1 (3%)	6 (5%)	7 (4%)		
481 C>T				0.183	¶
CC	15 (45%)	75 (60%)	90 (57%)		
CT	17 (52%)	43 (35%)	60 (38%)		
TT	1 (3%)	6 (5%)	7 (4%)		
590 G>A				0.612	¶
GG	18 (55%)	66 (53%)	84 (54%)		
GA	15 (45%)	52 (42%)	67 (43%)		
AA	0 (0%)	6 (5%)	6 (4%)		
803 A>G				0.439	§
AA	5 (15%)	32 (26%)	37 (24%)		
AG	19 (58%)	63 (51%)	82 (52%)		
GG	9 (27%)	29 (23%)	38 (24%)		
857 G>A				1.000	¶
GG	33 (100%)	123 (99%)	156 (99%)		
GA	0 (0%)	1 (1%)	1 (1%)		
AA	0 (0%)	0 (0%)	0 (0%)		

Dominant Genetic Model

SNP	ATN n (%)	No ATN n (%)	Total n (%)	p-value	
191 G>A				0.553	¶
GG	28 (85%)	110 (89%)	138 (88%)		
GA+AA	5 (15%)	14 (11%)	19 (12%)		
282 C>T				0.411	§
CC	14 (42%)	43 (35%)	57 (36%)		
CT+TT	19 (58%)	81 (65%)	100 (64%)		
341 T>C				0.129	§
TT	14 (42%)	71 (57%)	85 (54%)		
TC+CC	19 (58%)	53 (43%)	72 (46%)		
481 C>T				0.121	§
CC	15 (45%)	75 (60%)	90 (57%)		
CT+TT	18 (55%)	49 (40%)	67 (43%)		
590 G>A				0.893	§
GG	18 (55%)	66 (53%)	84 (54%)		
GA+AA	15 (45%)	58 (47%)	73 (47%)		
803 A>G				0.200	§
AA	5 (15%)	32 (26%)	37 (24%)		
AG+GG	28 (85%)	92 (74%)	120 (76%)		
857 G>A				1.000	¶
GG	33 (100%)	123 (99%)	156 (99%)		
GA+AA	0 (0%)	1 (1%)	1 (1%)		

Additive Genetic Model

SNP	ATN n (%)	No ATN n (%)	Total n (%)	p-value	
191 G>A				0.564	¶
G	61 (92%)	234 (94%)	295 (94%)		
A	5 (8%)	14 (6%)	19 (6%)		
282 C>T				0.423	§
C	44 (67%)	152 (61%)	196 (62%)		
T	22 (33%)	96 (39%)	118 (38%)		
341 T>C				0.279	§
T	46 (70%)	189 (76%)	235 (75%)		
C	20 (30%)	59 (24%)	79 (25%)		
481 C>T				0.261	§
C	47 (71%)	193 (78%)	240 (76%)		
T	19 (29%)	55 (22%)	74 (24%)		
590 G>A				0.608	§
G	51 (77%)	184 (74%)	235 (75%)		
A	15 (23%)	64 (26%)	79 (25%)		
803 A>G				0.294	§
A	29 (44%)	127 (51%)	156 (50%)		
G	37 (56%)	121 (49%)	158 (50%)		
857 G>A				1.000	¶
G	66 (100%)	247 (100%)	313 (100%)		
A	0 (0%)	1 (0%)	1 (0%)		

¶ Fisher's exact test

§ χ^2 test

Appendix P NAT2 acetylation comparisons after correcting for the effect of previous TB therapy

Table P-1: DSP odds ratios for allelic groups after correcting for previous TB therapy – DSP vs No DSP

General Genetic Model

SNP	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
191 G>A					
GG *	1.00		1.00		
GA	0.28	0.06 - 1.33	0.38	0.10 - 1.45	0.109
AA	N/A		N/A		
282 C>T					
CC *	1.00		1.00		
CT	1.24	0.56 - 2.76	1.13	0.63 - 2.00	0.600
TT	0.86	0.24 - 3.12	0.95	0.37 - 2.46	0.816
341 T>C					
TT *	1.00		1.00		
TC	2.33	1.08 - 5.03	1.72	1.00 - 2.96	***0.032***
CC	0.59	0.06 - 5.48	0.68	0.11 - 4.33	0.645
481 C>T					
CC *	1.00		1.00		
CT	1.28	0.60 - 2.74	1.19	0.69 - 2.04	0.528
TT	0.44	0.05 - 4.03	0.55	0.09 - 3.44	0.471
590 G>A					
GG *	1.00		1.00		
GA	1.44	0.67 - 3.06	1.33	0.78 - 2.28	0.349
AA	0.62	0.07 - 5.89	0.74	0.12 - 4.48	0.680
803 A>G					
AA *	1.00		1.00		
AG	1.05	0.42 - 2.65	1.03	0.54 - 1.96	0.913
GG	0.85	0.29 - 2.46	0.90	0.42 - 1.92	0.762
857 G>A					
GG *	1.00		1.00		
GA	N/A		N/A		
AA	N/A		N/A		

Dominant Genetic Model

SNP	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
191 G>A					
GG *	1.00		1.00		
GA+AA	0.28	0.06 - 1.33	0.38	0.10 - 1.45	0.109
282 C>T					
CC *	1.00		1.00		
CT+TT	1.16	0.54 - 2.50	1.09	0.63 - 1.91	0.710
341 T>C					
TT *	1.00		1.00		
TC+CC	2.07	0.98 - 4.40	1.61	0.93 - 2.78	0.058
481 C>T					
CC *	1.00		1.00		
CT+TT	1.16	0.55 - 2.44	1.11	0.65 - 1.89	0.699
590 G>A					
GG *	1.00		1.00		
GA+AA	1.35	0.64 - 2.83	1.28	0.75 - 2.17	0.429
803 A>G					
AA *	1.00		1.00		
AG+GG	0.98	0.41 - 2.32	0.98	0.53 - 1.80	0.957
857 G>A					
GG *	1.00		1.00		
GA+AA	N/A		N/A		

* Reference category

Table P-2: Symptomatic DSP odds ratios for allelic groups after correcting for previous TB therapy – SDSP vs No DSP

General Genetic Model						*Dominant Genetic Model*					
SNP	**Odds Ratio**	**Odds Ratio 95% CI**	**Prevalence Ratio**	**Prevalence Ratio 95% CI**	**p-value**	**SNP**	**Odds Ratio**	**Odds Ratio 95% CI**	**Prevalence Ratio**	**Prevalence Ratio 95% CI**	**p-value**
191 G>A						**191 G>A**					
GG *	1.00		1.00			GG *	1.00		1.00		
GA	0.41	0.09 - 1.94	0.50	0.13 - 1.91	0.261	GA+AA	0.41	0.09 - 1.94	0.50	0.13 - 1.91	0.261
AA	N/A		N/A								
282 C>T						**282 C>T**					
CC *	1.00		1.00			CC *	1.00		1.00		
CT	1.10	0.45 - 2.65	1.08	0.54 - 2.17	0.839	CT+TT	1.11	0.48 - 2.58	1.11	0.58 - 2.15	0.805
TT	1.18	0.31 - 4.40	1.23	0.46 - 3.30	0.809						
341 T>C						**341 T>C**					
TT *	1.00		1.00			TT *	1.00		1.00		
TC	1.90	0.82 - 4.40	1.49	0.78 - 2.83	0.133	TC+CC	1.75	0.77 - 3.98	1.42	0.75 - 2.69	0.180
CC	0.76	0.08 - 7.16	0.81	0.13 - 5.21	0.814						
481 C>T						**481 C>T**					
CC *	1.00		1.00			CC *	1.00		1.00		
CT	1.01	0.43 - 2.36	0.98	0.51 - 1.89	0.986	CT+TT	0.95	0.42 - 2.17	0.94	0.49 - 1.79	0.903
TT	0.57	0.06 - 5.25	0.66	0.10 - 4.12	0.622						
590 G>A						**590 G>A**					
GG *	1.00		1.00			GG *	1.00		1.00		
GA	1.55	0.67 - 3.57	1.47	0.77 - 2.79	0.303	GA+AA	1.49	0.66 - 3.36	1.42	0.76 - 2.68	0.342
AA	0.92	0.09 - 8.83	1.00	0.16 - 6.12	0.940						
803 A>G						**803 A>G**					
AA *	1.00		1.00			AA *	1.00		1.00		
AG	0.85	0.32 - 2.26	0.88	0.42 - 1.81	0.743	AG+GG	0.75	0.30 - 1.87	0.79	0.40 - 1.55	0.538
GG	0.59	0.18 - 1.90	0.64	0.26 - 1.59	0.374						
857 G>A						**857 G>A**					
GG *	1.00		1.00			GG *	1.00		1.00		
GA	N/A		N/A			GA+AA	N/A		N/A		
AA	N/A		N/A								

* Reference category

Table P-3: Incident symptoms odds ratios for allelic groups after correcting for previous TB therapy – Incident symptoms vs No symptoms

General Genetic Model

SNP	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
191 G>A					
GG *	1.00		1.00		
GA	1.72	0.55 - 5.40	1.49	0.66 - 3.37	0.355
AA	N/A		N/A		
282 C>T					
CC *	1.00		1.00		
CT	0.60	0.24 - 1.46	0.67	0.34 - 1.35	0.258
TT	0.77	0.18 - 3.21	0.79	0.26 - 2.40	0.717
341 T>C					
TT *	1.00		1.00		
TC	1.96	0.83 - 4.65	1.70	0.87 - 3.32	0.126
CC	1.07	0.11 - 10.14	1.09	0.17 - 7.06	0.951
481 C>T					
CC *	1.00		1.00		
CT	2.24	0.94 - 5.32	1.88	0.96 - 3.66	0.069
TT	1.13	0.12 - 10.66	1.14	0.18 - 7.36	0.916
590 G>A					
GG *	1.00		1.00		
GA	1.17	0.50 - 2.74	1.13	0.58 - 2.18	0.720
AA	N/A		N/A		
803 A>G					
AA *	1.00		1.00		
AG	1.36	0.44 - 4.19	1.27	0.51 - 3.17	0.595
GG	1.26	0.35 - 4.50	1.21	0.43 - 3.41	0.726
857 G>A					
GG *	1.00		1.00		
GA	N/A		N/A		
AA	N/A		N/A		

Dominant Genetic Model

SNP	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
191 G>A					
GG *	1.00		1.00		
GA+AA	1.72	0.55 - 5.40	1.49	0.66 - 3.37	0.355
282 C>T					
CC *	1.00		1.00		
CT+TT	0.63	0.27 - 1.46	0.70	0.36 - 1.33	0.278
341 T>C					
TT *	1.00		1.00		
TC+CC	1.87	0.80 - 4.36	1.65	0.85 - 3.20	0.150
481 C>T					
CC *	1.00		1.00		
CT+TT	2.11	0.90 - 4.94	1.81	0.93 - 3.51	0.086
590 G>A					
GG *	1.00		1.00		
GA+AA	1.04	0.45 - 2.43	1.03	0.53 - 2.00	0.923
803 A>G					
AA *	1.00		1.00		
AG+GG	1.32	0.45 - 3.88	1.25	0.52 - 3.01	0.610
857 G>A					
GG *	1.00		1.00		
GA+AA	N/A		N/A		

* Reference category

Table P-4: ATN odds ratios for Allelic odds ratios for allelic groups after correcting for previous TB therapy – ATN vs No ATN

General Genetic Model

SNP	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
191 G>A					
GG *	1.00		1.00		
GA	1.48	0.49 - 4.49	1.34	0.59 - 3.02	0.493
AA	N/A		N/A		
282 C>T					
CC *	1.00		1.00		
CT	0.73	0.32 - 1.65	0.78	0.42 - 1.47	0.449
TT	0.64	0.16 - 2.57	0.69	0.22 - 2.12	0.530
341 T>C					
TT *	1.00		1.00		
TC	1.90	0.86 - 4.21	1.67	0.90 - 3.09	0.112
CC	0.91	0.10 - 8.24	0.96	0.15 - 6.26	0.932
481 C>T					
CC *	1.00		1.00		
CT	1.98	0.90 - 4.39	1.72	0.94 - 3.17	0.090
TT	0.91	0.10 - 8.23	0.96	0.15 - 6.26	0.934
590 G>A					
GG *	1.00		1.00		
GA	1.02	0.47 - 2.22	1.02	0.56 - 1.87	0.968
AA	N/A		N/A		
803 A>G					
AA *	1.00		1.00		
AG	1.75	0.59 - 5.20	1.57	0.63 - 3.94	0.314
GG	1.92	0.57 - 6.41	1.70	0.63 - 4.58	0.292
857 G>A					
GG *	1.00		1.00		
GA	N/A		N/A		
AA	N/A		N/A		

Dominant Genetic Model

SNP	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
191 G>A					
GG *	1.00		1.00		
GA+AA	1.48	0.49 - 4.49	1.34	0.59 - 3.02	0.493
282 C>T					
CC *	1.00		1.00		
CT+TT	0.71	0.32 - 1.57	0.77	0.42 - 1.40	0.400
341 T>C					
TT *	1.00		1.00		
TC+CC	1.80	0.82 - 3.92	1.61	0.87 - 2.96	0.141
481 C>T					
CC *	1.00		1.00		
CT+TT	1.86	0.85 - 4.05	1.66	0.90 - 3.03	0.118
590 G>A					
GG *	1.00		1.00		
GA+AA	0.91	0.42 - 1.99	0.94	0.51 - 1.73	0.821
803 A>G					
AA *	1.00		1.00		
AG+GG	1.80	0.63 - 5.13	1.61	0.67 - 3.91	0.269
857 G>A					
GG *	1.00		1.00		
GA+AA	N/A		N/A		

* Reference category

Table P-5: DSP odds ratios for NAT2 acetylation after correcting for previous TB therapy – DSP vs No DSP

Group	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
Acetylation Classification 1					
Rapid *	1.00		1.00		
Slow	0.97	0.37 - 2.56	0.99	0.50 - 1.97	0.947
Acetylation Classification 2					
Rapid *	1.00		1.00		
Intermediate	1.05	0.39 - 2.80	1.05	0.52 - 2.09	0.928
Slow	0.46	0.08 - 2.69	0.58	0.14 - 2.37	0.389
Acetylation Classification 3					
Rapid *	1.00		1.00		
Intermediate	0.83	0.30 - 2.34	0.89	0.42 - 1.87	0.728
Slow/Intermediate	1.70	0.54 - 5.35	1.39	0.65 - 2.95	0.361
Slow	0.46	0.08 - 2.69	0.58	0.14 - 2.37	0.389

* Reference category

Table P-6: SDSP odds ratios for NAT2 acetylation after correcting for previous TB therapy – SDSP vs No SDSP

Group	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
Acetylation Classification 1					
Rapid *	1.00		1.00		
Slow	1.11	0.37 - 3.29	1.04	0.45 - 2.43	0.856
Acetylation Classification 2					
Rapid *	1.00		1.00		
Intermediate	1.16	0.39 - 3.50	1.08	0.46 - 2.53	0.789
Slow	0.72	0.12 - 4.46	0.78	0.18 - 3.38	0.722
Acetylation Classification 3					
Rapid *	1.00		1.00		
Intermediate	0.96	0.30 - 3.04	0.93	0.38 - 2.30	0.940
Slow/Intermediate	1.75	0.49 - 6.22	1.43	0.56 - 3.66	0.387
Slow	0.72	0.12 - 4.46	0.78	0.18 - 3.39	0.722

* Reference category

University of Cape Town

Table P-7: Incident symptoms odds ratios for NAT2 acetylation after correcting for previous TB therapy –Incident symptoms vs No symptoms

Group	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
Acetylation Classification 1					
*Rapid **	1.00		1.00		
Slow	6.72	0.86 - 52.40	5.28	0.76 - 36.86	0.069
Acetylation Classification 2					
*Rapid **	1.00		1.00		
Intermediate	7.34	0.94 - 57.39	5.65	0.81 - 39.47	0.058
Slow	2.13	0.12 - 37.83	2.03	0.14 - 29.47	0.605
Acetylation Classification 3					
*Rapid **	1.00		1.00		
Intermediate	7.76	0.98 - 61.70	5.87	0.83 - 41.37	0.053
Slow/Intermediate	6.20	0.68 - 56.29	5.03	0.66 - 38.65	0.105
Slow	2.13	0.12 - 37.82	2.03	0.14 - 29.46	0.605

* Reference category

Table P-8: ATN odds ratios for NAT2 acetylation after correcting for previous TB therapy ATN vs No ATN

Group	Odds Ratio	Odds Ratio 95% CI	Prevalence Ratio	Prevalence Ratio 95% CI	p-value
Acetylation Classification 1					
*Rapid **	1.00		1.00		
Slow	2.30	0.64 - 8.22	2.03	0.67 - 6.16	0.199
Acetylation Classification 2					
*Rapid **	1.00		1.00		
Intermediate	2.52	0.70 - 9.04	2.17	0.71 - 6.60	0.156
Slow	0.68	0.06 - 7.30	0.72	0.08 - 6.28	0.751
Acetylation Classification 3					
*Rapid **	1.00		1.00		
Intermediate	2.55	0.69 - 9.38	2.18	0.70 - 6.73	0.159
Slow/Intermediate	2.45	0.58 - 10.41	2.16	0.64 - 7.33	0.224
Slow	0.68	0.06 - 7.30	0.72	0.08 - 6.28	0.751

* Reference category

University of Cape Town

Appendix Q Vitamin B6 comparisons

Table Q-1: Baseline PLP concentrations between individuals with SDSP and those without DSP, and the influence of NAT2 acetylation phenotype

Acetylation type	Median PLP concentration (nmol/L)		p-value
	SDSP (N)	No DSP (N)	
Classification 1			
Rapid	27.3 (5)	23.4 (22)	0.516 [β]
Slow	20.1 (20)	24.4 (105)	0.607 [β]
Classification 2			
Rapid	27.3 (5)	23.4 (22)	0.516 [β]
Intermediate	19.5 (18)	24.1 (94)	0.842 [β]
Slow	44.5 (2)	26.7 (11)	0.333 [β]

[β] Student's *t*-test

Table Q-2: Baseline PLP concentrations between individuals with and without incident symptoms, and the influence of NAT2 acetylation phenotype

Acetylation type	Median PLP concentration (nmol/L)		p-value
	Incident symptoms (N)	No symptoms (N)	
Classification 1			
Rapid	13.4 (2)	24.0 (20)	0.294 [β]
Slow	22.6 (29)	27.1 (76)	0.393 [β]
Classification 2			
Rapid	13.4 (2)	24.0 (20)	0.294 [β]
Intermediate	21.4 (28)	27.4 (66)	0.267 [β]
Slow	32.3 (1)	23.6 (10)	N/A

[β] Student's *t*-test

University of Cape Town

Table Q-3: Distribution of grouped variables by baseline PLP quartiles

Group	Baseline PLP (nmol/L) Q1 (0 - 16.5) (n= 40)	Q2 (16.5 - 23.8) (n= 40)	Q3 (23.8 - 38.4) (n= 40)	Q4 (38.4+) (n= 39)	p-value
	N (% of group size)				
Female sex	29 (73)	31 (78)	29 (73)	21 (54)	0.111 §
Age > 40 years	9 (23)	10 (25)	10 (25)	10 (26)	1.000 ¶
WHO clinical stage < 2	21 (54)	25 (64)	32 (80)	22 (58)	0.078 §
IHDS score < 10	4 (14)	6 (22)	13 (38)	6 (23)	0.184 ¶
Previous / Current TB	17 (43)	13 (33)	12 (30)	17 (44)	0.488 §
Current TB	6 (15)	5 (13)	5 (13)	8 (21)	0.749 ¶
Previous TB	11 (32)	8 (23)	7 (20)	9 (29)	0.638 ¶
Vit Bco supplementation	39 (98)	39 (98)	39 (98)	38 (97)	1.000 ¶
Alcohol last year	10 (26)	14 (36)	12 (30)	8 (21)	0.492 §
Body Mass Index < 20 kg/m^2	10 (26)	6 (15)	4 (10)	14 (36)	***0.027*** ¶
Body Mass Index > 25 kg/m^2	10 (26)	14 (36)	20 (50)	13 (33)	0.170 §
CD4 T-cell count < 200 cells/mm^3	31 (82)	29 (73)	31 (79)	31 (79)	0.796 ¶
Fasting glucose > 5.6 mmol/L	2 (5)	1 (3)	5 (13)	0 (0)	0.054 ¶
Slow acetylation-phenotype	30 (77)	33 (87)	30 (81)	32 (84)	0.711 ¶

¶ Fisher's exact test

§ χ^2 test

Table Q-4: Distribution of baseline continuous variables by baseline PLP quartiles

Baseline variable	Units	Baseline PLP (nmol/L) Q1 (0 - 16.5) (n= 40)	Q2 (16.5 - 23.8) (n= 40)	Q3 (23.8 - 38.4) (n= 40)	Q4 (>38.4) (n= 39)	p-value
		Mean ± standard deviation				
Clinical						
Age	years	33 ± 9	34 ± 10	34 ± 8	34 ± 8	0.805 ε
Weight	kg	60.4 ± 9.9	63.1 ± 12.0	67.2 ± 13.6	64.1 ± 16.1	0.196 ε
Height	metre	1.62 ± 0.08	1.61 ± 0.08	1.61 ± 0.08	1.63 ± 0.08	0.362 ε
Body Mass Index	kg/m^2	23.0 ± 4.0	24.5 ± 5.1	26.0 ± 5.2	24.1 ± 5.9	0.062 ε
Waist : Hip ratio		0.85 ± 0.06	0.85 ± 0.07	0.86 ± 0.07	0.88 ± 0.06	0.133 ε
Systolic BP	mmHg	112 ± 17	111 ± 12	117 ± 16	113 ± 16	0.520 ε
Diastolic BP	mmHg	74 ± 11	72 ± 10	76 ± 12	70 ± 11	***0.030*** ε
Haematological						
CD4 T-cell count	$cells/mm^3$	159 ± 80	170 ± 71	151 ± 60	157 ± 50	0.814 ε
White cell count	$x10^9/L$	6.0 ± 2.8	5.6 ± 2.5	5.1 ± 1.6	6.1 ± 2.8	0.484 ε
C-reactive protein	mg/L	16.2 ± 24.0	8.6 ± 14.5	3.8 ± 10.3	9.8 ± 27.2	***0.001*** ε
Haemoglobin	g/dL	11.0 ± 1.7	11.4 ± 2.3	11.8 ± 2.3	12.1 ± 1.7	0.060 ε
MCV	fL	91.9 ± 6.6	90.5 ± 8.3	93.5 ± 6.6	91.0 ± 5.7	0.220 ε
Biochemical						
Albumin	g/L	35 ± 6	40 ± 5	41 ± 4	39 ± 4	***<0.001*** ε
ALT	IU/L	22 ± 12	19 ± 13	24 ± 11	29 ± 16	***0.001*** ε
Creatinine	µmol/L	66 ± 18	67 ± 20	65 ± 14	68 ± 17	0.981 ε
Metabolic						
Fasting glucose	mmol/L	4.7 ± 0.6	4.6 ± 0.4	4.8 ± 0.6	4.7 ± 0.4	0.830 ε
Fasting insulin	µU/mL	5.2 ± 3.6	5.5 ± 3.7	6.8 ± 4.7	6.1 ± 5.5	0.512 ε
Total cholesterol	mmol/L	3.5 ± 0.8	3.9 ± 0.8	4.0 ± 0.8	3.7 ± 0.7	0.115 ε
Triglycerides	mmol/L	1.0 ± 0.4	0.8 ± 0.3	1.1 ± 0.5	0.9 ± 0.4	***0.005*** ε
HDL	mmol/L	0.87 ± 0.35	1.01 ± 0.30	1.01 ± 0.35	0.99 ± 0.36	0.179 ε
LDL	mmol/L	2.2 ± 0.6	2.5 ± 0.7	2.4 ± 0.6	2.4 ± 0.7	0.343 ε
Lactate	mmol/L	2.4 ± 0.9	2.4 ± 1.3	2.6 ± 1.2	2.7 ± 1.3	0.752 ε
Pyridoxine						
4-PA	nmol/L	13.0 ± 5.2	16.4 ± 5.2	19.0 ± 9.8	37.1 ± 32.6	***<0.001*** ε

ε Wilcoxon rank-sum test

Table Q-5: Distribution of 24-week continuous variables by baseline PLP quartiles

24-week variable	Unit	Baseline PLP (nmol/L) Q1 (0 - 16.5) (n= 40)	Q2 (16.5 - 23.8) (n= 40)	Q3 (23.8 - 38.4) (n= 40)	Q4 (>38.4) (n= 39)	p-value
		Mean ± standard deviation				
Clinical						
Weight	kg	64.4 ± 10.9	65.4 ± 12.1	67.4 ± 13.6	65.1 ± 14.5	0.802 ε
Height	metre	1.62 ± 0.09	1.61 ± 0.08	1.62 ± 0.09	1.63 ± 0.08	0.377 ε
Body Mass Index	kg/m^2	24.7 ± 4.2	25.4 ± 5.1	25.8 ± 5.0	24.6 ± 5.8	0.567 ε
Waist : Hip ratio		0.85 ± 0.05	0.85 ± 0.07	0.84 ± 0.06	0.88 ± 0.07	0.143 ε
Systolic BP	mmHg	116 ± 16	117 ± 15	117 ± 15	116 ± 14	0.985 ε
Diastolic BP	mmHg	76 ± 12	75 ± 11	74 ± 10	73 ± 9	0.782 ε
Haematological						
C-reactive protein *	mg/L	15.6 ± 29.0	17.4 ± 43.6	14.0 ± 29.9	13.6 ± 30.5	0.461 ε
Metabolic						
Fasting glucose	mmol/L	4.8 ± 0.4	4.9 ± 0.3	5.0 ± 0.5	5.0 ± 0.5	0.390 ε
Fasting insulin	µU/mL	6.4 ± 6.1	6.4 ± 5.6	6.3 ± 4.0	6.6 ± 5.3	0.866 ε
Total cholesterol	mmol/L	4.2 ± 0.9	4.5 ± 0.9	4.3 ± 0.8	4.2 ± 0.9	0.547 ε
Triglycerides	mmol/L	0.8 ± 0.3	0.9 ± 0.3	1.0 ± 0.5	1.1 ± 1.9	0.292 ε
HDL	mmol/L	1.26 ± 0.35	1.39 ± 0.38	1.35 ± 0.47	1.35 ± 0.54	0.484 ε
LDL	mmol/L	2.5 ± 0.8	2.7 ± 0.9	2.6 ± 0.7	2.3 ± 0.6	0.480 ε
Pyridoxine						
4-PA *	nmol/L	20.7 ± 12.2	27.5 ± 39.7	26.3 ± 18.7	25.3 ± 35.3	0.315 ε

* 12-week blood samples

ε Wilcoxon rank-sum test

Table Q-6: Distribution of grouped variables by baseline 4-PA quartiles

Group	4-PA (nmol/L) Q1 (0 - 11.9) (n= 40)	Q2 (11.9 - 17.4) (n= 40)	Q3 (17.4 - 23.3) (n= 40)	Q4 (> 23.3) (n= 39)	p-value
	N (% of group size)				
Female sex	32 (80)	29 (73)	27 (68)	22 (56)	0.142 §
Age > 40 years	7 (18)	9 (23)	10 (25)	13 (33)	0.474 ¶
WHO clinical stage < 2	25 (66)	27 (68)	28 (70)	20 (53)	0.388 §
IHDS score < 10	6 (20)	4 (14)	9 (30)	10 (37)	0.211 ¶
Previous / Current TB	15 (38)	13 (33)	11 (28)	20 (51)	0.151 §
Current TB	4 (10)	2 (5)	6 (15)	12 (31)	***0.012*** ¶
Previous TB	11 (31)	11 (29)	5 (15)	8 (30)	0.369 ¶
Vit Bco supplementation	38 (95)	40 (100)	39 (98)	38 (97)	0.757 ¶
Alcohol last year	10 (26)	12 (30)	13 (33)	9 (23)	0.767 §
Body Mass Index < 20 kg/m^2	7 (18)	6 (15)	8 (21)	13 (33)	0.251 ¶
Body Mass Index > 25 kg/m^2	16 (42)	13 (33)	14 (36)	14 (36)	0.849 §
CD4 T-cell count < 200 cells/mm^3	29 (74)	31 (78)	30 (77)	32 (84)	0.771 ¶
Fasting glucose > 5.6 mmol/L	1 (3)	2 (5)	4 (11)	1 (3)	0.450 ¶
Slow acetylation-phenotype	33 (87)	30 (83)	29 (74)	33 (85)	0.542 ¶

¶ Fisher's exact test

§ χ^2 test

Table Q-7: Distribution of baseline continuous variables by baseline 4-PA quartiles

Baseline variable	Units	Baseline 4-PA (nmol/L) Q1 (0 - 11.9) (n= 40)	Q2 (11.9 - 17.4) (n= 40)	Q3 (17.4 - 23.3) (n= 40)	Q4 (> 23.3) (n= 39)	p-value
		Mean ± standard deviation				
Clinical						
Age	years	32 ± 8	34 ± 9	34 ± 10	34 ± 9	0.738 ε
Weight	kg	65.2 ± 13.2	63.2 ± 13.7	63.2 ± 11.4	63.4 ± 14.8	0.810 ε
Height	metre	1.62 ± 0.07	1.60 ± 0.08	1.62 ± 0.08	1.63 ± 0.09	0.257 ε
Body Mass Index	kg/m^2	24.9 ± 5.6	24.6 ± 5.0	24.4 ± 5.0	23.9 ± 5.4	0.790 ε
Waist : Hip ratio		0.86 ± 0.06	0.84 ± 0.08	0.85 ± 0.07	0.87 ± 0.06	0.117 ε
Systolic BP	mmHg	115 ± 15	112 ± 14	114 ± 17	112 ± 17	0.755 ε
Diastolic BP	mmHg	75 ± 11	74 ± 10	74 ± 10	70 ± 12	0.097 ε
Haematological						
CD4 T-cell count	$cells/mm^3$	159 ± 84	161 ± 64	168 ± 63	149 ± 49	0.660 ε
White cell count	$x10^9/L$	5.8 ± 3.2	5.6 ± 1.8	5.7 ± 2.2	5.8 ± 2.6	0.912 ε
C-reactive protein	mg/L	11.0 ± 18.3	9.9 ± 21.7	7.7 ± 12.6	9.6 ± 27.2	0.710 ε
Haemoglobin	g/dL	11.2 ± 1.6	11.8 ± 2.0	11.4 ± 2.5	11.9 ± 1.8	0.434 ε
MCV	fL	91.0 ± 6.9	90.5 ± 5.9	93.3 ± 7.7	91.9 ± 6.9	0.195 ε
Biochemical						
Albumin	g/L	38 ± 5	38 ± 6	39 ± 5	39 ± 4	0.933 ε
ALT	IU/L	19 ± 8	24 ± 14	22 ± 16	27 ± 14	***0.015*** ε
Creatinine	µmol/L	67 ± 17	64 ± 14	69 ± 21	67 ± 15	0.616 ε
Metabolic						
Fasting glucose	mmol/L	4.5 ± 0.5	4.7 ± 0.5	4.8 ± 0.5	4.7 ± 0.4	***0.026*** ε
Fasting insulin	µU/mL	5.9 ± 4.7	6.2 ± 5.3	5.7 ± 3.3	5.9 ± 4.4	0.992 ε
Total cholesterol	mmol/L	3.6 ± 0.8	3.9 ± 0.8	3.8 ± 0.7	3.8 ± 0.8	0.415 ε
Triglycerides	mmol/L	0.9 ± 0.5	1.0 ± 0.4	0.9 ± 0.3	1.0 ± 0.4	0.313 ε
HDL	mmol/L	0.98 ± 0.36	0.89 ± 0.31	1.01 ± 0.34	1.01 ± 0.36	0.431 ε
LDL	mmol/L	2.2 ± 0.6	2.6 ± 0.7	2.5 ± 0.6	2.4 ± 0.7	0.077 ε
Lactate	mmol/L	2.5 ± 1.2	2.8 ± 1.3	2.5 ± 1.2	2.3 ± 1.0	0.608 ε
Pyridoxine						
PLP	nmol/L	19.8 ± 14.4	23.6 ± 10.0	26.0 ± 12.9	52.8 ± 25.3	***<0.001*** ε

ε Wilcoxon rank-sum test

Table Q-8: Distribution of 24-week continuous variables by baseline 4-PA quartiles

24-week variable	Units	Baseline 4-PA (nmol/L) Q1 (0 - 11.9) (n= 40)	Q2 (11.9 - 17.4) (n= 40)	Q3 (17.4 - 23.3) (n= 40)	Q4 (> 23.3) (n= 39)	p-value
		Mean ± standard deviation				
Clinical						
Weight	kg	67.2 ± 12.6	63.3 ± 11.9	66.5 ± 11.2	65.2 ± 15.1	0.338 ε
Height	metre	1.63 ± 0.08	1.60 ± 0.08	1.61 ± 0.09	1.63 ± 0.08	0.151 ε
Body Mass Index	kg/m^2	25.5 ± 4.8	24.8 ± 5.0	25.9 ± 5.0	24.5 ± 5.4	0.519 ε
Waist : Hip ratio		0.85 ± 0.05	0.85 ± 0.08	0.86 ± 0.07	0.86 ± 0.06	0.569 ε
Systolic BP	mmHg	120 ± 16	120 ± 14	114 ± 16	113 ± 13	0.212 ε
Diastolic BP	mmHg	76 ± 11	77 ± 12	73 ± 10	72 ± 9	0.132 ε
Haematological						
C-reactive protein *	mg/L	7.3 ± 11.0	17.2 ± 39.5	22.9 ± 46.6	12.8 ± 23.7	0.803 ε
Metabolic						
Fasting glucose	mmol/L	4.8 ± 0.3	4.9 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	0.452 ε
Fasting insulin	µU/mL	6.3 ± 5.3	6.0 ± 5.6	6.7 ± 5.9	6.6 ± 4.4	0.650 ε
Total cholesterol	mmol/L	4.2 ± 0.9	4.5 ± 0.9	4.4 ± 0.9	4.1 ± 0.8	0.345 ε
Triglycerides	mmol/L	1.1 ± 1.9	0.9 ± 0.5	0.9 ± 0.3	0.9 ± 0.4	0.954 ε
HDL	mmol/L	1.39 ± 0.41	1.41 ± 0.46	1.28 ± 0.40	1.27 ± 0.47	0.439 ε
LDL	mmol/L	2.3 ± 0.9	2.7 ± 0.8	2.7 ± 0.7	2.4 ± 0.6	0.056 ε
Pyridoxine						
PLP *	nmol/L	25.1 ± 19.4	26.9 ± 18.1	24.6 ± 15.8	34.1 ± 38.0	0.959 ε

* 12-week blood samples

ε Wilcoxon rank-sum test

Table Q-9: Distribution of grouped variables by baseline PLP medians

Group	Baseline PLP (nmol/L)		p-value
	Below 23.8 (n = 80)	Above 23.8 (n = 79)	
Female sex	60 (75)	50 (63)	0.110 §
Age > 40 years	19 (24)	20 (25)	0.854 §
WHO clinical stage < 2	46 (59)	54 (69)	0.182 §
IHDS score < 10	10 (18)	19 (32)	0.096 §
Previous / Current TB	30 (38)	29 (37)	0.918 §
Current TB	11 (14)	13 (16)	0.634 §
Previous TB	19 (28)	16 (24)	0.662 §
Vit Bco supplementation	78 (98)	77 (97)	1.000 ¶
Alcohol last year	24 (31)	20 (25)	0.417 §
Body Mass Index < 20 kg/m^2	16 (21)	18 (23)	0.762 §
Body Mass Index > 25 kg/m^2	24 (31)	33 (42)	0.169 §
CD4 T-cell count < 200 cells/mm^3	60 (77)	62 (79)	0.698 §
Fasting glucose > 5.6 mmol/L	3 (4)	5 (7)	0.459 ¶
Slow acetylation-phenotype	63 (82)	62 (83)	0.891 §

¶ Fisher's exact test

§ χ^2 test

Table Q-10: Distribution of baseline continuous variables by baseline PLP medians

Baseline variable	Unit	Baseline PLP (nmol/L)		p-value
		Below 23.8 (n = 80)	Above 23.8 (n = 79)	
Clinical				
Age	years	33 ± 9	34 ± 8	0.438 ε
Weight	kg	61.8 ± 11.0	65.7 ± 14.9	0.178 ε
Height	metre	1.62 ± 0.08	1.62 ± 0.08	0.616 ε
Body Mass Index	kg/m^2	23.8 ± 4.7	25.1 ± 5.6	0.188 ε
Waist : Hip ratio		0.85 ± 0.07	0.87 ± 0.07	0.075 ε
Systolic BP	mmHg	111 ± 15	115 ± 16	0.231 ε
Diastolic BP	mmHg	73 ± 10	73 ± 12	0.925 ε
Haematological				
CD4 T-cell count	$cells/mm^3$	165 ± 75	154 ± 55	0.596 ε
White cell count	$x10^9/L$	5.8 ± 2.7	5.6 ± 2.3	0.759 ε
C-reactive protein	mg/L	12.3 ± 20.0	6.7 ± 20.4	***0.001*** ε
Haemoglobin	g/dL	11.2 ± 2.0	11.9 ± 2.0	***0.014*** ε
MCV	fL	91.2 ± 7.5	92.2 ± 6.2	0.315 ε
Biochemical				
Albumin	g/L	37 ± 6	40 ± 4	***0.003*** ε
ALT	IU/L	20 ± 13	26 ± 14	***0.001*** ε
Creatinine	µmol/L	67 ± 19	66 ± 15	0.819 ε
Metabolic				
Fasting glucose	mmol/L	4.6 ± 0.5	4.7 ± 0.5	0.622 ε
Fasting insulin	µU/mL	5.4 ± 3.6	6.4 ± 5.1	0.323 ε
Total cholesterol	mmol/L	3.7 ± 0.8	3.8 ± 0.7	0.270 ε
Triglycerides	mmol/L	0.9 ± 0.4	1.0 ± 0.4	0.051 ε
HDL	mmol/L	0.94 ± 0.33	1.00 ± 0.35	0.256 ε
LDL	mmol/L	2.4 ± 0.7	2.4 ± 0.7	0.800 ε
Lactate	mmol/L	2.4 ± 1.1	2.6 ± 1.3	0.426 ε
Pyridoxine				
4-PA	nmol/L	14.7 ± 5.4	27.9 ± 25.4	***<0.001*** ε

ε Wilcoxon rank-sum test

Table Q-11: Distribution of 24-week continuous variables by baseline PLP medians

24-week variable	Unit	Baseline PLP (nmol/L)		p-value
		Below 23.8 (n = 80)	Above 23.8 (n = 79)	
Clinical				
Weight	kg	64.9 ± 11.5	66.3 ± 14.0	0.900 ε
Height	metre	1.61 ± 0.08	1.63 ± 0.08	0.259 ε
Body Mass Index	kg/m^2	25.1 ± 4.7	25.2 ± 5.4	0.889 ε
Waist : Hip ratio		0.85 ± 0.06	0.86 ± 0.07	0.442 ε
Systolic BP	mmHg	117 ± 16	117 ± 14	0.937 ε
Diastolic BP	mmHg	75 ± 11	73 ± 9	0.345 ε
Haematological				
C-reactive protein *	mg/L	16.5 ± 37.0	13.8 ± 29.9	0.440 ε
Metabolic				
Fasting glucose	mmol/L	4.9 ± 0.4	5.0 ± 0.5	0.134 ε
Fasting insulin	µU/mL	6.4 ± 5.8	6.5 ± 4.7	0.495 ε
Total cholesterol	mmol/L	4.3 ± 0.9	4.3 ± 0.8	0.921 ε
Triglycerides	mmol/L	0.9 ± 0.3	1.0 ± 1.4	0.992 ε
HDL	mmol/L	1.32 ± 0.37	1.35 ± 0.50	0.821 ε
LDL	mmol/L	2.6 ± 0.8	2.4 ± 0.6	0.394 ε
Pyridoxine				
4-PA *	nmol/L	24.1 ± 29.4	25.8 ± 28.0	0.670 ε

* 12-week blood samples

ε Wilcoxon rank-sum test

Table Q-12: Distribution of grouped variables by baseline 4-PA medians

Group	Baseline 4-PA (nmol/L)		p-value
	Below 17.4 (n = 80)	Above 17.4 (n = 79)	
Female sex	61 (76)	49 (62)	0.052 §
Age > 40 years	16 (20)	23 (29)	0.197 §
WHO clinical stage < 2	52 (67)	48 (62)	0.504 §
IHDS score < 10	10 (17)	19 (33)	***0.047*** §
Previous / Current TB	28 (35)	31 (39)	0.580 §
Current TB	6 (8)	18 (23)	***0.007*** §
Previous TB	22 (30)	13 (21)	0.267 §
Vit Bco supplementation	78 (98)	77 (97)	1.000 ¶
Alcohol last year	22 (28)	22 (28)	1.000 §
Body Mass Index < 20 kg/m^2	13 (17)	21 (27)	0.121 §
Body Mass Index > 25 kg/m^2	29 (37)	28 (36)	0.868 §
CD4 T-cell count < 200 cells/mm^3	60 (76)	62 (81)	0.489 §
Fasting glucose > 5.6 mmol/L	3 (4)	5 (6)	0.719 ¶
Slow acetylation-phenotype	63 (85)	62 (79)	0.362 §

¶ Fisher's exact test

§ χ^2 test

Table Q-13: Distribution of baseline continuous variables by baseline 4-PA medians

Baseline variable	Unit	Baseline 4-PA (nmol/L)		p-value
		Below 17.4 (n = 80)	Above 17.4 (n = 79)	
Clinical				
Age	years	33 ± 8	34 ± 9	0.387 ε
Weight	kg	64.2 ± 13.4	63.3 ± 13.1	0.751 ε
Height	metre	1.61 ± 0.07	1.62 ± 0.08	0.409 ε
Body Mass Index	kg/m^2	24.7 ± 5.2	24.1 ± 5.1	0.404 ε
Waist : Hip ratio		0.85 ± 0.07	0.86 ± 0.06	0.455 ε
Systolic BP	mmHg	113 ± 14	113 ± 17	0.632 ε
Diastolic BP	mmHg	74 ± 11	72 ± 11	0.072 ε
Haematological				
CD4 T-cell count	$cells/mm^3$	160 ± 74	158 ± 57	0.855 ε
White cell count	$x10^9/L$	5.7 ± 2.6	5.7 ± 2.4	0.939 ε
C-reactive protein	mg/L	10.5 ± 19.9	8.6 ± 20.8	0.787 ε
Haemoglobin	g/dL	11.5 ± 1.9	11.6 ± 2.2	0.498 ε
MCV	fL	90.8 ± 6.4	92.6 ± 7.3	0.083 ε
Biochemical				
Albumin	g/L	38 ± 6	39 ± 5	0.699 ε
ALT	IU/L	22 ± 12	25 ± 15	0.310 ε
Creatinine	µmol/L	65 ± 16	68 ± 18	0.309 ε
Metabolic				
Fasting glucose	mmol/L	4.6 ± 0.5	4.8 ± 0.5	***0.019*** ε
Fasting insulin	µU/mL	6.1 ± 5.0	5.8 ± 3.9	0.868 ε
Total cholesterol	mmol/L	3.8 ± 0.8	3.8 ± 0.7	0.659 ε
Triglycerides	mmol/L	1.0 ± 0.4	0.9 ± 0.4	0.786 ε
HDL	mmol/L	0.94 ± 0.33	1.01 ± 0.35	0.180 ε
LDL	mmol/L	2.4 ± 0.7	2.4 ± 0.7	0.502 ε
Lactate	mmol/L	2.6 ± 1.3	2.4 ± 1.1	0.295 ε
Pyridoxine				
PLP	nmol/L	21.7 ± 12.5	39.2 ± 24.1	***<0.001*** ε

ε Wilcoxon rank-sum test

Table Q-14: Distribution of 24-week continuous variables by baseline 4-PA medians

24-week variable	Unit	Baseline 4-PA (nmol/L)		p-value
		Below 17.4 (n = 80)	Above 17.4 (n = 79)	
Clinical				
Weight	kg	65.3 ± 12.3	65.9 ± 13.3	0.805 ε
Height	metre	1.62 ± 0.08	1.62 ± 0.09	0.736 ε
Body Mass Index	kg/m^2	25.1 ± 4.9	25.2 ± 5.2	0.968 ε
Waist : Hip ratio		0.85 ± 0.06	0.86 ± 0.06	0.162 ε
Systolic BP	mmHg	120 ± 15	114 ± 14	***0.040*** ε
Diastolic BP	mmHg	77 ± 11	72 ± 9	***0.021*** ε
Haematological				
C-reactive protein *	mg/L	12.3 ± 29.4	17.9 ± 37.2	0.407 ε
Metabolic				
Fasting glucose	mmol/L	4.9 ± 0.4	5.0 ± 0.5	0.131 ε
Fasting insulin	µU/mL	6.2 ± 5.4	6.7 ± 5.2	0.273 ε
Total cholesterol	mmol/L	4.4 ± 0.9	4.2 ± 0.8	0.480 ε
Triglycerides	mmol/L	1.0 ± 1.4	0.9 ± 0.3	0.633 ε
HDL	mmol/L	1.40 ± 0.44	1.28 ± 0.44	0.101 ε
LDL	mmol/L	2.5 ± 0.8	2.5 ± 0.7	0.823 ε
Pyridoxine				
PLP *	nmol/L	26.0 ± 18.7	29.3 ± 29.2	0.905 ε

* 12-week blood samples

ε Wilcoxon rank-sum test

Table Q-15: Univariate analysis showing odds of PLP >25 nmol/L vs PLP <25 nmol/L

Variable	Odds Ratio in univariate analysis	Odds Ratio 95% Confidence Interval	p-value
Clinical			
Female sex	0.61	0.31 - 1.20	0.150
Age > 40 years	0.90	0.44 - 1.87	0.786
Previous/Current TB	0.94	0.49 - 1.80	0.859
Current TB	0.70	0.29 - 1.67	0.418
Previous TB	1.14	0.52 - 2.47	0.748
Body Mass Index > 25 kg/m^2	0.83	0.54 - 1.26	0.382
Waist : Hip ratio > 0.9	0.45	0.21 - 0.97	***0.041***
Diastolic BP > 80 mmHg	0.74	0.21 - 2.52	0.625
Haematological			
CD4 T-cell count > 100 cells/mm^3	0.97	0.42 - 2.26	0.943
C-reactive protein > 5 mg/L	2.22	1.08 - 4.55	***0.029***
Haemoglobin < 11.7 g/dL	0.48	0.25 - 0.90	***0.023***
Biochemical			
Albumin > 38 g/L	2.83	1.43 - 5.61	***0.003***
ALT > 19 IU/L	0.43	0.23 - 0.82	***0.011***
Metabolic			
Triglycerides > 0.9 mmol/L	0.40	0.21 - 0.76	***0.005***
Total cholesterol > 3.7 mmol/L	0.56	0.30 - 1.07	***0.081***
HDL < 1.0 mmol/L	1.85	0.58 - 5.92	0.303
Pyridoxine			
4-PA > 17.4 nmol/L	4.51	2.31 - 8.81	***<0.001***

Table Q-16: Baseline DSP status grouped by PLP medians

Baseline DSP status	Group size	Baseline PLP below median (< 23.8 nmol/L) (n = 80)	Baseline PLP above median (> 23.8 nmol/L) (n = 79)	p-value	
		N (% of group size)			
DSP	(n = 35)	17 (21%)	18 (23%)	0.815	§
SDSP	(n = 16)	14 (18%)	2 (3%)	0.778	§
ADSP	(n = 9)	3 (4%)	6 (8%)	0.328	¶

¶ Fisher's exact test

§ χ^2 test

Table Q-17: Baseline DSP status grouped by PLP quartiles

Baseline DSP status	Group size	Baseline PLP (nmol/L) Q1 (0 - 16.5) (n= 40)	Q2 (16.5 - 23.8) (n= 40)	Q3 (23.8 - 38.4) (n= 40)	Q4 (38.4+) (n= 39)	p-value	
		N (% of group size)					
DSP	(n = 35)	9 (23%)	8 (20%)	7 (18%)	11 (28%)	0.710	¶
SDSP	(n = 26)	8 (20%)	6 (15%)	3 (8%)	9 (23%)	0.290	¶
ADSP	(n = 9)	1 (3%)	2 (5%)	4 (10%)	2 (5%)	0.587	¶

¶ Fisher's exact test

§ χ^2 test

University of

Table Q-18: Baseline DSP status grouped by 4-PA medians

Baseline DSP status	Group size	Baseline 4-PA below median (< 17.4 nmol/L) (n = 80)	Baseline 4-PA above median (> 17.4 nmol/L) (n = 79)	p-value	
		N (% of group size)			
DSP	(n = 35)	14 (18%)	21 (27%)	0.167	§
SDSP	(n = 26)	11 (14%)	15 (19%)	0.311	§
ADSP	(n = 9)	3 (4%)	6 (8%)	0.328	¶

¶ Fisher's exact test

§ χ^2 test

Table Q-19: Baseline DSP status grouped by 4-PA quartiles

Baseline DSP status	Group size	Baseline 4-PA (nmol/L) Q1 (0 - 11.9) (n= 40)	Q2 (11.9 - 17.4) (n= 40)	Q3 (17.4 - 23.3) (n= 40)	Q4 (23.3+) (n= 39)	p-value	
		N (% of group size)					
DSP	(n = 35)	7 (18%)	7 (18%)	10 (25%)	11 (28%)	0.593	¶
SDSP	(n = 26)	6 (15%)	5 (13%)	7 (18%)	8 (21%)	0.745	¶
ADSP	(n = 9)	1 (3%)	2 (5%)	3 (8%)	3 (8%)	0.752	¶

¶ Fisher's exact test

§ χ^2 test

University of Southampton

Table Q-20: Week 12 DSP status grouped by PLP medians

Week 12 DSP status	Group size	Baseline PLP below median (< 23.8 nmol/L) (n = 78)	Baseline PLP above median (> 23.8 nmol/L) (n = 77)	p-value
		N (% of group size)		
ATN	(n = 33)	16 (21%)	17 (22%)	0.812 §
Incident symptoms	(n = 28)	15 (19%)	13 (17%)	0.636 §
Incident SDSP	(n = 20)	11 (14%)	9 (12%)	0.600 §

¶ Fisher's exact test

§ χ^2 test

Table Q-21: Week 12 DSP status grouped by PLP quartiles

Week 12 DSP status	Group size	Baseline PLP (nmol/L) Q1 (0 - 16.5) (n= 40)	Q2 (16.5 - 23.8) (n= 40)	Q3 (23.8 - 38.4) (n= 40)	Q4 (38.4+) (n= 39)	p-value
		N (% of group size)				
ATN	(n = 33)	8 (21%)	8 (21%)	10 (26%)	7 (18%)	0.900 ¶
Incident symptoms	(n = 28)	7 (18%)	8 (21%)	9 (23%)	4 (11%)	0.695 ¶
Incident SDSP	(n = 20)	4 (10%)	7 (18%)	5 (13%)	4 (11%)	0.794 ¶

¶ Fisher's exact test

§ χ^2 test

University of Wollongong

Table Q-22: Week 12 DSP status grouped by 4-PA medians

Week 12 DSP status	Group size	Baseline 4-PA below median (< 17.4 nmol/L) (n = 78)	Baseline 4-PA above median (> 17.4 nmol/L) (n = 77)	p-value
		N (% of group size)		
ATN	(n = 33)	19 (24%)	14 (18%)	0.348 §
Incident symptoms	(n = 28)	16 (21%)	12 (16%)	0.533 §
Incident SDSP	(n = 20)	12 (15%)	8 (10%)	0.432 §

¶ Fisher's exact test

§ χ^2 test

Table Q-23: Week 12 DSP status grouped by 4-PA quartiles

Week 12 DSP status	Group size	Baseline 4-PA (nmol/L) Q1 (0 - 11.9) (n= 39)	Q2 (11.9 - 17.4) (n= 39)	Q3 (17.4 - 23.3) (n= 39)	Q4 (23.3+) (n= 38)	p-value
		N (% of group size)				
ATN	(n = 33)	9 (23%)	10 (26%)	7 (18%)	7 (18%)	0.833 ¶
Incident symptoms	(n = 28)	7 (18%)	9 (23%)	7 (18%)	5 (13%)	0.801 ¶
Incident SDSP	(n = 20)	5 (13%)	7 (18%)	6 (15%)	2 (5%)	0.375 ¶

¶ Fisher's exact test

§ χ^2 test

University of ... own

Appendix R Cytokine analyses

Table R-1: Cross-sectional time series analysis for remainder of cytokines

Cytokine	Time effect[a]				Group effect[b]				
	Median (pg/mL)	Coefficient	Confidence interval (95%)	p-value	Incident symptoms Median (pg/mL)	No symptoms Median (pg/mL)	Coefficient	Confidence interval (95%)	p-value
Interleukin-5									
Baseline	0.56				0.54	0.58			0.880 [ε]
Week 2	1.94	1.33	(0.82 ; 1.85)	***<0.001***	1.28	2.30	-0.50	(-1.52 ; 0.52)	0.335
Week 4	1.83	1.46	(0.95 ; 1.98)	***<0.001***	2.01	1.83	-0.38	(-1.41 ; 0.65)	0.467
Week 12	0.77	0.48	(-0.01 ; 0.97)	0.055	0.76	0.85	-0.65	(-1.63 ; 0.33)	0.192
Interleukin-7									
Baseline	2.06				2.04	2.06			0.756 [ε]
Week 2	9.02	7.70	(6.00 ; 9.40)	***<0.001***	7.45	11.58	-2.07	(-5.44 ; 1.30)	0.228
Week 4	7.56	6.29	(4.57 ; 8.01)	***<0.001***	7.00	9.18	-2.57	(-5.98 ; 0.85)	0.141
Week 12	2.66	1.29	(-0.35 ; 2.93)	0.122	2.81	2.48	-0.88	(-4.13 ; 2.37)	0.596
Interleukin-8									
Baseline	3.77				3.38	4.30			0.534 [ε]
Week 2	3.28	-0.48	(-1.24 ; 0.27)	0.210	2.94	3.86	-0.91	(-2.40 ; 0.58)	0.233
Week 4	3.21	-0.58	(-1.34 ; 0.18)	0.136	3.13	3.21	0.56	(-0.95 ; 2.07)	0.468
Week 12	3.52	-0.88	(-1.60 ; -0.15)	***0.019***	3.16	3.68	0.46	(-0.97 ; 1.90)	0.527
Interleukin-12									
Baseline	0.01				0.01	0.02			0.372 [ε]
Week 2	13.69	13.96	(6.66 ; 21.27)	***<0.001***	8.55	17.84	-3.06	(-17.60 ; 11.48)	0.680
Week 4	9.46	14.10	(6.70 ; 21.50)	***<0.001***	8.42	12.53	-6.96	(-21.68 ; 7.77)	0.355
Week 12	1.11	6.63	(-0.41 ; 13.68)	0.065	1.11	1.29	-8.04	(-22.06 ; 5.99)	0.261
Interleukin-13									
Baseline	0.47				0.47	0.47			0.843 [ε]
Week 2	37.20	37.82	(30.56 ; 45.08)	***<0.001***	25.39	57.21	-15.80	(-30.01 ; -1.59)	***0.029***
Week 4	32.10	28.79	(21.44 ; 36.14)	***<0.001***	30.04	33.28	-5.58	(-19.97 ; 8.80)	0.447
Week 12	3.12	6.49	(-0.53 ; 13.51)	0.070	3.12	2.83	0.98	(-12.76 ; 14.72)	0.889
IFN-γ									
Baseline	1.20				1.05	1.38			0.621 [ε]
Week 2	12.53	14.94	(10.29 ; 19.60)	***<0.001***	8.42	12.82	1.99	(-7.30 ; 11.29)	0.674
Week 4	8.86	9.02	(4.32 ; 13.72)	***<0.001***	9.52	8.86	1.56	(-7.84 ; 10.96)	0.745
Week 12	2.41	2.35	(-2.16 ; 6.86)	0.307	3.32	1.54	3.05	(-5.96 ; 12.07)	0.507
GM-CSF									
Baseline	0.73				0.76	0.71			0.785 [ε]
Week 2	6.41	6.04	(4.67 ; 7.42)	***<0.001***	4.62	8.37	-2.48	(-5.18 ; 0.22)	0.072
Week 4	3.39	4.27	(2.88 ; 5.66)	***<0.001***	3.10	3.92	-0.74	(-3.47 ; 2.00)	0.598
Week 12	1.04	1.25	(-0.08 ; 2.59)	0.066	1.17	0.96	0.97	(-1.65 ; 3.58)	0.469

[a] Time effect is the effect of cART on mean cytokine concentration for the entire cohort

[b] Group effect is the difference in mean cytokine concentration between groups compared to baseline

[ε] Wilcoxon rank-sum test

University of Cape Town

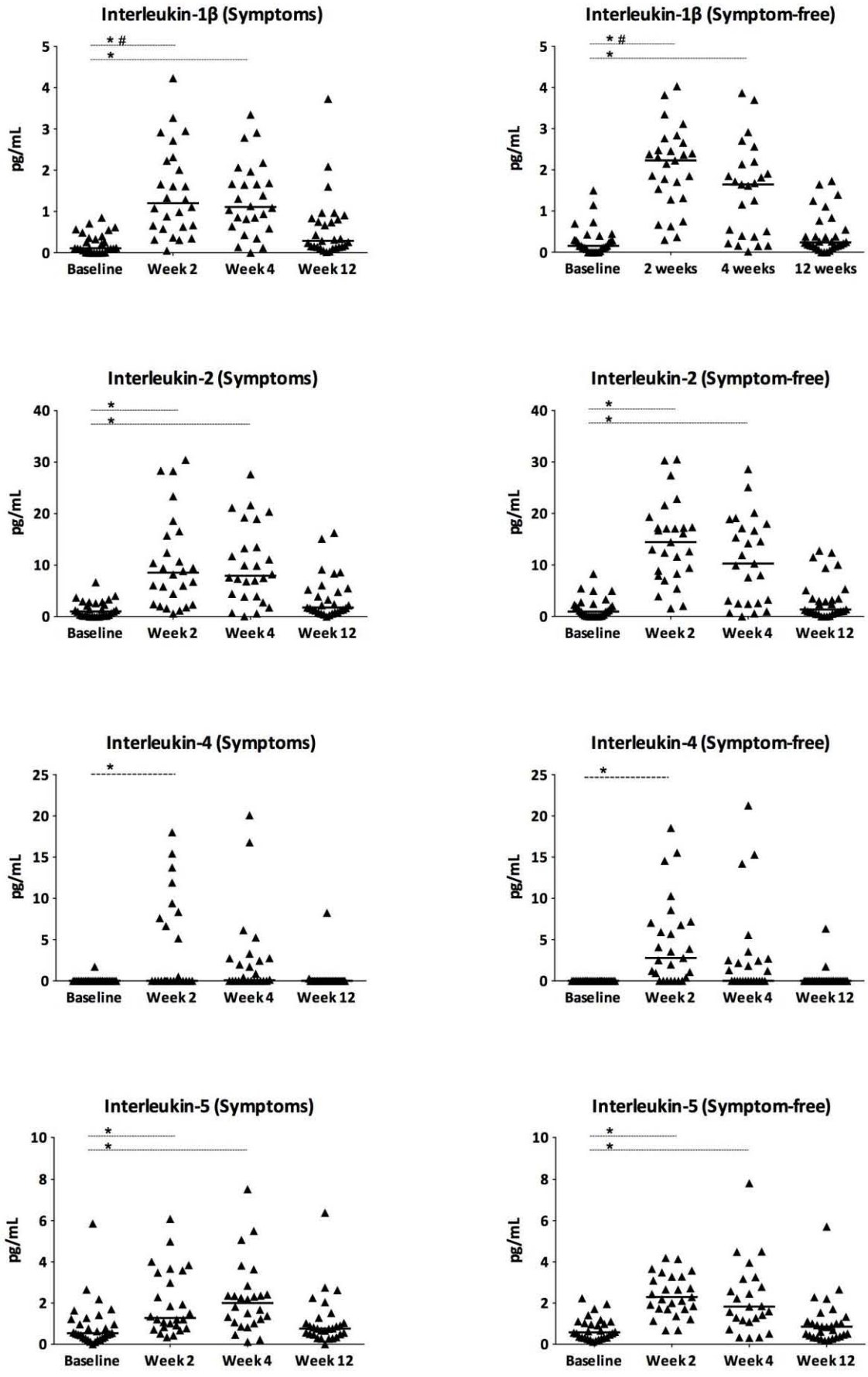


Figure R-1: Longitudinal cytokine concentrations categorized by incident symptom status (Part I)

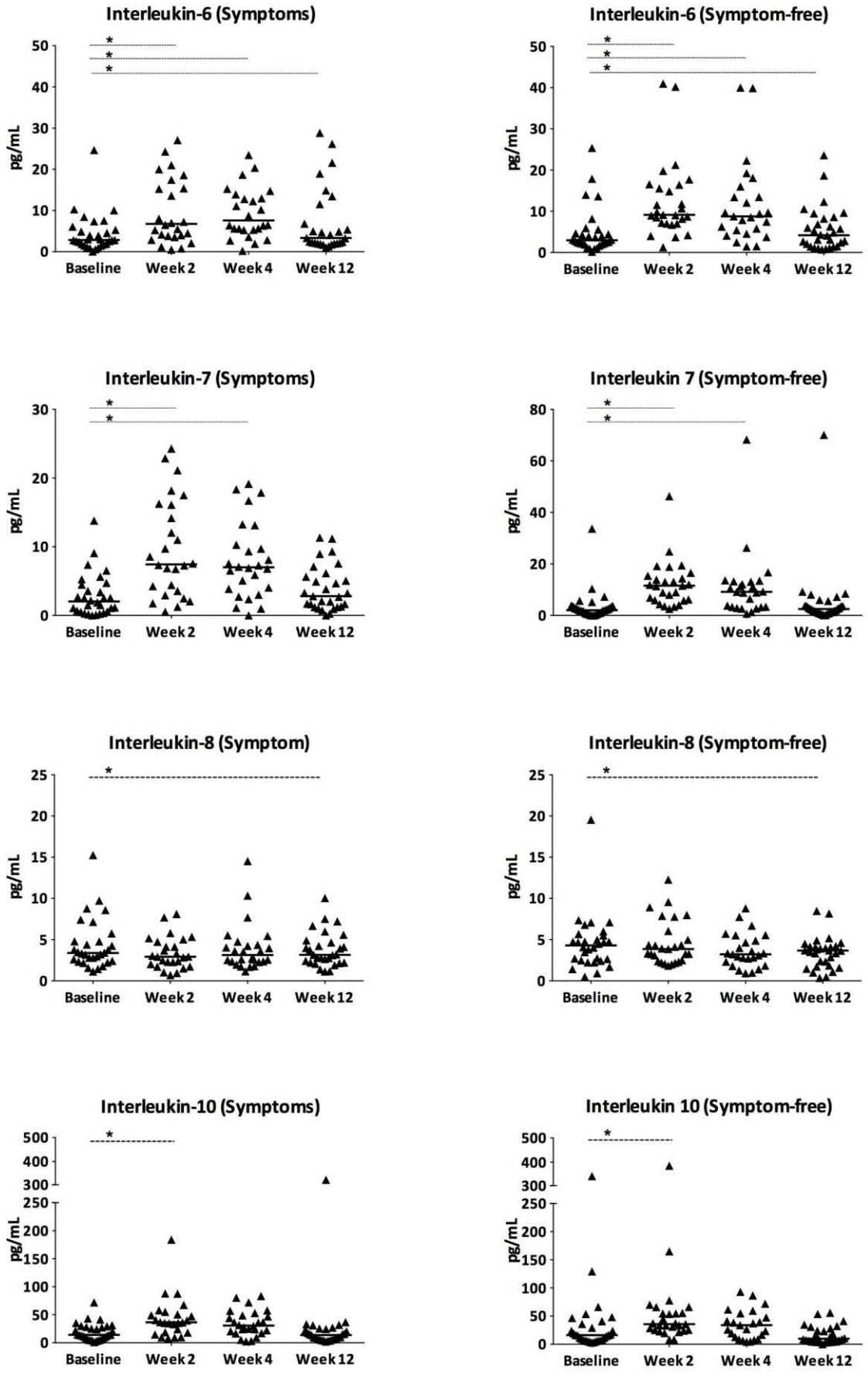


Figure R-2: Longitudinal cytokine concentrations categorized by incident symptom status (Part II)

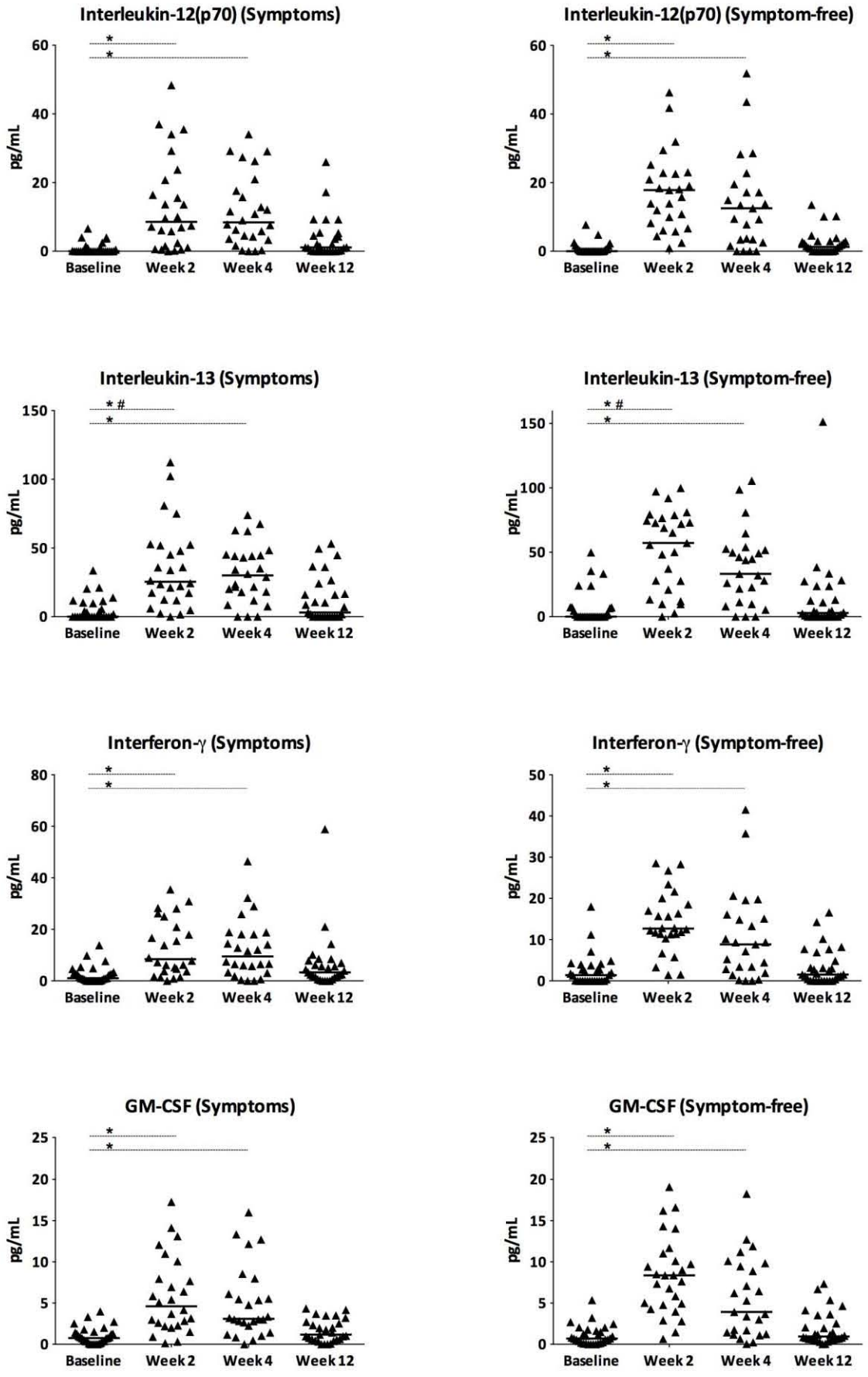


Figure R-3: Longitudinal cytokine concentrations categorized by incident symptom status (Part III)

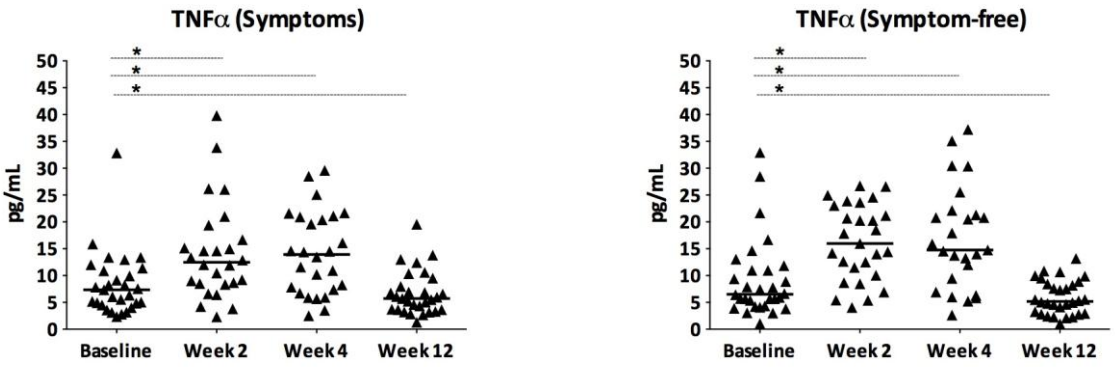


Figure R-4: Longitudinal cytokine concentrations categorized by incident symptom status (Part IV)

University of Cape Town

Table R-2: Cross-sectional time series analysis for cytokine ratios

Cytokine ratio	Visit	Time effect			Group effect		
		Coefficient	Confidence interval (95%)	p-value	Coefficient	Confidence interval (95%)	p-value
TNF-α / IL-4	Baseline						0.554
	Week 2	-30.61	(-47.33 ; -13.90)	***<0.001***	38.51	(5.56 ; 71.46)	***0.022***
	Week 4	-22.70	(-39.60 ; -5.80)	***0.008***	8.53	(-24.80 ; 41.85)	0.616
	Week 12	-23.50	(-39.70 ; -7.30)	***0.004***	14.81	(-17.12 ; 46.75)	0.363
IFN-γ / IL-4	Baseline						0.881
	Week 2	30.39	(-5.53 ; 66.31)	0.097	67.59	(-3.11 ; 138.29)	0.061
	Week 4	13.59	(-22.70 ; 49.88)	0.463	29.73	(-41.70 ; 101.16)	0.415
	Week 12	8.71	(-26.22 ; 43.64)	0.625	15.84	(-52.92 ; 84.60)	0.652
IL-6 / IL-4	Baseline						0.515
	Week 2	5.34	(-21.91 ; 32.59)	0.701	52.71	(-0.92 ; 106.34)	0.054
	Week 4	1.36	(-26.22 ; 28.95)	0.923	2.72	(-51.57 ; 57.00)	0.922
	Week 12	21.67	(-4.69 ; 48.03)	0.107	36.35	(-15.53 ; 88.23)	0.170
TNF-α / IL-10	Baseline						1.000
	Week 2	-0.30	(-0.82 ; 0.21)	0.250	0.01	(-1.02 ; 1.03)	0.987
	Week 4	-0.06	(-0.59 ; 0.46)	0.811	-0.18	(-1.22 ; 0.86)	0.734
	Week 12	0.20	(-0.30 ; 0.70)	0.442	-0.73	(-1.72 ; 0.27)	0.151
IFN-γ / IL-10	Baseline						0.308
	Week 2	0.22	(0.12 ; 0.33)	***<0.001***	-0.10	(-0.30 ; 0.11)	0.351
	Week 4	0.19	(0.08 ; 0.29)	***0.001***	0.00	(-0.21 ; 0.20)	0.970
	Week 12	0.19	(0.08 ; 0.29)	***<0.001***	0.20	(0.01 ; 0.40)	***0.044***
IL-6 / IL-10	Baseline						0.988
	Week 2	-0.07	(-2.24 ; 2.10)	0.949	-0.28	(-4.60 ; 4.04)	0.898
	Week 4	0.23	(-1.96 ; 2.43)	0.835	-0.27	(-4.63 ; 4.10)	0.905
	Week 12	1.69	(-0.42 ; 3.81)	0.116	-2.83	(-7.03 ; 1.38)	0.187
IL-1 / sIL1RI	Baseline						0.328
	Week 2	0.05	(0.04 ; 0.06)	***<0.001***	-0.03	(-0.06 ; -0.01)	***0.004***
	Week 4	0.03	(0.02 ; 0.04)	***<0.001***	-0.01	(-0.03 ; 0.01)	0.309
	Week 12	0.00	(-0.01 ; 0.02)	0.446	-0.01	(-0.03 ; 0.02)	0.642
IL-1 / sIL1RII	Baseline						0.976
	Week 2	0.00	(0.00 ; 0.00)	***<0.001***	0.00	(0.00 ; 0.00)	0.825
	Week 4	0.00	(0.00 ; 0.00)	0.054	0.00	(0.00 ; 0.00)	0.850
	Week 12	0.00	(0.00 ; 0.00)	0.942	0.00	(0.00 ; 0.00)	0.898
IL-2 / sIL2rα	Baseline						0.825
	Week 2	0.01	(0.00 ; 0.02)	0.084	-0.03	(-0.05 ; -0.01)	***0.014***
	Week 4	0.01	(0.00 ; 0.02)	0.283	-0.02	(-0.04 ; 0.00)	0.121
	Week 12	0.00	(-0.01 ; 0.01)	0.526	-0.01	(-0.03 ; 0.01)	0.232
IL-6 / sIL6R	Baseline						0.574
	Week 2	-0.03	(-0.06 ; 0.01)	0.199	-0.05	(-0.13 ; 0.02)	0.177
	Week 4	-0.03	(-0.06 ; 0.01)	0.198	-0.05	(-0.13 ; 0.02)	0.184
	Week 12	-0.03	(-0.06 ; 0.01)	0.177	-0.05	(-0.13 ; 0.02)	0.168
IL-1 / IL1ra	Baseline						0.060
	Week 2	0.31	(0.15 ; 0.46)	***<0.001***	-0.43	(-0.73 ; -0.13)	***0.006***
	Week 4	0.16	(0.00 ; 0.32)	0.051	0.00	(-0.30 ; 0.31)	0.990
	Week 12	0.03	(-0.12 ; 0.19)	0.670	0.02	(-0.28 ; 0.32)	0.895
TNF-α / sTNFRII	Baseline						0.585
	Week 2	-0.01	(-0.02 ; 0.01)	0.276	-0.02	(-0.06 ; 0.01)	0.134
	Week 4	-0.01	(-0.02 ; 0.01)	0.273	-0.02	(-0.05 ; 0.01)	0.169
	Week 12	-0.01	(-0.03 ; 0.00)	0.168	-0.02	(-0.05 ; 0.01)	0.162
TNF-α / sTNFRI	Baseline						0.952
	Week 2	0.00	(-0.03 ; 0.03)	0.851	-0.05	(-0.11 ; 0.00)	0.074
	Week 4	0.00	(-0.03 ; 0.02)	0.765	-0.03	(-0.09 ; 0.03)	0.276
	Week 12	-0.02	(-0.05 ; 0.00)	0.108	-0.03	(-0.09 ; 0.02)	0.224

Table R-3: Effect of d4T exposure on cytokine levels (selected cytokines)

Cytokine & Visit	D4T exposure group effect		
	Coefficient	Confidence interval (95%)	p-value
Interleukin-1β			
Week 2	-0.57	(-1.16 ; 0.01)	0.056
Week 4	0.21	(-0.39 ; 0.80)	0.499
Week 12	0.00	(-0.56 ; 0.55)	0.992
Interleukin-2			
Week 2	-3.67	(-7.99 ; 0.64)	0.095
Week 4	1.86	(-2.53 ; 6.25)	0.405
Week 12	-0.23	(-4.28 ; 3.82)	0.910
Interleukin-4			
Week 2	-0.67	(-7.16 ; 5.81)	0.839
Week 4	0.04	(-6.55 ; 6.64)	0.989
Week 12	2.30	(-3.84 ; 8.45)	0.462
Interleukin-6			
Week 2	0.86	(-5.26 ; 6.98)	0.783
Week 4	1.97	(-4.27 ; 8.22)	0.535
Week 12	4.26	(-1.44 ; 9.96)	0.143
Interleukin-10			
Week 2	8.35	(-26.72 ; 43.41)	0.641
Week 4	12.69	(-23.08 ; 48.46)	0.487
Week 12	19.21	(-13.35 ; 51.78)	0.247
TNF-α			
Week 2	-0.17	(-4.46 ; 4.12)	0.939
Week 4	1.45	(-2.92 ; 5.83)	0.515
Week 12	1.62	(-2.39 ; 5.62)	0.429

Table R-4: Effect of d4T exposure on cytokine levels (remainder of cytokines)

Cytokine & Visit	D4T exposure group effect		
	Coefficient	Confidence interval (95%)	p-value
Interleukin-5			
Week 2	-0.55	(-1.66 ; 0.55)	0.325
Week 4	0.70	(-0.42 ; 1.83)	0.220
Week 12	0.49	(-0.53 ; 1.52)	0.345
Interleukin-7			
Week 2	-1.69	(-5.39 ; 2.01)	0.371
Week 4	0.42	(-3.36 ; 4.19)	0.828
Week 12	1.20	(-2.24 ; 4.64)	0.493
Interleukin-8			
Week 2	0.65	(-0.95 ; 2.26)	0.425
Week 4	1.27	(-0.36 ; 2.91)	0.127
Week 12	2.23	(0.73 ; 3.73)	***0.004***
Interleukin-12			
Week 2	-4.97	(-20.84 ; 10.90)	0.539
Week 4	4.87	(-11.31 ; 21.06)	0.555
Week 12	7.34	(-7.44 ; 22.13)	0.330
Interleukin-13			
Week 2	-17.42	(-32.80 ; -2.03)	***0.026***
Week 4	7.21	(-8.46 ; 22.88)	0.367
Week 12	2.37	(-12.03 ; 16.76)	0.747
IFN-γ			
Week 2	3.76	(-6.37 ; 13.89)	0.467
Week 4	3.03	(-7.27 ; 13.33)	0.564
Week 12	0.90	(-8.64 ; 10.44)	0.853
GM-CSF			
Week 2	-1.73	(-4.70 ; 1.24)	0.253
Week 4	0.87	(-2.16 ; 3.89)	0.574
Week 12	0.76	(-2.04 ; 3.55)	0.595

Table R-5: Comparison of cytokine concentrations across TB groups

Cytokine	Previous TB (n = 14)	No Previous TB (n = 44)	p-value
Interleukin-1β	0.1 (0.1 - 0.4)	0.1 (0.0 - 0.3)	0.304 [ε]
Interleukin-2	1.1 (0.2 - 2.6)	0.9 (0.1 - 2.2)	0.501 [ε]
Interleukin-4	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.1)	0.421 [ε]
Interleukin-5	0.5 (0.3 - 1.2)	0.6 (0.3 - 1.1)	0.696 [ε]
Interleukin-6	2.7 (1.9 - 3.1)	3.0 (1.8 - 6.4)	0.467 [ε]
Interleukin-7	1.8 (0.9 - 3.6)	2.2 (0.6 - 3.8)	0.856 [ε]
Interleukin-8	3.8 (2.8 - 5.5)	3.8 (2.4 - 4.9)	0.737 [ε]
Interleukin-10	11.8 (5.4 - 23.9)	15.5 (7.7 - 29.7)	0.345 [ε]
Interleukin-12	0.0 (0.0 - 1.0)	0.0 (0.0 - 1.1)	0.673 [ε]
Interleukin-13	0.5 (0.5 - 4.6)	0.5 (0.5 - 7.4)	0.984 [ε]
IFN-γ	1.3 (0.0 - 3.1)	1.2 (0.0 - 3.7)	0.993 [ε]
GMCSF	1.0 (0.4 - 1.5)	0.7 (0.2 - 1.7)	0.792 [ε]
TNF-α	8.0 (5.7 - 13.1)	6.6 (4.1 - 11.0)	0.258 [ε]

[ε] Wilcoxon rank-sum test

Table R-6: Comparison of cytokine concentrations across baseline PLP groups

Cytokine	PLP < 25 nmol/L (n = 33)	PLP > 25 nmol/L (n = 25)	p-value
Interleukin-1β	0.1 (0.0 - 0.3)	0.2 (0.1 - 0.4)	0.362 [ε]
Interleukin-2	1.0 (0.1 - 2.0)	1.0 (0.2 - 2.9)	0.465 [ε]
Interleukin-4	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.1)	0.823 [ε]
Interleukin-5	0.5 (0.3 - 0.8)	0.9 (0.4 - 1.2)	0.255 [ε]
Interleukin-6	3.1 (2.0 - 6.1)	2.5 (1.6 - 4.0)	0.151 [ε]
Interleukin-7	2.2 (1.1 - 3.8)	1.9 (0.5 - 3.4)	0.495 [ε]
Interleukin-8	3.4 (2.4 - 4.8)	3.8 (2.7 - 5.5)	0.456 [ε]
Interleukin-10	14.6 (5.4 - 25.4)	16.1 (9.7 - 29.4)	0.319 [ε]
Interleukin-12	0.0 (0.0 - 1.1)	0.0 (0.0 - 0.6)	0.952 [ε]
Interleukin-13	0.5 (0.5 - 9.8)	0.5 (0.5 - 6.5)	0.739 [ε]
IFN-γ	2.2 (0.0 - 3.8)	0.6 (0.0 - 2.8)	0.234 [ε]
GMCSF	0.7 (0.1 - 1.5)	0.8 (0.4 - 1.3)	0.414 [ε]
TNF-α	8.2 (5.6 - 12.0)	6.0 (4.1 - 8.4)	0.113 [ε]

[ε] Wilcoxon rank-sum test

University of Cape Town

Table R-7: Effect of symptom duration and severity on cytokine levels (remainder of cytokines)

Cytokine	Symptom duration group effect[a]			Symptom severity group effect[b]		
	Coefficient	Confidence interval (95%)	p-value	Coefficient	Confidence interval (95%)	p-value
Interleukin-5						
Week 2	0.01	(-0.95 ; 0.98)	0.979	-0.32	(-1.22 ; 0.58)	0.486
Week 4	0.35	(-0.63 ; 1.32)	0.485	-0.48	(-1.37 ; 0.42)	0.298
Week 12	-0.35	(-1.34 ; 0.63)	0.484	-0.43	(-1.29 ; 0.43)	0.330
Interleukin-7						
Week 2	-4.47	(-9.50 ; 0.55)	0.081	-0.99	(-5.64 ; 3.65)	0.675
Week 4	-1.37	(-6.43 ; 3.69)	0.596	0.52	(-4.12 ; 5.17)	0.825
Week 12	-3.07	(-8.19 ; 2.05)	0.240	0.06	(-4.44 ; 4.56)	0.979
Interleukin-8						
Week 2	-0.51	(-2.70 ; 1.68)	0.648	-0.61	(-2.67 ; 1.45)	0.560
Week 4	-0.11	(-2.32 ; 2.10)	0.922	-0.53	(-2.59 ; 1.53)	0.614
Week 12	-0.26	(-2.49 ; 1.98)	0.822	-0.38	(-2.37 ; 1.61)	0.709
Interleukin-12						
Week 2	-5.99	(-15.39 ; 3.42)	0.212	-2.74	(-11.37 ; 5.90)	0.534
Week 4	-3.74	(-13.22 ; 5.74)	0.439	0.22	(-8.41 ; 8.86)	0.959
Week 12	0.16	(-9.42 ; 9.73)	0.974	1.91	(-6.47 ; 10.29)	0.655
Interleukin-13						
Week 2	-4.17	(-25.22 ; 16.87)	0.698	-6.77	(-26.04 ; 12.50)	0.491
Week 4	-0.60	(-21.81 ; 20.62)	0.956	8.83	(-10.44 ; 28.11)	0.369
Week 12	-14.37	(-35.82 ; 7.08)	0.189	3.50	(-15.20 ; 22.21)	0.713
IFN-γ						
Week 2	-12.73	(-32.32 ; 6.86)	0.203	15.19	(-2.07 ; 32.45)	0.085
Week 4	-4.25	(-23.98 ; 15.48)	0.673	0.32	(-16.94 ; 17.59)	0.971
Week 12	3.70	(-16.21 ; 23.62)	0.716	4.92	(-11.89 ; 21.72)	0.566
GM-CSF						
Week 2	-0.77	(-5.77 ; 4.24)	0.764	-1.03	(-5.49 ; 3.43)	0.651
Week 4	0.25	(-4.79 ; 5.29)	0.923	1.36	(-3.10 ; 5.82)	0.550
Week 12	2.77	(-2.32 ; 7.87)	0.286	2.71	(-1.63 ; 7.04)	0.221

[a] Difference in mean cytokine concentration between resolved symptoms and unresolved symptoms compared to baseline

[b] Difference in mean cytokine concentration between grade ≥ 2 severity and grade 1 severity compared to baseline

University of Cape Town

References

ABRAMS, B., DUNCAN, D. & HERTZ-PICCIOTTO, I. 1993. A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. *J Acquir Immune Defic Syndr,* 6**,** 949-58.

ACHARJEE, S., ZHU, Y., MAINGAT, F., PARDO, C., BALLANYI, K., HOLLENBERG, M. D. & POWER, C. 2011. Proteinase-activated receptor-1 mediates dorsal root ganglion neuronal degeneration in HIV/AIDS. *Brain,* 134**,** 3209-21.

ADAMS, C. H., WERELY, C. J., VICTOR, T. C., HOAL, E. G., ROSSOUW, G. & VAN HELDEN, P. D. 2003. Allele frequencies for glutathione S-transferase and *N*-acetyltransferase 2 differ in African population groups and may be associated with oesophageal cancer or tuberculosis incidence. *Clin Chem Lab Med,* 41**,** 600-5.

AFFANDI, J. S., PRICE, P., IMRAN, D., YUNIHASTUTI, E., DJAUZI, S. & CHERRY, C. L. 2008. Can we predict neuropathy risk before stavudine prescription in a resource-limited setting? *AIDS Res Hum Retroviruses,* 24**,** 1281-4.

AGUNDEZ, J. A., GOLKA, K., MARTINEZ, C., SELINSKI, S., BLASZKEWICZ, M. & GARCIA-MARTIN, E. 2008. Unraveling ambiguous *NAT2* genotyping data. *Clin Chem,* 54**,** 1390-4.

ALBERICI, L. C., OLIVEIRA, H. C., BIGHETTI, E. J., DE FARIA, E. C., DEGASPARI, G. R., SOUZA, C. T. & VERCESI, A. E. 2003. Hypertriglyceridemia increases mitochondrial resting respiration and susceptibility to permeability transition. *J Bioenerg Biomembr,* 35**,** 451-7.

ALEXEYEV, M. F., LEDOUX, S. P. & WILSON, G. L. 2004. Mitochondrial DNA and aging. *Clin Sci (Lond),* 107**,** 355-64.

ALFIREVIC, A., STALFORD, A. C., VILAR, F. J., WILKINS, E. G., PARK, B. K. & PIRMOHAMED, M. 2003. Slow acetylator phenotype and genotype in HIV-positive patients with sulphamethoxazole hypersensitivity. *Br J Clin Pharmacol,* 55**,** 158-65.

ALIER, K. A., ENDICOTT, J. A., STEMKOWSKI, P. L., CENAC, N., CELLARS, L., CHAPMAN, K., ANDRADE-GORDON, P., VERGNOLLE, N. & SMITH, P. A. 2008. Intrathecal administration of proteinase-activated receptor-2 agonists produces hyperalgesia by exciting the cell bodies of primary sensory neurons. *J Pharmacol Exp Ther,* 324**,** 224-33.

ALLARD, J. P., AGHDASSI, E., CHAU, J., SALIT, I. & WALMSLEY, S. 1998. Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection. *Am J Clin Nutr,* 67**,** 143-7.

ALOE, L., MORONI, R., ANGELUCCI, F. & FIORE, M. 1997. Role of TNF-alpha but not NGF in murine hyperalgesia induced by parasitic infection. *Psychopharmacology (Berl),* 134**,** 287-92.

AMES, B. N. 2004. Delaying the mitochondrial decay of aging. *Ann N Y Acad Sci,* 1019**,** 406-11.

AMIN, J., KAYE, M., SKIDMORE, S., PILLAY, D., COOPER, D. A. & DORE, G. J. 2004. HIV and hepatitis C coinfection within the CAESAR study. *HIV Med,* 5**,** 174-9.

ANABWANI, G. & NAVARIO, P. 2005. Nutrition and HIV/AIDS in sub-Saharan Africa: an overview. *Nutrition,* 21**,** 96-9.

ANCES, B. M., VAIDA, F., ROSARIO, D., MARQUIE-BECK, J., ELLIS, R. J., SIMPSON, D. M., CLIFFORD, D. B., MCARTHUR, J. C., GRANT, I., MCCUTCHAN, J. A. & GROUP, C. H. A. T. E. R. M. S. 2009. Role of metabolic syndrome components in HIV-associated sensory neuropathy. *AIDS,* 23**,** 2317-22.

University of Cape Town

ANDERSON, P. L., KAKUDA, T. N. & LICHTENSTEIN, K. A. 2004. The cellular pharmacology of nucleoside- and nucleotide-analogue reverse-transcriptase inhibitors and its relationship to clinical toxicities. *Clin Infect Dis,* 38**,** 743-53.

APOLONIO, E. G., HOOVER, D. R., HE, Y., SAAH, A. J., LYTER, D. W., DETELS, R., KASLOW, R. A. & PHAIR, J. P. 1995. Prognostic factors in human immunodeficiency virus-positive patients with a CD4+ lymphocyte count < 50/microL. *J Infect Dis,* 171**,** 829-36.

ARENAS-PINTO, A., BHASKARAN, K., DUNN, D. & WELLER, I. V. 2008. The risk of developing peripheral neuropathy induced by nucleoside reverse transcriptase inhibitors decreases over time: evidence from the Delta trial. *Antivir Ther,* 13**,** 289-95.

AREND, W. P., MALYAK, M., GUTHRIDGE, C. J. & GABAY, C. 1998. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol,* 16**,** 27-55.

ARGOV, Z. & MASTAGLIA, F. L. 1979. Drug-induced peripheral neuropathies. *Br Med J,* 1**,** 663-6.

ARNAUDO, E., DALAKAS, M., SHANSKE, S., MORAES, C. T., DIMAURO, S. & SCHON, E. A. 1991. Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. *Lancet,* 337**,** 508-10.

ARNETT, H. A., MASON, J., MARINO, M., SUZUKI, K., MATSUSHIMA, G. K. & TING, J. P. 2001. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci,* 4**,** 1116-22.

ATAMNA, H., KILLILEA, D. W., KILLILEA, A. N. & AMES, B. N. 2002. Heme deficiency may be a factor in the mitochondrial and neuronal decay of aging. *Proc Natl Acad Sci U S A,* 99**,** 14807-12.

ATAMNA, H., LIU, J. & AMES, B. N. 2001. Heme deficiency selectively interrupts assembly of mitochondrial complex IV in human fibroblasts: revelance to aging. *J Biol Chem,* 276**,** 48410-6.

AZIZ, N., NISHANIAN, P., TAYLOR, J. M., MITSUYASU, R. T., JACOBSON, J. M., DEZUBE, B. J., LEDERMAN, M. M., DETELS, R. & FAHEY, J. L. 1999. Stability of plasma levels of cytokines and soluble activation markers in patients with human immunodeficiency virus infection. *J Infect Dis,* 179**,** 843-8.

BACELLAR, H., MUNOZ, A., MILLER, E. N., COHEN, B. A., BESLEY, D., SELNES, O. A., BECKER, J. T. & MCARTHUR, J. C. 1994. Temporal trends in the incidence of HIV-1-related neurologic diseases: Multicenter AIDS Cohort Study, 1985-1992. *Neurology,* 44**,** 1892-900.

BACH, P. H., HIGGINS-OPITZ, S. B., BIMA, B. & LEARY, W. P. 1976. Isoniazid acetylator status of Black South African tuberculosis patients. *S Afr Med J,* 50**,** 1132-4.

BACKONJA, M. M., COE, C. L., MULLER, D. A. & SCHELL, K. 2008. Altered cytokine levels in the blood and cerebrospinal fluid of chronic pain patients. *J Neuroimmunol,* 195**,** 157-63.

BADRI, M., WILSON, D. & WOOD, R. 2002. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. *Lancet,* 359**,** 2059-64.

BAILEY, A. L., WRIGHT, A. J. & SOUTHON, S. 1999. High performance liquid chromatography method for the determination of pyridoxal-5-phosphate in human plasma: how appropriate are cut-off values for vitamin B6 deficiency? *Eur J Clin Nutr,* 53**,** 448-55.

BAILEY, R. O., BALTCH, A. L., VENKATESH, R., SINGH, J. K. & BISHOP, M. B. 1988. Sensory motor neuropathy associated with AIDS. *Neurology,* 38**,** 886-91.

BAKAYEV, V. V., MOHAMMADI, F., BAHADORI, M., SHEIKHOLSLAMI, M., JAVERI, A., MASJEDI, M. R. & VELAYATI, A. A. 2004. Arylamine *N*-

University of Cape Town

acetyltransferase 2 slow acetylator polymorphisms in unrelated Iranian individuals. *Eur J Clin Pharmacol,* 60**,** 467-71.

BANERJEE, S., MCCUTCHAN, J. A., ANCES, B. M., DEUTSCH, R., RIGGS, P. K., WAY, L. & ELLIS, R. J. 2011. Hypertriglyceridemia in combination antiretroviral-treated HIV-positive individuals: potential impact on HIV sensory polyneuropathy. *AIDS,* 25**,** F1-6.

BANI-SADR, F., CARRAT, F., POL, S., HOR, R., ROSENTHAL, E., GOUJARD, C., MORAND, P., LUNEL-FABIANI, F., SALMON-CERON, D., PIROTH, L., PIALOUX, G., BENTATA, M., CACOUB, P. & PERRONNE, C. 2005. Risk factors for symptomatic mitochondrial toxicity in HIV/hepatitis C virus-coinfected patients during interferon plus ribavirin-based therapy. *J Acquir Immune Defic Syndr,* 40**,** 47-52.

BARNES, A. 1998. Measurement of serum cytokines. *Lancet,* 352**,** 324-5.

BAROHN, R. J., GRONSETH, G. S., AMATO, A. A., MCGUIRE, S. A., MCVEY, A. L., LEFORCE, B. R. & KING, R. B. 1996. Cerebrospinal fluid and nerve conduction abnormalities in HIV positive individuals. *J Neurol Sci,* 136**,** 81-5.

BAROHN, R. J., GRONSETH, G. S., LEFORCE, B. R., MCVEY, A. L., MCGUIRE, S. A., BUTZIN, C. A. & KING, R. B. 1993. Peripheral nervous system involvement in a large cohort of human immunodeficiency virus-infected individuals. *Arch Neurol,* 50**,** 167-71.

BARTLEY, P. B., WESTACOTT, L., BOOTS, R. J., LAWSON, M., POTTER, J. M., HYLAND, V. J. & WOODS, M. L., 2ND 2001. Large hepatic mitochondrial DNA deletions associated with L-lactic acidosis and highly active antiretroviral therapy. *AIDS,* 15**,** 419-20.

BATES, C. J., PENTIEVA, K. D., MATTHEWS, N. & MACDONALD, A. 1999. A simple, sensitive and reproducible assay for pyridoxal 5'-phosphate and 4-pyridoxic acid in human plasma. *Clin Chim Acta,* 280**,** 101-11.

BAUM, M. K., MANTERO-ATIENZA, E., SHOR-POSNER, G., FLETCHER, M. A., MORGAN, R., EISDORFER, C., SAUBERLICH, H. E., CORNWELL, P. E. & BEACH, R. S. 1991. Association of vitamin B6 status with parameters of immune function in early HIV-1 infection. *J Acquir Immune Defic Syndr,* 4**,** 1122-32.

BAUM, M. K., SHOR-POSNER, G., LU, Y., ROSNER, B., SAUBERLICH, H. E., FLETCHER, M. A., SZAPOCZNIK, J., EISDORFER, C., BURING, J. E. & HENNEKENS, C. H. 1995. Micronutrients and HIV-1 disease progression. *AIDS,* 9**,** 1051-6.

BEACH, R. S., MANTERO-ATIENZA, E., SHOR-POSNER, G., JAVIER, J. J., SZAPOCZNIK, J., MORGAN, R., SAUBERLICH, H. E., CORNWELL, P. E., EISDORFER, C. & BAUM, M. K. 1992. Specific nutrient abnormalities in asymptomatic HIV-1 infection. *AIDS,* 6**,** 701-8.

BEADLES, W. I., JAHN, A., WEIGEL, R. & CLUTTERBUCK, D. 2009. Peripheral neuropathy in HIV-positive patients at an antiretroviral clinic in Lilongwe, Malawi. *Trop Doct,* 39**,** 78-80.

BEBELL, L. M., PASSMORE, J. A., WILLIAMSON, C., MLISANA, K., IRIOGBE, I., VAN LOGGERENBERG, F., KARIM, Q. A. & KARIM, S. A. 2008. Relationship between levels of inflammatory cytokines in the genital tract and CD4+ cell counts in women with acute HIV-1 infection. *J Infect Dis,* 198**,** 710-4.

BEIROWSKI, B., ADALBERT, R., WAGNER, D., GRUMME, D. S., ADDICKS, K., RIBCHESTER, R. R. & COLEMAN, M. P. 2005. The progressive nature of Wallerian degeneration in wild-type and slow Wallerian degeneration (WldS) nerves. *BMC Neurosci,* 6**,** 6.

University of Cape Town

BENATAR, M. 2006. Distal symmetric polyneuropathy: limitations of the proposed case definition. *Muscle Nerve,* 34**,** 131-4.

BENBOW, S. J., WALLYMAHMED, M. E. & MACFARLANE, I. A. 1998. Diabetic peripheral neuropathy and quality of life. *QJM,* 91**,** 733-7.

BERGER, A. R., AREZZO, J. C., SCHAUMBURG, H. H., SKOWRON, G., MERIGAN, T., BOZZETTE, S., RICHMAN, D. & SOO, W. 1993. 2',3'-dideoxycytidine (ddC) toxic neuropathy: a study of 52 patients. *Neurology,* 43**,** 358-62.

BERGSTEINSDOTTIR, K., KINGSTON, A., MIRSKY, R. & JESSEN, K. R. 1991. Rat Schwann cells produce interleukin-1. *J Neuroimmunol,* 34**,** 15-23.

BETTERIDGE, D. J. 2000. What is oxidative stress? *Metabolism,* 49**,** 3-8.

BHANGOO, S. K., RIPSCH, M. S., BUCHANAN, D. J., MILLER, R. J. & WHITE, F. A. 2009. Increased chemokine signaling in a model of HIV1-associated peripheral neuropathy. *Mol Pain,* 5**,** 48.

BIANCHI, M., DIB, B. & PANERAI, A. E. 1998. Interleukin-1 and nociception in the rat. *J Neurosci Res,* 53**,** 645-50.

BIEHL, J. P. & SKLAVEM, J. H. 1953. Toxicity of isoniazid. *Am Rev Tuberc,* 68**,** 296-7.

BIEHL, J. P. & VILTER, R. W. 1954. Effects of isoniazid on pyridoxine metabolism. *J Am Med Assoc,* 156**,** 1549-52.

BIRKUS, G., HITCHCOCK, M. J. & CIHLAR, T. 2002. Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. *Antimicrob Agents Chemother,* 46**,** 716-23.

BLUM, A. S., DAL PAN, G. J., FEINBERG, J., RAINES, C., MAYJO, K., CORNBLATH, D. R. & MCARTHUR, J. C. 1996. Low-dose zalcitabine-related toxic neuropathy: frequency, natural history, and risk factors. *Neurology,* 46**,** 999-1003.

BLUM, M., DEMIERRE, A., GRANT, D. M., HEIM, M. & MEYER, U. A. 1991. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. *Proc Natl Acad Sci U S A,* 88**,** 5237-41.

BOGDEN, J. D., BAKER, H., FRANK, O., PEREZ, G., KEMP, F., BRUENING, K. & LOURIA, D. 1990. Micronutrient status and human immunodeficiency virus (HIV) infection. *Ann N Y Acad Sci,* 587**,** 189-95.

BOLHAAR, M. G. & KARSTAEDT, A. S. 2007. A high incidence of lactic acidosis and symptomatic hyperlactatemia in women receiving highly active antiretroviral therapy in Soweto, South Africa. *Clin Infect Dis,* 45**,** 254-60.

BONNET, F., BONAREK, M., MORLAT, P., MERCIE, P., DUPON, M., GEMAIN, M. C., MALVY, D., BERNARD, N., PELLEGRIN, J. L. & BEYLOT, J. 2003. Risk factors for lactic acidosis in HIV-infected patients treated with nucleoside reverse-transcriptase inhibitors: a case-control study. *Clin Infect Dis,* 36**,** 1324-8.

BOR, M. V., REFSUM, H., BISP, M. R., BLEIE, O., SCHNEEDE, J., NORDREHAUG, J. E., UELAND, P. M., NYGARD, O. K. & NEXO, E. 2003. Plasma vitamin B6 vitamers before and after oral vitamin B6 treatment: a randomized placebo-controlled study. *Clin Chem,* 49**,** 155-61.

BOUBAKER, K., FLEPP, M., SUDRE, P., FURRER, H., HAENSEL, A., HIRSCHEL, B., BOGGIAN, K., CHAVE, J. P., BERNASCONI, E., EGGER, M., OPRAVIL, M., RICKENBACH, M., FRANCIOLI, P. & TELENTI, A. 2001. Hyperlactatemia and antiretroviral therapy: the Swiss HIV Cohort Study. *Clin Infect Dis,* 33**,** 1931-7.

BOUCHE, P., CATTELIN, F., SAINT-JEAN, O., LEGER, J. M., QUESLATI, S., GUEZ, D., MOULONGUET, A., BRAULT, Y., AQUINO, J. P. & SIMUNEK, P. 1993. Clinical and electrophysiological study of the peripheral nervous system in the elderly. *J Neurol,* 240**,** 263-8.

University of Cape Town

BOULLE, A., ORREL, C., KAPLAN, R., VAN CUTSEM, G., MCNALLY, M., HILDERBRAND, K., MYER, L., EGGER, M., COETZEE, D., MAARTENS, G. & WOOD, R. 2007. Substitutions due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral therapy in a large South African cohort. *Antivir Ther,* 12**,** 753-60.

BRADLEY, W. G., SHAPSHAK, P., DELGADO, S., NAGANO, I., STEWART, R. & ROCHA, B. 1998. Morphometric analysis of the peripheral neuropathy of AIDS. *Muscle Nerve,* 21**,** 1188-95.

BREEN, R. A., MILLER, R. F., GORSUCH, T., SMITH, C. J., SCHWENK, A., HOLMES, W., BALLINGER, J., SWADEN, L., JOHNSON, M. A., CROPLEY, I. & LIPMAN, M. C. 2006. Adverse events and treatment interruption in tuberculosis patients with and without HIV co-infection. *Thorax,* 61**,** 791-4.

BREW, B. J. 2003. The peripheral nerve complications of human immunodeficiency virus (HIV) infection. *Muscle Nerve,* 28**,** 542-52.

BRIN, M. 1978. Vitamin B6: Chemistry, absorption, metabolism, catabolism, and toxicity. *In:* FOOD AND NUTRITION BOARD, N. R. C. (ed.) *Human Vitamin B6 Requirements.* Washington, DC: National Academy of Sciences.

BRINKMAN, K. 2001. Management of hyperlactatemia: no need for routine lactate measurements. *AIDS,* 15**,** 795-7.

BRINKMAN, K., TER HOFSTEDE, H. J., BURGER, D. M., SMEITINK, J. A. & KOOPMANS, P. P. 1998. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. *AIDS,* 12**,** 1735-44.

BRINLEY, F. J., JR., PARDO, C. A. & VERMA, A. 2001. Human immunodeficiency virus and the peripheral nervous system workshop. *Arch Neurol,* 58**,** 1561-6.

BRUNK, U. T. & TERMAN, A. 2002. The mitochondrial-lysosomal axis theory of aging: accumulation of damaged mitochondria as a result of imperfect autophagocytosis. *Eur J Biochem,* 269**,** 1996-2002.

BUCKMASTER, E. A., PERRY, V. H. & BROWN, M. C. 1995. The rate of Wallerian degeneration in cultured neurons from wild type and C57BL/WldS mice depends on time in culture and may be extended in the presence of elevated K+ levels. *Eur J Neurosci,* 7**,** 1596-602.

BUGIANESI, E., GENTILCORE, E., MANINI, R., NATALE, S., VANNI, E., VILLANOVA, N., DAVID, E., RIZZETTO, M. & MARCHESINI, G. 2005. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol,* 100**,** 1082-90.

BUHL, R., JAFFE, H. A., HOLROYD, K. J., WELLS, F. B., MASTRANGELI, A., SALTINI, C., CANTIN, A. M. & CRYSTAL, R. G. 1989. Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet,* 2**,** 1294-8.

BUSS, J. L. & PONKA, P. 2003. Hydrolysis of pyridoxal isonicotinoyl hydrazone and its analogs. *Biochim Biophys Acta,* 1619**,** 177-86.

BUTT, A. A. 2003. Fatal lactic acidosis and pancreatitis associated with ribavirin and didanosine therapy. *AIDS Read,* 13**,** 344-8.

BYGRAVE, H., FORD, N., VAN CUTSEM, G., HILDERBRAND, K., JOUQUET, G., GOEMAERE, E., VLAHAKIS, N., TRIVINO, L., MAKAKOLE, L. & KRANZER, K. 2011. Implementing a tenofovir-based first-line regimen in rural Lesotho: clinical outcomes and toxicities after two years. *J Acquir Immune Defic Syndr,* 56**,** e75-8.

CALLARD, R. & GEARING, A. 1994. *The cytokine facts book,* London, Academic Press.

CANTER, J. A., ROBBINS, G. K., SELPH, D., CLIFFORD, D. B., KALLIANPUR, A. R., SHAFER, R., LEVY, S., MURDOCK, D. G., RITCHIE, M. D., HAAS, D. W. &

University of Cape Town

HULGAN, T. 2010. African mitochondrial DNA subhaplogroups and peripheral neuropathy during antiretroviral therapy. *J Infect Dis,* 201**,** 1703-7.

CARDONA, F., TUNEZ, I., TASSET, I., MURRI, M. & TINAHONES, F. J. 2008. Similar increase in oxidative stress after fat overload in persons with baseline hypertriglyceridemia with or without the metabolic syndrome. *Clin Biochem,* 41**,** 701-5.

CARR, A. & COOPER, D. A. 2000. Adverse effects of antiretroviral therapy. *Lancet,* 356**,** 1423-30.

CASCORBI, I., DRAKOULIS, N., BROCKMOLLER, J., MAURER, A., SPERLING, K. & ROOTS, I. 1995. Arylamine *N*-acetyltransferase (*NAT2*) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. *Am J Hum Genet,* 57**,** 581-92.

CASCORBI, I. & ROOTS, I. 1999. Pitfalls in *N*-acetyltransferase 2 genotyping. *Pharmacogenetics,* 9**,** 123-7.

CASULA, M., WEVERLING, G., DE BAAR, M., WIT, F., STEK, M., LANGE, J. & REISS, P. 2004. Longitudinal assessment of mitochondrial DNA and RNA in PBMC in a randomized comparative trial of NRTI-sparing and NRTI-containing antiretroviral combination therapy. *11th Conference on Retroviruses & Opportunistic Infections.* San Francisco, CA, USA.

CAVALETTI, G., BOGLIUN, G., MARZORATI, L., ZINCONE, A., PIATTI, M., COLOMBO, N., PARMA, G., LISSONI, A., FEI, F., CUNDARI, S. & ZANNA, C. 2003. Grading of chemotherapy-induced peripheral neurotoxicity using the Total Neuropathy Scale. *Neurology,* 61**,** 1297-300.

CAVALETTI, G., FRIGENI, B., LANZANI, F., PIATTI, M., ROTA, S., BRIANI, C., ZARA, G., PLASMATI, R., PASTORELLI, F., CARACENI, A., PACE, A., MANICONE, M., LISSONI, A., COLOMBO, N., BIANCHI, G. & ZANNA, C. 2007. The Total Neuropathy Score as an assessment tool for grading the course of chemotherapy-induced peripheral neurotoxicity: comparison with the National Cancer Institute-Common Toxicity Scale. *J Peripher Nerv Syst,* 12**,** 210-5.

CAVALETTI, G., JANN, S., PACE, A., PLASMATI, R., SICILIANO, G., BRIANI, C., COCITO, D., PADUA, L., GHIGLIONE, E., MANICONE, M. & GIUSSANI, G. 2006. Multi-center assessment of the Total Neuropathy Score for chemotherapy-induced peripheral neurotoxicity. *J Peripher Nerv Syst,* 11**,** 135-41.

CAYOTA, A., VUILLIER, F., SICILIANO, J. & DIGHIERO, G. 1994. Defective protein tyrosine phosphorylation and altered levels of p59fyn and p56lck in CD4 T cells from HIV-1 infected patients. *Int Immunol,* 6**,** 611-21.

CDC 2003. Centers for Disease Control and Prevention. Treatment of tuberculosis. Atlanta, GA, USA: American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America.

CDC 2009. Centers for Disease Control and Prevention. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents. Atlanta, GA, USA.

CENTNER, C. 2012. *Distal sensory polyneuropathy in HIV/TB co-infection: the role of vitamin B6 status and N-acetyltransferase 2 genetic variation.* Master of Science in Medicine, University of Cape Town, South Africa.

CERIELLO, A. & MOTZ, E. 2004. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol,* 24**,** 816-23.

CETTOMAI, D., KWASA, J., KENDI, C., BIRBECK, G. L., PRICE, R. W., BUKUSI, E. A., COHEN, C. R. & MEYER, A. C. 2010. Utility of quantitative sensory testing and

University of Cape Town

screening tools in identifying HIV-associated peripheral neuropathy in Western Kenya: pilot testing. *PLoS One,* 5**,** e14256.

CHAN, D. C. 2006. Mitochondria: dynamic organelles in disease, aging, and development. *Cell,* 125**,** 1241-52.

CHAN, E. D. & ISEMAN, M. D. 2002. Current medical treatment for tuberculosis. *BMJ,* 325**,** 1282-6.

CHANG, K. T., NIESCIER, R. F. & MIN, K. T. 2011. Mitochondrial matrix Ca^{2+} as an intrinsic signal regulating mitochondrial motility in axons. *Proc Natl Acad Sci U S A,* 108**,** 15456-61.

CHAVANET, GIROUD, M., SOLARY, E., WALDNER, A. & PORTER, H. 1989. Immune mechanisms in HIV-related neuropathies. *Lancet,* 2**,** 812-3.

CHENE, G., EASTERBROOK, P. J., JUSZCZAK, E., YU, L. M., POCOCK, S. J. & GAZZARD, B. G. 1997. Long-term survival in patients with advanced immunodeficiency. *AIDS,* 11**,** 209-16.

CHENG, C. H., CHANG, S. J., LEE, B. J., LIN, K. L. & HUANG, Y. C. 2006. Vitamin B6 supplementation increases immune responses in critically ill patients. *Eur J Clin Nutr,* 60**,** 1207-13.

CHERRY, C. L., AFFANDI, J. S., BREW, B. J., CREIGHTON, J., DJAUZI, S., HOOKER, D. J., IMRAN, D., KAMARULZAMAN, A., KAMERMAN, P., MCARTHUR, J. C., MOORE, R. D., PRICE, P., SMYTH, K., TAN, I. L., VANAR, S., WADLEY, A., WESSELINGH, S. L. & YUNIHASTUTI, E. 2010. Hepatitis C seropositivity is not a risk factor for sensory neuropathy among patients with HIV. *Neurology,* 74**,** 1538-42.

CHERRY, C. L., AFFANDI, J. S., IMRAN, D., YUNIHASTUTI, E., SMYTH, K., VANAR, S., KAMARULZAMAN, A. & PRICE, P. 2009. Age and height predict neuropathy risk in patients with HIV prescribed stavudine. *Neurology,* 73**,** 315-20.

CHERRY, C. L., MCARTHUR, J. C., HOY, J. F. & WESSELINGH, S. L. 2003. Nucleoside analogues and neuropathy in the era of HAART. *J Clin Virol,* 26**,** 195-207.

CHERRY, C. L., ROSENOW, A., AFFANDI, J. S., MCARTHUR, J. C., WESSELINGH, S. L. & PRICE, P. 2008. Cytokine genotype suggests a role for inflammation in nucleoside analog-associated sensory neuropathy (NRTI-SN) and predicts an individual's NRTI-SN risk. *AIDS Res Hum Retroviruses,* 24**,** 117-23.

CHERRY, C. L., SKOLASKY, R. L., LAL, L., CREIGHTON, J., HAUER, P., RAMAN, S. P., MOORE, R., CARTER, K., THOMAS, D., EBENEZER, G. J., WESSELINGH, S. L. & MCARTHUR, J. C. 2006. Antiretroviral use and other risks for HIV-associated neuropathies in an international cohort. *Neurology,* 66**,** 867-73.

CHERRY, C. L., WESSELINGH, S. L., LAL, L. & MCARTHUR, J. C. 2005. Evaluation of a clinical screening tool for HIV-associated sensory neuropathies. *Neurology,* 65**,** 1778-81.

CHEW, C. S., CHERRY, C. L., KAMARULZAMAN, A., YIEN, T. H., AGHAFAR, Z. & PRICE, P. 2011. A longitudinal study of the effects of ART on plasma chemokine levels in Malaysian HIV patients. *Dis Markers,* 31**,** 303-9.

CHIANG, C. Y., WANG, J., XIE, Y. F., ZHANG, S., HU, J. W., DOSTROVSKY, J. O. & SESSLE, B. J. 2007. Astroglial glutamate-glutamine shuttle is involved in central sensitization of nociceptive neurons in rat medullary dorsal horn. *J Neurosci,* 27**,** 9068-76.

CHIANG, E. P., SELHUB, J., BAGLEY, P. J., DALLAL, G. & ROUBENOFF, R. 2005a. Pyridoxine supplementation corrects vitamin B6 deficiency but does not improve inflammation in patients with rheumatoid arthritis. *Arthritis Res Ther,* 7**,** R1404-11.

University of Pretoria

CHIANG, E. P., SMITH, D. E., SELHUB, J., DALLAL, G., WANG, Y. C. & ROUBENOFF, R. 2005b. Inflammation causes tissue-specific depletion of vitamin B6. *Arthritis Res Ther,* 7**,** R1254-62.

CHIKANZA, I. C., ROUX-LOMBARD, P., DAYER, J. M. & PANAYI, G. S. 1993. Tumour necrosis factor soluble receptors behave as acute phase reactants following surgery in patients with rheumatoid arthritis, chronic osteomyelitis and osteoarthritis. *Clin Exp Immunol,* 92**,** 19-22.

CHILDS, E. A., LYLES, R. H., SELNES, O. A., CHEN, B., MILLER, E. N., COHEN, B. A., BECKER, J. T., MELLORS, J. & MCARTHUR, J. C. 1999. Plasma viral load and CD4 lymphocytes predict HIV-associated dementia and sensory neuropathy. *Neurology,* 52**,** 607-13.

CILLIERS, K., LABADARIOS, D., SCHAAF, H. S., WILLEMSE, M., MARITZ, J. S., WERELY, C. J., HUSSEY, G. & DONALD, P. R. 2010. Pyridoxal-5-phosphate plasma concentrations in children receiving tuberculosis chemotherapy including isoniazid. *Acta Paediatr,* 99**,** 705-10.

CLIFFORD, D. B., SIMPSON, D. M., BROWN, S., MOYLE, G., BREW, B. J., CONWAY, B., TOBIAS, J. K. & VANHOVE, G. F. 2012. A randomized, double-blind, controlled study of NGX-4010, a capsaicin 8% dermal patch, for the treatment of painful HIV-associated distal sensory polyneuropathy. *J Acquir Immune Defic Syndr,* 59**,** 126-33.

COICO, R. & SUNSHINE, G. 2009. *Immunology: A Short Course,* Hoboken New Jersey, John Wiley & Sons Inc.

COLEMAN, M. 2005. Axon degeneration mechanisms: commonality amid diversity. *Nat Rev Neurosci,* 6**,** 889-98.

COLHOUN, H. M., MCKEIGUE, P. M. & DAVEY SMITH, G. 2003. Problems of reporting genetic associations with complex outcomes. *Lancet,* 361**,** 865-72.

CONESA-BOTELLA, A., MEINTJES, G., COUSSENS, A. K., VAN DER PLAS, H., GOLIATH, R., SCHUTZ, C., MORENO-REYES, R., MEHTA, M., MARTINEAU, A. R., WILKINSON, R. J., COLEBUNDERS, R. & WILKINSON, K. A. 2012. Corticosteroid therapy, vitamin D status, and inflammatory cytokine profile in the HIV-tuberculosis immune reconstitution inflammatory syndrome. *Clin Infect Dis,* 55**,** 1004-11.

CONSTANS, J., PEUCHANT, E., PELLEGRIN, J. L., SERGEANT, C., HAMON, C., DUBOURG, L., THOMAS, M. J., SIMONOFF, M., PELLEGRIN, I., BROSSARD, G. & ET AL. 1995. Fatty acids and plasma antioxidants in HIV-positive patients: correlation with nutritional and immunological status. *Clin Biochem,* 28**,** 421-6.

CORNBLATH, D. R., CHAUDHRY, V., CARTER, K., LEE, D., SEYSEDADR, M., MIERNICKI, M. & JOH, T. 1999. Total neuropathy score: validation and reliability study. *Neurology,* 53**,** 1660-4.

CORNBLATH, D. R. & HOKE, A. 2006. Recent advances in HIV neuropathy. *Curr Opin Neurol,* 19**,** 446-50.

CORNBLATH, D. R. & MCARTHUR, J. C. 1988. Predominantly sensory neuropathy in patients with AIDS and AIDS-related complex. *Neurology,* 38**,** 794-6.

COTE, H. C. 2005. Possible ways nucleoside analogues can affect mitochondrial DNA content and gene expression during HIV therapy. *Antivir Ther,* 10 Suppl 2**,** M3-11.

COTE, H. C., BRUMME, Z. L., CRAIB, K. J., ALEXANDER, C. S., WYNHOVEN, B., TING, L., WONG, H., HARRIS, M., HARRIGAN, P. R., O'SHAUGHNESSY, M. V. & MONTANER, J. S. 2002. Changes in mitochondrial DNA as a marker of nucleoside toxicity in HIV-infected patients. *N Engl J Med,* 346**,** 811-20.

University of Cape Town

COX, S. E., ARTHUR, P., KIRKWOOD, B. R., YEBOAH-ANTWI, K. & RILEY, E. M. 2006. Vitamin A supplementation increases ratios of proinflammatory to anti-inflammatory cytokine responses in pregnancy and lactation. *Clin Exp Immunol,* 144**,** 392-400.

CREMONA, S., GOUJON, E., KELLEY, K. W., DANTZER, R. & PARNET, P. 1998a. Brain type I but not type II IL-1 receptors mediate the effects of IL-1 beta on behavior in mice. *Am J Physiol,* 274**,** R735-40.

CREMONA, S., LAYE, S., DANTZER, R. & PARNET, P. 1998b. Blockade of brain type II interleukin-1 receptors potentiates IL1beta-induced anorexia in mice. *Neurosci Lett,* 246**,** 101-4.

CRIBB, A. E. & SPIELBERG, S. P. 1992. Sulfamethoxazole is metabolized to the hydroxylamine in humans. *Clin Pharmacol Ther,* 51**,** 522-6.

CUNHA, F. Q., POOLE, S., LORENZETTI, B. B. & FERREIRA, S. H. 1992. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. *Br J Pharmacol,* 107**,** 660-4.

CUNNINGHAM, E. T., JR. & DE SOUZA, E. B. 1993. Interleukin 1 receptors in the brain and endocrine tissues. *Immunol Today,* 14**,** 171-6.

CURRIER, J. S. 2007. Sex differences in antiretroviral therapy toxicity: lactic acidosis, stavudine, and women. *Clin Infect Dis,* 45**,** 261-2.

DALAKAS, M. C., SEMINO-MORA, C. & LEON-MONZON, M. 2001. Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2'3'-dideoxycytidine (ddC). *Lab Invest,* 81**,** 1537-44.

DANDARA, C., MASIMIREMBWA, C. M., MAGIMBA, A., KAAYA, S., SAYI, J., SOMMERS, D. K., SNYMAN, J. R. & HASLER, J. A. 2003. Arylamine *N*-acetyltransferase (*NAT2*) genotypes in Africans: the identification of a new allele with nucleotide changes 481C>T and 590G>A. *Pharmacogenetics,* 13**,** 55-8.

DAO, C. N., PETERS, P. J., KIARIE, J. N., ZULU, I., MUIRURI, P., ONG'ECH, J., MUTSOTSO, W., POTTER, D., NJOBVU, L., STRINGER, J. S., BORKOWF, C. B., BOLU, O. & WEIDLE, P. J. 2011. Hyponatremia, hypochloremia, and hypoalbuminemia predict an increased risk of mortality during the first year of antiretroviral therapy among HIV-infected Zambian and Kenyan women. *AIDS Res Hum Retroviruses,* 27**,** 1149-55.

DE BEAUX, A. C., GOLDIE, A. S., ROSS, J. A., CARTER, D. C. & FEARON, K. C. 1996. Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. *Br J Surg,* 83**,** 349-53.

DE GOMEZ DUMM, N. T., GIAMMONA, A. M. & TOUCEDA, L. A. 2003. Variations in the lipid profile of patients with chronic renal failure treated with pyridoxine. *Lipids Health Dis,* 2**,** 7.

DE LA MONTE, S. M., GABUZDA, D. H., HO, D. D., BROWN, R. H., JR., HEDLEY-WHYTE, E. T., SCHOOLEY, R. T., HIRSCH, M. S. & BHAN, A. K. 1988. Peripheral neuropathy in the acquired immunodeficiency syndrome. *Ann Neurol,* 23**,** 485-92.

DEAN, G. L., EDWARDS, S. G., IVES, N. J., MATTHEWS, G., FOX, E. F., NAVARATNE, L., FISHER, M., TAYLOR, G. P., MILLER, R., TAYLOR, C. B., DE RUITER, A. & POZNIAK, A. L. 2002. Treatment of tuberculosis in HIV-infected persons in the era of highly active antiretroviral therapy. *AIDS,* 16**,** 75-83.

DELMAS-BEAUVIEUX, M. C., PEUCHANT, E., COUCHOURON, A., CONSTANS, J., SERGEANT, C., SIMONOFF, M., PELLEGRIN, J. L., LENG, B., CONRI, C. & CLERC, M. 1996. The enzymatic antioxidant system in blood and glutathione status

University of Cape Town

in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium or beta-carotene. *Am J Clin Nutr,* 64**,** 101-7.

DEVIGILI, G., TUGNOLI, V., PENZA, P., CAMOZZI, F., LOMBARDI, R., MELLI, G., BROGLIO, L., GRANIERI, E. & LAURIA, G. 2008. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. *Brain,* 131**,** 1912-25.

DIMAURO, S. & SCHON, E. A. 2003. Mitochondrial respiratory-chain diseases. *N Engl J Med,* 348**,** 2656-68.

DINARELLO, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood,* 87**,** 2095-147.

DODOO, D., OMER, F. M., TODD, J., AKANMORI, B. D., KORAM, K. A. & RILEY, E. M. 2002. Absolute levels and ratios of proinflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to Plasmodium falciparum malaria. *J Infect Dis,* 185**,** 971-9.

DOLL, M. A., FRETLAND, A. J., DEITZ, A. C. & HEIN, D. W. 1995. Determination of human *NAT2* acetylator genotype by restriction fragment-length polymorphism and allele-specific amplification. *Anal Biochem,* 231**,** 413-20.

DON, B. R. & KAYSEN, G. 2004. Serum albumin: relationship to inflammation and nutrition. *Semin Dial,* 17**,** 432-7.

DORSEY, S. G. & MORTON, P. G. 2006. HIV peripheral neuropathy: pathophysiology and clinical implications. *AACN Clin Issues,* 17**,** 30-6.

DOUEK, D. C., BETTS, M. R., HILL, B. J., LITTLE, S. J., LEMPICKI, R., METCALF, J. A., CASAZZA, J., YODER, C., ADELSBERGER, J. W., STEVENS, R. A., BASELER, M. W., KEISER, P., RICHMAN, D. D., DAVEY, R. T. & KOUP, R. A. 2001. Evidence for increased T cell turnover and decreased thymic output in HIV infection. *J Immunol,* 167**,** 6663-8.

DWORKIN, B. M., ROSENTHAL, W. S., WORMSER, G. P. & WEISS, L. 1986. Selenium deficiency in the acquired immunodeficiency syndrome. *JPEN J Parenter Enteral Nutr,* 10**,** 405-7.

ECK, H. P., GMUNDER, H., HARTMANN, M., PETZOLDT, D., DANIEL, V. & DROGE, W. 1989. Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. *Biol Chem Hoppe Seyler,* 370**,** 101-8.

EFFROS, R. B., FLETCHER, C. V., GEBO, K., HALTER, J. B., HAZZARD, W. R., HORNE, F. M., HUEBNER, R. E., JANOFF, E. N., JUSTICE, A. C., KURITZKES, D., NAYFIELD, S. G., PLAEGER, S. F., SCHMADER, K. E., ASHWORTH, J. R., CAMPANELLI, C., CLAYTON, C. P., RADA, B., WOOLARD, N. F. & HIGH, K. P. 2008. Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin Infect Dis,* 47**,** 542-53.

EIDUS, L., GLATTHAAR, E., HODGKIN, M. M., NEL, E. E. & KLEEBERG, H. H. 1979. Comparison of isoniazid phenotyping of black and white patients with emphasis on South African blacks. *Int J Clin Pharmacol Biopharm,* 17**,** 311-6.

ELLIS, R. J., EVANS, S. R., CLIFFORD, D. B., MOO, L. R., MCARTHUR, J. C., COLLIER, A. C., BENSON, C., BOSCH, R., SIMPSON, D., YIANNOUTSOS, C. T., YANG, Y. & ROBERTSON, K. 2005. Clinical validation of the NeuroScreen. *J Neurovirol,* 11**,** 503-11.

ELLIS, R. J., ROSARIO, D., CLIFFORD, D. B., MCARTHUR, J. C., SIMPSON, D., ALEXANDER, T., GELMAN, B. B., VAIDA, F., COLLIER, A., MARRA, C. M., ANCES, B., ATKINSON, J. H., DWORKIN, R. H., MORGELLO, S., GRANT, I. & GROUP, C. S. 2010. Continued high prevalence and adverse clinical impact of human immunodeficiency virus-associated sensory neuropathy in the era of combination antiretroviral therapy: the CHARTER Study. *Arch Neurol,* 67**,** 552-8.

University of Cape Town

EMPL, M., RENAUD, S., ERNE, B., FUHR, P., STRAUBE, A., SCHAEREN-WIEMERS, N. & STECK, A. J. 2001. TNF-alpha expression in painful and nonpainful neuropathies. *Neurology,* 56**,** 1371-7.

ENGLAND, J. D., GRONSETH, G. S., FRANKLIN, G., MILLER, R. G., ASBURY, A. K., CARTER, G. T., COHEN, J. A., FISHER, M. A., HOWARD, J. F., KINSELLA, L. J., LATOV, N., LEWIS, R. A., LOW, P. A. & SUMNER, A. J. 2005. Distal symmetric polyneuropathy: a definition for clinical research: report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Neurology,* 64**,** 199-207.

ESTANISLAO, L., CARTER, K., MCARTHUR, J., OLNEY, R. & SIMPSON, D. 2004a. A randomized controlled trial of 5% lidocaine gel for HIV-associated distal symmetric polyneuropathy. *J Acquir Immune Defic Syndr,* 37**,** 1584-6.

ESTANISLAO, L., THOMAS, D. & SIMPSON, D. 2004b. HIV neuromuscular disease and mitochondrial function. *Mitochondrion,* 4**,** 131-9.

ESTANISLAO, L. B., MORGELLO, S. & SIMPSON, D. M. 2005. Peripheral neuropathies associated with HIV and hepatitis C co-infection: a review. *AIDS,* 19 Suppl 3**,** S135-9.

EVANS, D. A., MANLEY, K. A. & MC, K. V. 1960. Genetic control of isoniazid metabolism in man. *Br Med J,* 2**,** 485-91.

EVANS, S. R., ELLIS, R. J., CHEN, H., YEH, T. M., LEE, A. J., SCHIFITTO, G., WU, K., BOSCH, R. J., MCARTHUR, J. C., SIMPSON, D. M. & CLIFFORD, D. B. 2011. Peripheral neuropathy in HIV: prevalence and risk factors. *AIDS,* 25**,** 919-28.

EVANS, S. R., LEE, A. J., ELLIS, R. J., CHEN, H., WU, K., BOSCH, R. J. & CLIFFORD, D. B. 2012. HIV peripheral neuropathy progression: protection with glucose-lowering drugs? *J Neurovirol,* 18**,** 428-33.

FARRAR, J. T., YOUNG, J. P., JR., LAMOREAUX, L., WERTH, J. L. & POOLE, R. M. 2001. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain,* 94**,** 149-58.

FAWZI, W. W., MSAMANGA, G. I., SPIEGELMAN, D., WEI, R., KAPIGA, S., VILLAMOR, E., MWAKAGILE, D., MUGUSI, F., HERTZMARK, E., ESSEX, M. & HUNTER, D. J. 2004. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med,* 351**,** 23-32.

FENG, J. Y., JOHNSON, A. A., JOHNSON, K. A. & ANDERSON, K. S. 2001. Insights into the molecular mechanism of mitochondrial toxicity by AIDS drugs. *J Biol Chem,* 276**,** 23832-7.

FERREIRA, S. H., LORENZETTI, B. B., BRISTOW, A. F. & POOLE, S. 1988. Interleukin-1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. *Nature,* 334**,** 698-700.

FINKEL, T. & HOLBROOK, N. J. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature,* 408**,** 239-47.

FISHBAIN, D. A., CUTLER, R., ROSOMOFF, H. L. & ROSOMOFF, R. S. 1997. Chronic pain-associated depression: antecedent or consequence of chronic pain? A review. *Clin J Pain,* 13**,** 116-37.

FLEISCHER, R., BOXWELL, D. & SHERMAN, K. E. 2004. Nucleoside analogues and mitochondrial toxicity. *Clin Infect Dis,* 38**,** e79-80.

FORD, E. S., MOKDAD, A. H., GILES, W. H. & BROWN, D. W. 2003. The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey. *Diabetes,* 52**,** 2346-52.

FORD, N. & CALMY, A. 2010. Improving first-line antiretroviral therapy in resource-limited settings. *Curr Opin HIV AIDS,* 5**,** 38-47.

University of Cape Town

FORNA, F., LIECHTY, C. A., SOLBERG, P., ASIIMWE, F., WERE, W., MERMIN, J., BEHUMBIIZE, P., TONG, T., BROOKS, J. T. & WEIDLE, P. J. 2007. Clinical toxicity of highly active antiretroviral therapy in a home-based AIDS care program in rural Uganda. *J Acquir Immune Defic Syndr,* 44**,** 456-62.

FRENCH, M. A. 2012. Immune reconstitution inflammatory syndrome: immune restoration disease 20 years on. *Med J Aust,* 196**,** 318-21.

FRISO, S., GIRELLI, D., MARTINELLI, N., OLIVIERI, O., LOTTO, V., BOZZINI, C., PIZZOLO, F., FACCINI, G., BELTRAME, F. & CORROCHER, R. 2004. Low plasma vitamin B-6 concentrations and modulation of coronary artery disease risk. *Am J Clin Nutr,* 79**,** 992-8.

FRISO, S., JACQUES, P. F., WILSON, P. W., ROSENBERG, I. H. & SELHUB, J. 2001. Low circulating vitamin B(6) is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. *Circulation,* 103**,** 2788-91.

FROMENTY, B. & PESSAYRE, D. 1995. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacol Ther,* 67**,** 101-54.

FUKUOKA, H., KAWATANI, M., HISAMITSU, T. & TAKESHIGE, C. 1994. Cutaneous hyperalgesia induced by peripheral injection of interleukin-1 beta in the rat. *Brain Res,* 657**,** 133-40.

FULLER, G. N., JACOBS, J. M. & GUILOFF, R. J. 1993. Nature and incidence of peripheral nerve syndromes in HIV infection. *J Neurol Neurosurg Psychiatry,* 56**,** 372-81.

GALASKO, D. & MONTINE, T. J. 2010. Biomarkers of oxidative damage and inflammation in Alzheimer's disease. *Biomark Med,* 4**,** 27-36.

GALER, B. S., GIANAS, A. & JENSEN, M. P. 2000. Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life. *Diabetes Res Clin Pract,* 47**,** 123-8.

GALLANT, J. E., STASZEWSKI, S., POZNIAK, A. L., DEJESUS, E., SULEIMAN, J. M., MILLER, M. D., COAKLEY, D. F., LU, B., TOOLE, J. J. & CHENG, A. K. 2004. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA,* 292**,** 191-201.

GALLI, M., VEGLIA, F., ANGARANO, G., SANTAMBROGIO, S., MENEGHINI, E., GRITTI, F., CARGNEL, A., MAZZOTTA, F. & LAZZARIN, A. 2003. Gender differences in antiretroviral drug-related adipose tissue alterations. Women are at higher risk than men and develop particular lipodystrophy patterns. *J Acquir Immune Defic Syndr,* 34**,** 58-61.

GARCIA-MARTIN, E. 2008. Interethnic and intraethnic variability of *NAT2* single nucleotide polymorphisms. *Curr Drug Metab,* 9**,** 487-97.

GARDINER, K. R., HALLIDAY, M. I., BARCLAY, G. R., MILNE, L., BROWN, D., STEPHENS, S., MAXWELL, R. J. & ROWLANDS, B. J. 1995. Significance of systemic endotoxaemia in inflammatory bowel disease. *Gut,* 36**,** 897-901.

GATTERMANN, N., DADAK, M., HOFHAUS, G., WULFERT, M., BERNEBURG, M., LOEFFLER, M. L. & SIMMONDS, H. A. 2004. Severe impairment of nucleotide synthesis through inhibition of mitochondrial respiration. *Nucleosides Nucleotides Nucleic Acids,* 23**,** 1275-9.

GEISS, A., VARADI, E., STEINBACH, K., BAUER, H. W. & ANTON, F. 1997. Psychoneuroimmunological correlates of persisting sciatic pain in patients who underwent discectomy. *Neurosci Lett,* 237**,** 65-8.

University of Cape Town

GENSER, B., COOPER, P. J., YAZDANBAKHSH, M., BARRETO, M. L. & RODRIGUES, L. C. 2007. A guide to modern statistical analysis of immunological data. *BMC Immunol,* 8**,** 27.

GERARD, Y., MAULIN, L., YAZDANPANAH, Y., DE LA TRIBONNIERE, X., AMIEL, C., MAURAGE, C. A., ROBIN, S., SABLONNIERE, B., DHENNAIN, C. & MOUTON, Y. 2000. Symptomatic hyperlactataemia: an emerging complication of antiretroviral therapy. *AIDS,* 14**,** 2723-30.

GERVASONI, C., RIDOLFO, A. L., TRIFIRO, G., SANTAMBROGIO, S., NORBIATO, G., MUSICCO, M., CLERICI, M., GALLI, M. & MORONI, M. 1999. Redistribution of body fat in HIV-infected women undergoing combined antiretroviral therapy. *AIDS,* 13**,** 465-71.

GIANNI, P., JAN, K. J., DOUGLAS, M. J., STUART, P. M. & TARNOPOLSKY, M. A. 2004. Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. *Exp Gerontol,* 39**,** 1391-400.

GIUNTA, B., FERNANDEZ, F., NIKOLIC, W. V., OBREGON, D., RRAPO, E., TOWN, T. & TAN, J. 2008. Inflammaging as a prodrome to Alzheimer's disease. *J Neuroinflammation,* 5**,** 51.

GODFRIED, M. H., VAN DER POLL, T., JANSEN, J., ROMIJIN, J. A., SCHATTENKERK, J. K., ENDERT, E., VAN DEVENTER, S. J. & SAUERWEIN, H. P. 1993. Soluble receptors for tumour necrosis factor: a putative marker of disease progression in HIV infection. *AIDS,* 7**,** 33-6.

GOLDMAN, A. L. & BRAMAN, S. S. 1972. Isoniazid: a review with emphasis on adverse effects. *Chest,* 62**,** 71-7.

GOLKA, K., BLASZKEWICZ, M., SAMIMI, M., BOLT, H. M. & SELINSKI, S. 2008. Reconstruction of *N*-acetyltransferase 2 haplotypes using PHASE. *Arch Toxicol,* 82**,** 265-70.

GOMEZ-GARCIA, A., MARTINEZ TORRES, G., ORTEGA-PIERRES, L. E., RODRIGUEZ-AYALA, E. & ALVAREZ-AGUILAR, C. 2007. Rosuvastatin and metformin decrease inflammation and oxidative stress in patients with hypertension and dyslipidemia. *Rev Esp Cardiol,* 60**,** 1242-9.

GONZALEZ-DUARTE, A., ROBINSON-PAPP, J. & SIMPSON, D. M. 2008. Diagnosis and management of HIV-associated neuropathy. *Neurol Clin,* 26**,** 821-32, x.

GONZALEZ-SCARANO, F. & MARTIN-GARCIA, J. 2005. The neuropathogenesis of AIDS. *Nat Rev Immunol,* 5**,** 69-81.

GORE, M., BRANDENBURG, N. A., DUKES, E., HOFFMAN, D. L., TAI, K. S. & STACEY, B. 2005. Pain severity in diabetic peripheral neuropathy is associated with patient functioning, symptom levels of anxiety and depression, and sleep. *J Pain Symptom Manage,* 30**,** 374-85.

GORI, A. M., SOFI, F., CORSI, A. M., GAZZINI, A., SESTINI, I., LAURETANI, F., BANDINELLI, S., GENSINI, G. F., FERRUCCI, L. & ABBATE, R. 2006. Predictors of vitamin B6 and folate concentrations in older persons: the InCHIANTI study. *Clin Chem,* 52**,** 1318-24.

GOULET, J. L., FULTZ, S. L., RIMLAND, D., BUTT, A., GIBERT, C., RODRIGUEZ-BARRADAS, M., BRYANT, K. & JUSTICE, A. C. 2007. Aging and infectious diseases: do patterns of comorbidity vary by HIV status, age, and HIV severity? *Clin Infect Dis,* 45**,** 1593-601.

GRAF, T., BROLY, F., HOFFMANN, F., PROBST, M., MEYER, U. A. & HOWALD, H. 1992. Prediction of phenotype for acetylation and for debrisoquine hydroxylation by DNA-tests in healthy human volunteers. *Eur J Clin Pharmacol,* 43**,** 399-403.

University of Cape Town

GRAHAM, S. M., BAETEN, J. M., RICHARDSON, B. A., WENER, M. H., LAVREYS, L., MANDALIYA, K., NDINYA-ACHOLA, J. O., OVERBAUGH, J. & MCCLELLAND, R. S. 2007. A decrease in albumin in early HIV type 1 infection predicts subsequent disease progression. *AIDS Res Hum Retroviruses,* 23**,** 1197-200.

GRANT, D. M., TANG, B. K. & KALOW, W. 1984. A simple test for acetylator phenotype using caffeine. *Br J Clin Pharmacol,* 17**,** 459-64.

GREENACRE, S., RIDGER, V., WILSONCROFT, P. & BRAIN, S. D. 1997. Peroxynitrite: a mediator of increased microvascular permeability? *Clin Exp Pharmacol Physiol,* 24**,** 880-2.

GRIFFIN, B. A., FREEMAN, D. J., TAIT, G. W., THOMSON, J., CASLAKE, M. J., PACKARD, C. J. & SHEPHERD, J. 1994. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. *Atherosclerosis,* 106**,** 241-53.

GRIFFIN, J. W. & MCARTHUR, J. C. 1998. Peripheral neuropathies associated with HIV infection. *In:* GENDELMAN, H. E. E., L.; SWINDELLS, S. (ed.) *The Neurology of AIDS.* . New York: Chapman & Hall.

GRIM, S. A. & ROMANELLI, F. 2003. Tenofovir disoproxil fumarate. *Ann Pharmacother,* 37**,** 849-59.

GRISHAM, M. B. 2004. Reactive oxygen species in immune responses. *Free Radic Biol Med,* 36**,** 1479-80.

GRUNDY, S. M., CLEEMAN, J. I., DANIELS, S. R., DONATO, K. A., ECKEL, R. H., FRANKLIN, B. A., GORDON, D. J., KRAUSS, R. M., SAVAGE, P. J., SMITH, S. C., JR., SPERTUS, J. A. & COSTA, F. 2005. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation,* 112**,** 2735-52.

HAGIWARA, E., GOURLEY, M. F., LEE, S. & KLINMAN, D. K. 1996. Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10:interferon-gamma-secreting cells in the peripheral blood. *Arthritis Rheum,* 39**,** 379-85.

HAHN, K., ARENDT, G., BRAUN, J. S., VON GIESEN, H. J., HUSSTEDT, I. W., MASCHKE, M., STRAUBE, M. E. & SCHIELKE, E. 2004. A placebo-controlled trial of gabapentin for painful HIV-associated sensory neuropathies. *J Neurol,* 251**,** 1260-6.

HAHN, K., ROBINSON, B., ANDERSON, C., LI, W., PARDO, C. A., MORGELLO, S., SIMPSON, D. & NATH, A. 2008. Differential effects of HIV infected macrophages on dorsal root ganglia neurons and axons. *Exp Neurol,* 210**,** 30-40.

HAMDY, S. I., HIRATSUKA, M., NARAHARA, K., ENDO, N., EL-ENANY, M., MOURSI, N., AHMED, M. S. & MIZUGAKI, M. 2003. Genotype and allele frequencies of *TPMT*, *NAT2*, *GST*, *SULT1A1* and *MDR-1* in the Egyptian population. *Br J Clin Pharmacol,* 55**,** 560-9.

HAN, T. S., SATTAR, N., WILLIAMS, K., GONZALEZ-VILLALPANDO, C., LEAN, M. E. & HAFFNER, S. M. 2002. Prospective study of C-reactive protein in relation to the development of diabetes and metabolic syndrome in the Mexico City Diabetes Study. *Diabetes Care,* 25**,** 2016-21.

HARMAN, D. 2001. Aging: overview. *Ann N Y Acad Sci,* 928**,** 1-21.

HARRIES, A. D., HARGREAVES, N. J., KEMP, J., JINDANI, A., ENARSON, D. A., MAHER, D. & SALANIPONI, F. M. 2001. Deaths from tuberculosis in sub-Saharan African countries with a high prevalence of HIV-1. *Lancet,* 357**,** 1519-23.

HARRISON, M. J. & MCARTHUR, J. C. 1995. *AIDS and Neurology,* Edinburgh, Churchill Livingstone.

University of Cape Town

HARRISON, T. B. & SMITH, B. 2011. Neuromuscular manifestations of HIV/AIDS. *J Clin Neuromuscul Dis,* 13**,** 68-84.

HAUG, C. J., AUKRUST, P., HAUG, E., MORKRID, L., MULLER, F. & FROLAND, S. S. 1998. Severe deficiency of 1,25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunological hyperactivity and only minor changes in calcium homeostasis. *J Clin Endocrinol Metab,* 83**,** 3832-8.

HAWKINS, C., ACHENBACH, C., FRYDA, W., NGARE, D. & MURPHY, R. 2007. Antiretroviral durability and tolerability in HIV-infected adults living in urban Kenya. *J Acquir Immune Defic Syndr,* 45**,** 304-10.

HAYS, R. D., CUNNINGHAM, W. E., SHERBOURNE, C. D., WILSON, I. B., WU, A. W., CLEARY, P. D., MCCAFFREY, D. F., FLEISHMAN, J. A., CRYSTAL, S., COLLINS, R., EGGAN, F., SHAPIRO, M. F. & BOZZETTE, S. A. 2000. Health-related quality of life in patients with human immunodeficiency virus infection in the United States: results from the HIV Cost and Services Utilization Study. *Am J Med,* 108**,** 714-22.

HEIN, D. W., DOLL, M. A., FRETLAND, A. J., LEFF, M. A., WEBB, S. J., XIAO, G. H., DEVANABOYINA, U. S., NANGJU, N. A. & FENG, Y. 2000. Molecular genetics and epidemiology of the *NAT1* and *NAT2* acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev,* 9**,** 29-42.

HELLERSTEIN, M. K., KAHN, J., MUDIE, H. & VITERI, F. 1990. Current approach to the treatment of human immunodeficiency virus-associated weight loss: pathophysiologic considerations and emerging management strategies. *Semin Oncol,* 17**,** 17-33.

HERBEIN, G., GRAS, G., KHAN, K. A. & ABBAS, W. 2010. Macrophage signaling in HIV-1 infection. *Retrovirology,* 7**,** 34.

HERBEIN, G. & KHAN, K. A. 2008. Is HIV infection a TNF receptor signalling-driven disease? *Trends Immunol,* 29**,** 61-7.

HERRMANN, D. N., GRIFFIN, J. W., HAUER, P., CORNBLATH, D. R. & MCARTHUR, J. C. 1999. Epidermal nerve fiber density and sural nerve morphometry in peripheral neuropathies. *Neurology,* 53**,** 1634-40.

HERRMANN, D. N., MCDERMOTT, M. P., SOWDEN, J. E., HENDERSON, D., MESSING, S., CRUTTENDEN, K. & SCHIFITTO, G. 2006. Is skin biopsy a predictor of transition to symptomatic HIV neuropathy? A longitudinal study. *Neurology,* 66**,** 857-61.

HERZBERG, U. & SAGEN, J. 2001. Peripheral nerve exposure to HIV viral envelope protein gp120 induces neuropathic pain and spinal gliosis. *J Neuroimmunol,* 116**,** 29-39.

HESSLER, J. R., ROBERTSON, A. L., JR. & CHISOLM, G. M., 3RD 1979. LDL-induced cytotoxicity and its inhibition by HDL in human vascular smooth muscle and endothelial cells in culture. *Atherosclerosis,* 32**,** 213-29.

HESTDAL, K., AUKRUST, P., MULLER, F., LIEN, E., BJERKELI, V., ESPEVIK, T. & FROLAND, S. S. 1997. Dysregulation of membrane-bound tumor necrosis factor-alpha and tumor necrosis factor receptors on mononuclear cells in human immunodeficiency virus type 1 infection: low percentage of p75-tumor necrosis factor receptor positive cells in patients with advanced disease and high viral load. *Blood,* 90**,** 2670-9.

HIRATSUKA, M., KISHIKAWA, Y., TAKEKUMA, Y., MATSUURA, M., NARAHARA, K., INOUE, T., HAMDY, S. I., ENDO, N., GOTO, J. & MIZUGAKI, M. 2002. Genotyping of the *N*-acetyltransferase2 polymorphism in the prediction of adverse drug reactions to isoniazid in Japanese patients. *Drug Metab Pharmacokinet,* 17**,** 357-62.

University of Cape Town

HO, D. D., ROTA, T. R., SCHOOLEY, R. T., KAPLAN, J. C., ALLAN, J. D., GROOPMAN, J. E., RESNICK, L., FELSENSTEIN, D., ANDREWS, C. A. & HIRSCH, M. S. 1985. Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *N Engl J Med,* 313**,** 1493-7.

HOFFMANN, C. J., FIELDING, K. L., CHARALAMBOUS, S., SULKOWSKI, M. S., INNES, C., THIO, C. L., CHAISSON, R. E., CHURCHYARD, G. J. & GRANT, A. D. 2008. Antiretroviral therapy using zidovudine, lamivudine, and efavirenz in South Africa: tolerability and clinical events. *AIDS,* 22**,** 67-74.

HOKE, A. & CORNBLATH, D. R. 2004. Peripheral neuropathies in human immunodeficiency virus infection. *Suppl Clin Neurophysiol,* 57**,** 195-210.

HOOVER, D. R., RINALDO, C., HE, Y., PHAIR, J., FAHEY, J. & GRAHAM, N. M. 1995. Long-term survival without clinical AIDS after CD4+ cell counts fall below 200 x 10^6/l. *AIDS,* 9**,** 145-52.

HOPKINS, S. J. & ROTHWELL, N. J. 1995. Cytokines and the nervous system. I: Expression and recognition. *Trends Neurosci,* 18**,** 83-8.

HORIE, H., SAKAI, I., AKAHORI, Y. & KADOYA, T. 1997. IL-1 beta enhances neurite regeneration from transected-nerve terminals of adult rat DRG. *Neuroreport,* 8**,** 1955-9.

HUGHES, H. B., BIEHL, J. P., JONES, A. P. & SCHMIDT, L. H. 1954. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. *Am Rev Tuberc,* 70**,** 266-73.

HULGAN, T., HAAS, D. W., HAINES, J. L., RITCHIE, M. D., ROBBINS, G. K., SHAFER, R. W., CLIFFORD, D. B., KALLIANPUR, A. R., SUMMAR, M. & CANTER, J. A. 2005. Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: an adult AIDS clinical trials group study. *AIDS,* 19**,** 1341-9.

HULGAN, T., HUGHES, M., SUN, X., SMEATON, L. M., TERRY, E., ROBBINS, G. K., SHAFER, R. W., CLIFFORD, D. B., MCCOMSEY, G. A., CANTER, J. A., MORROW, J. D. & HAAS, D. W. 2006. Oxidant stress and peripheral neuropathy during antiretroviral therapy: an AIDS clinical trials group study. *J Acquir Immune Defic Syndr,* 42**,** 450-4.

HUNG, C. F., GIBSON, S. A., LETENDRE, S. L., LONERGAN, J. T., MARQUIE-BECK, J. A., VAIDA, F. & ELLIS, R. J. 2008. Impact of long-term treatment with neurotoxic dideoxynucleoside antiretrovirals: implications for clinical care in resource-limited settings. *HIV Med,* 9**,** 731-7.

IKEGAMI, K. & KOIKE, T. 2003. Non-apoptotic neurite degeneration in apoptotic neuronal death: pivotal role of mitochondrial function in neurites. *Neuroscience,* 122**,** 617-26.

INGILIZ, P., VALANTIN, M. A., DUVIVIER, C., MEDJA, F., DOMINGUEZ, S., CHARLOTTE, F., TUBIANA, R., POYNARD, T., KATLAMA, C., LOMBES, A. & BENHAMOU, Y. 2009. Liver damage underlying unexplained transaminase elevation in human immunodeficiency virus-1 mono-infected patients on antiretroviral therapy. *Hepatology,* 49**,** 436-42.

ISANAKA, S., MUGUSI, F., HAWKINS, C., SPIEGELMAN, D., OKUMA, J., ABOUD, S., GUERINO, C. & FAWZI, W. W. 2012. Effect of high-dose vs standard-dose multivitamin supplementation at the initiation of HAART on HIV disease progression and mortality in Tanzania: a randomized controlled trial. *JAMA,* 308**,** 1535-44.

ISOMAA, B., HENRICSSON, M., ALMGREN, P., TUOMI, T., TASKINEN, M. R. & GROOP, L. 2001. The metabolic syndrome influences the risk of chronic complications in patients with type II diabetes. *Diabetologia,* 44**,** 1148-54.

University of Cape Town

ISRAEL, N., GOUGEROT-POCIDALO, M. A., AILLET, F. & VIRELIZIER, J. L. 1992. Redox status of cells influences constitutive or induced NF-kappa B translocation and HIV long terminal repeat activity in human T and monocytic cell lines. *J Immunol,* 149**,** 3386-93.

IVERS, L. C., KENDRICK, D. & DOUCETTE, K. 2005. Efficacy of antiretroviral therapy programs in resource-poor settings: a meta-analysis of the published literature. *Clin Infect Dis,* 41**,** 217-24.

JACOBS, J. M. & LOVE, S. 1985. Qualitative and quantitative morphology of human sural nerve at different ages. *Brain,* 108 (Pt 4)**,** 897-924.

JACOBS, J. M., MILLER, R. H., WHITTLE, A. & CAVANAGH, J. B. 1979. Studies on the early changes in acute isoniazid neuropathy in the rat. *Acta Neuropathol,* 47**,** 85-92.

JEONG, S. K., SEO, M. W., KIM, Y. H., KWEON, S. S. & NAM, H. S. 2005. Does waist indicate dyslipidemia better than BMI in Korean adult population? *J Korean Med Sci,* 20**,** 7-12.

JOHANNESSEN, A., NAMAN, E., NGOWI, B. J., SANDVIK, L., MATEE, M. I., AGLEN, H. E., GUNDERSEN, S. G. & BRUUN, J. N. 2008. Predictors of mortality in HIV-infected patients starting antiretroviral therapy in a rural hospital in Tanzania. *BMC Infect Dis,* 8**,** 52.

JOHN, M. & MALLAL, S. 2002. Hyperlactatemia syndromes in people with HIV infection. *Curr Opin Infect Dis,* 15**,** 23-9.

JOHN, M., MOORE, C. B., JAMES, I. R., NOLAN, D., UPTON, R. P., MCKINNON, E. J. & MALLAL, S. A. 2001. Chronic hyperlactatemia in HIV-infected patients taking antiretroviral therapy. *AIDS,* 15**,** 717-23.

JOHNSON, A. A., RAY, A. S., HANES, J., SUO, Z., COLACINO, J. M., ANDERSON, K. S. & JOHNSON, K. A. 2001. Toxicity of antiviral nucleoside analogs and the human mitochondrial DNA polymerase. *J Biol Chem,* 276**,** 40847-57.

JONES, C. Y., TANG, A. M., FORRESTER, J. E., HUANG, J., HENDRICKS, K. M., KNOX, T. A., SPIEGELMAN, D., SEMBA, R. D. & WOODS, M. N. 2006. Micronutrient levels and HIV disease status in HIV-infected patients on highly active antiretroviral therapy in the Nutrition for Healthy Living cohort. *J Acquir Immune Defic Syndr,* 43**,** 475-82.

JONES, G., ZHU, Y., SILVA, C., TSUTSUI, S., PARDO, C. A., KEPPLER, O. T., MCARTHUR, J. C. & POWER, C. 2005. Peripheral nerve-derived HIV-1 is predominantly CCR5-dependent and causes neuronal degeneration and neuroinflammation. *Virology,* 334**,** 178-93.

JONES, W. A. & JONES, G. P. 1953. Peripheral neuropathy due to isoniazid; report of two cases. *Lancet,* 1**,** 1073-4.

JTC 1998. Joint Tuberculosis Committee of the British Thoracic Society. Chemotherapy and management of tuberculosis in the United Kingdom: recommendations 1998. *Thorax.*

KAISER, J. D., CAMPA, A. M., ONDERCIN, J. P., LEOUNG, G. S., PLESS, R. F. & BAUM, M. K. 2006. Micronutrient supplementation increases CD4 count in HIV-infected individuals on highly active antiretroviral therapy: a prospective, double-blinded, placebo-controlled trial. *J Acquir Immune Defic Syndr,* 42**,** 523-8.

KAKUDA, T. N. 2000. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clin Ther,* 22**,** 685-708.

KALEBIC, T., KINTER, A., POLI, G., ANDERSON, M. E., MEISTER, A. & FAUCI, A. S. 1991. Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and *N*-acetylcysteine. *Proc Natl Acad Sci U S A,* 88**,** 986-90.

University of Cape Town

KAMATA, H., HONDA, S., MAEDA, S., CHANG, L., HIRATA, H. & KARIN, M. 2005. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell,* 120**,** 649-61.

KARYADI, E., SCHULTINK, W., NELWAN, R. H., GROSS, R., AMIN, Z., DOLMANS, W. M., VAN DER MEER, J. W., HAUTVAST, J. G. & WEST, C. E. 2000. Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. *J Nutr,* 130**,** 2953-8.

KAUFMANN, G. R., WENK, M., TAESCHNER, W., PETERLI, B., GYR, K., MEYER, U. A. & HAEFELI, W. E. 1996. *N*-acetyltransferase 2 polymorphism in patients infected with human immunodeficiency virus. *Clin Pharmacol Ther,* 60**,** 62-7.

KAUL, M. & LIPTON, S. A. 1999. Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proc Natl Acad Sci U S A,* 96**,** 8212-6.

KELLY, P. J., KISTLER, J. P., SHIH, V. E., MANDELL, R., ATASSI, N., BARRON, M., LEE, H., SILVEIRA, S. & FURIE, K. L. 2004. Inflammation, homocysteine, and vitamin B6 status after ischemic stroke. *Stroke,* 35**,** 12-5.

KEMPER, C. A., KENT, G., BURTON, S. & DERESINSKI, S. C. 1998. Mexiletine for HIV-infected patients with painful peripheral neuropathy: a double-blind, placebo-controlled, crossover treatment trial. *J Acquir Immune Defic Syndr Hum Retrovirol,* 19**,** 367-72.

KESWANI, S. C., PARDO, C. A., CHERRY, C. L., HOKE, A. & MCARTHUR, J. C. 2002. HIV-associated sensory neuropathies. *AIDS,* 16**,** 2105-17.

KEUSCH, G. T. & FARTHING, M. J. 1990. Nutritional aspects of AIDS. *Annu Rev Nutr,* 10**,** 475-501.

KIEBURTZ, K., SIMPSON, D., YIANNOUTSOS, C., MAX, M. B., HALL, C. D., ELLIS, R. J., MARRA, C. M., MCKENDALL, R., SINGER, E., DAL PAN, G. J., CLIFFORD, D. B., TUCKER, T. & COHEN, B. 1998. A randomized trial of amitriptyline and mexiletine for painful neuropathy in HIV infection. AIDS Clinical Trial Group 242 Protocol Team. *Neurology,* 51**,** 1682-8.

KINZIG-SCHIPPERS, M., TOMALIK-SCHARTE, D., JETTER, A., SCHEIDEL, B., JAKOB, V., RODAMER, M., CASCORBI, I., DOROSHYENKO, O., SORGEL, F. & FUHR, U. 2005. Should we use *N*-acetyltransferase type 2 genotyping to personalize isoniazid doses? *Antimicrob Agents Chemother,* 49**,** 1733-8.

KOMIYAMA, A. & SUZUKI, K. 1992. Age-related differences in proliferative responses of Schwann cells during Wallerian degeneration. *Brain Res,* 573**,** 267-75.

KOVARI, H., LEDERGERBER, B., BATTEGAY, M., RAUCH, A., HIRSCHEL, B., FOGUENA, A. K., VERNAZZA, P., BERNASCONI, E., MUELLER, N. J. & WEBER, R. 2010. Incidence and risk factors for chronic elevation of alanine aminotransferase levels in HIV-infected persons without hepatitis b or c virus co-infection. *Clin Infect Dis,* 50**,** 502-11.

KRESS, M. 2010. Nociceptor sensitization by proinflammatory cytokines and chemokines. *The Open Pain Journal,* 3**,** 97-107.

KRISHNAMURTHY, D. V., SELKON, J. B., RAMACHANDRAN, K., DEVADATTA, S., MITCHISON, D. A., RADHAKRISHNA, S. & STOTT, H. 1967. Effect of pyridoxine on vitamin B6 concentrations and glutamic-oxaloacetic transaminase activity in whole blood of tuberculous patients receiving high-dosage isoniazid. *Bull World Health Organ,* 36**,** 853-70.

KROEMER, G. & REED, J. C. 2000. Mitochondrial control of cell death. *Nat Med,* 6**,** 513-9.

KUJOTH, G. C., HIONA, A., PUGH, T. D., SOMEYA, S., PANZER, K., WOHLGEMUTH, S. E., HOFER, T., SEO, A. Y., SULLIVAN, R., JOBLING, W. A., MORROW, J. D., VAN REMMEN, H., SEDIVY, J. M., YAMASOBA, T., TANOKURA, M.,

University of Cape Town

WEINDRUCH, R., LEEUWENBURGH, C. & PROLLA, T. A. 2005. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science,* 309**,** 481-4.

KUMAR, N. 2007. Nutritional neuropathies. *Neurol Clin,* 25**,** 209-55.

LABADARIOS, D., ROSSOUW, J. E., MCCONNELL, J. B., DAVIS, M. & WILLIAMS, R. 1977. Vitamin B6 deficiency in chronic liver disease - evidence for increased degradation of pyridoxal-5'-phosphate. *Gut,* 18**,** 23-7.

LAURIA, G., CORNBLATH, D. R., JOHANSSON, O., MCARTHUR, J. C., MELLGREN, S. I., NOLANO, M., ROSENBERG, N. & SOMMER, C. 2005. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. *Eur J Neurol,* 12**,** 747-58.

LAURIA, G., MORBIN, M., LOMBARDI, R., BORGNA, M., MAZZOLENI, G., SGHIRLANZONI, A. & PAREYSON, D. 2003. Axonal swellings predict the degeneration of epidermal nerve fibers in painful neuropathies. *Neurology,* 61**,** 631-6.

LAWN, S. D., MYER, L., BEKKER, L. G. & WOOD, R. 2006. Burden of tuberculosis in an antiretroviral treatment programme in sub-Saharan Africa: impact on treatment outcomes and implications for tuberculosis control. *AIDS,* 20**,** 1605-12.

LAWN, S. D., WILKINSON, R. J., LIPMAN, M. C. & WOOD, R. 2008. Immune reconstitution and "unmasking" of tuberculosis during antiretroviral therapy. *Am J Respir Crit Care Med,* 177**,** 680-5.

LE CORRE, P., PARMER, R. J., KAILASAM, M. T., KENNEDY, B. P., SKAAR, T. P., HO, H., LEVERGE, R., SMITH, D. W., ZIEGLER, M. G., INSEL, P. A., SCHORK, N. J., FLOCKHART, D. A. & O'CONNOR D, T. 2004. Human sympathetic activation by alpha2-adrenergic blockade with yohimbine: Bimodal, epistatic influence of cytochrome P450-mediated drug metabolism. *Clin Pharmacol Ther,* 76**,** 139-53.

LEEM, J. G. & BOVE, G. M. 2002. Mid-axonal tumor necrosis factor-alpha induces ectopic activity in a subset of slowly conducting cutaneous and deep afferent neurons. *J Pain,* 3**,** 45-9.

LEHMANN, H. C., CHEN, W., BORZAN, J., MANKOWSKI, J. L. & HOKE, A. 2011. Mitochondrial dysfunction in distal axons contributes to human immunodeficiency virus sensory neuropathy. *Ann Neurol,* 69**,** 100-10.

LEKLEM, J. E. 1990. Vitamin B-6: a status report. *J Nutr,* 120 Suppl 11**,** 1503-7.

LEMBECK, F. & HOLZER, P. 1979. Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. *Naunyn Schmiedebergs Arch Pharmacol,* 310**,** 175-83.

LETENDRE, S. L., ELLIS, R. J., EVERALL, I., ANCES, B., BHARTI, A. & MCCUTCHAN, J. A. 2009. Neurologic complications of HIV disease and their treatment. *Top HIV Med,* 17**,** 46-56.

LEWIS, C. M. 2002. Genetic association studies: design, analysis and interpretation. *Brief Bioinform,* 3**,** 146-53.

LEWIS, W. 2005. Nucleoside reverse transcriptase inhibitors, mitochondrial DNA and AIDS therapy. *Antivir Ther,* 10 Suppl 2**,** M13-27.

LEWIS, W., DAY, B. J. & COPELAND, W. C. 2003. Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspective. *Nat Rev Drug Discov,* 2**,** 812-22.

LI, L., MAMPUTU, J. C., WIERNSPERGER, N. & RENIER, G. 2005. Signaling pathways involved in human vascular smooth muscle cell proliferation and matrix metalloproteinase-2 expression induced by leptin: inhibitory effect of metformin. *Diabetes,* 54**,** 2227-34.

University of Cape Town

LI, N. & STEPHENS, M. 2003. Modeling linkage disequilibrium and identifying recombination hotspots using single-nucleotide polymorphism data. *Genetics,* 165**,** 2213-33.

LICHTENSTEIN, K. A., ARMON, C., BARON, A., MOORMAN, A. C., WOOD, K. C., HOLMBERG, S. D. & INVESTIGATORS, H. I. V. O. S. 2005. Modification of the incidence of drug-associated symmetrical peripheral neuropathy by host and disease factors in the HIV outpatient study cohort. *Clin Infect Dis,* 40**,** 148-57.

LICHTENSTEIN, K. A., DELANEY, K. M., ARMON, C., WARD, D. J., MOORMAN, A. C., WOOD, K. C. & HOLMBERG, S. D. 2003. Incidence of and risk factors for lipoatrophy (abnormal fat loss) in ambulatory HIV-1-infected patients. *J Acquir Immune Defic Syndr,* 32**,** 48-56.

LIN, H. J., HAN, C. Y., LIN, B. K. & HARDY, S. 1993. Slow acetylator mutations in the human polymorphic *N*-acetyltransferase gene in 786 Asians, blacks, Hispanics, and whites: application to metabolic epidemiology. *Am J Hum Genet,* 52**,** 827-34.

LIU, S., TINKER, L., SONG, Y., RIFAI, N., BONDS, D. E., COOK, N. R., HEISS, G., HOWARD, B. V., HOTAMISLIGIL, G. S., HU, F. B., KULLER, L. H. & MANSON, J. E. 2007. A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Arch Intern Med,* 167**,** 1676-85.

LOKTIONOV, A., MOORE, W., SPENCER, S. P., VORSTER, H., NELL, T., O'NEILL, I. K., BINGHAM, S. A. & CUMMINGS, J. H. 2002. Differences in *N*-acetylation genotypes between Caucasians and Black South Africans: implications for cancer prevention. *Cancer Detect Prev,* 26**,** 15-22.

LOOK, M. P., RIEZLER, R., BERTHOLD, H. K., STABLER, S. P., SCHLIEFER, K., ALLEN, R. H., SAUERBRUCH, T. & ROCKSTROH, J. K. 2001. Decrease of elevated *N,N*-dimethylglycine and *N*-methylglycine in human immunodeficiency virus infection during short-term highly active antiretroviral therapy. *Metabolism,* 50**,** 1275-81.

LORENZO, C., WILLIAMS, K., HUNT, K. J. & HAFFNER, S. M. 2007. The National Cholesterol Education Program - Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. *Diabetes Care,* 30**,** 8-13.

LU, M., GROVE, E. A. & MILLER, R. J. 2002. Abnormal development of the hippocampal dentate gyrus in mice lacking the CXCR4 chemokine receptor. *Proc Natl Acad Sci U S A,* 99**,** 7090-5.

LUBING, H. N. 1953. Peripheral neuropathy in tuberculosis patients treated with isoniazid. *Am Rev Tuberc,* 68**,** 458-61.

LUI, A., LUMENG, L., ARONOFF, G. R. & LI, T. K. 1985. Relationship between body store of vitamin B6 and plasma pyridoxal-P clearance: metabolic balance studies in humans. *J Lab Clin Med,* 106**,** 491-7.

LUMA, H. N., TCHALEU, B. C., DOUALLA, M. S., TEMFACK, E., SOPOUASSI, V. N., MAPOURE, Y. N. & DJIENTCHEU, V. D. 2012. HIV-associated sensory neuropathy in HIV-1 infected patients at the Douala General Hospital in Cameroon: a cross-sectional study. *AIDS Res Ther,* 9**,** 35.

LUMENG, L., BRASHEAR, R. E. & LI, T. K. 1974. Pyridoxal 5'-phosphate in plasma: source, protein-binding, and cellular transport. *J Lab Clin Med,* 84**,** 334-43.

LUNETTA, K. L. 2008. Genetic association studies. *Circulation,* 118**,** 96-101.

LYNX, M. D., BENTLEY, A. T. & MCKEE, E. E. 2006. 3'-Azido-3'-deoxythymidine (AZT) inhibits thymidine phosphorylation in isolated rat liver mitochondria: a possible mechanism of AZT hepatotoxicity. *Biochem Pharmacol,* 71**,** 1342-8.

University of Cape Town

MAAGAARD, A., HOLBERG-PETERSEN, M., KOLLBERG, G., OLDFORS, A., SANDVIK, L. & BRUUN, J. N. 2006. Mitochondrial (mt)DNA changes in tissue may not be reflected by depletion of mtDNA in peripheral blood mononuclear cells in HIV-infected patients. *Antivir Ther,* 11**,** 601-8.

MACKEY, D. A., FINGERT, J. H., LUZHANSKY, J. Z., MCCLUSKEY, P. J., HOWELL, N., HALL, A. J., PIERCE, A. B. & HOY, J. F. 2003. Leber's hereditary optic neuropathy triggered by antiretroviral therapy for human immunodeficiency virus. *Eye (Lond),* 17**,** 312-7.

MACKINNON, D. P., KRULL, J. L. & LOCKWOOD, C. M. 2000. Equivalence of the mediation, confounding and suppression effect. *Prev Sci,* 1**,** 173-81.

MAH, V., VARTAVARIAN, L. M., AKERS, M. A. & VINTERS, H. V. 1988. Abnormalities of peripheral nerve in patients with human immunodeficiency virus infection. *Ann Neurol,* 24**,** 713-7.

MALLON, P. W., SEDWELL, R., UNEMORI, P., KELLEHER, A., COOPER, D. A. & CARR, A. 2005a. Methodological considerations in human studies of gene expression in HIV-associated lipodystrophy. *Antivir Ther,* 10 Suppl 2**,** M101-8.

MALLON, P. W., UNEMORI, P., SEDWELL, R., MOREY, A., RAFFERTY, M., WILLIAMS, K., CHISHOLM, D., SAMARAS, K., EMERY, S., KELLEHER, A., COOPER, D. A. & CARR, A. 2005b. In vivo, nucleoside reverse-transcriptase inhibitors alter expression of both mitochondrial and lipid metabolism genes in the absence of depletion of mitochondrial DNA. *J Infect Dis,* 191**,** 1686-96.

MANDEL, W. 1959. Pyridoxine and the isoniazid-induced neuropathy. *Dis Chest,* 36**,** 293-6.

MARITZ, J., BENATAR, M., DAVE, J. A., HARRISON, T. B., BADRI, M., LEVITT, N. S. & HECKMANN, J. M. 2010. HIV neuropathy in South Africans: frequency, characteristics, and risk factors. *Muscle Nerve,* 41**,** 599-606.

MARKS, D. J., DHEDA, K., DAWSON, R., AINSLIE, G. & MILLER, R. F. 2009. Adverse events to antituberculosis therapy: influence of HIV and antiretroviral drugs. *Int J STD AIDS,* 20**,** 339-45.

MARKS, J. 1975. *A guide to the vitamins: their role in health and disease,* Lancaster, UK, Medical and Technical Publishing Co.

MARKUS, R. & BREW, B. J. 1998. HIV-1 peripheral neuropathy and combination antiretroviral therapy. *Lancet,* 352**,** 1906-7.

MARTIN, A. M., HAMMOND, E., NOLAN, D., PACE, C., DEN BOER, M., TAYLOR, L., MOORE, H., MARTINEZ, O. P., CHRISTIANSEN, F. T. & MALLAL, S. 2003. Accumulation of mitochondrial DNA mutations in human immunodeficiency virus-infected patients treated with nucleoside-analogue reverse-transcriptase inhibitors. *Am J Hum Genet,* 72**,** 549-60.

MARTIN, C., SOLDERS, G., SONNERBORG, A. & HANSSON, P. 2000. Antiretroviral therapy may improve sensory function in HIV-infected patients: a pilot study. *Neurology,* 54**,** 2120-7.

MATAR, K. M., MAYET, A. Y., AYOOLA, E. A., BAWAZIR, S. A., AL-FALEH, F. Z. & AL-WAZZAN, A. 2004. Isoniazid acetylation phenotyping in Saudi Arabs. *J Clin Pharm Ther,* 29**,** 443-7.

MATARESE, L. E., DVORCHIK, I., COSTA, G., BOND, G. J., KORITSKY, D. A., FERRARIS, R. P., TOUGER-DECKER, R., O'SULLIVAN-MAILLET, J. K. & ABU-ELMAGD, K. M. 2009. Pyridoxal-5'-phosphate deficiency after intestinal and multivisceral transplantation. *Am J Clin Nutr,* 89**,** 204-9.

MATES, J. M., PEREZ-GOMEZ, C. & NUNEZ DE CASTRO, I. 1999. Antioxidant enzymes and human diseases. *Clin Biochem,* 32**,** 595-603.

University of Cape Town

MATXAIN, J. M., RISTILA, M., STRID, A. & ERIKSSON, L. A. 2006. Theoretical study of the antioxidant properties of pyridoxine. *J Phys Chem A,* 110**,** 13068-72.

MAURICE, M. M., NAKAMURA, H., VAN DER VOORT, E. A., VAN VLIET, A. I., STAAL, F. J., TAK, P. P., BREEDVELD, F. C. & VERWEIJ, C. L. 1997. Evidence for the role of an altered redox state in hyporesponsiveness of synovial T cells in rheumatoid arthritis. *J Immunol,* 158**,** 1458-65.

MCARTHUR, J. C., STOCKS, E. A., HAUER, P., CORNBLATH, D. R. & GRIFFIN, J. W. 1998. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. *Arch Neurol,* 55**,** 1513-20.

MCARTHUR, J. C., YIANNOUTSOS, C., SIMPSON, D. M., ADORNATO, B. T., SINGER, E. J., HOLLANDER, H., MARRA, C., RUBIN, M., COHEN, B. A., TUCKER, T., NAVIA, B. A., SCHIFITTO, G., KATZENSTEIN, D., RASK, C., ZABORSKI, L., SMITH, M. E., SHRIVER, S., MILLAR, L., CLIFFORD, D. B. & KARALNIK, I. J. 2000. A phase II trial of nerve growth factor for sensory neuropathy associated with HIV infection. AIDS Clinical Trials Group Team 291. *Neurology,* 54**,** 1080-8.

MCCOMSEY, G., TAN, D. J., LEDERMAN, M., WILSON, E. & WONG, L. J. 2002. Analysis of the mitochondrial DNA genome in the peripheral blood leukocytes of HIV-infected patients with or without lipoatrophy. *AIDS,* 16**,** 513-8.

MCCOMSEY, G. A., LIBUTTI, D. E., O'RIORDAN, M., SHELTON, J. M., STORER, N., GANZ, J., JASPER, J., HARRILL, D. & GERSCHENSON, M. 2008. Mitochondrial RNA and DNA alterations in HIV lipoatrophy are linked to antiretroviral therapy and not to HIV infection. *Antivir Ther,* 13**,** 715-22.

MCCOMSEY, G. A. & MORROW, J. D. 2003. Lipid oxidative markers are significantly increased in lipoatrophy but not in sustained asymptomatic hyperlactatemia. *J Acquir Immune Defic Syndr,* 34**,** 45-9.

MCKEOWN-EYSSEN, G., BAINES, C., COLE, D. E., RILEY, N., TYNDALE, R. F., MARSHALL, L. & JAZMAJI, V. 2004. Case-control study of genotypes in multiple chemical sensitivity: *CYP2D6*, *NAT1*, *NAT2*, *PON1*, *PON2* and *MTHFR*. *Int J Epidemiol,* 33**,** 971-8.

MCLAUGHLIN, T., ABBASI, F., CHEAL, K., CHU, J., LAMENDOLA, C. & REAVEN, G. 2003. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med,* 139**,** 802-9.

MCWILLIAMS, L. A., COX, B. J. & ENNS, M. W. 2003. Mood and anxiety disorders associated with chronic pain: an examination in a nationally representative sample. *Pain,* 106**,** 127-33.

MEHTA, S. A., AHMED, A., KARIUKI, B. W., SAID, S., OMASETE, F., MENDILLO, M., LAVERTY, M., HOLZMAN, R., VALENTINE, F. & SIVAPALASINGAM, S. 2010. Implementation of a validated peripheral neuropathy screening tool in patients receiving antiretroviral therapy in Mombasa, Kenya. *Am J Trop Med Hyg,* 83**,** 565-70.

MEHTA, S. A., AHMED, A., LAVERTY, M., HOLZMAN, R. S., VALENTINE, F. & SIVAPALASINGAM, S. 2011. Sex differences in the incidence of peripheral neuropathy among Kenyans initiating antiretroviral therapy. *Clin Infect Dis,* 53**,** 490-6.

MEINTJES, G., LAWN, S. D., SCANO, F., MAARTENS, G., FRENCH, M. A., WORODRIA, W., ELLIOTT, J. H., MURDOCH, D., WILKINSON, R. J., SEYLER, C., JOHN, L., VAN DER LOEFF, M. S., REISS, P., LYNEN, L., JANOFF, E. N., GILKS, C., COLEBUNDERS, R. & INTERNATIONAL NETWORK FOR THE STUDY OF, H. I. V. A. I. 2008. Tuberculosis-associated immune reconstitution

University of Cape Town

inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet Infect Dis,* 8**,** 516-23.

MEINTJES, G., RABIE, H., WILKINSON, R. J. & COTTON, M. F. 2009. Tuberculosis-associated immune reconstitution inflammatory syndrome and unmasking of tuberculosis by antiretroviral therapy. *Clin Chest Med,* 30**,** 797-810, x.

MELLI, G., KESWANI, S. C., FISCHER, A., CHEN, W. & HOKE, A. 2006. Spatially distinct and functionally independent mechanisms of axonal degeneration in a model of HIV-associated sensory neuropathy. *Brain,* 129**,** 1330-8.

MENARD, S. 2001. *Applied Logistic Regression Analysis,* Thousand Oaks, CA, Sage Publications.

MERRILL, A. H., JR. & HENDERSON, J. M. 1987. Diseases associated with defects in vitamin B6 metabolism or utilization. *Annu Rev Nutr,* 7**,** 137-56.

MONEY, G. L. 1959. Clinical aspects of tropical ataxic neuropathies related to malnutrition. *West Afr Med J,* 8**,** 3-17.

MOORE, R. D., WONG, W. M., KERULY, J. C. & MCARTHUR, J. C. 2000. Incidence of neuropathy in HIV-infected patients on monotherapy versus those on combination therapy with didanosine, stavudine and hydroxyurea. *AIDS,* 14**,** 273-8.

MORENO, A., QUEREDA, C., MORENO, L., PEREZ-ELIAS, M. J., MURIEL, A., CASADO, J. L., ANTELA, A., DRONDA, F., NAVAS, E., BARCENA, R. & MORENO, S. 2004. High rate of didanosine-related mitochondrial toxicity in HIV/HCV-coinfected patients receiving ribavirin. *Antivir Ther,* 9**,** 133-8.

MORGELLO, S., ESTANISLAO, L., SIMPSON, D., GERACI, A., DIROCCO, A., GERITS, P., RYAN, E., YAKOUSHINA, T., KHAN, S., MAHBOOB, R., NASEER, M., DORFMAN, D. & SHARP, V. 2004. HIV-associated distal sensory polyneuropathy in the era of highly active antiretroviral therapy: the Manhattan HIV Brain Bank. *Arch Neurol,* 61**,** 546-51.

MORGELLO, S., WOLFE, D., GODFREY, E., FEINSTEIN, R., TAGLIATI, M. & SIMPSON, D. M. 1995. Mitochondrial abnormalities in human immunodeficiency virus-associated myopathy. *Acta Neuropathol,* 90**,** 366-74.

MORIN, C. M., GIBSON, D. & WADE, J. 1998. Self-reported sleep and mood disturbance in chronic pain patients. *Clin J Pain,* 14**,** 311-4.

MORIOKA, N., TAKEDA, K., KUMAGAI, K., HANADA, T., IKOMA, K., HIDE, I., INOUE, A. & NAKATA, Y. 2002. Interleukin-1beta-induced substance P release from rat cultured primary afferent neurons driven by two phospholipase A2 enzymes: secretory type IIA and cytosolic type IV. *J Neurochem,* 80**,** 989-97.

MOSHAGE, H. J., JANSSEN, J. A., FRANSSEN, J. H., HAFKENSCHEID, J. C. & YAP, S. H. 1987. Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. *J Clin Invest,* 79**,** 1635-41.

MOSMANN, T. R. & COFFMAN, R. L. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol,* 7**,** 145-73.

MOYLE, G. 2000. Clinical manifestations and management of antiretroviral nucleoside analog-related mitochondrial toxicity. *Clin Ther,* 22**,** 911-36; discussion 898.

MOYLE, G. J., DATTA, D., MANDALIA, S., MORLESE, J., ASBOE, D. & GAZZARD, B. G. 2002. Hyperlactataemia and lactic acidosis during antiretroviral therapy: relevance, reproducibility and possible risk factors. *AIDS,* 16**,** 1341-9.

MOYLE, G. J., GAZZARD, B. G., COOPER, D. A. & GATELL, J. 1998. Antiretroviral therapy for HIV infection. A knowledge-based approach to drug selection and use. *Drugs,* 55**,** 383-404.

University of Cape Town

MOYLE, G. J. & SADLER, M. 1998. Peripheral neuropathy with nucleoside antiretrovirals: risk factors, incidence and management. *Drug Saf,* 19**,** 481-94.

MROZIKIEWICZ, P. M., DRAKOULIS, N. & ROOTS, I. 1994. Polymorphic arylamine *N*-acetyltransferase (*NAT2*) genes in children with insulin-dependent diabetes mellitus. *Clin Pharmacol Ther,* 56**,** 626-34.

MUIJSERS, R. B., FOLKERTS, G., HENRICKS, P. A., SADEGHI-HASHJIN, G. & NIJKAMP, F. P. 1997. Peroxynitrite: a two-faced metabolite of nitric oxide. *Life Sci,* 60**,** 1833-45.

MULLER, M., WANDEL, S., COLEBUNDERS, R., ATTIA, S., FURRER, H., EGGER, M., IE, D. E. A. S. & CENTRAL, A. 2010. Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis. *Lancet Infect Dis,* 10**,** 251-61.

MULLIN, S., TEMU, A., KALLUVYA, S., GRANT, A. & MANJI, H. 2011. High prevalence of distal sensory polyneuropathy in antiretroviral-treated and untreated people with HIV in Tanzania. *Trop Med Int Health,* 16**,** 1291-6.

NAGANO, I., SHAPSHAK, P., YOSHIOKA, M., XIN, K., NAKAMURA, S. & BRADLEY, W. G. 1996. Increased NADPH-diaphorase reactivity and cytokine expression in dorsal root ganglia in acquired immunodeficiency syndrome. *J Neurol Sci,* 136**,** 117-28.

NAKAHIRA, K., HASPEL, J. A., RATHINAM, V. A., LEE, S. J., DOLINAY, T., LAM, H. C., ENGLERT, J. A., RABINOVITCH, M., CERNADAS, M., KIM, H. P., FITZGERALD, K. A., RYTER, S. W. & CHOI, A. M. 2011. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol,* 12**,** 222-30.

NAKAMOTO, B. K., MCMURTRAY, A., DAVIS, J., VALCOUR, V., WATTERS, M. R., SHIRAMIZU, B., CHOW, D. C., KALLIANPUR, K. & SHIKUMA, C. M. 2010. Incident neuropathy in HIV-infected patients on HAART. *AIDS Res Hum Retroviruses,* 26**,** 759-65.

NICKLIN, M. J., HUGHES, D. E., BARTON, J. L., URE, J. M. & DUFF, G. W. 2000. Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. *J Exp Med,* 191**,** 303-12.

NOLAN, D., HAMMOND, E., MARTIN, A., TAYLOR, L., HERRMANN, S., MCKINNON, E., METCALF, C., LATHAM, B. & MALLAL, S. 2003. Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS,* 17**,** 1329-38.

O'CONNOR, M. J., TOMLINSON, M., LEROUX, I. M., STEWART, J., GRECO, E. & ROTHERAM-BORUS, M. J. 2011. Predictors of alcohol use prior to pregnancy recognition among township women in Cape Town, South Africa. *Soc Sci Med,* 72**,** 83-90.

O'NEIL, W. M., DROBITCH, R. K., MACARTHUR, R. D., FARROUGH, M. J., DOLL, M. A., FRETLAND, A. J., HEIN, D. W., CRANE, L. R. & SVENSSON, C. K. 2000. Acetylator phenotype and genotype in patients infected with HIV: discordance between methods for phenotype determination and genotype. *Pharmacogenetics,* 10**,** 171-82.

O'NEIL, W. M., GILFIX, B. M., DIGIROLAMO, A., TSOUKAS, C. M. & WAINER, I. W. 1997. *N*-acetylation among HIV-positive patients and patients with AIDS: when is fast, fast and slow, slow? *Clin Pharmacol Ther,* 62**,** 261-71.

OH, S. B., TRAN, P. B., GILLARD, S. E., HURLEY, R. W., HAMMOND, D. L. & MILLER, R. J. 2001. Chemokines and glycoprotein120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. *J Neurosci,* 21**,** 5027-35.

University of Cape Town

OH, S. Y., CHO, Y. K., KANG, M. S., YOO, T. W., PARK, J. H., KIM, H. J., PARK, D. I., SOHN, C. I., JEON, W. K., KIM, B. I., SON, B. H. & SHIN, J. H. 2006. The association between increased alanine aminotransferase activity and metabolic factors in nonalcoholic fatty liver disease. *Metabolism,* 55**,** 1604-9.

OHNO, M., YAMAGUCHI, I., YAMAMOTO, I., FUKUDA, T., YOKOTA, S., MAEKURA, R., ITO, M., YAMAMOTO, Y., OGURA, T., MAEDA, K., KOMUTA, K., IGARASHI, T. & AZUMA, J. 2000. Slow *N*-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. *Int J Tuberc Lung Dis,* 4**,** 256-61.

OKA, T., OKA, K., HOSOI, M. & HORI, T. 1995. Intracerebroventricular injection of interleukin-6 induces thermal hyperalgesia in rats. *Brain Res,* 692**,** 123-8.

OLIVER, B. G., ELLIOTT, J. H., PRICE, P., PHILLIPS, M., SAPHONN, V., VUN, M. C., KALDOR, J. M., COOPER, D. A. & FRENCH, M. A. 2010. Mediators of innate and adaptive immune responses differentially affect immune restoration disease associated with Mycobacterium tuberculosis in HIV patients beginning antiretroviral therapy. *J Infect Dis,* 202**,** 1728-37.

OSHINAIKE, O., AKINBAMI, A., OJO, O., OGBERA, A., OKUBADEJO, N., OJINI, F. & DANESI, M. 2012. Influence of Age and Neurotoxic HAART Use on Frequency of HIV Sensory Neuropathy. *AIDS Res Treat,* 2012**,** 961510.

OSTERLOH, J. M., YANG, J., ROONEY, T. M., FOX, A. N., ADALBERT, R., POWELL, E. H., SHEEHAN, A. E., AVERY, M. A., HACKETT, R., LOGAN, M. A., MACDONALD, J. M., ZIEGENFUSS, J. S., MILDE, S., HOU, Y. J., NATHAN, C., DING, A., BROWN, R. H., JR., CONFORTI, L., COLEMAN, M., TESSIER-LAVIGNE, M., ZUCHNER, S. & FREEMAN, M. R. 2012. dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. *Science,* 337**,** 481-4.

PACKER, L. 1993. Health effects of nutritional antioxidants. *Free Radic Biol Med,* 15**,** 685-6.

PADILLA, S., MASIA, M., GARCIA, N., JARRIN, I., TORMO, C. & GUTIERREZ, F. 2011. Early changes in inflammatory and pro-thrombotic biomarkers in patients initiating antiretroviral therapy with abacavir or tenofovir. *BMC Infect Dis,* 11**,** 40.

PALELLA, F. J., JR., BAKER, R. K., MOORMAN, A. C., CHMIEL, J. S., WOOD, K. C., BROOKS, J. T. & HOLMBERG, S. D. 2006. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *J Acquir Immune Defic Syndr,* 43**,** 27-34.

PARDO, C. A., MCARTHUR, J. C. & GRIFFIN, J. W. 2001. HIV neuropathy: insights in the pathology of HIV peripheral nerve disease. *J Peripher Nerv Syst,* 6**,** 21-7.

PARKIN, D. P., VANDENPLAS, S., BOTHA, F. J., VANDENPLAS, M. L., SEIFART, H. I., VAN HELDEN, P. D., VAN DER WALT, B. J., DONALD, P. R. & VAN JAARSVELD, P. P. 1997. Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. *Am J Respir Crit Care Med,* 155**,** 1717-22.

PATERSON, R. C. 1913. Pain as a Symptom in Pulmonary Tuberculosis. *Can Med Assoc J,* 3**,** 781-7.

PATIN, E., HARMANT, C., KIDD, K. K., KIDD, J., FROMENT, A., MEHDI, S. Q., SICA, L., HEYER, E. & QUINTANA-MURCI, L. 2006. Sub-Saharan African coding sequence variation and haplotype diversity at the *NAT2* gene. *Hum Mutat,* 27**,** 720.

PAYNE, B. A., WILSON, I. J., HATELEY, C. A., HORVATH, R., SANTIBANEZ-KOREF, M., SAMUELS, D. C., PRICE, D. A. & CHINNERY, P. F. 2011. Mitochondrial aging is accelerated by anti-retroviral therapy through the clonal expansion of mtDNA mutations. *Nat Genet,* 43**,** 806-10.

University of Pretoria

PETTERSEN, J. A., JONES, G., WORTHINGTON, C., KRENTZ, H. B., KEPPLER, O. T., HOKE, A., GILL, M. J. & POWER, C. 2006. Sensory neuropathy in human immunodeficiency virus/acquired immunodeficiency syndrome patients: protease inhibitor-mediated neurotoxicity. *Ann Neurol,* 59**,** 816-24.

PHILLIPS, T. J. C., CHERRY, C. L., MOSS, P. J. & RICE, A. S. C. 2010. Painful HIV-Associated Sensory Neuropathy. *Pain: Clinical Upadates - International Association for the Study of Pain,* Volume XVIII Issue 3.

PIALOUX, G., FOURNIER, S., MOULIGNIER, A., POVEDA, J. D., CLAVEL, F. & DUPONT, B. 1997. Central nervous system as a sanctuary for HIV-1 infection despite treatment with zidovudine, lamivudine and indinavir. *AIDS,* 11**,** 1302-3.

POLYDEFKIS, M., YIANNOUTSOS, C. T., COHEN, B. A., HOLLANDER, H., SCHIFITTO, G., CLIFFORD, D. B., SIMPSON, D. M., KATZENSTEIN, D., SHRIVER, S., HAUER, P., BROWN, A., HAIDICH, A. B., MOO, L. & MCARTHUR, J. C. 2002. Reduced intraepidermal nerve fiber density in HIV-associated sensory neuropathy. *Neurology,* 58**,** 115-9.

POZNIAK, A. 2008. Tenofovir: what have over 1 million years of patient experience taught us? *Int J Clin Pract,* 62**,** 1285-93.

PREZIOSI, P. 2007. Isoniazid: metabolic aspects and toxicological correlates. *Curr Drug Metab,* 8**,** 839-51.

PRICE, J. C. & THIO, C. L. 2010. Liver disease in the HIV-infected individual. *Clin Gastroenterol Hepatol,* 8**,** 1002-12.

PURVES, D., AUGUSTINE, G. J., FITZPATRICK, D., KATZ, L. C., LAMANTIA, A. S., MCNAMARA, J. O. & WILLIAMS, S. M. 2001. *Neuroscience,* Sunderland (MA), Sinauer Associates.

RALL, L. C. & MEYDANI, S. N. 1993. Vitamin B6 and immune competence. *Nutr Rev,* 51**,** 217-25.

RAMER, M. S., MURPHY, P. G., RICHARDSON, P. M. & BISBY, M. A. 1998. Spinal nerve lesion-induced mechanoallodynia and adrenergic sprouting in sensory ganglia are attenuated in interleukin-6 knockout mice. *Pain,* 78**,** 115-21.

RANCE, N. E., MCARTHUR, J. C., CORNBLATH, D. R., LANDSTROM, D. L., GRIFFIN, J. W. & PRICE, D. L. 1988. Gracile tract degeneration in patients with sensory neuropathy and AIDS. *Neurology,* 38**,** 265-71.

RASOLA, A., GRAMAGLIA, D., BOCCACCIO, C. & COMOGLIO, P. M. 2001. Apoptosis enhancement by the HIV-1 Nef protein. *J Immunol,* 166**,** 81-8.

REEVE, A. J., PATEL, S., FOX, A., WALKER, K. & URBAN, L. 2000. Intrathecally administered endotoxin or cytokines produce allodynia, hyperalgesia and changes in spinal cord neuronal responses to nociceptive stimuli in the rat. *Eur J Pain,* 4**,** 247-57.

RENAUD-THERY, F., NGUIMFACK, B. D., VITORIA, M., LEE, E., GRAAFF, P., SAMB, B. & PERRIENS, J. 2007. Use of antiretroviral therapy in resource-limited countries in 2006: distribution and uptake of first- and second-line regimens. *AIDS,* 21 Suppl 4**,** S89-95.

RIDKER, P. M., BURING, J. E., COOK, N. R. & RIFAI, N. 2003. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation,* 107**,** 391-7.

RITCHIE, J. M. 1973. Energetic aspects of nerve conduction: the relationships between heat production, electrical activity and metabolism. *Prog Biophys Mol Biol,* 26**,** 147-87.

RIZZUTO, N., CAVALLARO, T., MONACO, S., MORBIN, M., BONETTI, B., FERRARI, S., GALIAZZO-RIZZUTO, S., ZANETTE, G. & BERTOLASI, L. 1995. Role of HIV in the pathogenesis of distal symmetrical peripheral neuropathy. *Acta Neuropathol,* 90**,** 244-50.

University of Cape Town

ROBERTS, C. K. & SINDHU, K. K. 2009. Oxidative stress and metabolic syndrome. *Life Sci,* 84**,** 705-12.

ROBIN, E. D. & WONG, R. 1988. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. *J Cell Physiol,* 136**,** 507-13.

ROBINSON-PAPP, J., MORGELLO, S., VAIDA, F., FITZSIMONS, C., SIMPSON, D. M., ELLIOTT, K. J., AL-LOZI, M., GELMAN, B. B., CLIFFORD, D., MARRA, C. M., MCCUTCHAN, J. A., ATKINSON, J. H., DWORKIN, R. H., GRANT, I. & ELLIS, R. 2010. Association of self-reported painful symptoms with clinical and neurophysiologic signs in HIV-associated sensory neuropathy. *Pain,* 151**,** 732-6.

ROBINSON-PAPP, J., TAN, I. L. & SIMPSON, D. M. 2012. Neuromuscular complications in HIV: effects of aging. *J Neurovirol,* 18**,** 331-8.

ROMERO, F. J., MONSALVE, E., HERMENEGILDO, C., PUERTAS, F. J., HIGUERAS, V., NIES, E., SEGURA-AGUILAR, J. & ROMA, J. 1991. Oxygen toxicity in the nervous tissue: comparison of the antioxidant defense of rat brain and sciatic nerve. *Neurochem Res,* 16**,** 157-61.

ROSSITER, D., BLOCKMAN, M., BARNES, K., COHEN, K., DECLOEDT, E., DE WAAL, R., KREDO, T., MAARTENS, G., MCILLERON, H. & SINXADI, P. 2012. *South African medicines formulary,* Cape Town, South Africa, Health and Medical Publishing Group of the South African Medical Association.

ROUBENOFF, R., ROUBENOFF, R. A., SELHUB, J., NADEAU, M. R., CANNON, J. G., FREEMAN, L. M., DINARELLO, C. A. & ROSENBERG, I. H. 1995. Abnormal vitamin B6 status in rheumatoid cachexia. Association with spontaneous tumor necrosis factor alpha production and markers of inflammation. *Arthritis Rheum,* 38**,** 105-9.

RUCKEMANN, K., FAIRBANKS, L. D., CARREY, E. A., HAWRYLOWICZ, C. M., RICHARDS, D. F., KIRSCHBAUM, B. & SIMMONDS, H. A. 1998. Leflunomide inhibits pyrimidine de novo synthesis in mitogen-stimulated T-lymphocytes from healthy humans. *J Biol Chem,* 273**,** 21682-91.

RYBAK, M. E. & PFEIFFER, C. M. 2004. Clinical analysis of vitamin B(6): determination of pyridoxal 5'-phosphate and 4-pyridoxic acid in human serum by reversed-phase high-performance liquid chromatography with chlorite postcolumn derivatization. *Anal Biochem,* 333**,** 336-44.

SABBAGH, A. & DARLU, P. 2005. Inferring haplotypes at the *NAT2* locus: the computational approach. *BMC Genet,* 6**,** 30.

SABBAGH, A., LANGANEY, A., DARLU, P., GERARD, N., KRISHNAMOORTHY, R. & POLONI, E. S. 2008. Worldwide distribution of *NAT2* diversity: implications for *NAT2* evolutionary history. *BMC Genet,* 9**,** 21.

SACKTOR, N. 2002. The epidemiology of human immunodeficiency virus-associated neurological disease in the era of highly active antiretroviral therapy. *J Neurovirol,* 8 Suppl 2**,** 115-21.

SACKTOR, N., NAKASUJJA, N., SKOLASKY, R. L., ROBERTSON, K., MUSISI, S., RONALD, A., KATABIRA, E. & CLIFFORD, D. B. 2009. Benefits and risks of stavudine therapy for HIV-associated neurologic complications in Uganda. *Neurology,* 72**,** 165-70.

SACKTOR, N. C., WONG, M., NAKASUJJA, N., SKOLASKY, R. L., SELNES, O. A., MUSISI, S., ROBERTSON, K., MCARTHUR, J. C., RONALD, A. & KATABIRA, E. 2005. The International HIV Dementia Scale: a new rapid screening test for HIV dementia. *AIDS,* 19**,** 1367-74.

SAIBENI, S., CATTANEO, M., VECCHI, M., ZIGHETTI, M. L., LECCHI, A., LOMBARDI, R., MEUCCI, G., SPINA, L. & DE FRANCHIS, R. 2003. Low vitamin

University of Cape Town

B(6) plasma levels, a risk factor for thrombosis, in inflammatory bowel disease: role of inflammation and correlation with acute phase reactants. *Am J Gastroenterol,* 98**,** 112-7.

SAID, G. & HONTEBEYRIE-JOSKOWICZ, M. 1992. Nerve lesions induced by macrophage activation. *Res Immunol,* 143**,** 589-99.

SALAZAR-GONZALEZ, J. F., MARTINEZ-MAZA, O., AZIZ, N., KOLBERG, J. A., YEGHIAZARIAN, T., SHEN, L. P. & FAHEY, J. L. 1997. Relationship of plasma HIV-RNA levels and levels of TNF-alpha and immune activation products in HIV infection. *Clin Immunol Immunopathol,* 84**,** 36-45.

SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. 1989. *Molecular Cloning: A laboratory manual,* USA, Cold Spring Harbor Laboratory Press.

SARWAR, N., DANESH, J., EIRIKSDOTTIR, G., SIGURDSSON, G., WAREHAM, N., BINGHAM, S., BOEKHOLDT, S. M., KHAW, K. T. & GUDNASON, V. 2007. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. *Circulation,* 115**,** 450-8.

SCHAFERS, M., BRINKHOFF, J., NEUKIRCHEN, S., MARZINIAK, M. & SOMMER, C. 2001. Combined epineurial therapy with neutralizing antibodies to tumor necrosis factor-alpha and interleukin-1 receptor has an additive effect in reducing neuropathic pain in mice. *Neurosci Lett,* 310**,** 113-6.

SCHAFERS, M., GEIS, C., BRORS, D., YAKSH, T. L. & SOMMER, C. 2002. Anterograde transport of tumor necrosis factor-alpha in the intact and injured rat sciatic nerve. *J Neurosci,* 22**,** 536-45.

SCHAFERS, M., LEE, D. H., BRORS, D., YAKSH, T. L. & SORKIN, L. S. 2003a. Increased sensitivity of injured and adjacent uninjured rat primary sensory neurons to exogenous tumor necrosis factor-alpha after spinal nerve ligation. *J Neurosci,* 23**,** 3028-38.

SCHAFERS, M., SVENSSON, C. I., SOMMER, C. & SORKIN, L. S. 2003b. Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons. *J Neurosci,* 23**,** 2517-21.

SCHEFFLER, I. E. 2001. Mitochondria make a come back. *Adv Drug Deliv Rev,* 49**,** 3-26.

SCHIFITTO, G., MCDERMOTT, M. P., MCARTHUR, J. C., MARDER, K., SACKTOR, N., EPSTEIN, L. & KIEBURTZ, K. 2002. Incidence of and risk factors for HIV-associated distal sensory polyneuropathy. *Neurology,* 58**,** 1764-8.

SCHIFITTO, G., MCDERMOTT, M. P., MCARTHUR, J. C., MARDER, K., SACKTOR, N., MCCLERNON, D. R., CONANT, K., COHEN, B., EPSTEIN, L. G. & KIEBURTZ, K. 2005. Markers of immune activation and viral load in HIV-associated sensory neuropathy. *Neurology,* 64**,** 842-8.

SCHINDHELM, R. K., DIAMANT, M., DEKKER, J. M., TUSHUIZEN, M. E., TEERLINK, T. & HEINE, R. J. 2006. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab Res Rev,* 22**,** 437-43.

SCHOLNICK, P. L., HAMMAKER, L. E. & MARVER, H. S. 1972. Soluble -aminolevulinic acid synthetase of rat liver. II. Studies related to the mechanism of enzyme action and hemin inhibition. *J Biol Chem,* 247**,** 4132-7.

SEVIGNY, J. J., ALBERT, S. M., MCDERMOTT, M. P., MCARTHUR, J. C., SACKTOR, N., CONANT, K., SCHIFITTO, G., SELNES, O. A., STERN, Y., MCCLERNON, D. R., PALUMBO, D., KIEBURTZ, K., RIGGS, G., COHEN, B., EPSTEIN, L. G. & MARDER, K. 2004. Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia. *Neurology,* 63**,** 2084-90.

University of Cape Town

SHEDLOCK, D. J., HWANG, D., CHOO, A. Y., CHUNG, C. W., MUTHUMANI, K. & WEINER, D. B. 2008. HIV-1 viral genes and mitochondrial apoptosis. *Apoptosis,* 13**,** 1088-99.

SHEN, J., LAI, C. Q., MATTEI, J., ORDOVAS, J. M. & TUCKER, K. L. 2010. Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study. *Am J Clin Nutr,* 91**,** 337-42.

SHIKUMA, C. M., HU, N., MILNE, C., YOST, F., WASLIEN, C., SHIMIZU, S. & SHIRAMIZU, B. 2001. Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipoatrophy. *AIDS,* 15**,** 1801-9.

SHLAY, J. C., CHALONER, K., MAX, M. B., FLAWS, B., REICHELDERFER, P., WENTWORTH, D., HILLMAN, S., BRIZZ, B. & COHN, D. L. 1998. Acupuncture and amitriptyline for pain due to HIV-related peripheral neuropathy: a randomized controlled trial. Terry Beirn Community Programs for Clinical Research on AIDS. *JAMA,* 280**,** 1590-5.

SHUBAYEV, V. I., ANGERT, M., DOLKAS, J., CAMPANA, W. M., PALENSCAR, K. & MYERS, R. R. 2006. TNFalpha-induced MMP-9 promotes macrophage recruitment into injured peripheral nerve. *Mol Cell Neurosci,* 31**,** 407-15.

SHURIE, J. S. & DERIBEW, A. 2010. Assessment of the prevalence of distal symmetrical polyneuropathy and its risk factors among HAART-treated and untreated HIV infected individuals. *Ethiop Med J,* 48**,** 85-93.

SIMPSON, D. M., ESTANISLAO, L., BROWN, S. J. & SAMPSON, J. 2008. An open-label pilot study of high-concentration capsaicin patch in painful HIV neuropathy. *J Pain Symptom Manage,* 35**,** 299-306.

SIMPSON, D. M., HAIDICH, A. B., SCHIFITTO, G., YIANNOUTSOS, C. T., GERACI, A. P., MCARTHUR, J. C. & KATZENSTEIN, D. A. 2002. Severity of HIV-associated neuropathy is associated with plasma HIV-1 RNA levels. *AIDS,* 16**,** 407-12.

SIMPSON, D. M., KITCH, D., EVANS, S. R., MCARTHUR, J. C., ASMUTH, D. M., COHEN, B., GOODKIN, K., GERSCHENSON, M., SO, Y., MARRA, C. M., DIAZ-ARRASTIA, R., SHRIVER, S., MILLAR, L. & CLIFFORD, D. B. 2006. HIV neuropathy natural history cohort study: assessment measures and risk factors. *Neurology,* 66**,** 1679-87.

SIMPSON, D. M., MCARTHUR, J. C., OLNEY, R., CLIFFORD, D., SO, Y., ROSS, D., BAIRD, B. J., BARRETT, P. & HAMMER, A. E. 2003. Lamotrigine for HIV-associated painful sensory neuropathies: a placebo-controlled trial. *Neurology,* 60**,** 1508-14.

SIMPSON, D. M., OLNEY, R., MCARTHUR, J. C., KHAN, A., GODBOLD, J. & EBEL-FROMMER, K. 2000. A placebo-controlled trial of lamotrigine for painful HIV-associated neuropathy. *Neurology,* 54**,** 2115-9.

SIMPSON, D. M., SCHIFITTO, G., CLIFFORD, D. B., MURPHY, T. K., DURSO-DE CRUZ, E., GLUE, P., WHALEN, E., EMIR, B., SCOTT, G. N. & FREEMAN, R. 2010. Pregabalin for painful HIV neuropathy: a randomized, double-blind, placebo-controlled trial. *Neurology,* 74**,** 413-20.

SIMPSON, D. M. & TAGLIATI, M. 1995. Nucleoside analogue-associated peripheral neuropathy in human immunodeficiency virus infection. *J Acquir Immune Defic Syndr Hum Retrovirol,* 9**,** 153-61.

SIPE, H. J., JR., JASZEWSKI, A. R. & MASON, R. P. 2004. Fast-flow EPR spectroscopic observation of the isoniazid, iproniazid, and phenylhydrazine hydrazyl radicals. *Chem Res Toxicol,* 17**,** 226-33.

SITHINAMSUWAN, P., PUNTHANAMONGKOL, S., VALCOUR, V., ONSANIT, S., NIDHINANDANA, S., THITIVICHIANLERT, S. & SHIKUMA, C. 2008.

University of Cape Town

Frequency and characteristics of HIV-associated sensory neuropathy among HIV patients in Bangkok, Thailand. *J Acquir Immune Defic Syndr,* 49**,** 456-8.

SKURNICK, J. H., BOGDEN, J. D., BAKER, H., KEMP, F. W., SHEFFET, A., QUATTRONE, G. & LOURIA, D. B. 1996. Micronutrient profiles in HIV-1-infected heterosexual adults. *J Acquir Immune Defic Syndr Hum Retrovirol,* 12**,** 75-83.

SMYTH, K., AFFANDI, J. S., MCARTHUR, J. C., BOWTELL-HARRIS, C., MIJCH, A. M., WATSON, K., COSTELLO, K., WOOLLEY, I. J., PRICE, P., WESSELINGH, S. L. & CHERRY, C. L. 2007. Prevalence of and risk factors for HIV-associated neuropathy in Melbourne, Australia 1993-2006. *HIV Med,* 8**,** 367-73.

SNIDER, D. E., JR. 1980. Pyridoxine supplementation during isoniazid therapy. *Tubercle,* 61**,** 191-6.

SNIDER, W. D., SIMPSON, D. M., NIELSEN, S., GOLD, J. W., METROKA, C. E. & POSNER, J. B. 1983. Neurological complications of acquired immune deficiency syndrome: analysis of 50 patients. *Ann Neurol,* 14**,** 403-18.

SO, Y. T., HOLTZMAN, D. M., ABRAMS, D. I. & OLNEY, R. K. 1988. Peripheral neuropathy associated with acquired immunodeficiency syndrome. Prevalence and clinical features from a population-based survey. *Arch Neurol,* 45**,** 945-8.

SOMMER, C. 2001. Cytokines in neuropathic pain. *Anaesthesist,* 50**,** 416-26.

SOMMER, C. & KRESS, M. 2004. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett,* 361**,** 184-7.

SOMMER, C., LINDENLAUB, T., TEUTEBERG, P., SCHAFERS, M., HARTUNG, T. & TOYKA, K. V. 2001. Anti-TNF-neutralizing antibodies reduce pain-related behavior in two different mouse models of painful mononeuropathy. *Brain Res,* 913**,** 86-9.

SOMMER, C., PETRAUSCH, S., LINDENLAUB, T. & TOYKA, K. V. 1999. Neutralizing antibodies to interleukin 1-receptor reduce pain associated behavior in mice with experimental neuropathy. *Neurosci Lett,* 270**,** 25-8.

SOMMER, C., SCHMIDT, C. & GEORGE, A. 1998. Hyperalgesia in experimental neuropathy is dependent on the TNF receptor 1. *Exp Neurol,* 151**,** 138-42.

SORKIN, L. S., XIAO, W. H., WAGNER, R. & MYERS, R. R. 1997. Tumour necrosis factor-alpha induces ectopic activity in nociceptive primary afferent fibres. *Neuroscience,* 81**,** 255-62.

SOUTH AFRICAN DEPARTMENT OF HEALTH. 2004. *South African tuberculosis control programme practical guidelines 2004* [Online]. Department of Health. Available: http://www.kznhealth.gov.za/chrp/documents/Guidelines/Guidelines National/Tuberculosis/SA TB Guidelines 2004.pdf [Accessed January 11 2013].

STASZEWSKI, S., KEISER, P., MONTANER, J., RAFFI, F., GATHE, J., BROTAS, V., HICKS, C., HAMMER, S. M., COOPER, D., JOHNSON, M., TORTELL, S., CUTRELL, A., THORBORN, D., ISAACS, R., HETHERINGTON, S., STEEL, H., SPREEN, W. & TEAM, C. I. S. 2001. Abacavir-lamivudine-zidovudine vs indinavir-lamivudine-zidovudine in antiretroviral-naive HIV-infected adults: A randomized equivalence trial. *JAMA,* 285**,** 1155-63.

STASZEWSKI, S., MORALES-RAMIREZ, J., TASHIMA, K. T., RACHLIS, A., SKIEST, D., STANFORD, J., STRYKER, R., JOHNSON, P., LABRIOLA, D. F., FARINA, D., MANION, D. J. & RUIZ, N. M. 1999. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team. *N Engl J Med,* 341**,** 1865-73.

STEFANOVA, I., SAVILLE, M. W., PETERS, C., CLEGHORN, F. R., SCHWARTZ, D., VENZON, D. J., WEINHOLD, K. J., JACK, N., BARTHOLOMEW, C., BLATTNER, W. A., YARCHOAN, R., BOLEN, J. B. & HORAK, I. D. 1996. HIV

University of Cape Town

infection--induced posttranslational modification of T cell signaling molecules associated with disease progression. *J Clin Invest,* 98**,** 1290-7.

STEICHEN, O., MARTINEZ-ALMOYNA, L. & DE BROUCKER, T. 2006. Isoniazid induced neuropathy: consider prevention. *Rev Mal Respir,* 23**,** 157-60.

STEPHENS, M. & DONNELLY, P. 2003. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet,* 73**,** 1162-9.

STEPHENS, M., SMITH, N. J. & DONNELLY, P. 2001. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet,* 68**,** 978-89.

STOCKER, P., LESGARDS, J. F., VIDAL, N., CHALIER, F. & PROST, M. 2003. ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochim Biophys Acta,* 1621**,** 1-8.

STRINGER, J. S., ZULU, I., LEVY, J., STRINGER, E. M., MWANGO, A., CHI, B. H., MTONGA, V., REID, S., CANTRELL, R. A., BULTERYS, M., SAAG, M. S., MARLINK, R. G., MWINGA, A., ELLERBROCK, T. V. & SINKALA, M. 2006. Rapid scale-up of antiretroviral therapy at primary care sites in Zambia: feasibility and early outcomes. *JAMA,* 296**,** 782-93.

STROMSKA, D. P. & OCHS, S. 1982. Axoplasmic transport in aged rats. *Exp Neurol,* 77**,** 215-24.

STRUIK, G. M., DEN EXTER, R. A., MUNTHALI, C., CHIPETA, D., VAN OOSTERHOUT, J. J., NOUWEN, J. L. & ALLAIN, T. J. 2011. The prevalence of renal impairment among adults with early HIV disease in Blantyre, Malawi. *Int J STD AIDS,* 22**,** 457-62.

STYLIANOU, E., AUKRUST, P., KVALE, D., MULLER, F. & FROLAND, S. S. 1999. IL-10 in HIV infection: increasing serum IL-10 levels with disease progression - down-regulatory effect of potent anti-retroviral therapy. *Clin Exp Immunol,* 116**,** 115-20.

SZELENYI, J. 2001. Cytokines and the central nervous system. *Brain Res Bull,* 54**,** 329-38.

TADOKERA, R., MEINTJES, G., SKOLIMOWSKA, K. H., WILKINSON, K. A., MATTHEWS, K., SELDON, R., CHEGOU, N. N., MAARTENS, G., RANGAKA, M. X., REBE, K., WALZL, G. & WILKINSON, R. J. 2011. Hypercytokinaemia accompanies HIV-tuberculosis immune reconstitution inflammatory syndrome. *Eur Respir J,* 37**,** 1248-59.

TAGLIATI, M., GRINNELL, J., GODBOLD, J. & SIMPSON, D. M. 1999. Peripheral nerve function in HIV infection: clinical, electrophysiologic, and laboratory findings. *Arch Neurol,* 56**,** 84-9.

TAKADA, Y., MUKHOPADHYAY, A., KUNDU, G. C., MAHABELESHWAR, G. H., SINGH, S. & AGGARWAL, B. B. 2003. Hydrogen peroxide activates NF-kappa B through tyrosine phosphorylation of I kappa B alpha and serine phosphorylation of p65: evidence for the involvement of I kappa B alpha kinase and Syk protein-tyrosine kinase. *J Biol Chem,* 278**,** 24233-41.

TAMAKOSHI, K., YATSUYA, H., KONDO, T., HORI, Y., ISHIKAWA, M., ZHANG, H., MURATA, C., OTSUKA, R., ZHU, S. & TOYOSHIMA, H. 2003. The metabolic syndrome is associated with elevated circulating C-reactive protein in healthy reference range, a systemic low-grade inflammatory state. *Int J Obes Relat Metab Disord,* 27**,** 443-9.

TANG, A. M., GRAHAM, N. M., CHANDRA, R. K. & SAAH, A. J. 1997a. Low serum vitamin B-12 concentrations are associated with faster human immunodeficiency virus type 1 (HIV-1) disease progression. *J Nutr,* 127**,** 345-51.

TANG, A. M., GRAHAM, N. M., KIRBY, A. J., MCCALL, L. D., WILLETT, W. C. & SAAH, A. J. 1993. Dietary micronutrient intake and risk of progression to acquired

University of Pretoria

immunodeficiency syndrome (AIDS) in human immunodeficiency virus type 1 (HIV-1)-infected homosexual men. *Am J Epidemiol,* 138**,** 937-51.

TANG, A. M., GRAHAM, N. M., SEMBA, R. D. & SAAH, A. J. 1997b. Association between serum vitamin A and E levels and HIV-1 disease progression. *AIDS,* 11**,** 613-20.

TANG, A. M. & SMIT, E. 1998. Selected vitamins in HIV infection: a review. *AIDS Patient Care STDS,* 12**,** 263-73.

TANG, A. M., SMIT, E., SEMBA, R. D., SHAH, N., LYLES, C. M., LI, D. & VLAHOV, D. 2000. Improved antioxidant status among HIV-infected injecting drug users on potent antiretroviral therapy. *J Acquir Immune Defic Syndr,* 23**,** 321-6.

TASKINEN, H. S., OLSSON, T., BUCHT, A., KHADEMI, M., SVELANDER, L. & ROYTTA, M. 2000. Peripheral nerve injury induces endoneurial expression of IFN-gamma, IL-10 and TNF-alpha mRNA. *J Neuroimmunol,* 102**,** 17-25.

TASKINEN, H. S. & ROYTTA, M. 2000. Increased expression of chemokines (MCP-1, MIP-1alpha, RANTES) after peripheral nerve transection. *J Peripher Nerv Syst,* 5**,** 75-81.

TAVEE, J. & ZHOU, L. 2009. Small fiber neuropathy: A burning problem. *Cleve Clin J Med,* 76**,** 297-305.

TEGEDER, I., COSTIGAN, M., GRIFFIN, R. S., ABELE, A., BELFER, I., SCHMIDT, H., EHNERT, C., NEJIM, J., MARIAN, C., SCHOLZ, J., WU, T., ALLCHORNE, A., DIATCHENKO, L., BINSHTOK, A. M., GOLDMAN, D., ADOLPH, J., SAMA, S., ATLAS, S. J., CARLEZON, W. A., PARSEGIAN, A., LOTSCH, J., FILLINGIM, R. B., MAIXNER, W., GEISSLINGER, G., MAX, M. B. & WOOLF, C. J. 2006. GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. *Nat Med,* 12**,** 1269-77.

THEA, D. M., PORAT, R., NAGIMBI, K., BAANGI, M., ST LOUIS, M. E., KAPLAN, G., DINARELLO, C. A. & KEUSCH, G. T. 1996. Plasma cytokines, cytokine antagonists, and disease progression in African women infected with HIV-1. *Ann Intern Med,* 124**,** 757-62.

TIMPERIO, A. M., RINALDUCCI, S. & ZOLLA, L. 2005. Hydrazide derivatives produce active oxygen species as hydrazine. *Bioorg Chem,* 33**,** 459-69.

TISCHNER, D., WOESS, C., OTTINA, E. & VILLUNGER, A. 2010. Bcl-2-regulated cell death signalling in the prevention of autoimmunity. *Cell Death Dis,* 1**,** e48.

TOUZANI, O., BOUTIN, H., LEFEUVRE, R., PARKER, L., MILLER, A., LUHESHI, G. & ROTHWELL, N. 2002. Interleukin-1 influences ischemic brain damage in the mouse independently of the interleukin-1 type I receptor. *J Neurosci,* 22**,** 38-43.

TOWER, D. B. 1956. Neurochemical aspects of pyridoxine metabolism and function. *Am J Clin Nutr,* 4**,** 329-45.

TRAN, P. B. & MILLER, R. J. 2003. Chemokine receptors: signposts to brain development and disease. *Nat Rev Neurosci,* 4**,** 444-55.

TRIFILIEFF, A., FUJITANI, Y., MENTZ, F., DUGAS, B., FUENTES, M. & BERTRAND, C. 2000. Inducible nitric oxide synthase inhibitors suppress airway inflammation in mice through down-regulation of chemokine expression. *J Immunol,* 165**,** 1526-33.

TUCKER, T. J., VOIGT, M., BIRD, A., ROBSON, S., GIBBS, B., KANNEMEYER, J., GALLOWAY, M., KIRSCH, R. E. & SMUTS, H. 1997. Hepatitis C virus infection rate in volunteer blood donors from the Western Cape--comparison of screening tests and PCR. *S Afr Med J,* 87**,** 603-5.

TYOR, W. R., WESSELINGH, S. L., GRIFFIN, J. W., MCARTHUR, J. C. & GRIFFIN, D. E. 1995. Unifying hypothesis for the pathogenesis of HIV-associated dementia

University of Cape Town

complex, vacuolar myelopathy, and sensory neuropathy. *J Acquir Immune Defic Syndr Hum Retrovirol,* 9**,** 379-88.

UBBINK, J. B., SERFONTEIN, W. J., BECKER, P. J. & DE VILLIERS, L. S. 1987. Effect of different levels of oral pyridoxine supplementation on plasma pyridoxal-5'-phosphate and pyridoxal levels and urinary vitamin B-6 excretion. *Am J Clin Nutr,* 46**,** 78-85.

UCEYLER, N., ROGAUSCH, J. P., TOYKA, K. V. & SOMMER, C. 2007. Differential expression of cytokines in painful and painless neuropathies. *Neurology,* 69**,** 42-9.

UNAIDS. 2011. *World AIDS day report* [Online]. Available: http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/JC2216_WorldAIDSday_report_2011_en.pdf [Accessed January 13 2013].

UNCINI, A., DI MUZIO, A., DI GUGLIELMO, G., DE ANGELIS, M. V., DE LUCA, G., LUGARESI, A. & GAMBI, D. 1999. Effect of rhTNF-alpha injection into rat sciatic nerve. *J Neuroimmunol,* 94**,** 88-94.

UNIVERSITY OF LOUISVILLE. 2011. *Human NAT2 alleles (Haplotypes) (updated July 22, 2011)* [Online]. Available: http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/nat_pdf_files/Human_NAT2_alleles.pdf [Accessed September 18 2012].

UPTON, A. M., MUSHTAQ, A., VICTOR, T. C., SAMPSON, S. L., SANDY, J., SMITH, D. M., VAN HELDEN, P. V. & SIM, E. 2001. Arylamine *N*-acetyltransferase of *Mycobacterium tuberculosis* is a polymorphic enzyme and a site of isoniazid metabolism. *Mol Microbiol,* 42**,** 309-17.

URAKAWA, H., KATSUKI, A., SUMIDA, Y., GABAZZA, E. C., MURASHIMA, S., MORIOKA, K., MARUYAMA, N., KITAGAWA, N., TANAKA, T., HORI, Y., NAKATANI, K., YANO, Y. & ADACHI, Y. 2003. Oxidative stress is associated with adiposity and insulin resistance in men. *J Clin Endocrinol Metab,* 88**,** 4673-6.

VALLEJO, R., TILLEY, D. M., VOGEL, L. & BENYAMIN, R. 2010. The role of glia and the immune system in the development and maintenance of neuropathic pain. *Pain Pract,* 10**,** 167-84.

VAN DER WATT, G. F. 2011. Mitochondrial dysfunction and human immunodeficiency virus infection. *JEMDSA,* 16**,** 94-100.

VAN DER WATT, J. J., HARRISON, T. B., BENATAR, M. & HECKMANN, J. M. 2011. Polyneuropathy, anti-tuberculosis treatment and the role of pyridoxine in the HIV/AIDS era: a systematic review. *Int J Tuberc Lung Dis,* 15**,** 722-8.

VAN LETTOW, M., FAWZI, W. W. & SEMBA, R. D. 2003. Triple trouble: the role of malnutrition in tuberculosis and human immunodeficiency virus co-infection. *Nutr Rev,* 61**,** 81-90.

VAN OOSTERHOUT, J. J., BODASING, N., KUMWENDA, J. J., NYIRENDA, C., MALLEWA, J., CLEARY, P. R., DE BAAR, M. P., SCHUURMAN, R., BURGER, D. M. & ZIJLSTRA, E. E. 2005. Evaluation of antiretroviral therapy results in a resource-poor setting in Blantyre, Malawi. *Trop Med Int Health,* 10**,** 464-70.

VANDERFORD, T. H., ADAMSKI, J. & SILVESTRI, G. 2010. HIV-associated chronic immune activation: current understandings and therapeutic intervention. *HIV Therapy,* 4**,** 161-177.

VERGNOLLE, N., BUNNETT, N. W., SHARKEY, K. A., BRUSSEE, V., COMPTON, S. J., GRADY, E. F., CIRINO, G., GERARD, N., BASBAUM, A. I., ANDRADE-GORDON, P., HOLLENBERG, M. D. & WALLACE, J. L. 2001. Proteinase-activated receptor-2 and hyperalgesia: A novel pain pathway. *Nat Med,* 7**,** 821-6.

University of Cape Town

VERMA, A. 2001. Epidemiology and clinical features of HIV-1 associated neuropathies. *J Peripher Nerv Syst,* 6**,** 8-13.

VERMA, S., ESTANISLAO, L., MINTZ, L. & SIMPSON, D. 2004. Controlling neuropathic pain in HIV. *Curr HIV/AIDS Rep,* 1**,** 136-41.

VERMA, S., ESTANISLAO, L. & SIMPSON, D. 2005. HIV-associated neuropathic pain: epidemiology, pathophysiology and management. *CNS Drugs,* 19**,** 325-34.

VILLAMOR, E., MUGUSI, F., URASSA, W., BOSCH, R. J., SAATHOFF, E., MATSUMOTO, K., MEYDANI, S. N. & FAWZI, W. W. 2008. A trial of the effect of micronutrient supplementation on treatment outcome, T cell counts, morbidity, and mortality in adults with pulmonary tuberculosis. *J Infect Dis,* 197**,** 1499-505.

VINCENT, A. M., HAYES, J. M., MCLEAN, L. L., VIVEKANANDAN-GIRI, A., PENNATHUR, S. & FELDMAN, E. L. 2009. Dyslipidemia-induced neuropathy in mice: the role of oxLDL/LOX-1. *Diabetes,* 58**,** 2376-85.

VINIK, A. I., STROTMEYER, E. S., NAKAVE, A. A. & PATEL, C. V. 2008. Diabetic neuropathy in older adults. *Clin Geriatr Med,* 24**,** 407-35, v.

VISSER, M. E., TEXEIRA-SWIEGELAAR, C. & MAARTENS, G. 2004. The short-term effects of anti-tuberculosis therapy on plasma pyridoxine levels in patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis,* 8**,** 260-2.

WADLEY, A. L., CHERRY, C. L., PRICE, P. & KAMERMAN, P. R. 2011. HIV neuropathy risk factors and symptom characterization in stavudine-exposed South Africans. *J Pain Symptom Manage,* 41**,** 700-6.

WAGNER, R. & MYERS, R. R. 1996. Schwann cells produce tumor necrosis factor alpha: expression in injured and non-injured nerves. *Neuroscience,* 73**,** 625-9.

WALKER, U. A. & VENHOFF, N. 2001. Multiple mitochondrial DNA deletions and lactic acidosis in an HIV-infected patient under antiretroviral therapy. *AIDS,* 15**,** 1449-50.

WALLACE, D. C. 1999. Mitochondrial diseases in man and mouse. *Science,* 283**,** 1482-8.

WALLACE, V. C., BLACKBEARD, J., PHEBY, T., SEGERDAHL, A. R., DAVIES, M., HASNIE, F., HALL, S., MCMAHON, S. B. & RICE, A. S. 2007. Pharmacological, behavioural and mechanistic analysis of HIV-1 gp120 induced painful neuropathy. *Pain,* 133**,** 47-63.

WALLER, A. 1850. Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Philosophical Transactions of the Royal Society of London,* 140**,** 423-429.

WALMSLEY, S., BERNSTEIN, B., KING, M., ARRIBAS, J., BEALL, G., RUANE, P., JOHNSON, M., JOHNSON, D., LALONDE, R., JAPOUR, A., BRUN, S., SUN, E. & TEAM, M. S. 2002. Lopinavir-ritonavir versus nelfinavir for the initial treatment of HIV infection. *N Engl J Med,* 346**,** 2039-46.

WALRAVEN, J. M., ZANG, Y., TRENT, J. O. & HEIN, D. W. 2008. Structure/function evaluations of single nucleotide polymorphisms in human *N*-acetyltransferase 2. *Curr Drug Metab,* 9**,** 471-86.

WALSH, D. T., WEG, V. B., WILLIAMS, T. J. & NOURSHARGH, S. 1995. Substance P-induced inflammatory responses in guinea-pig skin: the effect of specific NK1 receptor antagonists and the role of endogenous mediators. *Br J Pharmacol,* 114**,** 1343-50.

WANG, H., LEMIRE, B. D., CASS, C. E., WEINER, J. H., MICHALAK, M., PENN, A. M. & FLIEGEL, L. 1996. Zidovudine and dideoxynucleosides deplete wild-type mitochondrial DNA levels and increase deleted mitochondrial DNA levels in cultured Kearns-Sayre syndrome fibroblasts. *Biochim Biophys Acta,* 1316**,** 51-9.

University of Cape Town

WANG, S. S., SHULTZ, J. R., BURISH, M. J., HARRISON, K. H., HOF, P. R., TOWNS, L. C., WAGERS, M. W. & WYATT, K. D. 2008. Functional trade-offs in white matter axonal scaling. *J Neurosci,* 28**,** 4047-56.

WATKINS, L. R., GOEHLER, L. E., RELTON, J., BREWER, M. T. & MAIER, S. F. 1995. Mechanisms of tumor necrosis factor-alpha (TNF-alpha) hyperalgesia. *Brain Res,* 692**,** 244-50.

WATKINS, L. R. & MAIER, S. F. 2002. Beyond neurons: evidence that immune and glial cells contribute to pathological pain states. *Physiol Rev,* 82**,** 981-1011.

WATTERS, M. R., POFF, P. W., SHIRAMIZU, B. T., HOLCK, P. S., FAST, K. M., SHIKUMA, C. M. & VALCOUR, V. G. 2004. Symptomatic distal sensory polyneuropathy in HIV after age 50. *Neurology,* 62**,** 1378-83.

WEI, Y. H. & LEE, H. C. 2002. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp Biol Med (Maywood),* 227**,** 671-82.

WESSELINGH, S. L., GLASS, J., MCARTHUR, J. C., GRIFFIN, J. W. & GRIFFIN, D. E. 1994. Cytokine dysregulation in HIV-associated neurological disease. *Adv Neuroimmunol,* 4**,** 199-206.

WESTREICH, D. J., SANNE, I., MASKEW, M., MALOPE-KGOKONG, B., CONRADIE, F., MAJUBA, P., FUNK, M. J., KAUFMAN, J. S., VAN RIE, A. & MACPHAIL, P. 2009. Tuberculosis treatment and risk of stavudine substitution in first-line antiretroviral therapy. *Clin Infect Dis,* 48**,** 1617-23.

WHITE, A. J. 2001. Mitochondrial toxicity and HIV therapy. *Sex Transm Infect,* 77**,** 158-73.

WHO. 2009. *World Health Organization. Treatment of tuberculosis: guidelines for national programmes* [Online]. Geneva, Switzerland. Available: http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf [Accessed January 13 2013].

WHO. 2011. *World Health Organization. Global summary of the AIDS epidemic* [Online]. Available: http://www.who.int/hiv/data/2012_epi_core_en.png [Accessed January 13 2013].

WHO. 2012. *World Health Organization. Global tuberculosis report* [Online]. Available: http://www.who.int/tb/publications/global_report/en/index.html [Accessed January 13 2013].

WILKINSON, K. A., SELDON, R., MEINTJES, G., RANGAKA, M. X., HANEKOM, W. A., MAARTENS, G. & WILKINSON, R. J. 2009. Dissection of regenerating T-Cell responses against tuberculosis in HIV-infected adults sensitized by Mycobacterium tuberculosis. *Am J Respir Crit Care Med,* 180**,** 674-83.

WILLIS-CARR, J. I. & ST PIERRE, R. L. 1978. Effects of vitamin B6 deficiency on thymic epithelial cells and T lymphocyte differentiation. *J Immunol,* 120**,** 1153-9.

WOLKENSTEIN, P., LORIOT, M. A., ARACTINGI, S., CABELGUENNE, A., BEAUNE, P. & CHOSIDOW, O. 2000. Prospective evaluation of detoxification pathways as markers of cutaneous adverse reactions to sulphonamides in AIDS. *Pharmacogenetics,* 10**,** 821-8.

WOOD, R., MIDDELKOOP, K., MYER, L., GRANT, A. D., WHITELAW, A., LAWN, S. D., KAPLAN, G., HUEBNER, R., MCINTYRE, J. & BEKKER, L. G. 2007. Undiagnosed tuberculosis in a community with high HIV prevalence: implications for tuberculosis control. *Am J Respir Crit Care Med,* 175**,** 87-93.

WOOLF, K. & MANORE, M. M. 2008. Elevated plasma homocysteine and low vitamin B-6 status in nonsupplementing older women with rheumatoid arthritis. *J Am Diet Assoc,* 108**,** 443-53; discussion 454.

University of Cape Town

WOOLLEY, I., FARAGHER, M. & SPELMAN, D. 1997. Association between HIV distal symmetric polyneuropathy and Mycobacterium avium complex infection. *J Neurol Neurosurg Psychiatry,* 63**,** 557.

WOSKA, J. R., JR. & MAGRAM, J. 2005. Overview of approaches to the identification of inhibitors of cytokine action. *Curr Protoc Pharmacol,* Chapter 1**,** Unit 1 31.

WRIGHT, E., BREW, B., ARAYAWICHANONT, A., ROBERTSON, K., SAMINTHARAPANYA, K., KONGSAENGDAO, S., LIM, M., VONTHANAK, S., LAL, L., SARIM, C., HUFFAM, S., LI, P., IMRAN, D., LEWIS, J., LUN, W. H., KAMARULZAMAN, A., TAU, G., ALI, S. T., KISHORE, K., BAIN, M. P., DWYER, R., MCCORMACK, G., HELLARD, M., CHERRY, C., MCARTHUR, J. & WESSELINGH, S. 2008. Neurologic disorders are prevalent in HIV-positive outpatients in the Asia-Pacific region. *Neurology,* 71**,** 50-6.

WULFF, E. A., WANG, A. K. & SIMPSON, D. M. 2000. HIV-associated peripheral neuropathy: epidemiology, pathophysiology and treatment. *Drugs,* 59**,** 1251-60.

XU, X. J., HAO, J. X., ANDELL-JONSSON, S., POLI, V., BARTFAI, T. & WIESENFELD-HALLIN, Z. 1997. Nociceptive responses in interleukin-6-deficient mice to peripheral inflammation and peripheral nerve section. *Cytokine,* 9**,** 1028-33.

YE, X., MARAS, J. E., BAKUN, P. J. & TUCKER, K. L. 2010. Dietary intake of vitamin B-6, plasma pyridoxal 5'-phosphate, and homocysteine in Puerto Rican adults. *J Am Diet Assoc,* 110**,** 1660-8.

YOULE, M., OSIO, M. & GROUP, A. S. 2007. A double-blind, parallel-group, placebo-controlled, multicentre study of acetyl L-carnitine in the symptomatic treatment of antiretroviral toxic neuropathy in patients with HIV-1 infection. *HIV Med,* 8**,** 241-50.

ZACHARIAH, R., FITZGERALD, M., MASSAQUOI, M., PASULANI, O., ARNOULD, L., MAKOMBE, S. & HARRIES, A. D. 2006. Risk factors for high early mortality in patients on antiretroviral treatment in a rural district of Malawi. *AIDS,* 20**,** 2355-60.

ZANGERLE, R., GALLATI, H., SARCLETTI, M., WEISS, G., DENZ, H., WACHTER, H. & FUCHS, D. 1994. Increased serum concentrations of soluble tumor necrosis factor receptors in HIV-infected individuals are associated with immune activation. *J Acquir Immune Defic Syndr,* 7**,** 79-85.

ZHENG, J., GHORPADE, A., NIEMANN, D., COTTER, R. L., THYLIN, M. R., EPSTEIN, L., SWARTZ, J. M., SHEPARD, R. B., LIU, X., NUKUNA, A. & GENDELMAN, H. E. 1999. Lymphotropic virions affect chemokine receptor-mediated neural signaling and apoptosis: implications for human immunodeficiency virus type 1-associated dementia. *J Virol,* 73**,** 8256-67.

ZHONG, M., CHENG, G. F., WANG, W. J., GUO, Y., ZHU, X. Y. & ZHANG, J. T. 1999. Inhibitory effect of resveratrol on interleukin 6 release by stimulated peritoneal macrophages of mice. *Phytomedicine,* 6**,** 79-84.

ZHOU, L., KITCH, D. W., EVANS, S. R., HAUER, P., RAMAN, S., EBENEZER, G. J., GERSCHENSON, M., MARRA, C. M., VALCOUR, V., DIAZ-ARRASTIA, R., GOODKIN, K., MILLAR, L., SHRIVER, S., ASMUTH, D. M., CLIFFORD, D. B., SIMPSON, D. M. & MCARTHUR, J. C. 2007. Correlates of epidermal nerve fiber densities in HIV-associated distal sensory polyneuropathy. *Neurology,* 68**,** 2113-9.

ZHOU, L., LI, J., ONTANEDA, D. & SPERLING, J. 2011. Metabolic syndrome in small fiber sensory neuropathy. *J Clin Neuromuscul Dis,* 12**,** 235-43.

ZUIDEMA, J., HILBERS-MODDERMAN, E. S. & MERKUS, F. W. 1986. Clinical pharmacokinetics of dapsone. *Clin Pharmacokinet,* 11**,** 299-315.

University of Cape Town