A Clinical Investigation of Painful HIV-Associated Sensory Neuropathy

Thesis presented for the degree of

Doctor Of Medicine

Imperial College London

Dr Tudor Joseph Colin Phillips

The Pain Group,
Section of Anaesthetics, Pain Medicine and Intensive Care, Chelsea and Westminster Hospital Campus, London.

Supervisor: Professor ASC Rice

June 2013

Acknowledgements

I would first like to thank my supervisor, Andrew Rice for his encouragement to get involved in the world of pain research, and his subsequent patience and guidance. As with many things in life, small co-incidences can change your world; all of this would not have happened but for the seating arrangement on a BA flight. I am thankful to whoever picked Andrew's seat for that flight.

Many thanks to the Derek Butler Trust who funded this research. It was a leap into the unknown for them. Their enthusiasm for helping people living with HIV is inspiring.

Thanks to the Pain Lab guys who were (and are) a breath of fresh air after years in clinical medicine. Talk of break-beats, Romanian gypsy music and photography were not normal in hospital medicine. Many thanks to Tim who always had sensible advice and would often help more than he needed to. Thanks also to Faruq for helping me to negotiate the red tape of a clinical research project by always knowing which form I needed to fill in. I'd like to thank Matt for his enthusiasm and help with the data entry; who along with Juan prepared the skin biopsy slides. Bingo will never be the same again. Thanks Yohannes for getting me enthusiastic again when I was loosing steam!

Many thanks to Amanda Williams for helping me understand what I was trying to achieve when all I could see was an ocean of data. I'd like to thank Dave Bennett and Sarah Cox for helping with the project and for the memories of the most interesting clinics I've ever been to.

Thanks to Jim for his hard work with the database. Jim was never rude to me once, despite my incessant emails regarding the king of all unfathomably complicated quality of life instruments. We all know which one that is.

Many thanks to all the participants who gave up their time and agreed to leave a bit of themselves behind to test. Thanks to Dr Mark Nelson; his patients' respect for him made my recruitment life easy. Many thanks to the clinical and administrative staff at The St Stephens' Centre who accepted me into their team.

Research has also given my future wife Amparo, and Blai – our beautiful baby boy who I dedicate this thesis to. Thank you Amparo for being the best thing to happen to me and for your love and support through about all the weekends and holidays I spent number crunching for this. It would have been impossible without you.

It's nearly done……..

Declaration and Author Contributions

All the work contained herein this thesis is mine, with the following exceptions.

Chapter 1:

Professor Andrew S. C. Rice (The Pain Research Group, Imperial College London), Dr Catherine L. Cherry (Head of Neuropathy and Drug Toxicity Group, Monash University, Melbourne, Australia) and Philippa Moss (PhD Student London Pain Consortium Student, The Pain Research Group, Imperial College) edited and made suggestions to the content of this chapter.

Chapter 2:

As part of the Systematic Review the following authors also conducted data quality assessments: Dr Catherine L. Cherry, Dr Sarah Cox (Palliative Care Physician, Chelsea and Westminster NHS Trust Hospital, London) and Professor Andrew S. C. Rice who also acted as final arbitrator.

Chapter 3:

Dr Dave L. H. Bennett (Clinical Neurosciences Department, Oxford University) aided with the protocol preparation, help preparing the Structure Neurological Examination (SNE) and expertise in assessing participant electrophysiology data.

Dr Matthew R.D. Brown (NIHR Academic Clinical Fellow, The Pain Research Group Imperial College) who collected 18 of the 36 healthy volunteer DFNS QST datasets, and helped with data entry. Dr Matthew Brown and Dr Juan Ramirez Rozo (DPhil Student, Clinical Neurosciences Dept Oxford University), prepared and stained the skin biopsies I took, and determined intra-epidermal nerve fibre densities (IENFD).

Dr Amanda C. Williams (Clinical Health Psychology Department, University College London) for help with selecting the Psychological Instruments for the protocol and for help with the SPSS data analysis of these results.

James Perkins (PhD Student London Pain Consortium, The Bioinformatics Group, University College London) for preparation and maintenance of the web-based data-entry portal. James Perkins conducted the hierarchical cluster analysis of QST data.

Dr Yohannes Woldeamanuel (IASP Fellow, Imperial College) for conducting the ROC analysis for the neuropathy screening tools and preparation of the QST data scatter plots (Figures 3.1 and 3.2) as an alternative method to present the data in Figure 3.3.

Abstract

HIV-associated sensory polyneuropathy (HIV-SN) is a distal symmetrical polyneuropathy that is a prevalent (approximately 40%), and often painful complication of HIV infection. The introduction of combination antiretroviral treatment (cART), transformed HIV infection from a high mortality condition into a chronic disease. Despite this, and the phasing-out of neurotoxic antiretroviral drugs, HIV-SN continues to be a significant morbidity experienced in HIV infection. Consequently, the understanding, prevention and management of HIV-SN is increasingly important.

A systematic review and meta-analysis was undertaken to examine the evidence for the efficacy of pharmacological agents in the management of pain in HIV-SN. Of 44 studies identified, 19 were randomized controlled trials, and only 14 were of sufficient quality. Interventions demonstrating greater efficacy than placebo included smoked cannabis and recombinant nerve growth factor (rhNGF). However rhNGF is not clinically available and smoked cannabis cannot be recommended as routine therapy.

A second study was undertaken to phenotype a cohort of HIV infected participants with and without HIV-SN. This study examined demographic factors, symptomatology, metabolic factors, sensory nerve dysfunction through quantitative sensory testing (QST), and intra-epidermal nerve fibre density (IENFD); quality of life, sleep dysfunction and psychological co-morbidity. A novel triumvirate definition using QST, IENFD and a structured neurological examination was used to define HIV-SN.

This study showed that patterns of sensory nerve dysfunction are heterogeneous in HIV-SN; the most common feature being loss of sensory function in mechanical and vibration detection modalities. Gain of function sensory changes were infrequent, but were seen in a small sub-group demonstrating wind-up phenomenon; this group demonstrated higher levels of catastrophizing behaviours. Measures of quality of life were generally lower

than in other painful peripheral neuropathies. Sleep dysfunction was common in HIV-SN. Participants with HIV-SN had elevated triglycerides (TRG), providing further evidence for the role of dyslipidaemia in HIV-SN pathogenesis.

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Publications arising from this work

Abstracts

A Meta-analysis of Randomised Controlled Clinical Trials Assessing the Effectiveness of Pharmacological Interventions for Painful HIV-Associated Sensory Neuropathy. **Tudor J. C. Phillips**, Catherine L. Cherry, Sarah Cox, Sarah Marshall, Andrew S. C. Rice

Abstract and poster presentation IASP 13th World Congress on Pain, Montreal, Canada. September 2010.

Sensory Profiles and Epidermal Innervation in Patients with HIV-Associated Sensory Neuropathy. Matthew R. D. Brown, **Tudor J. C. Phillips**, Juan D. Ramirez, James R. Perkins, Christine Orengo, Yohannes W. Woldeamanuel, Sarah Cox, Istvan Bodi, Christoph Maier, Elena Krumova, David L. H. Bennett, Andrew S. C. Rice.

Abstract and poster presentation NeupSIG World Congress, Toronto Canada. May 2013.

Assessment of the Utility of Clinical Instruments for Diagnosing HIV-associated Sensory Neuropathy. Yohannes W. Woldeamanuel, **Tudor J. C. Phillips**, Matthew R. D. Brown, Juan D. Ramirez, James R. Perkins, Christine Orengo, Sarah Cox, David L. H. Bennett, Andrew S. C. Rice.

Abstract and poster presentation NeupSIG World Congress, Toronto Canada. May 2013

Original Papers

Phillips, T. J. C., Cherry, C. L. , Cox, S. , Marshall, S. J. , *et al.* (2010) Pharmacological treatment of painful HIV-associated sensory neuropathy: a systematic review and meta-analysis of randomised controlled trials. *PloS one*. [Online] 5 (12), e14433.

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ARV	Anti-Retroviral agent
AUC	Area Under the Curve
ATN	Antiretroviral Toxic Neuropathy
BMI	Body Mass Index
BPNS	Brief Peripheral Neuropathy Screen
BPI	Brief Pain Inventory
cART	combination Anti-Retroviral Therapy
CDT	Cold Detection Threshold
CIDP	Chronic Inflammatory Demyelinating Polyradiculopathy
CPT	Cold Pain Threshold
CRF	Clinical Record File
DAPOS	Depression Anxiety Positive-Outlook Scale
ddC	2',3'-dideoxycytidine (Zalcitabine)
ddI	2',3'-dideoxyinosine (Didanosine)
DDS	Descriptor Differential Scale
DMA	Dynamic Mechanical Allodynia
dNRTI	2',3'-dideoxy moiety containing class of NRTI drug
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
DSP	Distal Sensory Polyneuropathy
d4T	2',3'-didehydro-2',3'-dideoxythymidine (Stavudine)

EFNS	European Federation of Neurological Societies
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HIV-No SN	HIV infection with No HIV-Associated Sensory Neuropathy
HIV-SN	HIV-Associated Sensory Neuropathy
HPT	Heat Pain Threshold
IENFD	Intra-Epidermal Nerve Fibre Density
ISI	Insomnia Severity Index
ITT	Intention-To-Treat
LDL	Low Density Lipoprotein
MDT	Mechanical Detection Threshold
MPT	Mechanical Pain Threshold
MPS	Mechanical Pain Sensitivity
NCS	Nerve Conduction Studies
NICE	National Institute for Health and Care Excellence
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NNT	Number Needed to Treat
NPRS	Numerical Pain Rating Scale
NRES	National Research Ethics Service
NRS	Numerical Rating Scale
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OCT	Optimal Cutting Temperature compound
PASS-20	Short Version of the Pain Anxiety Symptoms Scale

PCS	Pain Catastrophizing Scale
PEP	Post Exposure Prophylaxis
PHS	Paradoxical Heat Sensation
PI	Protease Inhibitor
PP	Per Protocol population
PPT	Pressure Pain Threshold
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QST	Quantitative Sensory Testing
RCT	Randomised Controlled Trial
rhNGF	recombinant human Nerve Growth Factor
ROC	Receiver Operating Characteristic
RR	Relative Risk
SD	Standard Deviation
SF-36	Short Form (36) Health Survey
SN	Sensory Neuropathy
SNE	Structured Neurological Examination
TCSS	Toronto Clinical Neuropathy Score
TNF-α	Tumour Necrosis Factor α
TRG	Triglycerides
TSL	Thermal Sensory Limen
UENS	Utah Early Neuropathy Scale

Chapter 1

Introduction to Painful HIV-Associated Sensory Neuropathy

Introduction to Painful HIV-Associated Sensory Neuropathy

Pain and HIV disease

Pain associated with HIV disease is common and multifactorial (Cox & Rice, 2008) with different causes possible at different stages of the disease. A frequent cause of pain, at all stages of the disease, is neuropathic pain associated with a symmetrical sensory polyneuropathy; HIV associated sensory neuropathies (HIV-SN) attributable to HIV itself and to some HIV treatments (Verma, 2001).

HIV-SN is common. Prior to the availability of antiretroviral drugs, estimates of HIV-SN prevalence varied from <2% if those with very early HIV disease (R. Barohn et al., 1993) to 35% among hospitalized patients with AIDS (So, Holtzman, Abrams, & Olney, 1988). When ambulatory HIV patients (all stages of disease) were surveyed, around 13% were found to have HIV-SN (Woolley, Faragher, Ugoni, & Spelman, 1993). The prevalence of HIV-SN attributable to the virus itself increases with advancing HIV disease (Childs et al., 1999).

The introduction of combination antiretroviral therapy (cART) in the mid 1990s has dramatically reduced the morbidity and mortality associated with HIV among patients who have access to treatment (Mocroft et al., 2003). Life expectancy with HIV in well-resourced countries is now estimated to be up to two-thirds of the general population (Collaboration., 2008; Lohse et al., 2007). While the incidence of most neurological complications of HIV has fallen with the introduction of effective therapy, rates of HIV-SN have been rising since the first effective antiretroviral drugs were developed (Bacellar et al., 1994). It is now clear that some HIV treatments, notably particular nucleoside reverse transcriptase inhibitors (NRTI) – stavudine (d4T), didanosine (ddI) and zalcitabine (ddC) – are potentially neurotoxic (Keswani, Pardo, Cherry, Hoke, & Mcarthur, 2002; Moyle & Sadler, 1998). Recent estimates of HIV-SN prevalence among cohorts with access to cART range from 20% to >50% (Konchalard, K. Wangphonpattanasiri, 2007; Wright et al., 2008). Importantly, the available evidence suggests that HIV-SN prevalence remains high among cART-treated patients, even in countries where known neurotoxic antiretroviral drugs such as stavudine are no longer commonly used (Smyth et al., 2007). In less well-resourced centres, use of stavudine, an inexpensive and effective antiretroviral, in first-line HIV

treatment remains common despite high rates of toxicity (Joint United Nations Programme on HIV/AIDS, 2013).

Current estimates of global HIV prevalence stand at 33 million, with 2.5 million new infections each year and more patients gaining access to cART (Joint United Nations Programme on HIV/AIDS, 2013). With high rates of HIV-SN now reported globally, and up to 90% of affected patients experiencing potentially debilitating neuropathic pain, HIV-SN represent a large, and potentially worsening, source of world morbidity. Therefore, there is an urgent need to understand better the pathogenesis of HIV-SN, identify risk factors, develop effective preventative strategies and improve symptom control among existing sufferers.

Clinical Presentation of HIV-SN

The clinical presentation of HIV-SN is similar regardless of whether the syndrome is caused by HIV itself (often termed Distal Sensory Polyneuropathy – DSP) or by toxicity from antiretroviral drugs (Antiretroviral Toxic Neuropathy – ATN). In many cases, DSP and ATN may co-exist and indeed there may be a level of interdependence in their aetiologies.

Patients with HIV-SN typically present with the classical distal bilateral sensory symptoms of an axonal length dependant polyneuropathy, in a "sock and glove" distribution with the feet being first affected. Symptom onset is variable; with some data suggesting ATN may be more likely to be sudden in onset and rapidly progressive, and DSP more insidious (D. Cornblath & McArthur, 1988; Justin C McArthur, Brew, & Nath, 2005).

Quantitative sensory testing of patients with HIV-SN reveals a loss of thermo-sensation characteristic of a loss of C- fibres (Bouhassira, Attal, Willer, & Brasseur, 1999; Martin, Solders, Sönnerborg, & Hansson, 2003). This "die back" pattern of degeneration of C fibres has been confirmed by analysis of epidermal innervation in skin biopsies. Profound motor weakness is a relatively infrequent feature of HIV-SN, and if present, is usually a late finding restricted to the intrinsic muscles of the feet (D. Cornblath & McArthur, 1988). Indeed, its presence should prompt a search for an alternative diagnosis such as Chronic Inflammatory Demyelinating Polyradiculopathy (CIDP).

Risk Factors For HIV-SN

Risk factors for HIV-SN in patients not exposed to antiretroviral drug

Studies examining rates and risk factors for HIV-SN among AVR–naive patients typically found that the prevalence of HIV-SN increases with disease progression (see Table 1.1). Precise associations with HIV-SN vary among the available studies, but have included lower CD4 T-cell count, higher viral load or surrogate markers of disease progression, such as *Mycobacterium avium* infection.

Risk factors for SN among patients exposed to antiretroviral drugs

The role of nucleoside reverse transcriptase inhibitors (NRTI)

Despite the association between HIV-SN and advanced HIV disease in patients not receiving antiretroviral therapy, the introduction of effective HIV treatment has not been associated with the predicted fall in HIV-SN prevalence. Indeed, where data are available before and after the introduction of cART, the prevalence of HIV-SN has risen, probably due ATN (Bacellar et al., 1994; Smyth et al., 2007).

The potential neurotoxicity of particular NRTI and the development ATN was observed in early clinical trials, where SN was a dose limiting toxicity for the NRTIs d4T, ddI and ddC (Moyle & Sadler, 1998; Yarchoan et al., 1988). The profound neurotoxicity of d4T, the dNRTI most widely used today, was highlighted in a study of Post Exposure Prophylaxis (PEP) where healthy, HIV uninfected men were offered 28 days of antiretroviral therapy as prophylaxis following a possible HIV exposure. Six percent of HIV negative adults developed symptoms consistent with neuropathy within 28 days of d4T-containing PEP (Winston, McAllister, Amin, Cooper, & Carr, 2005). In HIV infected individuals on long-term cART, ATN has been observed to occur as long as one year after commencement of therapy including a dNRTI (Lichtenstein et al., 2005). In the clinical situation, the temporal association between dNRTI exposure and the onset of neuropathy symptoms is typically the sole feature that suggests a diagnosis of ATN rather than DSP.

cART-Era risk factors for HIV-SN

Whilst exposure to dNRTIs, high viral load and low CD4 counts have been previously established in the pre-cART era as risk factors (see Table 1.1), in the cART era this has become less clear (see Table 2.2).

One study has suggested that the use of dNRTI may be *protective* against SN after a year of use (Lichtenstein et al., 2005). This paradox might be explained by the observation that individuals susceptible to ATN will often become symptomatic within a few months of dNRTI exposure. Thus, ATN susceptible individuals may be switched to alternative ARV when they develop neuropathy symptoms, leaving only those who are at intrinsically low risk for ATN on dNRTI therapy after a year.

The neurotoxic potential of dNRTI is clear but in recent years there has been a growing interest in *other* factors that may place HIV patients at risk for neuropathy. This is important for at least two reasons:

1. Although ddC, the most profoundly neurotoxic NRTI (A. Berger et al., 1993), is not longer in clinical use, d4T remains a common component of first-line HIV treatment in many low and middle-income countries, largely for economic reasons. Understanding which patients are at highest risk for ATN if treated with d4T would allow rational prioritization of the highest risk individuals for access to more expensive alternatives.

2. As set out above, rates of HIV-SN remain high even in high-income country centres where use of dNRTI has largely ceased. More work is needed to understand why particular patients are developing HIV-SN despite effective HIV treatment with cART not known to be neurotoxic so that preventative strategies and pathogenesis based therapies can be developed (Smyth et al., 2007).

Increasing age is associated with HIV-SN risk in almost every published cohort, both before and after the introduction of cART (see Tables 1.1-1.3). This is consistent with the known vulnerability of the ageing peripheral nervous system to most types of polyneuropathy, including diabetic and toxin-induced neuropathies.

Height is an HIV-SN risk factors (Affandi et al., 2008; C L Cherry et al., 2006, 2009; Morgello et al., 2004; Pettersen et al., 2006; Antonia L Wadley, Cherry, Price, & Kamerman, 2011; Watters et al., 2004) as it is for other polyneuropathies (Cheng et al., 2006). Rates of HIV-SN were associated with age and height in Australian, Indonesian and Malaysian patients prescribed stavudine. Using cut off values of 170cm and 40 years, the authors showed that the rate of stavudine-associated

neuropathy was 20% in younger, shorter patients compared with 66% of older, taller patients (C L Cherry et al., 2009). It was suggested that these simple criteria could be used to determine who should be given priority access to less neurotoxic but more expensive ARVs in resource poor settings.

Demographic and clinical features, other than age, in untreated HIV patients have not been consistently associated with SN risk among patients on cART (Table 1.2). For example, neither viral load nor CD4 T-cell counts are independently associated with SN risk among treated patients (Morgello et al., 2004; Smyth et al., 2007). In contrast, evidence of advanced HIV infection *prior* to initiation of therapy may still be relevant, with nadir CD4 count and a history of an AIDS defining illness associating with SN risks in some cohorts. As more studies are performed in a range of settings, new risk factors for HIV-SN are also emerging. For example, race may be an important determinant of the individual's HIV-SN risk (Jessica Robinson-Papp, Gonzalez-Duarte, Simpson, Rivera-Mindt, & Morgello, 2009).

Host genotype has been associated with SN risk among dNRTI-exposed patients by several groups. Consistent with putative pathogenic mechanisms of HIV-SN, associations include mitochondrial haplogroups (Hulgan et al., 2005) and genes associated with inflammation (Affandi et al., 2008; Catherine L Cherry et al., 2008). Associations with haemochromatosis gene polymorphisms are also reported (Kallianpur et al., 2006).

Emerging treatment associations with HIV-SN in the era of cART

Despite a reduction in the use of dNRTI in well-resourced settings, the available data suggest that the expected decline in HIV-SN prevalence has not occurred. For example, repeated cross sectional surveys of an ambulatory Australian HIV population showed that SN prevalence rose from 14% in 1993 (pre-cART) to 44% in 2001 (era of frequent use of dNRTI). Despite a change to mostly dNRTI sparing cART regimens, and a reduction in the proportion of patients who had ever been exposed to dNRTI, the prevalence of HIV-SN remained unchanged at 42% in 2006 (Smyth et al., 2007). This and other recent studies suggest that risk factors *other* than dNRTI exposure are important. Exposure to certain protease inhibitors, especially indinavir, is associated with SN risk (Lichtenstein et al., 2005; Pettersen et al., 2006; Smyth et al., 2007). This is supported by *in vitro* studies using cultured rodent dorsal

root ganglia DRGs from rats expressing the human chemokine receptors required for HIV cell entry demonstrate indinavir-associated neurotoxicity from indinavir exposure (Pettersen et al., 2006). Whether this is a class effect of PI or is isolated to indinavir has yet to be fully explored.

Putative mechanisms of pathogenesis in HIV-SN development

The exact mechanism/s by which HIV-SN develops continues to be subject of debate. It also remains unclear whether or not DSP and ATN may share some degree of interaction and/or synergy in their pathophysiology (Bhangoo, Ripsch, Buchanan, Miller, & White, 2009; Wallace, Blackbeard, Pheby, et al., 2007).

Proposed pathogenesis of DSP

Direct infection of neurons by HIV rarely, if ever, occurs; consequently hypotheses of HIV-SN have focused on neuronal toxicity of HIV secreted proteins and the immune dysregulation seen in HIV disease.

The HIV viral envelope glycoprotein gp120 is required to interact with both CD4 receptors and with the chemokine receptors CXCR4 or CCR5 in order to enter immune cells and replicate. Gp120 shed from HIV virions is thought to interact with neuronally expressed CCR5 and/or CXCR4 to induced the neuronal apoptosis which is characteristic of HIV dementia (Kaul, Garden, & Lipton, 2001; Kaul & Lipton, 1999; Kirsh et al., 1990). There is now a reasonable body of evidence to suggest that a similar pathological mechanism exists for HIV-SN (Bhangoo et al., 2009; Maratou et al., 2009; Melli, Keswani, Fischer, Chen, & Höke, 2006; Oh et al., 2001). GP120 induces a TNF-α mediated apoptosis and axonal degeneration in sensory neurones both directly and indirectly via the stimulation of Schwann cells and macrophages that results in the aberrant release of neurotoxins (Kaul & Lipton, 1999; Keswani et al., 2003; Melli et al., 2006). Chemokines have been shown to play a key role in the pathophysiology of neuropathic pain in the context of multiple diseases (White, Jung, & Miller, 2007). TNF-α 1031*2 was independently associated with an increased risk of HIV-SN in both, a population of predominately European descent and an Indonesian population (Affandi et al., 2008). In contrast a cytokine polymorphism of the proinflammatory IL-12β cytokine was associated with increased risk of developing HIV-SN but this was not replicated in an Indonesian population. A recent large epidemiological study in a South African population has further established the

role of inflammatory response and HIV-SN development by identifying the importance of a IL-4 inflammatory cytokine polymorphism (IL-4 590*T) as a risk factor for the development of HIV-SN (A L Wadley et al., 2013).

Proposed pathogenesis of ATN

dNRTIs have been shown to be neurotoxic to sensory neurones and result in features reflecting neuropathic pain in animal models (Bhangoo et al., 2009; Chen & Levine, 2007; Joseph, Chen, Khasar, & Levine, 2004; Joseph & Levine, 2004, 2006; Wallace, Blackbeard, Segerdahl, et al., 2007a): both chemokine-induced and mitochondrial toxicity have been implicated in this pathology. There is also some evidence of indinavir mediated neurotoxicity of sensory neurones (Pettersen et al., 2006). Recently established animal models of both stavudine (Wenlong Huang et al., 2013) and indinavir mediated neurotoxicity (W Huang, Pheby, & Rice, 2013) will be important in further elucidating the pathogenesis of ARV mediated neurotoxicity.

Clinical assessment of HIV-SN

HIV-SN is primarily a clinical diagnosis and the general principles of assessing any patient with peripheral neuropathic pain should be followed (Cruccu et al., 2010; Haanpää et al., 2009). Use of diagnostic tests to investigate primary sensory neurone morphology or function (such as quantification of epidermal nerve fibre density, thermal threshold testing or nerve conduction studies) may be helpful (Bouhassira et al., 1999; Griffin, McArthur, & Polydefkis, 2001; Lauria et al., 2005, 2010; Martin et al., 2003; Rolke et al., 2006), but such testing is not always available outside specialist centres. Importantly, normal nerve conduction studies do not necessarily exclude the diagnosis of HIV-SN, as they primarily survey the function of large diameter afferent nerve fibres. Such testing is most often indicated when atypical features (such as motor involvement) necessitate the exclusion of alternative diagnoses, such as a demyelinating polyneuropathy (Keswani et al., 2002).

Clinical diagnosis of HIV-SN

HIV-SN is typically a small fibre, sensory neuropathy. Common symptoms are often accompanied by neuropathic signs such as reduced or absent ankle reflexes, diminished vibration sense and altered sensitivity to light tactile or thermal stimuli. In a patient with HIV infection, a diagnosis of HIV-SN is supported by symptoms and signs that are largely symmetrical and distal in nature, and the absence of significant

motor involvement. Investigating a temporal association between symptom onset and exposure to potentially neurotoxic ARV is the only method available to assess whether ATN is perhaps contributing to a case of HIV-SN (Keswani et al., 2002).

Screening tools for the diagnosis of HIV-SN in research context have been described, including the widely used AIDS Clinical Trials Group Brief Peripheral Neuropathy Screen (BPNS). This tool is based on a directed symptom questionnaire and limited clinical examination. It takes only minutes to administer and requires no specialized equipment. Importantly, this tool has been validated against objective measures including both thermal threshold testing and epidermal nerve fibre density (Catherine L Cherry, Wesselingh, Lal, & McArthur, 2005). However, while these features make the BPNS a useful research tool (for example, in studies examining risk factors associated with HIV-SN) it cannot replace a careful clinical assessment of the HIV infected patient presenting with neuropathic symptoms or signs.

A critical part of the clinical assessment of HIV-SN is to exclude *alternative* diagnoses that may explain the patient's symptoms – looking particularly for reversible causes of peripheral neuropathy. This includes a careful drug history (including ARV exposures, use of isoniazid or cytotoxic chemotherapy, use of large doses of vitamins that may be neurotoxic, such as pyridoxine (Gdynia et al., 2008), and alcohol/recreational drug use) and the exclusion of metabolic disturbances such as diabetes, thyroid dysfunction or vitamin B_{12} deficiency. Treatment of any other contributing condition (or removal of a contributing toxin) is important, including in patients where HIV-SN is thought to be present. Exclusion of non-neuropathy causes of pain such as arthropathy or plantar fascitis is also important.

Clinical management of HIV-SN

The clinical management of HIV-SN is primarily directed towards minimising the progression of pathology and managing the patient's symptoms.

Remove the underlying cause

When HIV-SN develops in a patient naive to antiretroviral therapy, symptomatic improvement may be observed with commencement of (non-neurotoxic) cART to control HIV replication. However, this situation is uncommon today in well-

resourced settings, with DSP being a disease of advanced HIV and earlier commencement of cART recommended in current treatment guidelines.

When HIV-SN develops in a patient who is on treatment with known neurotoxic ARV (again, an uncommon situation in well-resourced settings today), a change to non-neurotoxic agents may be required. However, this is a complex decision that only those with expertise in the management of HIV disease should make. In early clinical trials, immediate cessation of dNRTI therapy upon development of neuropathic symptoms was associated with symptomatic improvement in around two thirds of cases (Blum et al., 1996; Moore, Wong, Keruly, & McArthur, 2000; Pettersen et al., 2006). Importantly, an initial worsening of symptoms, or "coasting", commonly occurs prior to any improvement and may continue for months (Moyle & Sadler, 1998). Following this, some improvement in neuropathic symptoms may be seen up to a year after discontinuation of the causative agent. Beyond this time, further improvement is unlikely.

As above, minimizing (or removing) any other potential peripheral nerve insults (e.g. poorly controlled diabetes, vitamin B_{12} deficiency) is important to prevent any additive or synergistic effect this might have on the continued progression of HIV-SN.

Provision of analgesia

The pharmacological management of pain associated with HIV-SN is an area of therapeutic need with few therapies being supported by robust evidence. Until the systematic review presented in Chapter 2 was prepared, there were no systematic reviews or meta-analysis of evidence for the efficacy of pharmacological agents in treating pain in HIV-SN.

Summary and conclusions

Sensory neuropathy is a frequent complication of HIV and some HIV treatments. With an estimated 33 million people living with HIV, more people gaining access to antiretroviral therapy every day, and HIV-SN prevalence ranging from 20% to >50% in cART treated cohorts, the burden of HIV-SN pain is a problem of enormous global importance. No routinely available therapy has been unequivocally shown to be effective for treating HIV-SN pain. There is an urgent need to understand better the

pathogenesis of HIV-SN, develop strategies to prevent new cases of this debilitating condition and find effective treatments to control the symptoms associated with existing cases of HIV-SN.

Table 1.1. Summary of clinical studies examining associated factors and the development of HIV-SN pre-cART introduction.

Study	Studied years	Population	Type	*n*	Prevalence of HIV-SN	Observed Associations with HIV-SN	Comment
No ARV exposure							
Barohn *et al.,* 1993	1985-89	US Air force recruits. Mostly asymptomatic of HIV disease.		798	12 (1.5%)	Advanced HIV disease: reduced CD4 count <300 cells/mm^3	
So *et al.,* 1988	1987	Advanced HIV disease. Hospital in-patients USA.	Cross sectional	37	13 (35%)	Advanced HIV disease as indicated by: >5 months systemic illness	
Woolley *et al.,* 1997	1993	Ambulatory patients at a tertiary referral centre Australia	Cross sectional	94	13 (14%)	Advanced HIV disease as indicated by: *Mycobacterium avium* complex infection	
ARV exposure but pre-cART							
Childs *et al.,* 1999	1985-95	Multicentre AIDS Cohort Study (MACS) USA.	Longitudinal	1604	213 (13%)	Advanced HIV disease: CD4<200 cells/mm^3 and HIV RNA levels >3000 copies/ml	
Bacellar *et al.,* 1994	1988-92	Multicentre AIDS Cohort Study (MACS) US	Longitudinal	1992	138 (7%) cases over 5 yr period	Advanced HIV disease: CD4 <200 cells/mm^3 ddI, ddC and d4T use.	Risk of HIV-SN associated with dNRTIs not statistically significant.
Tagliati *et al.,* 1999	1990-94	Primary care referred USA – dNRTI exposure excluded	Longitudinal	166	63 (38%)	Advancing age CD4 <100 cells/mm^3 Reduced haemoglobin, albumin, and weight.	Reduced Hb, albumin and weight were most probably surrogate indicators of advanced HIV disease.
Schifitto *et al.,* 2002	1994-96	Dana cohort: HIV patients with early symptoms of HIV dementia (HIVD)	Longitudinal	272	93(35%) symptomatic	Advanced HIV disease: AIDS diagnosis, low CD4 count	

Table 1.2. Summary of clinical studies examining associated factors and the development of HIV-SN following introduction of cART (2004 to 2009).

Study	Studied years	Population	*Type*	*n*	Prevalence of HIV-SN	Observed Associations with HIV-SN	Comment
cART era							
Morgello *et al.,* 2004	1999-2002	Manhattan HIV Brain Bank – US advanced HIV disease	Longitudinal	187	99 (53%)	Advancing age Male sex	No association with: viral load, CD4 count, or dNRTI exposure.
Lichtenstein *et al.,* 2005	1992-2003	HIV Outpatients Study (HOPS) Multiple clinics US ambulatory patients	Longitudinal	2525	329 (13.5%)	Advancing age >40yrs Peak viral load Nadir CD4 count diabetes mellitus white race In 1st year of exposure: didanosine, stavudine, naviripine efavirenz, and protease inhibitors:(lamivudine, ritonavir, nelfinavir, saquinavir)	Following 1 yr exposure all ARVs associated with *reduced* risk HIV-SN.
Pettersen *et al.,* 2006	1998-2004	Ambulatory patients Canada. Neurological disease at entry (including HIV-SN)	Longitudinal	221	101 (46%)	Advancing age CD4 Peak Viral load Alcohol consumption dNRTI exposure (d4T, ddC and ddI) Protease Inhibitor (PI) -Indinavir	
Morgello *et al.,* 2004	1999-2002	Manhattan HIV Brain Bank – US advanced HIV disease		187	99 (53%)	Age Male sex	No association with: viral load, CD4 count, or dNRTI exposure.
Cherry *et al.,* 2006	2000-04	2 ambulatory cohorts: US and AUS	Cross sectional	147	77(52%)	Advancing age >40yrs ddI, d4T,	Failed to show HCV, lactate or viral load to be associated.
Simpson *et al.,* 2006	2000-02	AIDS Clinical Trial Group (ACTG): patients with advanced HIV with exposure to ARV.	Longitudinal	101	53 (52%)	Progression associated with: white race	No association of progression with: dNRTI exposure viral load, and CD4 count.
Schifitto *et al.,* 2002	1994-96	Dana cohort: HIV patients with early symptoms of HIV dementia (HIVD)	Longitudinal	272	93(35%) symptomatic	Advanced HIV disease: AIDS diagnosis, low CD4 count	
Schifitto *et al.,* 2005		NEAD cohort (North East AIDS Dementia) -patients with advanced HIV disease and/or evidence of HIV dementia (HIVD)	Longitudinal	376	141 (38%)	dNRTIs associated with *reduced* risk HIV-SN	No association with: Viral load CD4 count
Smyth *et al.,* 2007	2006	Ambulatory patients: Australia	Cross sectional	100	42 (42%)	Advancing age d4T indinavir	No association with: Other dNRTIs CD4 counts Viral load
Cherry *et al.,* 2009	2006	Ambulatory patients: Australia, Indonesia and Malaysia.	Cross sectional	294	94 (32%)	Advancing age Increased height d4T, ddC, indinavir	No association with: Viral load, AIDS diagnosis, HCV isoniazide

Table 1.3. Summary of clinical studies examining associated factors and the development of HIV-SN following introduction of cART (2009 to 2011)

Study	Population	*Type*	*n*	Prevalence of HIV-SN	Observed Associations with HIV-SN
Beadles *et al.,* 2009	Malawi stavudine cART treated group	Retrospective chart review	3341	35%	All had stavudine. No other associations tested.
Saktor *et al.,* 2009	Uganda stavudine cART treated group	Prospective cohort	102	38%	All had stavudine. No associations found.
Maritz *et al.,* 2010	South Africa community based HIV clinic	Cross-sectional	598	30%	Older age cART use TB treatment
Ellis *et al.,* 2010	USA ambulatory HIV patients (CHARTER)	Cross-sectional	1539	57%	Older age, cART use, lower CD4 nadir, past dNRTI exposure.
Evans *et al.,* 2011	USA patients initiating cART (ACTG trials)	Longitudinal cohort, Annual screening	2141	8.6%	Older age, diabetes, past dNRTI exposure, use of PIs
Wadley *et al.,* 2011	South Africa hospital based HIV clinic	Cross-sectional	395	57%	Older age, use of cART, stavudine, TB treatment, height
Banerjee *et al.,* 2011	USA Ambulatory HIV population	Cross-sectional	436	27%	Older age, lower CD4 nadir, elevated triglycerides, type II diabetes mellitus

Aims of the thesis

Many of the important risk factors for the development of HIV-SN identified in the pre-cART era were related to poor HIV virus suppression (nadir CD4 counts, high viral loads and AIDS) and its treatment with earlier neurotoxic ARV agents (e.g. 'd' NRTI agents). It was therefore anticipated, with good reason that the prevalence of HIV-SN would fall significantly with the introduction of effective HIV management with cART in the mid-1990s. However despite both the improved HIV virus control and crucially the phasing out of neurotoxic dNRTI drugs, especially in well-resourced countries, the prevalence of HIV-SN in both high and low resource HIV populations has remained consistently at around 40%. This suggests strongly that drug induced neurotoxicity may not be a major aetiological factor for HIV-SN in the cART-era (Catherine Louise Cherry, Wadley, & Kamerman, 2012; Ellis et al., 2010; Smyth et al., 2007).

Current estimates of global HIV prevalence stand at 33 million, with 2.5 million new infections each year and more patients gaining access to cART (Joint United Nations Programme on HIV/AIDS, 2013). With high rates of HIV-SN now reported globally, and with between 40 to 90% of affected patients experiencing potentially debilitating neuropathic pain, HIV-SN represent a large, and potentially worsening, source of world morbidity.

There is an urgent need to better understand the pathogenesis of HIV-SN, identify risk factors, develop effective preventative strategies and improve symptom control among existing sufferers. In Chapter 2 this thesis will explore the current evidence base for the treatment of pain in HIV-SN by means of a systematic review and meta-analysis of available randomised controlled trials of pharmacological agents used in the treatment of painful HIV-SN. This work is important, as it will aid decision making by clinicians treating painful HIV-SN and will potentially direct future clinical efficacy trials by identifying any paucity in the literature that might need to be addressed.

Chapter 3 of this thesis will explore in great detail the nature of the phenotype associated with HIV-SN, with a view to gain a better understanding of the relationships between pain symptomatology, sensory nerve dysfunction, intra-epidermal nerve fibre density, health related quality of life, psychological co-

morbidity and sleep disturbance. In addition to collecting detailed medical and drug histories, this phenotyping study will employ a battery of techniques:

- Quantitative Sensory Testing (QST) using the established DFNS protocol.
- Patient self-reporting instruments to exam the characteristics and existence of psychological, health-related quality of life and sleep disturbance co-morbidity.
- Intra-epidermal sensory nerve fibre densities (IENFD) through the taking of small skin biopsies.

This study is the first time a single cohort of HIV infected individuals have been extensively phenotyped in this manner. Whilst there have been a number of small detailed QST studies of HIV-SN in the pre-cART era (Bouhassira et al., 1999; Martin et al., 2003), this study will be important as it will explore if the phenotype of HIV-SN has changed in parallel with the change in risk factors that has occurred in the transition from pre-cART to cART eras. The potential identification of phenotypically distinct sub-groups, particularly with QST measures, is important as there has been some limited evidence to suggest that such sub-groups might have a differential response to analgesic drug therapy (D M Simpson, Scott, & Freeman, 2010).

Useful comparisons between different QST studies have been limited by the lack of an established common QST protocol, however by using the DFNS QST protocol the work that results from this study will be comparable to other neuropathic pain states aiding both the study of HIV-SN and neuropathic pain *per se*.

There is also a need for easy to use, robust screening tools for the identification of HIV-SN that can be translated for use into resource poor settings. Part of the phenotyping study examined the efficacy of a number of neuropathy screening tools (and their constituent components), some of which have not been previously validated in the context of HIV-SN. This study will hopefully form the basis for future work directed at producing a composite screening tool for use in resource poor settings.

As part of this study it is hoped that a blood and tissue bank for highly phenotyped individuals with HIV-SN would be established, which would inform future work exploring genetic risk factors for the development of HIV-SN and painful HIV-SN.

Chapter 2

Pharmacological treatment of painful HIV-associated sensory neuropathy

Pharmacological treatment of painful HIV-associated sensory neuropathy: a systematic review and meta-analysis of randomised controlled trials.

Introduction

Evidence-based guidelines for the pharmacological management of neuropathic pain tend to focus on a "blanket" approach of recommending therapies across the spectrum of neuropathic pain, irrespective of the underlying condition (Finnerup, Sindrup, & Jensen, 2010; RH, Dworkin et al., 2007). NICE guidance for the management of neuropathic pain in "non-specialist settings" have adopted this approach (Tan, Barry, Reken, Baker, & Guideline Development Group, 2010). This may not be appropriate for HIV-SN for three main reasons. Firstly, neuropathic pain is a heterogeneous phenomenon, both within and across underlying conditions, and evidence obtained from the study of an analgesic in one condition cannot necessarily be applied to another (Baron, Tölle, Gockel, Brosz, & Freynhagen, 2009; Scholz et al., 2009). Secondly, in high, middle and low income countries the pain associated with HIV-SN will usually be managed outside of specialist pain management clinics, so appropriate, disease specific guidance may be required. Finally, there are a number of randomised controlled trials (RCTs) conducted in HIV-SN, which were not identified in the NICE guidance. Therefore, I have conducted a systematic review and meta-analysis to elucidate the evidence base for pharmacological management of neuropathic pain in HIV-SN.

Methods

Eligibility, data sources and search strategy

PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses)

Systematic reviews and meta-analysis are important tools for the summary of evidence accurately and reliably. They help to keep clinicians up to date, gather together and summarise related research for patients and carers; provide a resource for the development of clinical practice guidelines; provide summaries of previous research to identify paucity of evidence for both researchers and funders wishing to support new research. However, prior to the development of QUOROM, and subsequently PRISMA, there had been much criticism of the reporting of systematic reviews and meta-analysis; in particular poor literature searches, poor evaluation of

the quality of RCTs and the poor synthesis of results (Mulrow, 1987; Sacks, Berrier, Reitman, Ancona-Berk, & Chalmers, 1987).

The QUOROM (QUality Of Reporting Of Meta-analyses) Statement, published in 1999 (Moher et al., 1999), was conceived as a guidance for authors reporting meta-analysis of randomized trials. The QUOROM statement was developed by an international group of thirty clinical epidemiologists, clinicians, statisticians, editors and experienced researchers. The original QUOROM statement was subsequently updated in 2009 to address conceptual advances in outcome level assessment (detailed evaluation of the reliability and validity of the data for each important outcome by determining the methods used to assess them in each individual study), and risk of bias relevant to systematic review and meta-analysis. This updated statement PRISMA (*Preferred Reporting Items for Systematic reviews and Meta-Analyses*) was published in 2009 and comprises of a 27 item check-list and a flow-diagram pertaining to the content of a systematic review and meta-analysis, which includes the title, abstract, methods, results, discussion and funding. The flow diagram is four-phased and depicts the flow of information through the different phases of a systematic review and helps to display information about the number of records identified in the literature searches, the number of studies included and excluded, and the reasons for exclusions in a clear manner.

The PRISMA statement is not a quality assessment instrument for medical intervention RCTs.

In accordance with PRISMA (Liberati et al., 2009), I sought to identify RCTs that included patients with painful HIV-SN and reported at least one clinically relevant pain outcome measure.

A systematic search, without language restrictions, was conducted on 20 June 2008, and a follow-up search on 22 February 2010, with the following databases: Medline (from 1966 to date searched), The Cochrane central register of controlled trials (Cochrane Library 2010, Issue 2), www.clinicaltrials.gov (a US registry of clinical trials) and www.controlled-trials.com (a meta-registry of controlled trials). By searching registries of clinical trials it was hoped to reduce the risk of missing 'negative' results from unpublished trials, as this is a potential source of publication-bias in systematic review and meta-analysis reporting.

Search terms

Search and Boolean logic terms used were: {"HIV" or "AIDS"} and {"pain" or "painful"} and {"neuropathy" or "neuropathic"}, and {"treatment" or "trial" or "random" or "randomised" or "double-blinded"}. Further trials were identified by hand searching the reference lists of identified trials and review articles, relevant NICE guidelines and Health Technology Assessment reports.

Study selection and risk of bias assessment

I excluded animal studies, reviews, letters, abstract-only trials, open-label trials, and trials that were not randomised. The identified RCTs then underwent independent quality assessment by me and another three reviewers (C. L. Cherry, S. Cox and A. S. C. Rice) using the 7-point modified "Jadad" scoring system.

The modified Jadad scale

The Jadad assessment scale, also known as the Oxford quality scoring system, was developed as a method to systematically assess the quality of medical intervention RCTs (Jadad et al., 1996). The Jadad scale is a widely used RCT quality instrument in the medical literature (Haynes, 2005).

The Jadad scale assesses three areas of an RCT that have potential to 'bias' its conclusions from a 'true' result: randomisation, blinding and participant withdrawals/ drop-outs (the fate of all participants is known).

The Jadad scale was modified by the supervisor of this thesis (A.S.C.Rice) in collaboration with the original authors of the Jadad scale to include an assessment of adequate powering of an RCT. This scale is called the *modified* Jadad scale (A. Rice, Lever, & Zarnegar, 2008).

The modified Jadad scale asks reviewers to answer the following for each RCT:

1. Was the study described as randomised (this includes the use of words such as randomly, random, and randomisation)?
2. Was the study described as double blind?
3. Was there a description of withdrawals and drop-outs?
4. Was there a description of a power calculation?

A ‘yes’ answer to each of the above questions receives one point. Questions 1, 2 and 4 receive an extra point each if the methods used for each is appropriate. A point is deducted from 1, 2 or 4 if the method is inappropriate or its description is unclear. A maximum score is seven points (Supplemental Document 1: The Modified Jadad Score).

RCTs with a score of less than five points and studies that enrolled fewer than five HIV-SN patients were excluded from the systematic review. Scoring discrepancies between reviewers were resolved through discussion and consensus.

Data extraction

I extracted the data from eligible RCTs. Data extracted included: year of publication; study design and duration; study sample population and characteristics; withdrawals; interventions; doses; pain and non-pain related primary and secondary outcome measures; and adverse events.

Where possible, dichotomous pain improvement outcome data were extracted from RCTs that reported efficacy superior to placebo. Intention to treat (ITT) responder rates for 30% and 50% pain relief were sought for the longest follow-up period reported in each study. If required, authors were contacted for missing or unreported data.

RCTs in which the primary pain outcome of a studied intervention did not show efficacy greater than placebo in the intention to treat population, were not included in a subsequent meta-analysis.

Statistical analysis

For each intervention the extracted dichotomous outcomes were used to calculate numbers needed to treat (NNT) by me and another reviewer (A. S. C. Rice), with 95% confidence intervals for 30% and 50% pain improvement responders.

Meta-analysis

In the context of medical RCTs, meta-analysis are methods that focus on combining results from different studies in order to obtain a quantified synthesis which increases the power of statistical analysis by using pooled results.

There are three main parts to a meta-analysis:

1. Production of a pooled estimate and confidence interval for the treatment effect after combining all the studies.
2. To test if the treatment effects seen are statistically significant or not (i.e. does the treatment effect differ from no effect more than would be expected by chance?)
3. To test heterogeneity of the effects outcome between the included studies (i.e. does the effect vary across the studies more than would be expected by chance?)

To combine the results of more than one study a summary statistic needs to be calculated for each. This determines the treatment effect seen in each individual trail. This treatment effect then needs to be weighted according to the importance of each trial. In the context of RCTs this is most often the size of the trail.

Weighted average

There are two statistical models of meta-analysis:

1. Fixed effect model:

The fixed effect model provides a weighted average of a series of study effects. The model assumes that all included studies investigate the same population, use the same variable and outcome definitions, etc. This assumption is typically unrealistic as RCTs are often prone to several sources of heterogeneity; e.g. treatment effects may differ according to population studies, drug dosage, study conditions, etc .

2. Random effects model:

A common model used to synthesize heterogeneous research is the random effects model of meta-analysis. This is simply the weighted average of the effect sizes of a group of studies. The weight that is applied in this process of weighted averaging with a random effects meta-analysis is achieved in two steps:

Weight = $1/\sqrt{}$ variance of the trial estimate

$1/\sqrt{}$Standard Error (SE) of the trial

This ‘weighting’ is then used to multiple by the effects difference seen in each trial to give the weighted average, which can then be used to pool with other studies.

Heterogeneity

Ideally, those studies whose results are being combined in the meta-analysis should all be conducted in the same way and to the same protocol: study heterogeneity is a term used to indicate that this ideal is not fully met. There are several sources of heterogeneity. Clinical heterogeneity can be the result of different treatment protocols or different study populations being used. Measuring study heterogeneity is important, as it indicates where it might not be valid to pool the results of different studies.

Identifying statistical heterogeneity requires testing the null hypothesis that the different trials all have the same treatment effect in the population. The test looks at the differences between observed treatment effects for the trials and the pooled treatment effect estimate. By using the Chi-squared test with degrees of freedom (df) and a ‘p’ value, heterogeneity can be estimated. When there are few studies, the test is not very good at detecting heterogeneity if it is present. For this reason, a p value of less than 0.10 is often used to indicate heterogeneity rather than the conventional cut point of $p = 0.05$. A useful way to identify heterogeneity without having to use statistical tables to look up p values is to compare the chi-square statistic with its degrees of freedom. If the statistic is bigger than its degrees of freedom then there is evidence of heterogeneity (The Cochrane Collaborators Open learning material).

I originally planned to assess heterogeneity as described above, however, as only three studies were used in the meta-analysis, this was felt to be inappropriate by the supervisor of this thesis, for reasons outlined above. Similarly, a sensitivity analysis was not performed, as there were insufficient data. All calculations were undertaken using Microsoft Excel 2007

Determination of Number Needed to Treat (NNT)

The NNT is a useful way of expressing the results of a medical intervention trial as it indicates the number of patients that would have to be treated in order for one to have benefit. To calculate NNT dichotomous outcome measures need to be available. For

this systematic review/meta-analysis I used the dichotomous outcome measure of > 30% or 50% improvement on a pain VAS for a given treatment and its placebo.

NNT is the inverse of the Absolute Risk Reduction (ARR).

The ARR = Placebo event rate – Treatment event rate.

NNT=1/ARR

Results

I identified 44 potentially relevant articles (Figure 2.1). Twenty-five articles were excluded after screening identified these as being a review article, letter, open-label study, case report or other non-RCT study. The remaining 19 RCTs were retrieved and independently reviewed by me and other three reviewers (C. L. Cherry, S. Cox and A. S. C. Rice). Four articles were excluded at this stage by scoring <5 out of 7-points with the modified Jadad score. A further RCT was excluded as having <5 HIV-SN patients enrolled. Details of these excluded RCTs, and therefore of interventions that must be regarded as not having been adequately tested, are shown in Table 2.1.

The remaining 14 RCTs were retained for further analysis (Table 2.2). Of the 14 trials retained for further analysis, 13 were of a parallel group design and one a cross-over design. All were placebo controlled with one using "active" placebo (David M Simpson, Brown, & Tobias, 2008). Data extraction was for the longest follow-up period reported by the article. In most cases this was to the end of the treatment phase, except for a study of a topical 8% capsaicin (David M Simpson, Brown, et al., 2008) that reported data for 12 weeks after a single treatment application.

In two studies (Paice et al., 2000 and Hahn et al., 2004) no reference to ITT analysis was made. In one of these RCTs studying topical capsaicin 0.075% efficacy (Paice et al., 2000) no primary outcome data were published, as it was reported that no superiority to placebo was seen. In a study of lamotrigine efficacy (D M Simpson et al., 2003) only a per protocol (PP) population data analysis was undertaken. This was reported to show no superiority over placebo; however no primary outcome data were reported.

Of the four trials that reported superiority of an intervention over placebo, three reported dichotomous pain outcome measures. Where possible I used responder rate

data for ≥30% and ≥50% improvement in pain as measured using Visual Analogue Scale (VAS) or Numerical Pain Rating Scale (NPRS). These data were requested from the authors if they had not been reported.

Acetyl –L-carnitine

Whilst acetyl-L-carnitine has been the subject of six articles (Chiechio, Copani, Gereau, & Nicoletti, 2007; Hart et al., 2004; Herzmann, Johnson, & Youle, 2005; Osio et al., 2006; Scarpini, Sacilotto, Baron, Cusini, & Scarlato, 1997; M Youle & Osio, 2007) in the treatment of painful HIV-SN, only one was an RCT (M Youle & Osio, 2007) and eligible for inclusion. This was a parallel group trial of acetyl-L-carnitine (1000mg/day) and placebo intramuscular injections. In this RCT acetyl-L-carnitine, in an analysis of the PP population, showed a modest superiority to placebo. However an analysis of the ITT population did not show superiority to placebo: mean change in VAS (0-10cm)(SD) from baseline to the end of week 2: acetyl-L-carnitine -1.32 (1.84); placebo -0.61 (1.55) p=0.07. Consequently we undertook no further analysis of this trial.

Amitriptyline and Mexilitine

Two trials (Shlay et al., 1998 and Kieburtz et al., 1998) that were included studied the efficacy of amitriptyline. Both trials compared amitriptyline to placebo and another intervention. One RCT (Shlay et al., 1998) examined efficacy of amitriptyline as part of a trial also assessing acupuncture treatment. However despite being described as a parallel group, placebo controlled RCT, its design was complex. Consequently the results of this trial are difficult to evaluate. In particular bias may have been introduced because of unconventional randomisation procedures and because true placebo controls were not used. Specifically, patients were allowed to 'opt-out' of being randomised to the amitriptyline arms of the trial based on personal preference. In addition, many participants included in the analysis of amitriptyline efficacy, had also received acupuncture or sham acupuncture, further complicating analysis. Ignoring the methodological concerns, amitriptyline demonstrated no superiority to placebo in the primary outcome measure. The mean change in Gracely pain scores from baseline to week 14 was -0.26 with amitriptyline (maximum dose 75mg/day) and -0.30 with placebo. The difference between amitriptyline and placebo was: 0.00 95%CI(-0.18 to 0.19) p=0.99.

The second trial (Kieburtz et al., 1998) compared amitriptyline, mexilitine and placebo. This trial was terminated early following an interim review of results. It was deemed by the trial monitoring board that further enrolment into the study was unlikely to detect significant differences in either amitriptyline or mexilitine arms compared to placebo. No superiority was reported in reducing mean Gracely pain scores (SD) from baseline to the end of treatment week 8 for: amitriptyline (maximum dose 100mg/day) -0.31 (0.31); mexilitine -0.23 (0.41); compared to placebo -0.20 (0.30).

Smoked Cannabis

The original literature search found four articles related to cannabinoid use and painful HIV-SN. Only two were RCTs (Abrams et al., 2007; Ellis et al., 2009). The excluded articles included one clinical survey (Woolridge et al., 2005) and one review article (Beaulieu & Ware, 2007).

One of these included articles (Ellis et al., 2009) was a cross-over study that compared the efficacy of smoked cannabis (maximum tolerated dose 1 to 8% Δ-9-tetrahydrocannabinol q.d.s.) to placebo cigarettes in reducing subjects pain measured using the Descriptor Differential Scale (DDS). The DDS is a ratio scale (0 to 20) containing 24 words describing pain intensity and unpleasantness. Smoked cannabis was reported to be superior to placebo in reducing DDS from baseline to end of treatment day five in the PP population. The median difference between cannabis and placebo was -3.3 out of 20; p=0.016. No data were reported for the ITT analysis, however the authors stated that the PP analysis was similar to the ITT analysis with p=0.02. VAS data not reported by the authors, but was supplied on request, relating to cannabis and placebo subjects who reported a ≥30% (18/34 and 7/34 respectively) and ≥50% (13/34 and 4/34 respectively) improvement in pain intensity.

This trial reported a high proportion of inadvertent unblinding amongst subjects following dose titration with smoked cannabis cigarettes in the treatment arms, but not with placebo cigarettes.

A second study (Abrams et al., 2007) compared smoked cannabis (3.56% Δ-9-tetrahydrocannabinol t.d.s.) to placebo cigarettes in a parallel group RCT. Smoked cannabis was shown to be superior to placebo in reducing pain from baseline to end of treatment day 5 in the ITT analysis: cannabis -34% (IQR -71 to -16), placebo -17%

(IQR -29 to 8) p=0.03. More subjects reported ≥30% VAS improvement with smoked cannabis compared to the placebo: 13/27 and 6/27 respectively.

Inclusion into the study required subjects to have had previous exposure to cannabis, with current users asked to discontinue prior to the study. Of note no attempt was made to assess unintentional unblinding during the course of the study, which may have been high due to subjects' previous experience with smoked cannabis.

Using the ITT analysis dichotomous VAS data from both trials, an NNT for smoked cannabis was calculated as 3.38 95%CI (2.19 to 7.50) (Table 2.3)

Topical Capsaicin

Four trials (D. B. Clifford et al., 2012; Paice et al., 2000; David M Simpson, Estanislao, Brown, & Sampson, 2008 and D. Clifford, Simpson, Brown, Moyle, & Brew, 2010) were found that assessed topical capsaicin efficacy in painful HIV-SN. Two reports were excluded from further analysis; one was an open-label study (David M Simpson, Brown, et al., 2008) and the other has been reported in abstract form only (D. Clifford et al., 2010). Of the included trials, one (Paice et al., 2000) examined the efficacy of topical capsaicin 0.075% cream in a parallel group RCT. The authors stated that no superiority of capsaicin 0.075% over placebo in mean improvement in a numeric rating score (NRS) (0-10) was seen, however only graphical data were presented.

A second study (David M Simpson, Brown, et al., 2008) examined topical capsaicin 8%. Patients received either the 8% patch or an active placebo (capsaicin 0.04%) in a single application lasting either 30, 60 or 90 minutes. Following this single application patients were followed-up for 12 weeks. Capsaicin 8% was found to be superior to placebo in the percentage reduction of the NPRS (SD) from baseline to week 2 to 12: 8% capsaicin: -22.8 (30.6); compared to placebo: -10.7 (30.8), (p=0.0026). The study also reported responder rates as percentage of patients measured on the NPRS who experienced ≥30% mean reduction in pain: capsaicin 8%: 76/225; placebo (capsaicin 0.04%): 15/82; p=0.0092. It is not possible to calculate an NNT that is strictly comparable to those calculated for other studies included in this review since the placebo control used here was not pharmacologically inactive. However, as an informative exercise using these data, and presuming that the control

capsaicin 0.04% is a true placebo, an NNT of 6.46 95%CI(3.86-19.69) was calculated for treatment with capsaicin 8% patch.

Gabapentin

Only one retrieved report related to treatment of painful HIV-SN with gabapentin was an RCT. Four additional articles were excluded. Two were letters (Newshan, 1998; Vadivelu & Berger, 1999) one an abstract (Gatti, Antonella, Jann, Stefano, & Sandro, 1998), and one a case series (La Spina, Porazzi, Maggiolo, Bottura, & Suter, 2001). The included study (Hahn et al., 2004) compared gabapentin (titrated to a maximum of 2400mg/day) to placebo in a parallel group RCT. At the longest treatment period assessed, no difference in efficacy was reported between gabapentin and placebo groups for the primary outcome measure, median change in VAS (0-100mm) baseline to end of week 4: gabapentin: - 44.1, placebo: -29.8. No indication of variance or p value was documented.

It is noteworthy that this trial demonstrated an unusual placebo response. The placebo subjects' pain VAS baseline remained unchanged for the first two weeks, after which a stronger placebo response followed to week 4. This unusual placebo response may have contributed to the apparent superiority of gabapentin over placebo at week 2, which was not evident at week 4.

Pregabalin

One large multi-centre RCT (D M Simpson, Schifitto, et al., 2010)[50] examined the efficacy of pregabalin, titrated over 2 weeks to a maximum tolerated dose up to 1200mg/day, in a multicentre, 14 week parallel group, placebo controlled RCT. No superiority of pregabalin over placebo in the primary pain outcome measure was reported: mean change in NPRS baseline to end of week 14: pregabalin -2.88; placebo -2.63, p=0.39.

Lamotrigine

Three trials assessing the efficacy of lamotrigine in painful HIV-SN were identified (Silver, Blum, Grainger, Hammer, & Quessy, 2007; D M Simpson et al., 2000 and D M Simpson et al., 2003). One trial (Silver et al., 2007), enrolled only one painful HIV-SN patient (to the placebo control group) and was therefore excluded from further analysis. The included lamotrigine trials (D M Simpson et al., 2000) and (D M

Simpson et al., 2003) were both conducted by the same group; with (D M Simpson et al., 2000) being smaller and preceding (D M Simpson et al., 2003). The smaller study (D M Simpson et al., 2000) did demonstrate some efficacy superior to placebo when the primary outcome for the PP population was analysed. However in the ITT analysis with 'last value carried forward' (LVCF), lamotrigine was not superior to placebo: improvement in mean Gracely pain score (SE): lamotrigine: – 0.242 (0.009); placebo: -0.183 (0.087); (p=0.65). The large number of dropouts in the lamotrigine group (n= 11 of 20) compared to placebo (n=3 of 22) suggest a narrow therapeutic index and make interpretation of the trial results difficult.

Similarly the larger trial (D M Simpson et al., 2003), where participants were stratified according to previous exposure to neurotoxic ARVs, did not demonstrate a superiority of lamotrigine over placebo for the primary outcome measure (mean improvement in Gracely pain score) in the total cohort or in either stratum. However lamotrigine did show superiority to placebo in the neurotoxic ARV-exposed stratum in a secondary outcome measure, mean improvement in VAS (0-100mm) baseline to end of treatment: lamotrigine: -27.1; compared to placebo: -9.0; p=0.003.

For each stratum the number of responders (≥30% improvement in VAS) were calculated from the published data. For the neurotoxic ARV stratum: lamotrigine 36/62, placebo 7/30 (p=0.02) and for no exposure to neurotoxic ART: lamotrigine 46/88, placebo 21/47. As an informative exercise using these data the NNT for lamotrigine was calculated for each stratum, and for the overall trial. Subjects with exposure to neurotoxic ARVs: 2.88 95%CI(1.84 to 6.57); no exposure to neurotoxic ARVs: 13.17 95%CI(3.96 to -9.95) and for the unstratified population: 6.09 95%CI(3.51 to 23.08)(Not included in Table 2.3 as no superiority of lamotrigine over placebo was demonstrated for any primary endpoint).

Recombinant human Nerve Growth Factor (rhNGF)

One RCT (J C McArthur et al., 2000) examined the efficacy of subcutaneous recombinant human Nerve Growth Factor (rhNGF) in the treatment of painful HIV-SN. This study assessed two doses (0.1 and 0.3μg/kg) given twice weekly compared with placebo for 18 weeks. rhNGF was superior to placebo for the primary outcome measure in the ITT analysis; median change of the Gracely pain score from baseline

to end of week 18: rhNGF 0.1µg/kg: -0.18 (-0.10 to -0.25) p=0.05, 0.3µg/kg: -0.21 (-0.14 to -0.29) p=0.04, and placebo: 0.06 (+0.01 to -0.14)

No significant dose effect was reported and no differential effect was seen based on baseline stratification of subjects according to neurotoxic ARV drug exposure. As rhNGF was reported to be associated with myalgia, there may have been inadvertent breaking of the blinding.

Dichotomous data were requested from the authors however we were unable to calculate RR and NNT values for rhNGF from the data provided.

Prosaptide and Peptide –T

Two trials (Evans et al., 2007; D M Simpson et al., 1996) examined the efficacy of the novel agents in placebo controlled parallel group RCTs. One (Evans et al., 2007) reported the use of subcutaneous prosaptide (maximum dose of 16mg/day) over 6 treatment weeks and did not report efficacy superior to placebo in the primary outcome measure; mean change in Gracely pain score baseline to week 6. The study was terminated after a planned interim futility analysis. Another trial (D M Simpson et al., 1996) studied efficacy of intranasal peptide T (maximum dose 6mg/day), over 12 treatment weeks, but reported no superiority over placebo in the primary outcome measure; mean change in a modified Gracely pain score baseline to end of week 12.

Discussion

This systematic review found that RCT evidence of analgesic efficacy superior to placebo in the context of HIV-SN pain exists only for smoked cannabis, rhNGF and high dose (8%) topical capsaicin. Several other agents have been examined in high quality RCTs and found to be no more effective than placebo for managing HIV-SN pain in the doses used, specifically acetyl-L carnitine (1g/day), amitriptyline (100mg/day), topical capsaicin 0.075%, gabapentin (2.4g/day), mexilitine (600mg/day), peptide –T (6mg/day), pregabalin (1200mg/day), lamotrigine (600mg/day) and prosaptide (16mg/day). Therefore, there is evidence that both of the first line therapies (pregabalin and amitriptyline) recommended in the NICE guidance for non-specialist management of neuropathic pain show no superiority to placebo in the management of pain in HIV-SN (Tan et al., 2010).

Of the pharmacological interventions shown to be effective for HIV-SN in RCTs, only topical capsaicin 8% is currently approved for marketing for neuropathic pain indications. In Europe 8% capsaicin has been approved for the treatment of peripheral neuropathic pain in non-diabetic adults, whilst the U.S. Food and Drug Administration (FDA) has approved its use only for the indication of post herpetic neuralgia. However, it should also be borne in mind that we located a preliminary report (conference abstract only and therefore excluded from the systematic review) of another parallel group RCT which included 494 patients with HIV-SN in which topical 8% capsaicin was compared to 0.04% topical capsaicin (D. Clifford et al., 2010). No analgesic superiority of 8% capsaicin over 0.04% was demonstrated. rhNGF therapy is not currently clinically available and both legal and mental health issues preclude routine recommendation of long term smoked cannabis for pain management (A. S. C. Rice, 2008).

This systematic review represents a comprehensive review of the literature relating to the pharmacological management of painful HIV-SN. It used a predefined protocol for the initial literature search, data extraction and analysis. There was also strict adherence to inclusion quality criteria as assessed by four independent reviewers using the modified Jadad score, a tool that assesses each study for potential bias as well as evaluating study power.

This systematic review was limited by the paucity of high quality RCTs examining pharmacological treatment of painful HIV-SN. Additionally the heterogeneity of the included studies design and size made evaluation and comparison of trials difficult. In particular, use of the Gracely pain scale (GPS) in five of the 14 included RCTs made evaluation and inter-study comparison complicated. The GPS is a log unit pain outcome measure that is not a frequently used measure outside trials of HIV-SN. In a recent consensus statement regarding core chronic pain outcome measures (Dworkin, Katz, & Gitlin, 2005) it was not one of the recommended pain scales. Several of the studies utilising the Gracely pain score also included more validated secondary pain outcome measures such as either a VAS score or a NPRS. These were used here in preference to the Gracely pain score in the calculation of NNT and RR.

The Jadad tool has been validated and used widely to identify common and major sources of experimental bias in RCTs identified in systematic reviews. Nevertheless, whilst the use of the modified Jadad score improves the probability that only high

quality RCTs were included in the systematic review, its use may conceivably have biased our systematic review in favour of more recently tested agents. The RCTs associated with these agents now routinely report the information required by the modified Jadad tool, because of the nature of the evolution of RCT methodology over the past few years.

Both of the RCTs that examined the efficacy of smoked cannabis, were of high quality, however the apparent marked superiority of smoked cannabis to placebo cigarettes should be tempered by the high proportion of potential unblinding measured in (Ellis et al., 2009) (92% correctly guessing treatment allocation after treatment cross-over), and its lack of measurement in (Abrams et al., 2007) despite participants having all had previous experience of smoked cannabis. In a similar manner, the RCT investigating recombinant human NGF demonstrated a high degree of unblinding related to injection site myalgia, which when accounted for in a separate analysis reported a more attenuated treatment-related difference which consequently lost statistical significance.

Lamotrigine was the subject of two high quality RCTs. Both failed to show superiority over placebo in the primary pain outcome measure, improvement in the GPS in the ITT population. However, in the larger of the two RCTs, analysis of a secondary pain outcome measure, mean improvement in VAS, did demonstrate efficacy superior to placebo in the subpopulation of subjects who had been previously exposed to neurotoxic ARTs. If this stratum alone is examined an NNT of 2.88 is calculated.

Most of the included RCTs did not stratify subjects with painful HIV-SN according to their exposure to neurotoxic ARTs. This stratification was instrumental in demonstrating an efficacy of lamotrigine in neurotoxic ART exposed painful HIV-SN subjects. It is possible to speculate that a similar strategy of stratifying other RCTs might have elucidated other agents with sub-group efficacy, despite lack of observed analgesic efficacy in an unstratified painful HIV-SN subject population. Additionally, the included RCTs were not uniform in their approach to the use of concomitant analgesics; whilst most allowed continued use of drugs at stable doses, two elected to stop them (Evans et al., 2007; Hahn et al., 2004). The use of such concomitant analgesics, and also the inclusion of participants with previously failed therapies, may conceivably have influenced the outcomes of these RCTs.

Gabapentin and pregabalin were the subject of two high quality RCTs in which neither agent was shown to be superior to placebo. This contrasts with the efficacy of these agents demonstrated in other peripheral neuropathic pain conditions (Finnerup et al., 2010; Hempenstall, Nurmikko, Johnson, A'Hern, & Rice, 2005; RH, Dworkin et al., 2007; Tan et al., 2010). However the gabapentin study was small, with only 30 patients randomised (Hahn et al., 2004). This finding may therefore represent a 'failed trial' rather than a true lack of efficacy.

Amitriptyline efficacy was examined in two large RCTs. The evaluation of one study (Shlay et al., 1998) was made difficult by a complicated study design that may have not been truly randomised or placebo controlled. However the finding that amitriptyline did not display superior analgesic efficacy than placebo in the context of HIV-SN is supported by a similar finding a second, higher quality RCT (Kieburtz et al., 1998). Again, this finding directly contrasts with evidence of efficacy for tricyclic antidepressants in a range of other peripheral neuropathic pain conditions (Finnerup et al., 2010; Hempenstall et al., 2005; RH, Dworkin et al., 2007; Tan et al., 2010).

Capsaicin 0.075% cream was the subject of a small RCT enrolling only 26 subjects. The authors stated that capsaicin 0.075% did not demonstrate statistically significant superiority to placebo in the primary pain outcome measure. However, outcome data were published only in a graphical representation of mean current pain scores from baseline to the end of treatment. From this graph there does appear to be a trend for capsaicin to be superior to placebo at this final time point measured at week 4. However a high drop-out rate in both arms resulted in only 6/11 patients remaining in the capsaicin group, and only 8/15 in the placebo group. It is therefore difficult to determine from this study if capsaicin 0.075% was indeed without efficacy. This has two implications: the first being that capsaicin 0.075% might have some degree of clinically relevant efficacy in painful HIV-SN; and secondly, if capsaicin 0.075% is indeed efficacious, then the use of a similar concentration (capsaicin 0.04%) as an active placebo in the large capsaicin 8% patch RCT would change the design of this study from a placebo controlled to a superiority approach.

In the treatment of painful HIV-SN, the lack of efficacy compared with placebo of many agents with proven efficacy in other forms of neuropathic pain has implications in the understanding of neuropathic pain in general. These findings further support the hypothesis that neuropathic pain cannot be considered as a single symptom with a

single pathogenesis (Baron et al., 2009; Scholz et al., 2009). A more mechanistic approach to the treatment of specific types of neuropathic pain is therefore warranted as has been established in trigeminal neuralgia and post herpetic neuralgia. Equally, caution should be exercised in the use of neuropathic pain treatment algorithms that do not consider these potential mechanistic differences, as their rationale may be fundamentally flawed.

The absence of studies examining the efficacy of opioid use in painful HIV-SN is notable and mandates additional research. Opioids have shown efficacy in other neuropathic pain conditions (Finnerup et al., 2010; Hempenstall et al., 2005; RH, Dworkin et al., 2007). Furthermore, the efficacy of duloxetine in diabetic neuropathy, a condition that has similarities to HIV-SN, may suggest that it is worth investigating (Tan et al., 2010). In addition, the efficacy of cannabis in HIV-SN would suggest that cannabinoids with an appropriate therapeutic index when delivered by a mechanism other than smoking might be worthy of investigation (A. S. C. Rice, 2008).

Summary

On the basis of current published evidence, topical capsaicin 8%, smoked cannabis and Nerve Growth Factor have evidence of efficacy in pain associated with HIV-SN. However the apparent efficacy of capsaicin is contentious, as a larger RCT, reported in abstract form only at the time of this systematic review, suggested this treatment is not superior to placebo (D. Clifford et al., 2010). Some commonly recommended analgesics, including opioids, have not been formally studied for the management of painful HIV-SN.

The current evidence base available for the treatment of painful HIV-SN is at odds with the recommendations made by NICE for neuropathic pain management in the non-specialist situation. This indicates the potential dangers of extrapolating efficacy from one neuropathic pain condition to another where efficacy has not been directly assessed. In particular amitriptyline, pregabalin, and gabapentin have been demonstrated to have no superiority to placebo in the treatment of painful HIV-SN.

With an estimated 33 million people living with HIV and more gaining access to ARV every day, the management of HIV-SN associated neuropathic pain is a problem of major global significance. There is an urgent need for the development of effective; evidence based analgesic strategies for this common condition. On-going evaluation

of both novel analgesics and existing untested strategies for HIV-SN is a clear research priority.

Update Search

On December 15th 2013 follow-up literature search limited to Medline (from 1966 to date searched) was conducted using the same search terms used for the above systematic review.

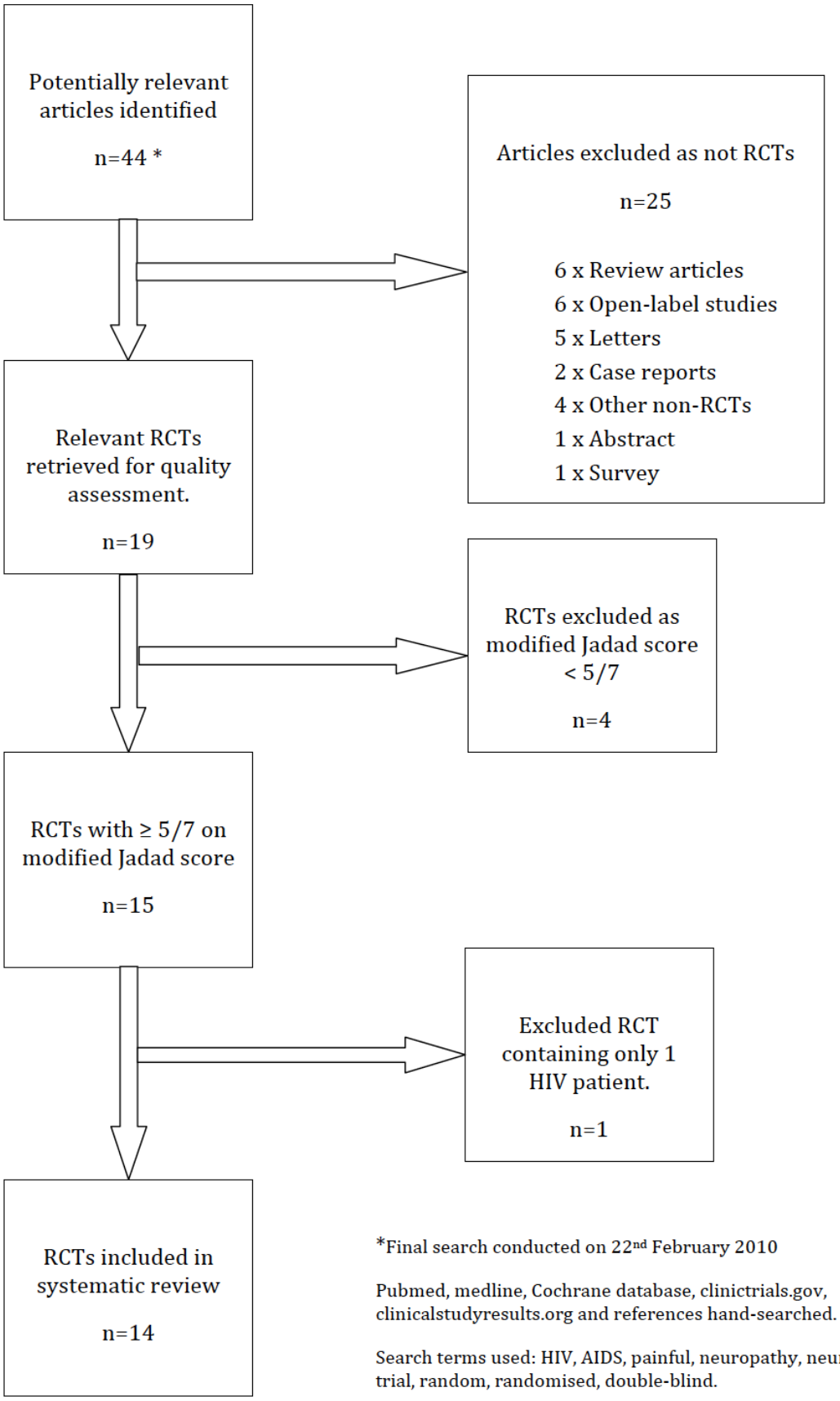
Only two additional RCTs were identified as being potentially relevant to the above systematic review.

One was a high quality RCT that had been previously only reported in abstract form, but mentioned in the systematic review discussion (D. B. Clifford et al., 2012). This RCT was a high quality active placebo controlled with n=332 painful HIV-SN participants for capsaicin 8% patch for either 30 or 60min and n=162 painful HIV-SN who received active placebo (0.04% capsaicin patch). The treatment group showed no statistically significant superiority to placebo at any time point from week 2 to week 12 for the primary outcome Numeric Pain Rating Scale score or for the secondary outcome measure of Patients Impression of Change at week 12.

A second RCT of capsaicin 8% patch was identified comparing capsaicin with three topical anaesthetic preparations (Webster, Peppin, Murphy, Tobias, & Vanhove, 2012). This RCT included n=117 participants with neuropathic pain, however only one participant with painful HIV-SN was entered; this RCT would therefore not be suitable for inclusion in the above systematic review.

Figure 2.1

PRISMA flow diagram of included randomised controlled trails



*Final search conducted on 22nd February 2010

Pubmed, medline, Cochrane database, clinictrials.gov, clinicalstudyresults.org and references hand-searched.

Search terms used: HIV, AIDS, painful, neuropathy, neuropathic, trial, random, randomised, double-blind.

Table 2.1. Studies excluded from the systematic review.

Reference	Treatment	Primary Reason for Exclusion
(Chiechio et al., 2007)	Acetyl-L-carnitine	Review
(Mike Youle, 2007)	Acetyl-L-carnitine	Review
(Woolridge et al., 2005)	Cannabinoids	Review
(Wiffen & Rees, 2007)	Lamotrigine	Review
(Saarto & Wiffen, 2010)	Antidepressants	Review
(Liu, Manheimer, & Yang, 2005)	Herbal medicine	Review
(David M Simpson, Estanislao, et al., 2008)	8% capsaicin patch	Open-label
(D. Clifford et al., 2010)	8% capsaicin patch	Abstract
(Osio et al., 2006)	Acetyl-L-carnitine	Open-label
(Scarpini et al., 1997)	Acetyl-L-carnitine	Open-label
(G Schifitto et al., 2001)	Recombinant human NGF	Open-label
(von Gunten et al., 2007)	Flecainide	Open-label
(Dorfman et al., 1999)	5% lidocaine patch	Open-label
(King, 1999)	Acupuncture	Letter
(Ulett, 1999)	Acupuncture	Letter
(Kaptchuk, 1999)	Acupuncture	Letter
(Valdivelu & Berger, 1999)	Gabapentin	Letter
(Newshan, 1998)	Gabapentin	Letter
(La Spina et al., 2001)	Gabapentin	Case report
(Bradley & Verma, 1996)	Prednisolone	Case report
(Gatti et al., 1998)	Gabapentin	Abstract
(Woolridge et al., 2005)	Smoked cannabis	Other non-RCT
(Herzmann et al., 2005)	Acetyl-L-carnitine	Other non-RCT
(Phillips, Skelton, & Hand, 2004)	Acupuncture	Other non-RCT
(Hart et al., 2004)	Acetyl-L-carnitine	Other non-RCT
(Estanislao, Carter, McArthur, Olney, & Simpson, 2004)	5% lidocaine patch	Modified Jadad score <5
(Kemper, Kent, Burton, & Deresinski, 1998)	Mexiletine	Modified Jadad score <5
(Giovanni Schifitto et al., 2006)	Memantine	Modified Jadad score <5
(Navia et al., 1998)	Nimodipine	Modified Jadad score <5
(Silver et al., 2007)	Lamotrigine	< 5 patients enrolled

Table 2.2. Characteristics and results of included studies. **]**

ference	Participants recruited (completed)	Design and duration	Intervention (n= patient episodes)	Maximum dose studied	Primary Outcome	Data (ITT)	Superior to placebo?
Youle M *et al.,* 2007 [36]	90(76)	Parallel: 2 wks	Acetyl-L-carnitine 500 mg bd i.m. (n=43); placebo (n=47)	1000mg/day	VAS (0-10cm) change: baseline to wk 2.	ITT: Acetyl-L-carnitine: -1.32 (SD 1.84); placebo -0.61 (SD 1.55)(p=0.07)	No
Shlay JC *et al.,* 1998 [38]	136 (105)	Parallel: 14 wks	Amitriptyline (n=71); placebo (n=65)	75mg/day	GP score: change baseline to wk 14.	ITT with LOCF: Amitriptyline: -0.26; placebo: -0.30;difference 0.00 95%CI(-0.18 to 0.19)(p=0.99)	No
Paice JA *et al.,* 2000 [29]	26(14)	Parallel: 4 wks.	Capsaicin 0.075% cream q.d.s. (n=15); placebo (n=11)	0.075% q.d.s.	NRS (0-10): change from baseline to wk 4.	No numeric data given. Stated no statistically significant difference between capsaicin 0.075% and placebo (p>0.05)	No
Simpson DM *et al.,* 2008 [28]	307(274)	Parallel: 12 wks follow-up.	Capsaicin 8% patch for 30min (n=72); 60min (n=78); 90min (n=75); placebo (capsaicin 0.04%) (n=82)	8% for 90min.	NPRS: % change baseline to wk 12.	ITT with LOCF: Capsaicin: -22.8 (SD 30.6); placebo -10.7 (SD 30.8); (p=0.0026)	Yes
Abrams DI *et al.,* 2007 [40]	55(50)	Parallel: 5 days	Smoked cannabis (n=27); placebo (n=28)	3.56% Δ-9-tetrahydrocannabinol t.d.s.	VAS: % change from baseline to day 5.	ITT: Cannabis: -34% (IQR -71 to -16); placebo -17% (IQR – 29 to 8) (p=0.03)	Yes
Ellis RJ *et al.,* 2009 [41]	34(27)	Crossover: 5 days, 2 wks washout, 5 days treatment.	Smoked cannabis (n=28); placebo (n= 28)	Max tolerable: 1 to 8% Δ-9-tetrahydrocannabinol q.d.s.	DDS (0-20): median change from baseline to day 5.	Difference in DDS reduction cannabis *vs* placebo for PP: -3.3 p=0.016, no data for ITT: said to be 'similar' with p=0.020	Yes
Hahn K *et al.,* 2004 [30]	26(24)	Parallel: 4 wks treatment.	Gabapentin (n=15); placebo (n=11)	2400mg/day	VAS: median change: baseline to wk 4.	Gabapentin: -44.1; placebo: -29.8. Stated as being not statistically significant.	No
Simpson DM *et al.,* 2010 [50]	302(241)	Parallel: 14 wks treatment.	Pregabalin (n=151); placebo (n=151)	1200mg/day	NPRS: mean change: baseline to wk 14.	ITT: Pregabalin: -2.88; placebo -2.63 (p=0.39)	No
Simpson DM *et al.,* 2000 [51]	42(29)	Parallel: 14 wks treatment	Lamotrigine (n=20); placebo (n=22)	300mg/day	GP score: mean change: baseline to wk 14.	ITT with LOCF: Lamotrigine: -0.242 (SE 0.092); placebo: -0.183 (SE 0.087) (p=0.65)	No
Simpson *et al.,* 2003 [31]	227(172)	Parallel:12 wks treatment.	Lamotrigine (n=150); placebo (n=77)	600mg/day	GP score: change: baseline to wk 12.	PP: Lamotrigine *vs* placebo. No data given, stated no statistically significant difference seen in all or ARV stratum.	No
Keiburtz K *et al.,* 1998 [39]	145(104)	Parallel: 8 wks	Mexilitine (n=48); amitriptyline (n=47); placebo (n=50)	Mexilitine: 300mg/day Amitriptyline: 100mg/day	GP score: mean change: baseline to wk 8.	ITT: Amitriptyline: -0.31 (SD 0.31); mexilitine: -0.23 (SD 0.41); placebo -0.20 (SD 0.30) No p value given, stated no statistical significance	No
McArthur JC *et al.,* 2000 [53]	270(235)	Parallel:18 wks	Recombinant human NGF (n=180); placebo (n=90)	0.3µg/kg s.c. twice weekly	GP score: median change: baseline to wk 18	ITT with LOCF: NGF 0.1µg/kg: -0.18 (-0.10 to -0.25)(p=0.05); NGF 0.3µg/kg: -0.21 (-0.14 to -0.29)(p=0.04); placebo: 0.06 (+0.01 to -0.14)	Yes
Simpson DM *et al.,* 1996 [54]	104(81)	Parallel: 12 wks	Peptide-T (n=40); placebo (n=41)* PP data	6mg/day intranasal	Modified GP score: mean change: baseline to wk 12.	PP: Peptide-T: -0.24 (±0.45); placebo -0.39 (±0.19) (p=0.32). ITT results not presented but stated showed the 'same pattern'.	No

GP - Gracely Pain Score, VAS – Visual Analogue Scale, ITT – Intention To Treat population, PP -Per Protocol population, NRS – Numerical Rating Scale, NPRS- Numerical Pain Rating Scale, DDS – Descriptor Differential Scale, LOCF - Last Observation Carried Forward

Table 2.3. Summary of RCTs which demonstrated treatment superior to placebo, for which Relative Risk and Number Needed to Treat values could be calculated.

	Active Treatment (maximum tested dose)	Number of patient Episodes	Benefit Efficacy on Treatment (≥30% improvement VAS	Efficacy on Placebo (≥30% improvement VAS)	RR (95% CI)	NNT (95% CI)
Smoked cannabis						
Abrams *et al.,* 2007	Smoked cannabis: 3.56% Δ-9-tetrahydrocannabinol	55 (50)	13/27	6/28	2.17 (0.97 to 4.86)	3.86 (1.98 to 71.11)
Ellis *et al.,* 2009	Smoked cannabis: 8% Δ-9-tetrahydrocannabinol	68 (56)	18/34	7/34	2.57 (1.24 to 5.35)	3.09 (1.98 to 9.30)
Abrams *et al.,* 2007 Ellis et al 2009	Combined smoked cannabis studies	122 (106)	31/ 61	15/ 61	2.38 (1.38 to 4.10)	3.38 (2.19 to 7.50)

Chapter 3

Patterns of sensory, psychological and metabolic dysfunction in HIV positive patients with and without HIV-associated peripheral neuropathy.

Patterns of sensory, psychological, and metabolic dysfunction in HIV positive patients with and without HIV-associated peripheral neuropathy.

Introduction.

Established risk factors associated with the development of HIV-SN include advancing age, height, lower nadir CD4 counts, high viral titres, and exposure to the neurotoxic 'd' NRTI class of antiretroviral agents. Crucially, despite the phasing out of neurotoxic dNRTI drugs, especially in well-resourced countries, the prevalence of HIV-SN has remained consistently at around 40%, suggesting that drug induced neurotoxicity may not be a major aetiological factor for HIV-SN in the cART-era (Catherine Louise Cherry et al., 2012; Ellis et al., 2010; Smyth et al., 2007). More recent cART-era population and genetic association studies have identified additional patient related risk factors such as ethnicity, elevated plasma triglycerides (Banerjee et al., 2011), the expression of genes involved in the peripheral inflammatory response and those affecting mitochondrial function (Kamerman, Wadley, & Cherry, 2012). Whilst there have been several epidemiological studies documenting the prevalence and risk factors for HIV-SN in the current cART era; profiling studies directed at describing in detail the HIV-SN phenotype are lacking. Specifically, factors which are crucial to understanding the nature of HIV-SN, such as the relationships between sensory nerve dysfunction, symptomatology, clinical signs, patient quality of life, psychological co-morbidity and sleep disturbance in HIV-SN are required.

There have been few studies that have attempted to describe in detail the HIV-SN phenotype in the cART era (Bouhassira et al., 1999; Martin et al., 2003).

I have therefore conducted detailed profiling of a cohort of HIV positive patients, with and without HIV-SN, using a battery of techniques directed at understanding the nature and pattern of sensory nerve dysfunction in HIV-SN and its relationship to symptomatology, psychological and circadian rhythm disruption.

Methods.

Study design and participants

The Pain In Neuropathy Study - HIV (PINS-HIV) was an observational single cohort cross-sectional study conducted at Chelsea and Westminster Hospital NHS Trust London. The study protocol was assessed and approved by a local ethics committee (Riverside Research Ethics Committee; NRES number: 09/H0706/24). Subjects participated in the study after giving written informed consent.

Participants were recruited between July 7th 2009 and January 25th 2011 from ambulatory HIV infected patients attending the St Stephen's Centre, Chelsea and Westminster Hospital NHS Trust London. In order to reflect the population treated at one of Europe's major HIV centres, the inclusion criteria were as inclusive as possible. Eligible persons were all HIV infected adults (≥18 yrs of age) irrespective of concurrent or previous ARV treatment, or the presence of symptoms of a peripheral neuropathy. Exclusion criteria included pregnancy, co-incident major psychiatric disorders (DSM-IV), poor or no English language skills, pain ≥4/10 on a numerical rating scale (NRS) from a cause other than a peripheral neuropathy at recruitment, patients with central nervous system lesions, subjects with insufficient mental capacity to obtain informed consent from or complete the study questionnaires. Skin biopsies were not conducted on anticoagulated patients, or who had any other contra-indication to skin biopsy.

The study design consisted of a single clinical assessment appointment, at the end of which participants were given a questionnaire pack to complete and return to the study centre by post.

During the clinical assessment appointment participants had detailed medical and drug histories taken by a study investigator recording: gender, age, ethnicity, past medical history, date of HIV diagnosis, presence of a family history of neuropathy, presence of other potential causes of neuropathy (hypothyroidism, diabetes, alcohol abuse, vitamin B12 deficiency, isoniazid and chemotherapy drug exposure); smoking and alcohol consumption assessed using UK Department of Health method (Hedges, 1996).

Following this, basic clinical parameters were measured for each participant: weight, height and lying/standing blood pressures. Participants then underwent a Structured Neurological Examination (SNE), a detailed Quantitative Sensory Testing (QST) assessment and skin biopsy sampling as described below. Each participant had 30mls of blood drawn and stored for future genotype studies.

Following the clinical assessment appointment the study investigator collected further drug, laboratory and clinical investigation data from the clinical records. The following clinical data was collected: detailed ARV drug histories; nerve conduction study data; the most recent routine haematological and biochemical tests: HIV-viral load, CD4+ counts, plasma electrolytes, liver function enzymes, vitamin B_{12}, thyroid function, blood glucose, plasma lipid profiles, and Hepatitis B, Hepatitis C and Syphilis serology (Supplemental Document 1: PINS-HIV CRF).

Quantitative sensory testing (QST)

Sensory profiles were measured using the German Research Network on Neuropathic Pain (DFNS) QST protocol. Measurements were performed bilaterally in the S1 dermatome at the dorsum of feet. The DFNS has developed and validated a comprehensive QST battery which uses standardised equipment, paradigms and verbal instructions as described (Rolke, 2006; Rolke et al., 2006). This method has been used in multiple investigations of different neuropathic pain conditions to phenotypically characterise patterns of sensory nerve dysfunction (Gierthmühlen et al., 2012; Konopka et al., 2012; Maier et al., 2010).

The DFNS QST protocol assesses the functional characteristics of both small and large afferent fibres by recording responses to 13 thermal and mechanical stimuli. These are described elsewhere in detail and have not been duplicated here (Rolke, 2006; Rolke et al., 2006). In brief, the DFNS QST battery test the following modalities in the order: cold detection threshold (CDT), warm detection threshold (WDT), thermal sensory limen (TSL), the presence of paradoxical heat sensations (PHS), cold pain threshold (CPT), heat pain threshold (HPT), mechanical detection threshold (MDT), mechanical pain threshold (MPT), a stimulus response function for pinprick sensitivity (MPS: mechanical pain sensitivity), allodynia (DMA: dynamic mechanical allodynia), wind-up ratio (WUR), vibration detection threshold (VDT), and blunt pressure pain

threshold (PPT).

Me and the other QST operator (M. R. D. Brown) underwent a formal course of instruction in conducting the DFNS QST protocol at Mannheim University using healthy volunteers. On return to the UK investigation centre, for quality control purposes each investigator was required to produce QST data sets for 18 healthy volunteer controls which were age and sex matched to DFNS requirements: equal number of male and female participants. Healthy volunteers were recruited through local departmental advertisement targeting students and employees of Imperial College London. Inclusion as healthy volunteer data required participants to have responses to vibration, warm and cold threshold detection, within the normal standardised DFNS values. Potential participants were excluded if they presented with a neurological, vascular or psychiatric disorder or a chronic pain condition; diabetes mellitus; or were taking medication with potential central or peripheral nervous system effects, this included benzodiazepines, triptanes or cortisone preparations.

The healthy volunteer control data were subsequently analysed and critiqued by the DFNS to provide quality assurances for the study centre and QST investigators. Additionally normative data for suprathreshold heat testing (as outlined below) were collected from each healthy control subject after the completion of the DFNS QST protocol.

QST equipment

DFNS specification compliant QST equipment was used in the study as described below.

Thermal threshold detection

Sensory and nociceptive thermal thresholds were determined using a SOMEDIC MSA100 thermal stimulator (SOMEDIC AB, Hörby, Sweden) using a fluid cooled Peltier element thermode measuring 25 x 50mm. Base line temperature 32^0C; ramped stimuli at 1^0Cs^{-1} using method of limits. Threshold values of cold detection (CDT) and warm detection (WDT) were first determined in triplicate. A thermal sensory limen of alternating warm and cold stimuli determined the presence of paradoxical heat sensations to cold stimuli (PHS).

Mechanical detection threshold

MDT was assessed using a standardized set of modified von Frey filaments (Optihair2-Set, Marstock Nervtest, Schreisheim, Germany) exerting forces between 0.25 and 512 mN. The contact area of the von Frey hairs with the skin was a rounded tip (0.5 mm in diameter) to avoid sharp edges that would cause nociceptor activation.

Mechanical pain threshold

MPT was assessed using custom-made weighted pinprick stimuli with fixed stimulus intensities (8, 16, 32, 64, 128, 256, 512 mN; flat contact area of 0.25 mm diameter; The PinPrick, MRC Systems GmbH, Heidelberg, Germany).

Mechanical pain sensitivity and dynamic mechanical allodynia

A stimulus-response function for MPS was determined using the same weighted pinprick stimuli as for mechanical pain threshold. Additionally, pain in response to light touch (DMA) was tested by light stroking with a cotton wisp (3 mN), a cotton wool tip fixed to an elastic strip (100 mN), and a brush (200–400 mN).

Vibration detection threshold

VDT was assessed with a Rydel–Seiffert tuning fork (128/64 Hz, 8/8 scale; Model:AB-125A, A. Barthelmes & Co. GmbH, Tuttlingen, Germany) applied at suprathreshold vibration intensity over the subjects medial malleolus until the participant indicated the sensation of vibration had ceased.

Pressure pain threshold

PPT was assessed by using a pressure gauge device (FDN200, Wagner Instruments, Greenwich, CT, USA) with a probe area of 1 cm^2 that exerts pressure up to 2000 kPa.

QST data analysis

QST data entry was into an Excel-based (Excel 2007, Microsoft USA) data analysis system (EQUISTA, Casquar GmbH Germany) provided by the DFNS. This system allowed entry of basic patient demographics and QST data. EQUISTA performed z-score transformations of raw QST data values by comparing against normative reference data published by the DFNS (n=180 subjects, bilateral assessment of 560 test

areas; Magerl et al., 2010). The DFNS normative reference data is age, gender and anatomical test site matched. The mathematical transformation of QST data to z-scores has been described elsewhere in detail (Rolke, 2006)

Heat suprathreshold nociceptive testing

The DFNS protocol is largely threshold based, but includes a stimulus response function for detection of dynamic mechanical allodynia. However, it does not include such a function for thermal stimuli. This was considered necessary by the supervisor of this thesis (A. S. C. Rice) on the basis of his clinical observations from employing QST for routine clinical diagnosis of HIV-SN for several years. This includes HPT, and sometimes WDT, often being above the range the maximum temperature ($> 50^0$C) in the DFNS protocol and that hyperpathia is reported once threshold was reached. Therefore, after completion of the standard DFNS QST protocol, we conducted an additional bespoke heat suprathreshold testing protocol designed in collaboration with Prof Didier Bouhassira. Participants were subjected to 14 suprathreshold heat stimuli of 2s duration each in a temperature range between 44^0C and 52^0C, at 2^0C intervals (Supplemental Document 3: Heat Suprathreshold Protocol). During each stimulus, patients recorded pain experienced using an electronic visual analogue scale (eVAS) transducer (SENSEbox™ 450-010, SOMEDIC AB, Sweden). The anchors used were 0mm - 'No Pain' and 100mm - 'Maximum pain imaginable'. The protocol ensured that each temperature was tested twice, in a fixed, but random order to prevent participants anticipating stimulus temperatures. All the patient responses for QST were recorded in the QST CRF (Supplemental Document 4: QST CRF).

Hierarchical cluster analysis

The extent to which specific groups of participants with similar QST profiles also associate according to other measures was tested using hierarchical cluster analysis. DAISY function of R (version 2.1.2; R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org/) was used to calculate a distance matrix based on Gower's general dissimilarity coefficient. Binary variables were treated as asymmetric. Ward's method was applied to perform a hierarchical cluster analysis on the distance matrix. In the resulting dendrogram, an arbitrary separation threshold was set so that the resulting clusters would not include fewer than 10% of the total participants.

Structured Neurological Examination (SNE)

A comprehensive structured upper and lower limb neurological examination was devised to detect clinical signs of a peripheral neuropathy. The examination was performed on each patient and included assessment of: light touch and pin-prick sensation, joint position proprioception, vibration perception, deep-tendon reflexes, muscle wasting and motor power (Supplemental Document 5: Structured Neurological Examination).

Sympathetic nervous system function was examined by testing for the presence of orthostatic hypotension, as assessed by measuring lying and standing blood pressure in accordance with established protocols (The Consensus Committee of the American Autonomic Society and the American Academy of Neurology, 1996). Lying and standing blood pressures were each measured in triplicate using a non-invasive blood pressure measuring system (Patient Transport Monitor HP M1275A, Hewlett-Packard, USA). Lying blood pressures were measured first, following which the subject was asked to stand for a timed three minutes after which standing blood pressures were measured. Orthostatic hypotension was determined to be present in subjects in whom either at least a 20mmHg reduction in systolic or a 10mmHg reduction in diastolic blood pressure was observed.

Intra-epidermal nerve fibre density testing (IENFD)

The determination of IENFD in skin biopsies is a validated and sensitive diagnostic tool for the assessment of small fibre neuropathies including HIV-SN (Griffin et al., 2001; Lauria et al., 2005, 2010; M. Polydefkis et al., 2002; Michael Polydefkis, 2006).

Punch biopsies of skin were taken immediately after the completion of QST. Biopsies were taken in accordance with the consensus document produced by the European Federation of Neurological Societies/ Peripheral Nerve Society Guideline (Lauria et al., 2005, 2010) on the utilization of skin biopsies in the diagnosis of peripheral neuropathies. Following the local infiltration of tissues with 1% lidocaine and under sterile conditions, skin biopsies were taken from a site 10cm proximal to the lateral malleolus with a disposable 3mm punch biopsy circular blade (Stiefel Laboratories Inc/ GSK Plc NC, USA).

The freshly harvested biopsies were fixed for 12 to 24 hours in 2% paraformaldehyde/ lysine/ periodate (PLP) fixative at 4°C and rinsed with 0.08% Sorensen's phosphate buffer. Samples underwent cryoprotection with 15% and then 30% sucrose solutions for 24 hours each. Samples were then embedded in OCT (Fisher Scientific UK Ltd), snap-frozen by submersion in liquid nitrogen and stored at -20^{o}C. Each biopsy was cut into 50μm sections using a sliding microtome. Immunohistochemistry for PGP 9.5 (Ultraclone Ltd, Isle of White, UK; dilution 1:15000) was performed on sections using the immunoperoxidase method. Sections from each patient were processed in two separate staining runs to eliminate the risk of tissue loss and to ensure staining quality. All sections were allocated an individual code and three sections per subject were selected randomly to undergo analysis of IENFD using random sequences generated by http://www.random.org/.

IENFD was assessed using a double bright-field microscope at 40× magnification using established counting rules (Lauria et al., 2005, 2010). The two IENFD assessors (M. R. D. Brown and J. D. Ramirez) underwent instruction and technical validation in IENFD assessment at a clinical diagnostic laboratory run by Dr I. Bodi. The study biopsy samples were coded, so that the assessors were unaware of the participant's neuropathy status. For the study materials, each assessor independently determined IENFD values on three biopsy sections for each participant; the resulting mean value was used. Inter-observer Pearson's product-moment correlation co-efficacies were determined for all the samples between the two microscopists; in addition intra-observer reliability scores were also determined for 20 of these study sample selected randomly (http://www.random.org/).

In accordance with previously published data, IENFD values below 7.63 fibre/mm were considered to be abnormal (Bakkers et al., 2009). This value has been shown to be associated with a specificity of 90% and sensitivity of 82.8% for the diagnosis of small fibre neuropathies (Devigili et al., 2008).

Clinical Electrophysiology testing

Electrophysiological data were extracted where available from the electronic hospital record of participants who had had lower limb electrophysiology clinical tests as part

of their routine medical care. This clinical data was evaluated for peripheral neuropathies by an experienced neurologist (D. L. H. Bennett) who was blinded to the peripheral neuropathy status of the participants evaluated.

Neuropathy Screening and Severity Tools

The Brief Peripheral Neuropathy Screen (BPNS)

The Brief Peripheral Neuropathy Screening tool was developed to detect HIV-SN and has been used in several studies of HIV-SN (Affandi et al., 2008; C L Cherry et al., 2009; Catherine L Cherry et al., 2008, 2005; Wright et al., 2008). The BPNS was designed to be utilised by non-specialist medical personnel for the detection of HIV-SN and consists of a set of questions relating to patient reported symptoms of a peripheral neuropathy and a brief examination of the distal lower limb vibration perception and deep tendon reflexes. The sensitivity and specificity of the BPNS has been measured on several occasions, most notably by Simpson *et. al,* 2006 in a group of 101 subjects: sensitivity 73% and specificity of 68% (D M Simpson et al., 2006).

The subjective elicited symptoms questions ask patients to report (i) the presence of pain, aching, burning in the feet and/or legs and (ii) the presence of 'pins and needles' and numbness in feet and/or legs. Symptom severity is scored on an 11-point Likert scale 0 (absent) to 10 (severe). The total response is then graded Grade 0 (score 0), Grade 1 (score 1 -3), Grade 2 (score 4 – 6), Grade 3 (score 7-10).

The lower limb examination consists of vibration perception evaluation using a 128 Hz tuning fork maximally struck and placed on the top of the distal inter-phalangeal (DIP) joint on one great toe. The time for a patient to lose vibration perception is graded: Grade 0 - >10 sec, Grade 1 - felt for 6 -10 sec, Grade 2 - felt for 5 sec or less, Grade 3 - no perception of vibration. Ankle reflexes are assessed relative to knee reflexes and graded: Grade 0 - absent, Grade 1 - hypoactive, Grade 2 - normal, Grade 4 - clonus.

Subjects are considered to have HIV-SN if they have at least one symptom *and* either reduced or absent vibration sensation *or* ankle reflexes using the BPNS as revised by Simpson *et al* 2006 (D M Simpson et al., 2006).

The Toronto Clinical Scoring System (TCSS)

The TCSS was developed as a screening tool for diabetic peripheral neuropathy (B. a Perkins, Olaleye, Zinman, & Bril, 2001). Subsequent studies validated its utility in reflecting diabetic neuropathy severity, its correlation with clinical electrophysiological measures and microscopic morphological changes in peripheral nerve biopsies (Bril & Perkins, 2002) . The TCSS utilises a simplified neurological examination assessing peripheral sensory perception, deep tendon reflexes, and the presence of neuropathy symptoms.

Sensory testing is performed on the first toe for: pinprick sensation using a Neurotip™ (Owen Mumford, Oxford U.K.); temperature discrimination (warm and cool water filled test tubes); light touch 10g monofilament and vibration with a maximally struck 128Hz tuning fork; position and rated as normal or abnormal, 0 or 1 point respectively. Scores range from 0 to a maximum of 19.

Deep tendon reflexes scores are graded as loss - 2, reduced -1 and normal - 0 for each side. The presence of each following neuropathy symptom scores 1 point: pain, numbness, tingling and weakness in the feet; the presence of similar upper limb symptoms and the presence of unsteadiness on ambulation.

The Utah Early Neuropathy Scale (UENS)

The UENS is a physical examination scale developed specifically for the detection of early, sensory predominant, diabetic polyneuropathy (Singleton et al., 2008). The UENS emphasis is on the severity and spatial distribution of pin sharp sensation loss in the lower limb. The scale was developed and validated in a population with early diabetic peripheral neuropathy.

The UENS assesses sharp sensation in the lower leg relative to an unaffected portion of skin. A Neurotip™ pin (Owen Mumford, Oxford U.K.) is applied to the dorsum of the first toe and, working centripetally in 2cm increments, the subject is asked at each application if they feel “any sharpness” and if they do “is it as sharp as they would expect?” This is performed on both lower limbs. The lower limbs are divided into 6 regions for testing; 2 points are scored for each region in which the subject fails to feel

any sharpness. This is conducted and scored on both lower limbs. One additional point is scored for each additional region in which the pin feels less sharp than expected. Vibration is tested using a 128Hz tuning fork maximally struck and applied to the dorsum of the great toe at the interphalangeal joint.

The UENS has also been correlated with data from electrophysiological and QST testing (Singleton et al., 2008). Moreover, the UENS has recently been used in a small HIV-SN population, where it was shown to correlate with autonomic dysfunction measures (quantitative sudomotor axon reflex testing) and pain severity, but it was not validated in the diagnosis of HIV-SN (Boger et al., 2012).

Pain, sleep disturbance, quality of life and psychological co-morbidity measures

Participants were given a questionnaire pack to complete and return to the investigation centre following their clinical assessment appointment.

7-day Pain diary

Patients were also asked to keep a pain diary for seven days, recording pain at 8am and 8pm daily on an 11-point Likert scale with 0 being 'no pain' and 10 the 'worst pain imaginable'.

Brief Pain Inventory (BPI) 7-item pain interference sub-scale

Pain related interference in activities of daily living was assessed using the 7-item Pain Interference Sub-scale of the Brief Pain Inventory (BPI-PIS; Cleeland & Ryan, 1994). The BPI-PIS assesses pain interference within 7 domains: general activity, walking, work, relationships, mood, life enjoyment and sleep which participants score on a 11 point Likert scale ranging from 0 to 10. The composite score was calculated as the sum of the 7 interference items.

SF-36 instrument

The short-form 36 questions (SF-36) is an established instrument used for the assessment of health-related quality of life (Ware & Sherbourne, 1992). Participants complete a questionnaire consisting of 36 questions. As per convention, SF-36

responses were regrouped, into 8 domains of physical functioning: physical functioning, role - physical, bodily pain, general health, vitality, social functioning, role - emotional, and mental health. SF-36 scores range from 0 to 100: representing extreme dysfunction/ symptom severity to optimal function respectively.

Pain Anxiety Symptom Scale-20 (PASS-20)

Pain-related anxiety was assessed with the Pain Anxiety Symptom Scale-20 (PASS-20; L. McCracken & Dhingra, 2002) which is a shortened version of PASS (M. L. McCracken, Zayfert, & Gross, 1992). It is comprised of 20 questions assessing four facets of pain-related anxiety: fearfulness of pain, cognitive anxiety, escape/avoidance, and physiological anxiety. Each subscale score ranges from 0 'no interference' to 25 'maximum interference'. Additionally summation of the sub-scales provides a general measure of pain related anxiety.

Amended Depression Anxiety Positive-Outlook instrument (DAPOS)

We used the depression and anxiety sub-scales of the Depression Anxiety Positive-Outlook (DAPOS) instrument (Pincus, Williams, Vogel, & Field, 2004). The DAPOS instrument was designed to measure mood in pain populations, and consists of 3 subscales. The subscale for depression (DAPOS-D) contains 5 items with scores ranging from 5 to 25, indicating normal mood to severe depression. The subscale for anxiety (DAPOS-A) contains 3 items, which ranges from 3 to 15 (no anxiety to maximal anxiety).

The Pain Catastrophizing Scale (PCS)

Pain Catastrophizing Scale assesses the cognitive process by which pain is seen as an extreme threat and from which the patient suffers exaggerated negative consequences (Osman et al., 1997). The PCS questionnaire consists of 13 statements of descriptions of pain experiences. Respondents are asked to indicate whether they agree with these statements by using a 5-point rating scale scored from 0 (not at all) to 4 (always). A high total score indicates a high level of pain catastrophizing. The instrument comprises three dimensions: rumination, magnification and helplessness. Rumination

refers to the patients' inability to apart the pain from his mind; magnification expresses the exaggerated cognitions of pain as a threat and hopelessness is the estimation that the patient cannot do anything to influence in his pain.

Insomnia Severity Index (ISI)

To assess the prevalence of sleep dysfunction we used the Insomnia Severity Index (ISI; Bastien, Vallières, & Morin, 2001). The ISI was specifically designed to assess insomnia and is a brief self-report instrument, which measures a patient's perception, subjective symptoms and consequences of their insomnia. Its content corresponds, in part, to the diagnostic criteria of insomnia. The ISI comprises of seven items assessing the severity of sleep-onset and sleep maintenance difficulties, satisfaction with sleep patterns, interference with daily functioning, noticeability of impairment due to sleep dysfunction and the degree of distress experienced by the patient.

Case definition of HIV-SN for PINS-HIV; rationale and implications for the study.

There is no gold standard test for the presence of HIV-SN (Brew, 2003; Catherine L Cherry et al., 2005). This lack of gold standard for HIV-SN and other predominately small fibre neuropathies, has been identified as a factor hindering the progress in understanding HIV-SN (Evans et al., 2008) and small fibre neuropathy pathophysiologies *per se* (Devigili et al., 2008).

The most frequently employed neuropathy screening instruments used to assess the presence of HIV-SN, were the Total Neuropathy Score (TNS) and Brief Peripheral Neuropathy Screen (BPNS) (Catherine L Cherry et al., 2008; Maritz et al., 2010; J Robinson-Papp et al., 2010; D M Simpson et al., 2006; Smyth et al., 2007). These instruments were conceived for use in larger epidemiological studies and are designed to be brief and require little specialist equipment or training to use. These screening tools focus predominately on large sensory nerve fibre function, such as vibration sensation and deep tendon reflexes. Consequently these scales may be less sensitive to the small fibre dysfunction characteristics of HIV-SN (Boger et al., 2012) which have been hypothesized to be important in the early pathogenesis of HIV-SN (Catherine L Cherry et al., 2005; D. R. Cornblath et al., 1999) which is an area of interest in the PINS-HIV study. The use of these established screening instruments would potentially

lead to an over representation of participants with later, established HIV-SN and fail to diagnose early HIV-SN.

Other diagnostic tests such as nerve conduction studies, (R. J. Barohn et al., 1996; P. Y. Chavanet et al., 1988; P. Chavanet et al., 1989; Fuller, Jacobs, & Guiloff, 1991; Husstedt et al., 2000; Jakobsen, Smith, Gaub, Helweg-Larsen, & Trojaborg, 1989; Malessa et al., 1996; T. Smith, Jakobsen, Gaub, Helweg-Larsen, & Trojaborg, 1988) QST testing, (Bouhassira et al., 1999; Huengsberg, Winer, Ross, & Shahmanesh, 1998; Tagliati, Grinnell, Godbold, & Simpson, 1999; Winer et al., 1992) and IENFD quantification (D N Herrmann, Griffin, Hauer, Cornblath, & McArthur, 1999; Holland et al., 1997; McCarthy et al., 1995; Pittenger et al., 2004; M. Polydefkis et al., 2002; Michael Polydefkis et al., 2004) may be abnormal in the context of HIV-SN. However, normal results to these tests occur frequently in patients with clinically apparent HIV-SN (M. Polydefkis et al., 2002; Tagliati et al., 1999; Winer et al., 1992). Similarly, some subclinical abnormalities, not necessarily indicative of neuropathy pathophysiology are also described in these tests (R. J. Barohn et al., 1996; Bouhassira et al., 1999; P. Y. Chavanet et al., 1988; P. Chavanet et al., 1989; Fuller et al., 1991; David N Herrmann et al., 2004; Huengsberg et al., 1998; Husstedt et al., 2000; Jakobsen et al., 1989; Malessa et al., 1996; T. Smith et al., 1988; Tagliati et al., 1999).

As no single testing paradigm could be identified that would have an acceptable reliability, sensitivity and specificity for the aims of the PINS-HIV study, a novel composite definition was constructed in discussion with the collaborating neurologist.

The PINS-HIV study used a definition of HIV-SN requiring ≥2 out of the following triumvirate:

- Clinical signs of distal neuropathy on the Structured Neurological Exam (SNE).
- ≥2 abnormal QST findings using DFNS protocol.
- Intra-epidermal-nerve-fibre-density of ≤7.63 fibres/mm on skin biopsy examination.

Such a composite definition has been previously proposed for use in the study of other small fibre neuropathies, but has not been used in studies of HIV-SN (Devigili et al., 2008).

By using three different test modalities and requiring more than one 'positive' finding for defining HIV-SN, it was hoped to negate many of the shortcomings of the individual tests if used in isolation.

A limitation of using this method is that there is potential for a circular argument for some of the outcome measures of interest in the study, which partly rely on the diagnostic tests used in the case definition. To explore this potential problem a *post-hoc* analysis of altering the case definition was planned to investigate the impact on individual participant neuropathy diagnosis designation (Table 3.10). A further exploration of using different definitions of HIV-SN on the QST results has also been conducted *post-hoc* below (Table 3.11, 3.12 and Figures 3.2.a,b,c and d).

Sample Size Calculation

At the time of the study there were few data available regarding the diagnostic value for HIV-SN for each of the two primary measurements of interest (QST and IENFD) on which to base a sample size calculation. Nevertheless, such a calculation in relation to these measures has been performed in order to guide study conduct (t test for power calculation, Sigma v 3.5, Systat Software Inc).

For QST the ΔWDT data for HIV positive patients *versus* healthy controls from Martin et al were used (Martin et al., 2003). This calculation revealed a minimum sample size of 11 was required per group for a power > 0.8 (Power 0.828; Difference in Means 4.3; Standard Deviation 3.3; α 0.05).

For IENFD data for patients with small fibre neuropathies verses healthy controls from Nebuchennykh *et al* were used (Nebuchennykh, Løseth, Lindal, & Mellgren, 2009). This calculation revealed a minimum sample size of 16 was required per group for a power > 0.8 (Power 0.816; Difference in Means 4.7; Standard Deviation 4.5; α 0.05). However, because of the uncertain assumptions inherent in such calculations a minimum group size of 25 was used.

Results

Participants

Between July 7th 2009 and January 25th 2011 66 volunteers who were living with HIV were recruited to participate in the study. They were all receiving routine clinical care for their HIV disease as ambulatory patients attending the St Stephen's Centre out patients department, Chelsea and Westminster Hospital NHS Foundation Trust, London, United Kingdom

All 66 participants attended the clinical assessment appointment. Two participants (one from the HIV-SN and the other from the HIV-No SN group) produced only partial QST datasets (missing thermal parameters) due an isolated equipment problem that was resolved. These subjects were excluded from the subsequent thermal parameter QST data analysis.

IENFD were determined on 57 participants out of the total 66; 6 participants did not consent to a skin biopsy (4 from the HIV-No SN and 2 from the HIV-SN group), one participant from the HIV-SN group was anticoagulated with warfarin and so a skin biopsy was contra-indicated. Two samples (one from each group) were of insufficient quality to allow IENFD determination. Participants with missing IENFD data were not included in the IENFD data analysis.

Demographics

Participant demographic data are presented in Table 3.1. The groups were evenly matched in terms of demographic and related clinical factors. The majority of participants were white (86.4%), male (86.4%) and middle aged (mean 49.23 yrs SD 8.78), broadly reflecting the patient population of the highly resourced United Kingdom recruitment centre in the current cART era (personal communication Dr Marta Boffito and Dr David Asboe). Using the triumvirate criteria for HIV-SN diagnosis the study group was divided into 'HIV-SN' and 'HIV-No SN' groups. Twenty-eight (42.4%) of participants were determined to have HIV-SN and 38 (57.6%) to have HIV-No SN. Twenty-one (75.0%) of the 28 HIV-SN patients complained of a painful peripheral neuropathy. No statistically significant difference

was seen between the HIV-SN and HIV-No SN groups for sex, ethnicity, height, or weight. There was also no difference between the groups in terms of age, height and years since HIV diagnosis.

There was also no difference between the two groups for the frequency of co-morbidities which might potentially affect the peripheral neuropathy status of participants; diabetes, hepatitis B and C, syphilis or exposure to chemotherapy agents.

Quantitative Sensory Testing healthy control participants

Healthy control data was collected to meet the DFNS group's requirements for investigation centre quality assurance, and to provide normal values for suprathreshold heat responses. To fulfil DFNS requirements 36 healthy controls were recruited with a mean age of 34.60 yrs of age (SD 9.45) of which 21 (58.3%) were male. These participants underwent the DFNS QST protocol after which the additional suprathreshold heat testing was conducted on each.

Anti-retroviral therapy history

ARV therapy histories of the participants are presented in Table 3.2. Only 2 participants (3%) had no previous exposure to ARVs; both were in the HIV-No SN group. The majority had previous ARV exposure and were all still currently receiving cART (n=64, 97%). No statistical difference was observed between the two groups for either, individual agents or classes of agents in relation to exposure or mean years exposed. There was also no difference between the two groups for the total number of 'drug years' a patient received a class of agent for, or the maximum number of consecutive years of exposure to a given drug class (data not shown).

There was no difference between the two groups for exposure to NRTI agents. Between 52% and 71% of participants had been exposed at some point to neurotoxic "dNRTIs" and the average exposure was about 12 years. This was independent to the duration of time since HIV diagnosis. The groups were also matched for exposure to the potentially neurotoxic protease inhibitor indinavir (Catherine Louise Cherry et al., 2012; Ellis et al., 2010; Pettersen et al., 2006; Smyth et al., 2007) with 13-32 % of subjects having been exposed to the drug for an average of about 13 years.

Quantitative Sensory Testing results

Distribution of mean QST z-scores across groups

Mean z-score values for HIV-No SN and HIV-SN groups (Figure 3.1, Figure 3.3 and Table 3.3) showed a loss of sensory function for all three thermal sensory QST parameters CDT, WDT, and TSL compared to DFNS values, indicating a degree of thermal detection hypothesia ($p<0.05$ Kruskal-Wallis, Dunn's *post-hoc*; HIV-No SN and HIV-SN and HC groups). This general shift across all thermal parameters towards hypothesia was largest in the HIV-SN group. A similar shift in thermal nociceptive parameters was not seen, with only HPT demonstrating a shift in mean z–scores towards loss of function. Mean z-scores for CPT were similar to DFNS control values in both groups.

Mechanical detection parameters also showed a shift of the group mean towards loss of function in the HIV groups; again, the largest difference was observed in the HIV-SN group (see Figures 3.1 and 3.3 and Table 3.3). This was particularly marked in the HIV-SN group for MDT and VDT mean values compared to the HIV-No SN, HC and DFNS control values ($p<0.05$ Kruskal-Wallis, Dunn's *post-hoc*). MDT and VDT were the only QST parameters that showed a mean group value outside of the DFNS defined normal range.

Whilst the difference in mean WUR was small in the HIV-SN compared to the other groups it had a large SD value (1.66) suggestive of a high degree of heterogeneity for this parameter within the HIV-SN group. This heterogeneity was evident in the scatter plot of WUR for HIV-SN, where a small group of 4 HIV-SN participants (14.3%) number showed high (gain of function) z-scores for WUR ($p <0.05$ Chi squared test of association compared to HIV-No SN group).

Mean healthy control QST participant data closely matched that expected by DFNS control values for 12 of the 13 DFNS QST parameters (see Table 3.3). This is indicated by the fact that the z-transformed QST mean values in the healthy control group are all close to zero, with standard deviations of near to one or less. Healthy control MPS values showed a slight shift in mean values, with 5 (13.9%) participants more than expected showing abnormal values compared to DFNS healthy control data.

Frequency of individual QST measure abnormalities: loss and gain of sensory function.

The frequency of abnormal QST values in each group are shown in Figure 3.4 and Table 3.3. The majority of abnormal parameters were loss of sensory function in any of the QST parameters with 34.2% and 85.7% in the HIV-No SN and HIV-SN groups respectively. A smaller number of participants in the HIV-No SN and HIV-SN group showed gain of function in any parameter: 26.3% and 28.6% respectively. – but this was similar to the Healthy control groups at 30.6%, there was no statistical difference between all three groups.

The most frequent sensory abnormalities seen in the HIV-SN group were loss of MDT and VDT both of which were independently absent in 50% of the HIV-SN subjects, with 32.14% demonstrating the loss of both. In contrast, the healthy control and HIV-No SN group showed a loss of MDT in only 5.6% and 5.3%, and VDT in 5.6% and 10.5% respectively.

Within the thermal sensory parameters the HIV-SN participants showed a loss of CDT function (28.6%) compared to HIV-No SN group (5.3%), most frequently followed by WDT and TSL (14.3%, 2.6% in both HIV groups). Loss of HPT was the most frequent abnormal thermal nociceptor parameter in the HIV-SN group (32.1%) and in the HIV-No SN group (7.9%). No participants in either HIV group displayed abnormalities of CPT. The presence of PHS was considered a loss of thermal discrimination and therefore a loss of sensory function (Rolke, 2006). PHS phenomena were observed in 7.9% and 14.3% of the HIV-No SN and HIV-SN groups respectively.

The presence of gain of function in QST parameters was rare across all groups, but was seen most frequently in the mechanical WUR parameter, with 14.3% of HIV-SN patients demonstrating it, compared to only 5.3% and 5.6% of the healthy control and HIV-No SN groups respectively ($p<0.05$ Chi squared test of association against HIV-No SN group). No participants in any of the groups were found to have DMA.

Patterns of loss and gain of sensory function

We utilized the DFNS coding system (Maier et al., 2010) to display combinations of sensory dysfunction in HIV-SN (Table 3.4). Accordingly, a value for a QST parameter

within the normal DFNS reference range was designated '0'; the presence of thermal hypoesthesia (i.e. loss of WDT or CDT) was designated as L1; and the presence of hypoesthesia to mechanical modalities (i.e. loss of MDT or VDT) was designated L2. Gain of sensory function to thermal modalities was designated G1 and gain of sensory function to mechanical modalities designated as G2. When both thermal and mechanical abnormalities were present they were designated as L3 and G3 respectively.

HIV-SN abnormal QST parameters were dominated by loss of sensory function. The majority of HIV-SN participants (85.7%) had at least one abnormality on QST testing, and had, at a minimum, the loss of one sensory parameter. Different combinations of sensory loss and gain did occur across the HIV-SN group, however the majority involved just sensory loss (L1+L2+L3: 78.6%). Indeed, the most frequent combination was the loss of at least one thermal and one mechanical QST parameter in 42.9% of participants (L3G0). HIV-No SN participants demonstrated loss or gain of thermal modalities in isolation (L1 or G1; see Table 3.4). The second most frequent combination was loss of just mechanical sensory modalities (L2G0: 21.4%).

Suprathreshold heat testing

Figure 3.5 displays the cumulative mean pain VAS responses to suprathreshold heat stimuli from 48^{o}C to 52^{o}C for the three groups: healthy controls, HIV-No SN and HIV-SN groups. Health control patients had an earlier take off in pain VAS responses – i.e. this occurred at lower temperatures, however at higher suprathreshold temperatures there was little difference in the VAS responses elicited from all the three groups tested. No statistically significant difference was seen between the three groups, or in the painful HIV-SN subgroup of HIV-SN (not shown) for pain response at any suprathreshold temperature.

Hierarchical cluster analysis of QST data

The results of hierarchical cluster analysis of individual participant's DFNS QST data did show a tendency for HIV-SN patients to cluster according to neuropathy and pain status (see Figure 3.6). A distinct cluster of 25 subjects, containing 20 (71.4%) of the HIV-SN participants, of whom the majority (80%) complained of pain symptoms, was

observed. No other discernible clusters were found relating to any other participant outcome measures examined using hierarchical cluster analysis of QST data (Figure 3.6).

Intra Epidermal Fibre Densities (IENFD)

A total of 57 skin punch biopsy samples were available for IENFD measurement from the 66 participants (see above for details). Figure 3.7 shows representative images of two participants, one with normal fibre counts (Figure 3.7A) and the other with reduced IENFD counts (Figure 3.7B).

The associated intra-observer correlation co-efficients for 20 randomly selected samples for quality assurance exercise was 0.88 (J. D. Ramirez) and 0.89 (M. R. D. Brown), both of which are high. A high inter-observer Pearson's product-moment correlation co-efficient of 0.93 was determined between the two microscopists for all study samples (n=55).

The median values of IENFD for HIV-No SN group was 9.2 fibres/mm (range 1.7 - 14.4 fibres/mm) compared to 6.3 fibres/mm (range 0.7 - 12.4 fibres/mm) for the HIV-SN group ($p<0.001$). In accordance with previously published literature, an IENFD value of < 7.63 fibres/mm was taken as being abnormal (Lauria et al., 2005); this was seen in 8 subjects (21.1%) of the HIV-No SN and in 17 subjects (60.7%) of the HIV-SN group ($p<0.05$) with an associated sensitivity and specificity of detecting HIV-SN in isolation of 61% and 79% respectively.

Table 3.5 shows Pearson's correlation coefficients for individual QST parameters and IENFD. Little correlation between the measured QST parameters and IEFND can be seen.

As would be expected, IENFD was inversely correlated to the severity of the peripheral neuropathy as measured by the TCSS instrument (r=-0.343, 95%CI -0.56 to -0.88; $p<0.01$).

Electrophysiological testing

Suitable lower limb electrophysiology clinical studies were available for 18 (27.3%) of the 66 HIV positive participants; 5 subjects (13.2%) in the HIV-No SN group and 13 subjects (46.4%) in the HIV-SN group. Within the HIV-SN group, nine of the thirteen participants (64.3%) were diagnostic of an axonal peripheral neuropathy in comparison to only one in the HIV-No SN group.

Pain and patient reported symptoms

Results of the structured patient symptom and medical history interview, and 7-day pain diary are presented in Table 3.6.

More patients reported experiencing pain in their hands and/or feet in the HIV-SN compared to HIV-No SN (75% *vs* 28.95% respectively; $p<0.001$). The mean 7-day pain score for patients experiencing pain was also greater in the HIV-SN group (5.65, SD 1.76) compared to the HIV-No SN group (2.8, SD 2.34; $p<0.001$). The mean number of years from HIV diagnosis and the mean duration of pain were similar in the two groups. Although there was no difference between groups in the reporting of 'pins and needles', the reporting of 'numbness' in feet and/or hands was more frequent in the HIV-SN group (75%) compared to the HIV-No SN group (36.8%; $p<0.05$).

More participants with HIV-SN compared to those without HIV-SN, reported suffering from symptoms of postural hypotension (50% *vs* 24.6% respectively; $p <0.01$), however participants' reported symptoms of orthostatic hypotension correlated to measured orthostatic hypotension in only 33.3% of HIV-No SN and 7.1% of HIV-SN participants. No difference was seen in measured orthostatic hypotension between the two groups (14.8% *vs* 18.4% for HIV-SN and HIV-No SN groups respectively; $p=0.702$).

Patients reported symptoms of urinary dysfunction more commonly in the HIV-SN group (42.9%) compared to participants in the HIV-No SN (15.8%; $p<0.05$). The frequency of participants reporting erectile dysfunction and nocturnal diarrhoea were similar for both groups respectively (Table 3.6)

Psychological co-morbidity, sleep disturbance and health related quality of life.

The return of questionnaires and completion of individual scores within each group are shown in Table 3.7. Of the 66 participants, 57 (86.3%) returned completed questionnaire booklets to the test centre for analysis. Results from the psychological and insomnia instrument battery are presented in Table 3.8.

Pain interference and health related quality of life

As expected the Brief Pain Interference (BPI) subscale mean total score was significantly higher in the painful HIV-SN group (46.11, SD 13.69) compared to the HIV-No SN group (15.20, SD 16.22; $p<0.001$; see Table 3.8). The high scores present in the painful HIV-SN group indicate that participants' pain has an important detrimental impact on their day to day living and their quality of life.

The SF-36 data further emphasises this point, showing participants with painful HIV-SN displayed significantly more morbidity when compared to the HIV-No SN group across all of the domains of the SF-36 (Table 3.8). In particular HIV-SN patient's physical functioning (RP), perceptions of their physical selves (RP), general vitality (VT) and social functioning (SF) were dramatically poorer in the participants in the painful HIV-SN group compared to patients in the HIV-No SN group.

Sleep dysfunction

Analysis of data from the self-report ISI instrument indicated that both groups suffered from mild sub-clinical insomnia. However, at the group level there was no independent effect of HIV-SN in this regard. Nevertheless, at the individual level, more participants in the painful HIV-SN group reported degrees of sleep disturbance equating to clinical insomnia (ISI $\geq$15): 13 participants (68.4%), with 7 (36.8% overall) of these suffering severe insomnia (ISI $\geq$ 22). In contrast, 7 participants (22.6%, $p<0.001$) of the HIV-No SN group suffered from clinical insomnia, and 3 participants (9.7% overall, $p<0.001$) with severe insomnia (Table 3.8).

Depression, Anxiety and Catastrophizing

Participants with painful HIV-SN were more depressed (11.21, SD 4.22 *vs* 8.38, SD 4.10, $p<0.05$) and anxious (4.47 SD 2.97 *vs* 5.45 SD 2.89, $p<0.05$) as measured with the DAPOS instrument, than those in the HIV-No SN group (see Table 3.8).

Overall, there was no difference between the groups with regards to the total PASS-20 scores (see Table 3.8). However, differences were evident in some domains of the PASS-20. The HIV-SN group reported more features of cognitive impairment in response to pain compared to the HIV-No SN group (PASS-20 cognitive sub-scale - mean 8.45, SD 5.28 *vs* 14.95, SD 5.64 respectively; $p < 0.001$). There was more psychological anxiety and fear as measured with the PASS subscales in the painful HIV-SN group compared to the HIV-No SN group: physiological anxiety (9.21 SD 7.54 *vs* 4.83 SD 6.45 respectively; $p < 0.05$) and fear (11.79, SD 6.75 *vs* 6.97, SD 7.11 respectively: $p < 0.05$).

Catastrophizing as measured using the PCS instrument revealed a significant difference in PCS total scores between the groups (see Table 3.8; 14.3, SD 11.81 in the HIV-No SN group compared to 23.74, SD 12.64 in the painful HIV-SN group; $p=0.009$). This difference was mostly attributable to a difference in the PCS helplessness sub-scale (6.03, SD 5.52 in the HIV-No SN group compared to 11.16, SD 6.13 in the HIV-SN group; $p=0.004$). No statistically significant differences were seen in the other PCS sub-scores for rumination or magnification.

Plasma lipid profiles, random glucose, BMI and waist-hip circumference ratios.

A higher mean triglyceride plasma (TRG) concentration was measured in the HIV-SN group compared to the than the HIV-No SN group (see Table 3.9; 2.18mmol/l, SD 1.09 *vs* 1.61mmol/l, SD 0.77 respectively; $p <0.05$). There was no significant difference between the two groups in statin drug use. Despite this difference in TRG concentrations we did not find a correlation between the severity of neuropathy (using the TCSS instrument) and plasma TRG concentrations ($r_s = -0.155$ (-0.514 to 0.250); $p = 0.439$). No significant differences were found between the two groups for other plasma lipids, BMI or waist-hip circumference ratios, although all were the upper-end of normal (see Table 3.9).

Peripheral neuropathy screening and severity instruments: BPNS, UENS and TCSS.

Mean scores were determined and ROC plots were generated for each of the three neuropathy screening instruments examined in the study. Optimal cut-off values for the diagnosis of HIV-SN in the study population were determined from these plots, giving equal weight for sensitivity and specificity.

Using the standard BPNS scoring method (D M Simpson et al., 2006), which adds weight to the loss of deep tendon reflexes and vibration perception, the BPNS instrument shows a specificity and sensitivity in the detection of HIV-SN of 75% and 79% respectively ($p<0.001$).For the ROC analysis the numerical component of the BPNS was used in its raw form, weighting sensitivity and specificity equally. A cut-off value of 19 was determined as optimal which was associated with a sensitivity and specificity of 76%.

The mean TCSS values in the HIV-SN were higher when compared to those in the HIV-No SN group (9.26, SD 3.28 *vs* 3.36, SD 3.65 respectively; $p<0.001$). ROC analysis found a value of 8 provided the best accuracy in HIV-SN detection; sensitivity/ specificity 81%.

The UENS median values were also significantly higher in the HIV-SN group when compared to the HIV-No SN group (11.26, SD 5.66 *vs* 3.64, SD 2.98 respectively; $p<0.001$). Following a ROC analysis a cut-off value of 7 was selected as providing the best accuracy in our study population. This provides a sensitivity and specificity of 84% in detection of HIV-SN.

Using the ROC plots for each instrument, AUC was used as a measure of accuracy; with a smaller AUC equating to greater accuracy. The instrument found to have the greatest accuracy was the UENS (AUC 0.91), followed by TCSS (AUC 0.86) and then the BPNS (AUC 0.69).

Testing the HIV-SN triumvirate definition and the consequences of using different HIV-SN definitions.

As this study's case definition of HIV-SN used a triumvirate utilising QST, IENFD and a Structured Neurological Examination (SNE), we tested the consequences of

using different combinations of the triumvirate on the HIV-SN designation of participants in the study (see Table 3.10). Removal of QST and IENFD individually from the triumvirate resulted in sensitivity and specificity of 71%, 100% and 79%, 100% respectively when compared to using the full triumvirate diagnosis. Not using QST in the diagnosis resulted in 8 participants (12.1%) having a different diagnosis; not using IENFD results in a change in 6 subject's diagnosis (9.1%) and removal of SNE resulted in the largest number of diagnosis changes at 17 (25.8%).

The criteria most effective in isolation is SNE - with a sensitivity and specificity of 1.00 and 0.740 respectively, followed by QST, (0.786 sensitivity, 0.816 specificity) and IENFD (0.607 sensitivity, 0.790 specificity).

Discussion

Quantitative Sensory Testing

To our knowledge, this is the first detailed profiling of the HIV-SN phenotype in a cART era population. This study has shown that the pattern of sensory nerve dysfunction that occurs in HIV-SN is profoundly heterogeneous. Across sensory and nociceptive mechanical and thermal modalities, the predominant picture was that of loss of sensory function; 86% of HIV-SN participants were observed to have a loss of function of at least one sensory modality. However the degree to which individual modalities were affected across the HIV-SN group did not correlate to either the symptoms experienced (including pain) or the severity of neuropathy. Equally, no single sensory QST parameter was found to have a useful diagnostic utility for HIV-SN diagnosis in isolation.

Unexpectedly it was the large fibre mediated modalities of MDT and VDT (both mediated by larger myelinated Aβ fibres) which were most frequently lost in HIV-SN (see Table 3.4; loss of 50% for each modality). Moreover 32% of HIV-SN participants demonstrated a loss of both MDT and VDT modalities together. HIV-SN has often been described to be a predominately small fibre neuropathy, i.e. affecting mostly small unmyelinated C and Aδ sensory fibres. Therefore, it would be expected that this would be reflected in QST testing as more in a loss of thermal detection thresholds (CDT and WDT) and an elevation in the heat pain threshold (HPT), as these modalities

are mediated by the smaller unmyelinated sensory fibres. Whilst these modalities are indeed affected on a group level (see Figures 3.2-3.3 and Table 3.3), HIV-SN mean CDT and WDT showed a significant reduction in function compared to both the HIV-No SN and DFNS values. Individually only a smaller number of HIV-SN participants showed a large enough loss in function of these modalities to be considered as 'abnormal' by the DFNS definitions used. As mechanical QST parameters are more often affected in HIV-SN, the inclusion of thermal QST parameters as part of diagnostic testing for HIV-SN would appear to not be necessary. This has significant resource implications, as the equipment involved in such thermal testing is, to date, expensive and not easily portable. Consequently the use of QST outside of well-resourced research centres has been limited. VDT can be tested with an appropriate 128Hz tuning fork and MDT with von Frey filaments, both of which are relatively inexpensive and do not require specialist training to use.

Whilst the diagnostic limitations of electrophysiological methods for small fibre neuropathies is well know, this study shows the limitations of QST for the diagnosis of HIV-SN. Forteen per cent of participants determined to have HIV-SN by the multimodal definition used in this study did not demonstrate dysfunction of any of the QST modalities measured. This is broadly similar to an earlier DFNS study examining the QST phenotype of painful polyneuropathies (HIV- SN participants), which found 17% of subjects that did not present QST abnormalities (Maier et al., 2010).

An observation in routine clinical QST testing by the supervisor of this thesis (A.S.C. Rice) was that patients with HIV-SN appeared to express higher pain on the routine testing of heat pain thresholds (HPT). The heat suprathreshold testing protocol was designed to detect the presence of thermal hyperpathia in HIV-SN participants. No statistically significant difference was seen between the three groups (Healthy volunteers, HIV-No SN and HIV-SN) at any of the temperatures tested. The HC and HIV-No SN groups did have early takes offs of the VAS/ temperature stimulus response curves (see Figure 3.5), but at higher temperatures little difference was seen between the groups. A similar finding was seen in an earlier study, which employed an analogous heat suprathreshold protocol during the pre cART era (Bouhassira et al., 1999).

This current study found no evidence of gain of function features of neuropathic pain for dynamic mechanical allodynia (DMA) or static mechanical hyperalgesia (MPS), but did find a small group (14%) of HIV-SN participants who demonstrated high wind-up ratios (WUR) to pin-prick stimuli (discussed below). In contrast, the group of Bouhassira demonstrated mechanical hyperalgesia in HIV-SN (Bouhassira et al., 1999), however, the current study did not replicate this finding. There are two potential reasons for this: firstly the DFNS QST protocol tests for mechanical hyperalgesia uses a method that is different to Bouhassira's group; secondly, and more fundamentally, the HIV-SN participants examined in Bouhassira's study were from the pre-cART era. It has been demonstrated that different risk factors for the development of HIV-SN operated in the pre-cART era to present day (e.g. CD4 counts, d NRTI drug exposure; Catherine Louise Cherry et al., 2012), consequently the HIV-SN phenotype might be substantially different now in the c-ART era.

Simpson and collaborators found a raised pin-prick punctate hyperalgesia present in 13% of HIV-SN participants screened for a large pregabalin efficacy RCT (D M Simpson, Scott, et al., 2010). This study measured the pain response to a single (non-standardised) strength pin-prick stimulus to measure pin-prick hyperalgesia. In contrast, the DFNS protocol uses a stimulus pain response curve to different (standardised strength) pin-prick stimuli, consequently comparing the two measures is difficult. However one of the tests used in the DFNS QST protocol does exam the pain response to a single strength pin-prick applied multiple times to exam for the presence of wind-up phenomenon; and whilst not strictly the same, this is perhaps a more comparable test to the one used by Simpson and collaborators. Interestingly the current study's sub-group of raised WUR in 14% subjects (n=4) in painful HIV-SN participants, is of similar proportion to the pin-prick hyperalgesia sub-group seen in Simpson and collaborators' work (13%) and therefore the raised WUR sub-group might represent the same pin-prick hyperalgesia sub-group seen in the Simpson and collaborators' study. The pin-prick hyperalgesia group of the Simpson and collaborators' study is important as this group demonstrated a greater therapeutic response to pregabalin. Future studies would need to determine if these two findings indeed represent the same sub-group, as it would potentially have important mechanistic and therapeutic implications for HIV-SN and neuropathic pain *per se*.

As higher levels of catastrophization have been previously shown to correlate with the presence of wind-up phenomenon (Vase et al., 2011), a *post-hoc* analysis of catastrophization was undertaken for this high wind-up sub-group. It was found that this sub-group of participants demonstrated higher levels of catastrophization (PCS total=26.0 (IQR 22.5 - 31.5) *vs* the other participants, PCS total=2.0 (IQR 0 - 22.0); $p < 0.05$; Mann Whitney Rank Sum Test). A correlation between raised wind-up phenomena and catastophising traits has previously been seen in a study of an upper-limb amputees with phantom limb pain, but this is, to our knowledge, the first time this has been shown in a painful peripheral neuropathy population. This is additionally interesting as it shows that wind-up and catastrophizing are associated in two very different forms of neuropathic pain, perhaps showing a common mechanism of interaction between spinal pain processing (wind-up) and higher centres (catastrophizing) in different types of neuropathic pain.

The hierarchical cluster dendrogram of QST data (Figure 3.6) shows that the majority of the participants divide at a high level broadly into HIV-SN and HIV-No SN: 71% of all HIV-SN participants are contained within a cluster of 25 subjects. The hierarchical cluster analysis shows that HIV-SN neuropathy participants tend to cluster together. Interestingly participants with HIV-SN but no pain (n=7) were more evenly distributed and did not appear to associate with the HIV-SN dominant cluster or each other. This suggests that non-painful HIV-SN perhaps have different QST characteristics to painful HIV-SN. Whilst its difficult to say that this is a robust finding as the study was not powered primarily for such a hierarchical cluster analysis, the differences between painful and non-painful HIV-SN seen here suggest that this should be investigated by future larger studies.

Intra-epidermal nerve fibre densities

Mean IENFD counts were significantly lower in the HIV-SN group (9.2 fibres/mm *vs* 6.3fibres/mm), and the severity of neuropathy was inversely correlated to IENFD. However the diagnostic utility of IENFD counts <7.63 fibres/mm can only be considered moderate at best with a sensitivity and specificity of 61% and 79% in isolation of detecting HIV-SN (Table 3.10). Consistent with several other studies, no convincing correlation was found between IENFD and sensory nerve fibre dysfunction

for individual QST parameters (Table 3.5), even in the small fibre modalities of CDT, WDT, HPT, MPT and PPT (Cruccu et al., 2010).

Metabolic factors

This study found significantly higher random plasma TRG concentrations in the HIV-SN group compared to the HIV-No SN group. Whilst no correlation was identified between TRG concentrations and the neuropathy severity, this finding further underlines the emerging understanding in the literature that dyslipidaemia is important in the development of HIV-SN (Ances et al., 2009; Banerjee et al., 2011) as well as diabetic peripheral neuropathy (Vincent, Hinder, Pop-busui, & Feldman, 2009; Wiggin et al., 2009). In this study there was no difference in statin use between the two groups, however, an important future question is if more effective or earlier suppression of raised TRGs might reduce the risk or delay the progression of HIV-SN development. The role of other metabolic factors in this study is less clear, as no differences were seen in BMI, random plasma glucose, waist-hip circumferences or other plasma lipids between the HIV-SN and HIV-No SN groups.

Psychological co-morbidity and sleep disturbance

Participants with painful HIV-SN show significant interference from pain on the BPI instrument and had significantly reduced quality of life compared to participants without neuropathy across most of the domains of the SF-36. The largest differences seen between the two groups were in domains of physical functioning (PF) and social functioning (SF); indicating that painful HIV-SN profoundly interferes with everyday tasks, social interaction and relationships. This is comparable to changes seen in other painful peripheral neuropathies, however, HIV-SN appear to have lower mean scores across all domains implying greater disability (Poliakov & Toth, 2011). Compared to other painful peripheral neuropathies, a difference was seen in participants perception of general health (GH; HIV-SN= 26.58, SD 19.8 *vs* HIV-No SN=50.3, SD 10.7) This low general health perception has been previously identified as characteristic of individuals with HIV infection (Drewes, Gusy, & Rüden, 2013), but appears to be further reduced by HIV-SN. Interestingly bodily pain (BP) perception values were very comparable to other painful peripheral neuropathies that did not include HIV-SN, this is despite HIV-SN participants having a higher mean VAS (HIV-SN=5.65, SD 1.76 *vs* HIV-No SN=4.6, SD 2.4) to that study.

Pain related anxiety symptoms measured using the PASS-20 were similar across most domains to that previously seen in a recent study using the same instrument comparing lower back pain (LBP) and post-herpetic neuralgia (PHN) patients (Daniel et al., 2008). However HIV-SN patients would appear to be less likely to have escape/avoidance behaviour in response to pain than either PHN or LBP patients.

Whilst depression and anxiety symptoms were more common in the HIV-SN group, a greater difference was seen in catastrophizing behaviours in the HIV-SN compared to those who did not suffer with a painful neuropathy. The majority of this difference was seen in the PCS helplessness domain. This may well be a reflection of the relatively low levels of less escape/avoidance behaviour seen in HIV-SN compared to other pain states (Daniel et al., 2008; Poliakov & Toth, 2011).

There have been numerous sleep laboratory and self report studies examining insomnia in HIV infected individuals (Reid & Dwyer, 2005). However the current significance of insomnia in HIV-infection is unclear, as its reported prevalence ranges from the same as the general population (D. Perkins et al., 1995) to 73% of HIV infected individuals (Rubinstein & Selwyn, 1998). Early pre-cART era studies suggested that CD4 count (Brown, Mitler, & Atkinson, 1991), cognitive impairment (Rubinstein & Selwyn, 1998) and ARV therapy (Nokes & Kendrew, 2001) were important risk factors for insomnia in HIV positive patients. More recent studies continue to demonstrate insomnia in HIV positive individuals, but do not correlate as convincingly with earlier risk factors (Cruess et al., 2003; Lee, Portillo, & Miramontes, 2001). Few studies have discussed the role of pain or neuropathy in HIV-associated insomnia. In the present study we have shown a large difference in the incidence of insomnia between the patients with HIV-SN (68%) and those without (23%; $p < 0.001$). As it is established that other forms of neuropathic pain have a high co-incidence of insomnia (Langley, Van Litsenburg, Cappelleri, & Carroll, 2013), the role neuropathic pain and insomnia in HIV-SN would appear to be a useful area to explore in future studies.

The PINS-HIV study demonstrates that the presence of painful HIV-SN has a marked impact on individuals' quality of life as measured by the SF-36, DAPOS, ISI and BPI. In particular participants with painful HIV-SN have reduced vitality, social functioning, emotional functioning.

If time had allowed I would have liked to have performed a more detailed analysis of the psychological outcomes to see if there was indeed any clustering of certain psychological attributes or dysfunction that could be corresponded to severity of pain or neuropathy. The presence of high catastrophising scores (PCS) within the high WUR group supports the idea that perhaps testing for high WUR groups through the use of simple psychological testing is possible. It is clear that further exploration of this area would be useful.

Diagnostic tools

The use of a robust composite HIV-SN definition is useful for research purposes, but would prove too time consuming and require expensive diagnostic equipment for routine use in most clinical practices. This is especially so in poorly resourced settings. Part of the objectives of this study was to investigate the utility of neuropathy screening tools that might be useful in such situations. Therefore in this study the BPNS, TCSS and the UENS were compared.

The BPNS has been used as the screening tool for several HIV-SN studies (C L Cherry et al., 2009; Catherine L Cherry et al., 2008; Smyth et al., 2007; Antonia L Wadley et al., 2011). Whilst its utility is clear, we found that both, the TCSS and the UENS were superior in the diagnosis of HIV-SN in our population in a head-to-head comparison against this study's triumvirate diagnostic criteria. The UENS has been used in a proportion of one study of HIV-SN where it showed correlation with measures of cutaneous autonomic function and pain (Boger et al., 2012). This current study is, to our knowledge, the first validation of the UENS and TCSS in HIV-SN diagnosis.

The original validation study for the UENS did not suggest a cut-off score for determining the presence of a peripheral neuropathy (Singleton et al., 2008). However, in the present study, giving equal weight to sensitivity and specificity, a cut off value of 7 was selected. In that studies population it is associated with a sensitivity and specificity of 84%. The utility of the UENS was superior to both the TCSS (79%) and the BPNS (76%) in detecting HIV-SN when tested against the triumvirate multimodal scoring system. The utility of the BPNS as determined in this population is similar to that seen in previous larger studies (D M Simpson et al., 2006) and underlines that this

relatively small study population behaved as expected, and also that the triumvirate definition used as a gold standard in the study was a valid one.

Epidemiological studies

Using the triumvirate criteria definition for HIV-SN, in this study, HIV-SN showed a prevalence of 43% with 75% of these participants suffering with a painful neuropathy. This is very similar to that seen in recent large epidemiological studies of HIV populations in both well and poor resourced countries (Catherine Louise Cherry et al., 2012). In the cART era HIV-SN prevalence is consistently found to be approximately 40% in many different HIV populations (Wenlong Huang et al., 2013; Kamerman, Moss, et al., 2012; Maratou et al., 2009; Wallace, Blackbeard, Pheby, et al., 2007; Wallace, Blackbeard, Segerdahl, et al., 2007b). This reinforces the face validity of the triumvirate criteria used in this work and makes it likely that this study's findings would be applicable to larger HIV populations and in different settings. However, this study did not find important some of the well-established risk factors for the development of HIV-SN in the cART era in this study population. In particular, no significant difference or correlation with HIV-SN severity was seen between the HIV-SN and HIV-No SN group for height and age. The reasons for these are probably two-fold: Firstly, the study reflects the studied population, which is generally white, middle aged, male and tall and therefore, it would be difficult for this study to elucidate risk factors that have been found in much more heterogeneous epidemiological studies. Secondly, the current study was powered as a detailed phenotyping study and was not large enough to elucidate these risk factors compared to substantially larger epidemiological studies (C L Cherry et al., 2009; Ellis et al., 2010; Maritz et al., 2010; Smyth et al., 2007; Wright et al., 2008).

Summary

This study has demonstrated that the sensory nerve dysfunction that occurs in HIV-SN is heterogenic as measured with QST. The most frequent changes seen in QST where loss of function in the large fibre parameters of mechanical and vibration detection. This further emphasises the large sensory nerve fibre involvement that occurs in HIV-SN despite it frequently being considered a small fibre neuropathy in the literature. The diagnostic utility and limitation of QST, IENFD and clinical examination in isolation

has been demonstrated. This study has validated the use of UENS and TCSS in the diagnosis of HIV-SN both of which were found to be superior in HIV-SN diagnosis in this study population. This study also further supported the growing evidence that lipid dyslipidaemia is an important factor in the development of HIV-SN. Moreover, painful HIV-SN also appears to have a greater negative impact on quality of life when compared to other painful polyneuropathies, post-herpetic neuralgia and lower back pain.

Future work should determine the triumvirate diagnostic criteria's validity and utility in other HIV populations. Detailed examination of individual components of diagnostic tools should be investigated as there is an urgent need to produce a simple and robust diagnostic tool for both large epidemiological studies in low resource environments and for the use in future therapeutic efficacy trials.

Study limitations

A potential criticism of the study is that after the start of study recruitment, the joint EFNS/ PNS guidelines on the use of skin biopsies for the diagnosis of small fibre neuropathies was updated (Cruccu et al., 2010) to recommend the use of sex and age matched healthy control data (Bakkers et al., 2009). However, Nebuchennykh and collaborators identified that the method used in determining IENFD cut-off values is very important to the IENFD test's final sensitivity and specificity (Nebuchennykh et al., 2009). In this study, comparisons were made using less than fifth-percentile values, a ROC analysis or using fifth-percentile of age and decade matched normal values (z-scores) as "cut-off" values as recommended by the EFNS/PNS 2010 guidelines. Age and sex adjusted cut-off values produced the best specificity (98%) but resulted in much poorer sensitivity (31%); using a ROC analysis with a higher "cut-off" value of 10.3 fibres/mm produced improved sensitivity (78%) but resulted in a poorer specificity (64%). The lower "cut-off" value of 7.63 fibres/mm proposed by Devigili and collaborators (Devigili et al., 2008) is associated with a specificity of 90% and a sensitivity of 80% and represents a compromise between the z-score and ROC methods of Nebuchennykh and collaborators' study.

My opinion was that the use of age and sex matched "cut-offs" would improve specificity from 90 to 98%, but sensitivity would be excessively reduced to

approximately 30% and this was not acceptable for the aims of the current study. Consequently, 7.63 fibres/mm continued to be used as the cut-off value in the triumvirate HIV-SN definition.

To explore the impact of using the newer guidelines on IENFD use a *post-hoc* analysis of using the EFNS/PNS 2010 guideline age and sex matched values was performed using the recommended normative values (Bakkers et al., 2009) in conjunction with this study's diagnostic triumvirate. This analysis demonstrated no change in the participants originally allocated to HIV-No SN group, however five participants (7.6%) previously allocated to HIV-SN group were now allocated to HIV-No SN. This was felt to be acceptable as the difference is proportional to that which would be expected using this more conservative definition of HIV-SN. Additionally, 3 of the 5 (60%) participants who's diagnosis was altered by the re-analysis to HIV-No SN complained of a painful peripheral neuropathy, confirming that the diagnosis of HIV-SN was indeed the correct one for these participants.

A further potential limitation of the study is that several items of the multimodal triumvirate definition for HIV-SN are compared and contrasted between the HIV-No SN group and the HIV-SN group. There is therefore potential for a circular argument relating to the QST and IENFD findings of this study; however this is minimal, as a *post-hoc* re-analysis using different combinations of the triumvirate (Table 3.10) shows that the neuropathy diagnosis of individual participants was not dramatically altered by the removal of individual items from the definition. Removal of QST resulted in 12% of the study participants having an altered diagnosis, and IENFD only 9%. The single most important factor of the definition was found to be the SNE which when removed from the definition altered the diagnosis of 26% of the participants.

Further post-hoc exploration of using different HIV-SN definitions on QST results

A *post-hoc* exploration of the impact of the HIV-SN case definition on one of the main outcome measures of the study, QST evaluation was undertaken.

Three additional different case definitions were used:

1. Using the BPNS definition of HIV-SN alone to determine neuropathy status.
2. Using the Structured Neurological Examination (SNE) alone to determine neuropathy status.

3. Using only the reported symptoms of paraesthesia and/or numbness to determine neuropathy status.

BPNS

The BPNS was chosen for further exploration, as it is a well-established screening tool for HIV-SN that has been used in many previous studies of HIV-SN. Using the BPNS to determine HIV-SN status resulted in similar proportions to that when using PINS-HIV criteria: n=37 for HIV-No SN and n=29 for HIV-SN (Table 3.11 and Figure 3.2.b). However there are larger differences in individual neuropathy allocations: eight individual allocations are changed from HIV-No SN to HIV-SN, and seven from HIV-SN to HIV-No SN. The mean z score values are similar for HIV-SN and HIV-No SN however the proportion of *individuals* demonstrating loss and gain of sensory function on QST is different. Interestingly the high WUR 'cluster' seen with the PINS criteria is larger using the BPNS (five/six with BPNS versus four participants with PINS). The high WUR cluster also appears more separated from the lower WUR HIV-SN group. Whilst the overall conclusions regarding QST changes seen in HIV-SN would be similar

The finding that using the BPNS as a HIV-SN criteria produces similar results, and demonstrates a similar interesting feature of a cluster of HIV-SN participants with raised WUR is supportive of the findings seen in the PINS study, as it demonstrates that this novel high WUR is not a result of the new HIV-SN definition used.

Structured Neurological Examination (SNE)

The use of the SNE tended to 'over' diagnose HIV-SN in comparison to the PINS criteria, with ten more subjects being allocated to HIV-SN compared to PINS; n=38 for HIV-SN and n=28 for HIV-No SN. The allocation of individuals with HIV-SN however resulted in a smaller cluster of high WUR in the HIV-SN.

Paraesthesia and numbness symptoms

Using self reported symptoms of peripheral neuropathy (paraesthesia and /or numbness symptoms) tended to over diagnose HIV-SN compared to using the PINS criteria. HIV-SN n=43 and HIV-No SN n=23. Despite a larger group of HIV-SN the high WUR remained similar with four participants present.

Pain

Using pain as the definition of HIV-SN interestingly produced identical proportions of patients allocated to HIV-SN and HIV-No SN; however seven participants were reallocated from HIV-SN to No SN and seven from HIV-No SN to HIV-SN so the situation is not identical. Once again similar mean z score values were seen and a similar high WUR cluster in HIV-SN group

The overall QST findings for each of the different criteria tested are similar to using the PINS criteria: i.e. a 'loss' of sensory function dominated picture, with the most affected modalities being MDT and VDT in the HIV-SN groups (Table 3.11 and 3.12). More affected by the changes of definition however is the detail of the number of individuals who show loss and gain in areas of interest such as WUR. The relatively small number of participants in the PINS-HIV study probably heightens this sensitivity to the definition of HIV-SN.

Figure 3.1. Scatter plot of z scores for QST parameters in the Healthy control, HIV-No SN and HIV-SN groups using the PINS-HIV triumvirate criteria. *Kruskal-Wallis, Dunn's post-hoc one way analysis of variance: * p <0.05, NS = p > 0.05.

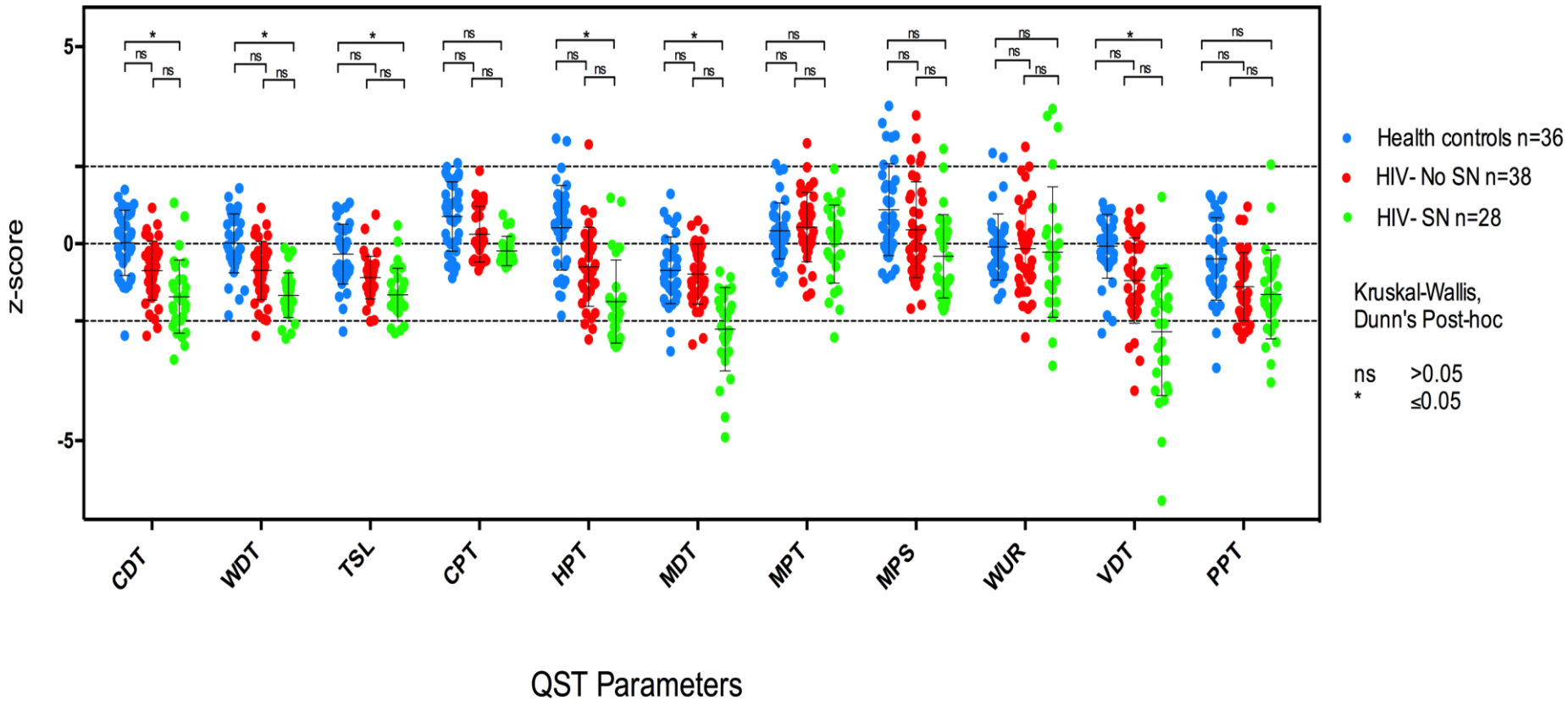


Figure 3.2.a. Scatter plot of z scores for QST parameters in the Healthy control, HIV-No SN and HIV-SN groups using an alternative definition for HIV-SN using the BPNS only. *Kruskal-Wallis, Dunn's post-hoc one way analysis of variance: * $p < 0.05$, NS = $p > 0.05$

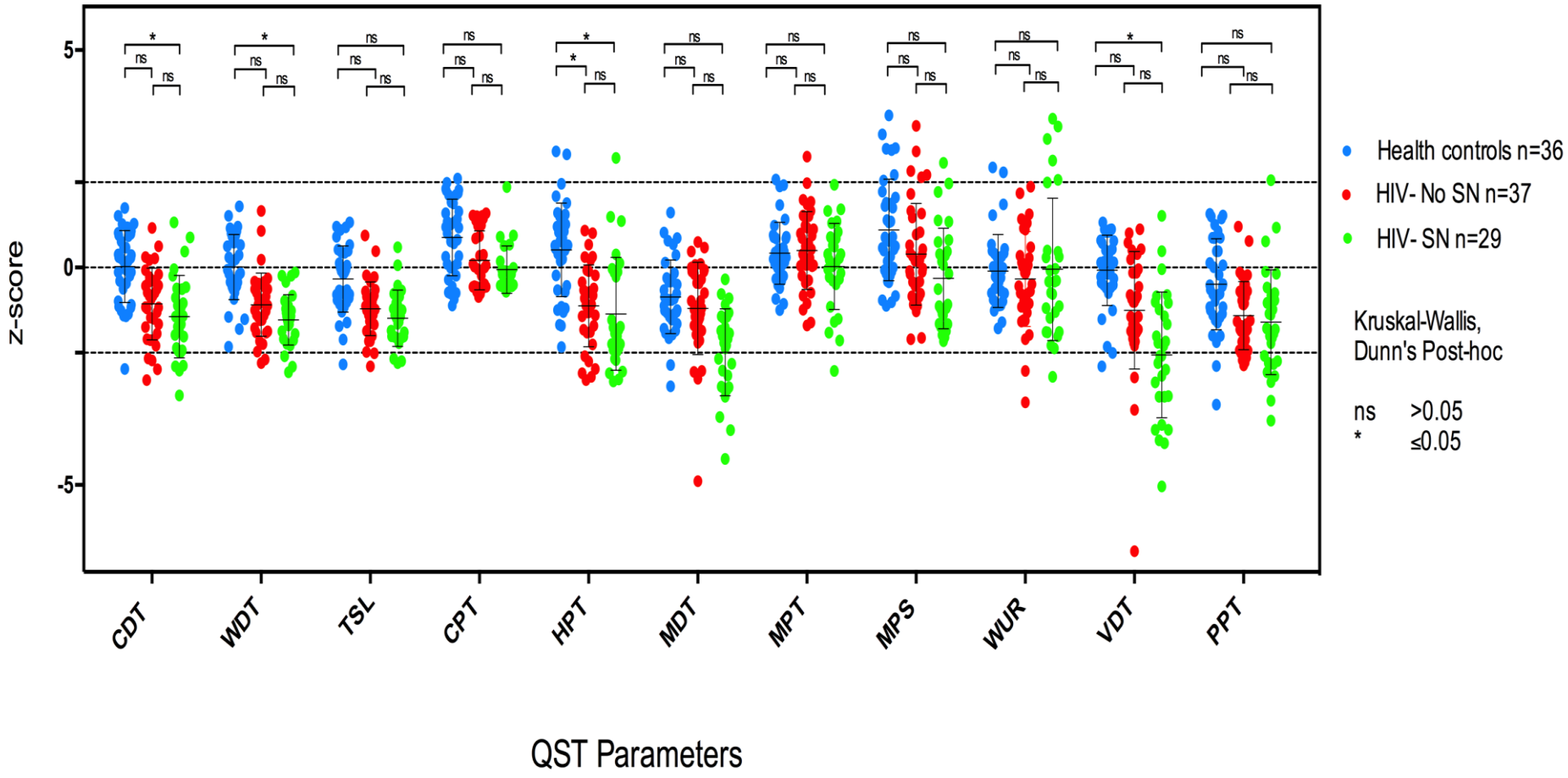


Figure 3.2.b. Scatter plot of z scores for QST parameters in the Healthy control, HIV-No SN and HIV-SN groups using an alternative definition for HIV-SN using Structured Neurological Examination (SNE) only. *Kruskal-Wallis, Dunn's post-hoc one way analysis of variance: * $p < 0.05$, NS = $p > 0.05$

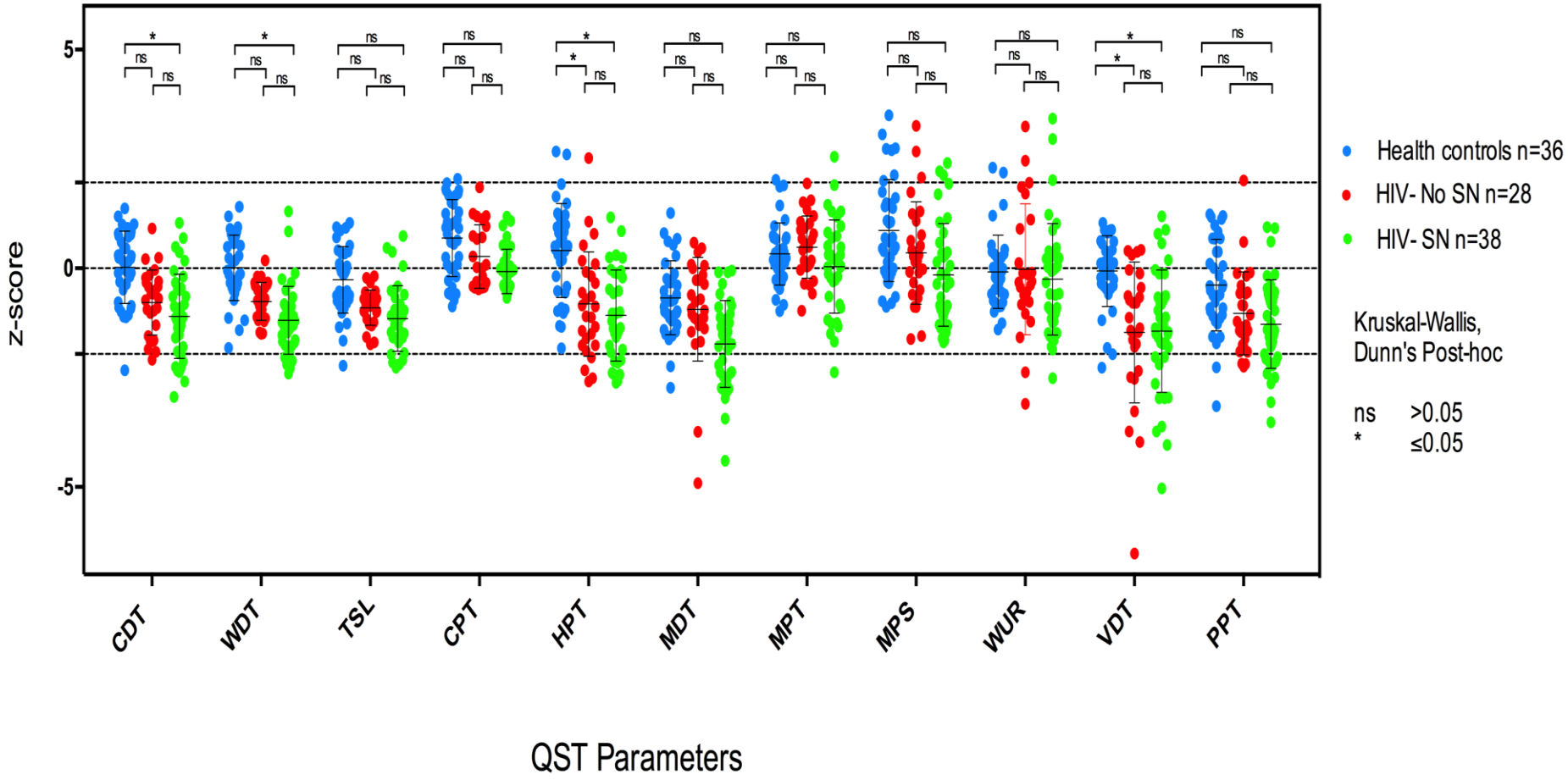


Figure 3.2.c. Scatter plot of z scores for QST parameters in the Healthy control, HIV-No SN and HIV-SN groups using an alternative definition for HIV-SN using paraesthesia and numbness symptoms only. *Kruskal-Wallis, Dunn's post-hoc one way analysis of variance: * p <0.05, NS = p > 0.05

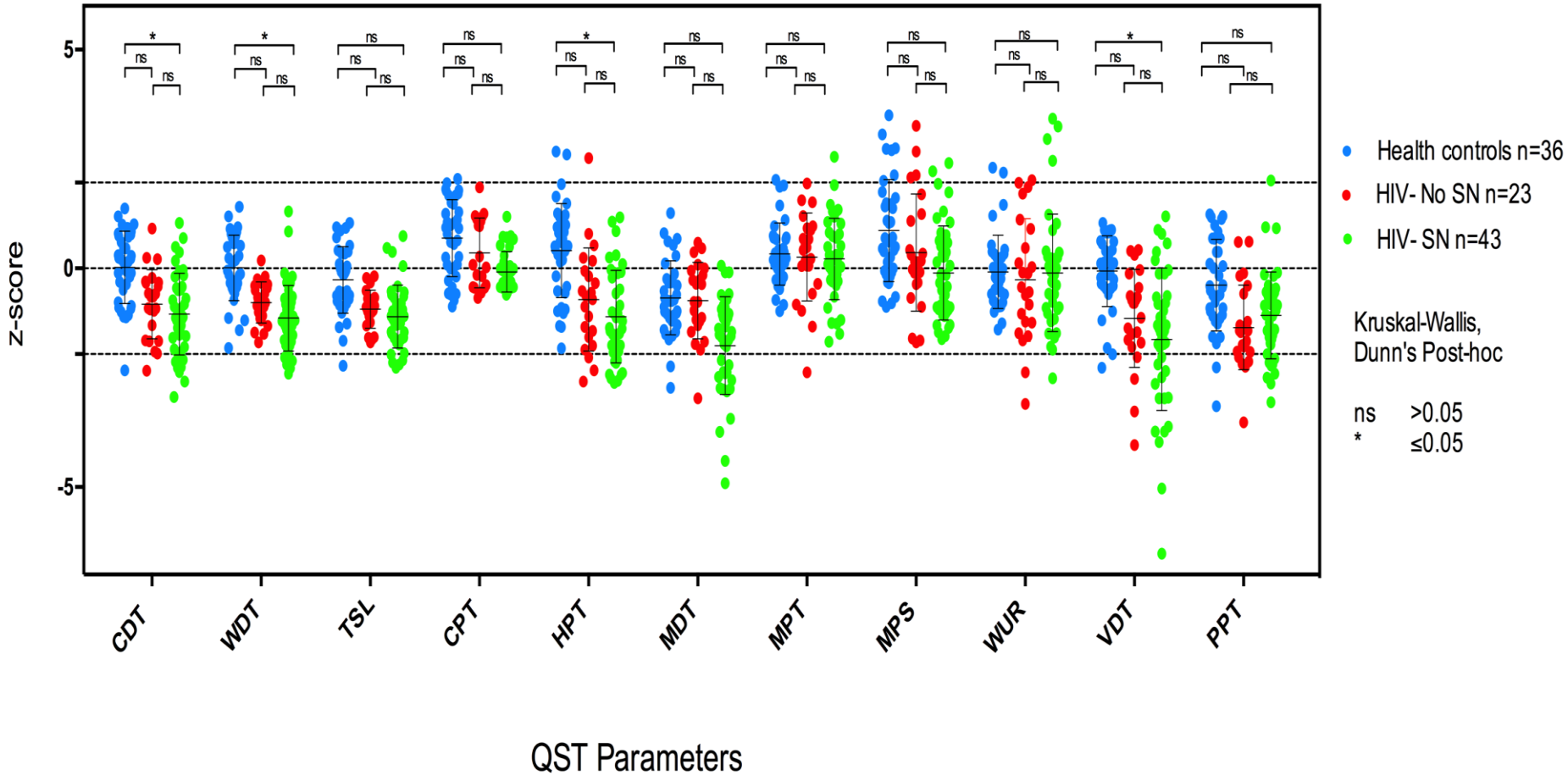


Figure 3.2.d. Scatter plot of z scores for QST parameters in the Healthy control, HIV-No SN and HIV-SN groups using an alternative definition for HIV-SN using pain symptoms only. *Kruskal-Wallis, Dunn's post-hoc one way analysis of variance: * p <0.05, NS = p > 0.05

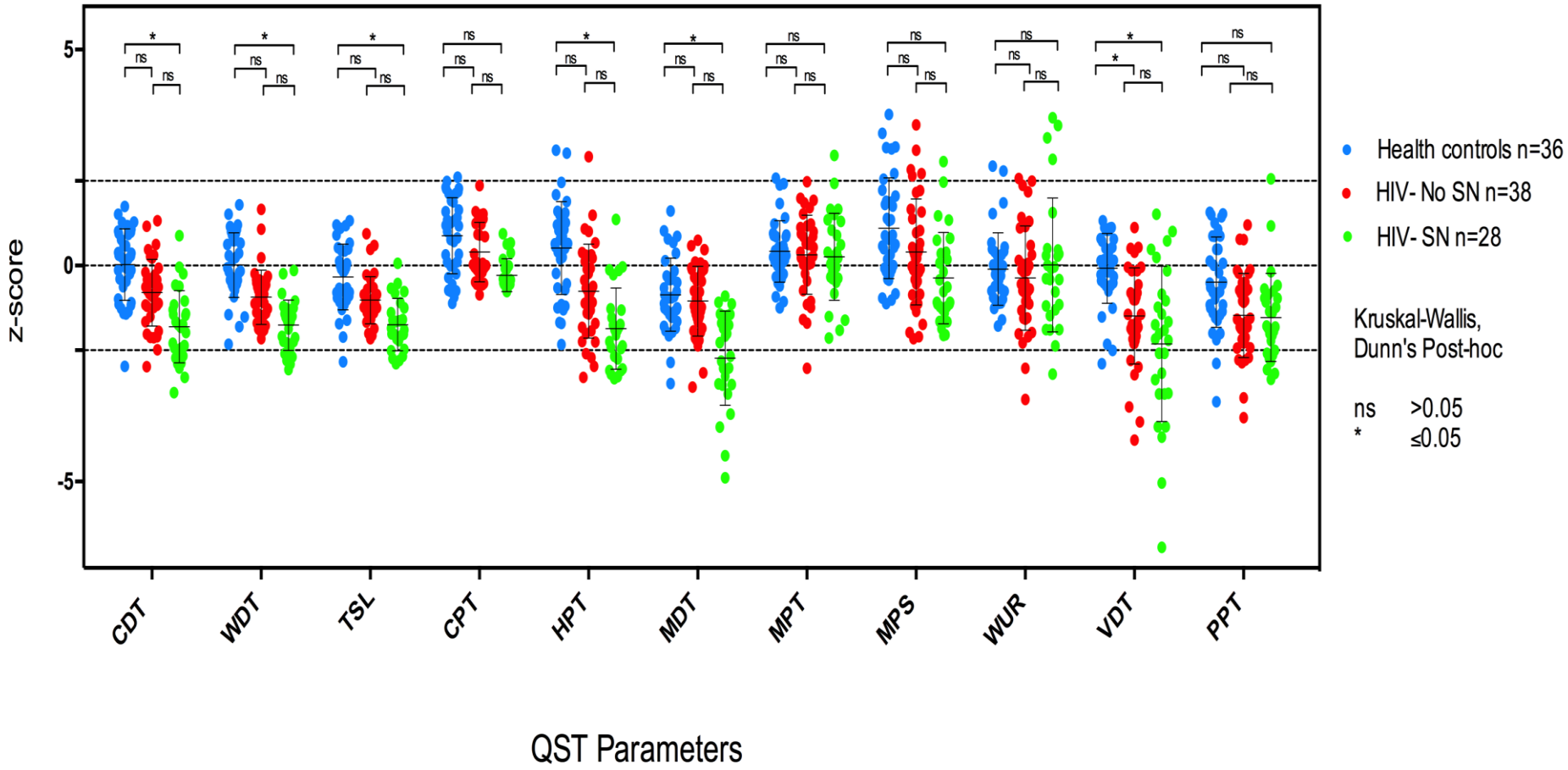


Figure 3.3. (i): Comparison of mean QST z-scores (SD) of the HIV-SN, No-HIV-SN and Healthy control groups. (ii): Occurrence of paradoxical heat sensation (PHS) and dynamic mechanical allodynia (DMA). Cold detection threshold (CDT), warm detection threshold (WDT), thermal sensory limen (TSL), cold pain threshold (CPT), heat pain threshold (HPT), pressure pain threshold (PPT), mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), wind-up ratio (WUR), mechanical detection thresholds (MDT), vibration detection threshold (VDT) and numerical rating scale (NRS). * $p<0.05$ Threeway ANOVA (Holm-Sidak).

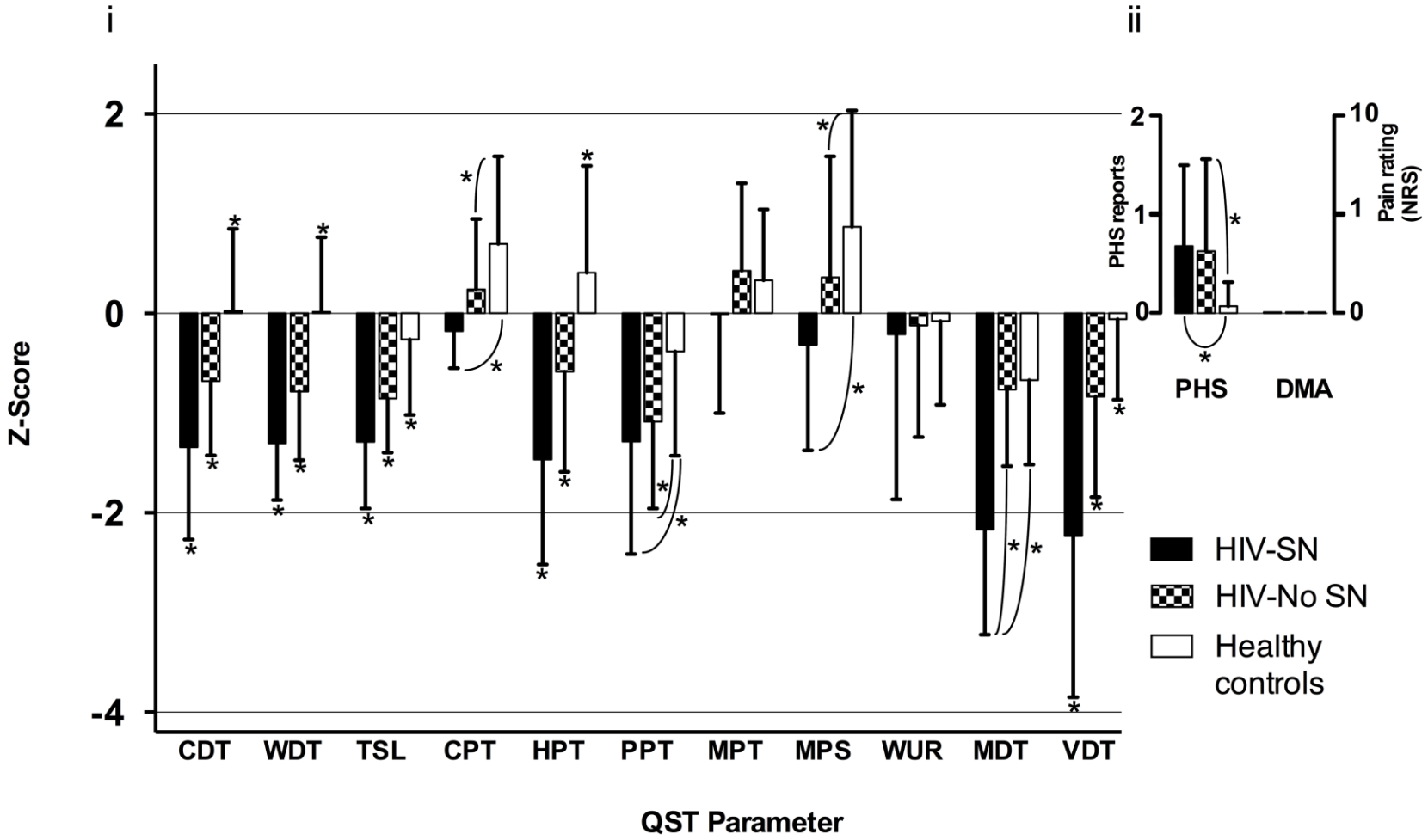


Figure 3.4. Loss and Gain of sensory function: A comparison of the frequency of QST abnormalities in participants in the Healthy control, HIV-No SN and HIV-SN groups which have QST values outside the 95% CI of the DFNS reference database. The *y*-axis shows the percentage of patients in each group (HIV-SN *n*=38, No HIV-SN *n*=28 and Healthy controls n=36) with 'gain' of sensory function plotted upwards, and 'loss' of sensory function plotted downwards. Chi squared test of association: * P < 0.05, ** P < 0.01 comparison to Healthy controls; † P < 0.05, ††P < 0.01 comparison to HIV-No SN.

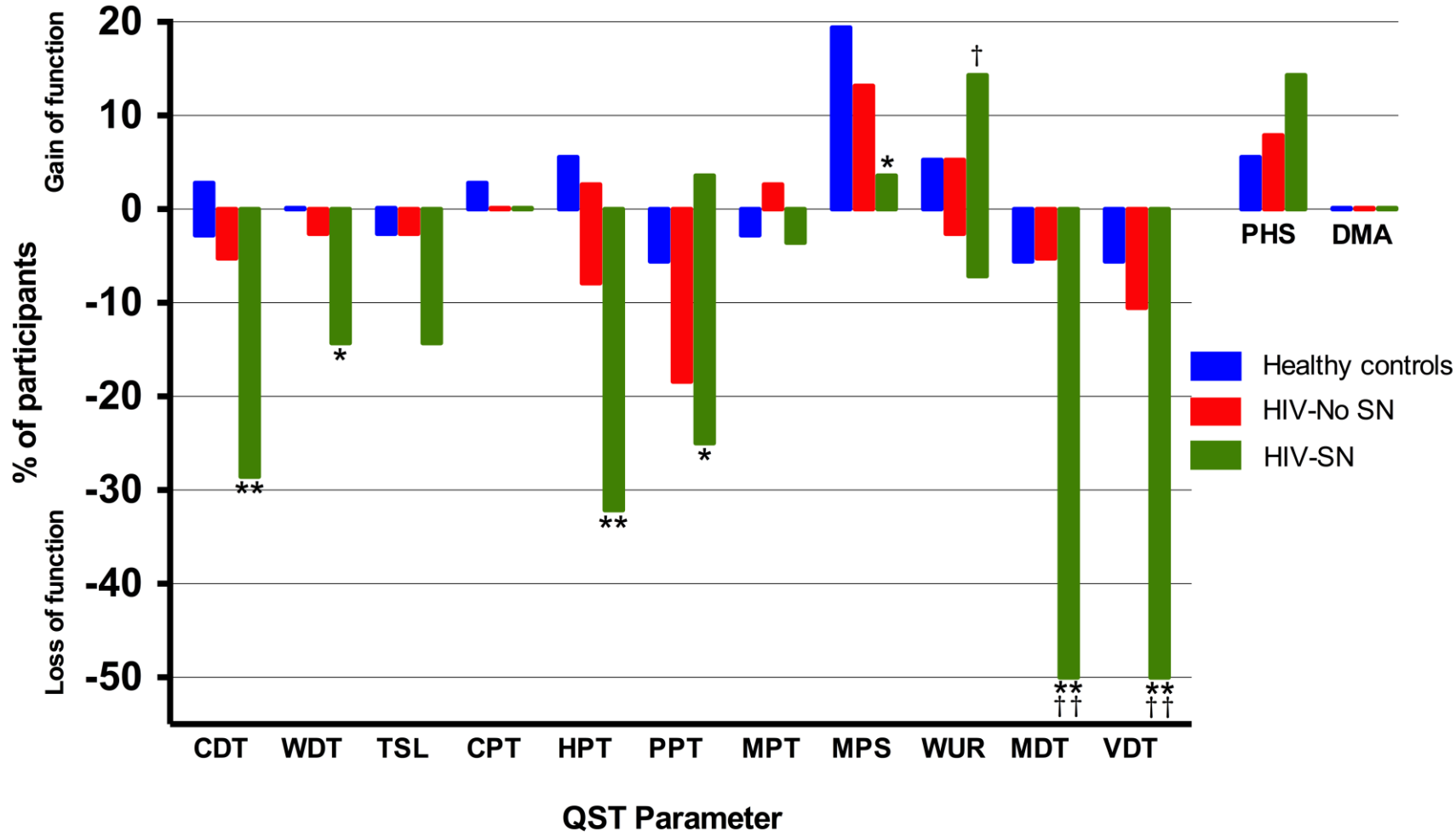


Figure 3.5. Pain intensity response curves for suprathreshold heat stimuli in Healthy control subjects (*n*=36), HIV-No SN group (*n*=38) and HIV-SN (*n*=28) group. Data are presented as mean (SD). No statistically significant difference present between groups.

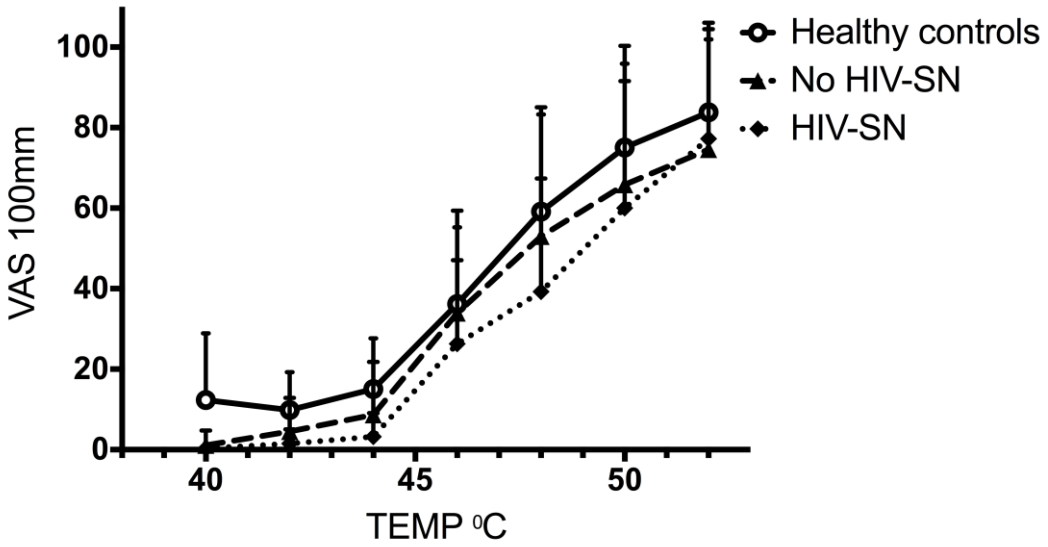


Figure 3.6. Hierarchical cluster analysis of participants' peripheral neuropathy and pain status defined by participants QST data. Individual patients are identified as circles at the bottom of the dendrogram; Open circles: HIV-No SN, Partly shaded circles: HIV-SN with no pain symptoms; Solid circles: HIV-SN with pain symptoms. Horizontal lines represent similarities between participants and vertical lines represent differences. The two groups identified at the highest separation threshold (arrow) accounted for the majority QST differences seen between the HIV-SN and HIV-No SN groups.

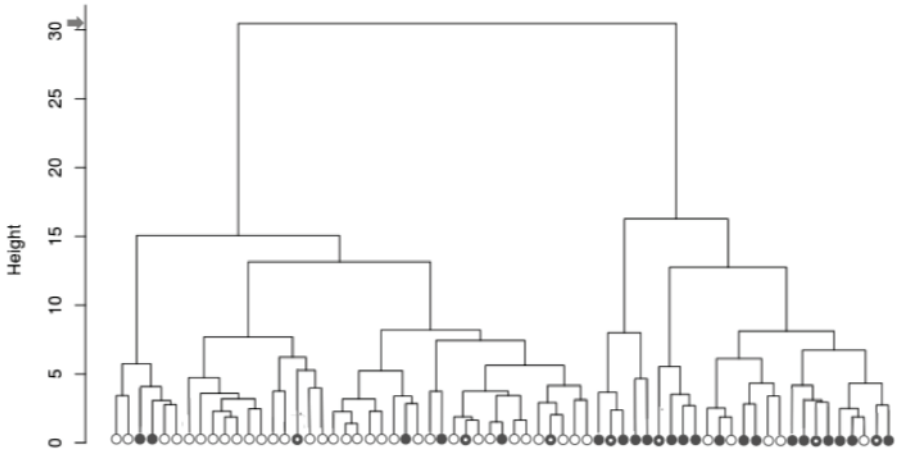


Figure 3.7. Two representative skin biopsy samples of HIV infected participants.

A: A participant from the 'HIV-SN' peripheral neuropathy group demonstrating complete absence of small unmyelinated sensory nerve fibres reaching the epidermis. Sub-epidermal dermal plexus fibres are present (blue arrow) as identified with the pan neuronal marker PGP 9.5.

B: A participant from the 'HIV-No SN' group with normal counts of small unmyelinated nerve fibres (black arrows) reaching the epidermis beyond the dermal epidermal junction (red dotted line), and positive dermal plexus staining (blue arrows). Objective 40X. Scale bar: 50μm

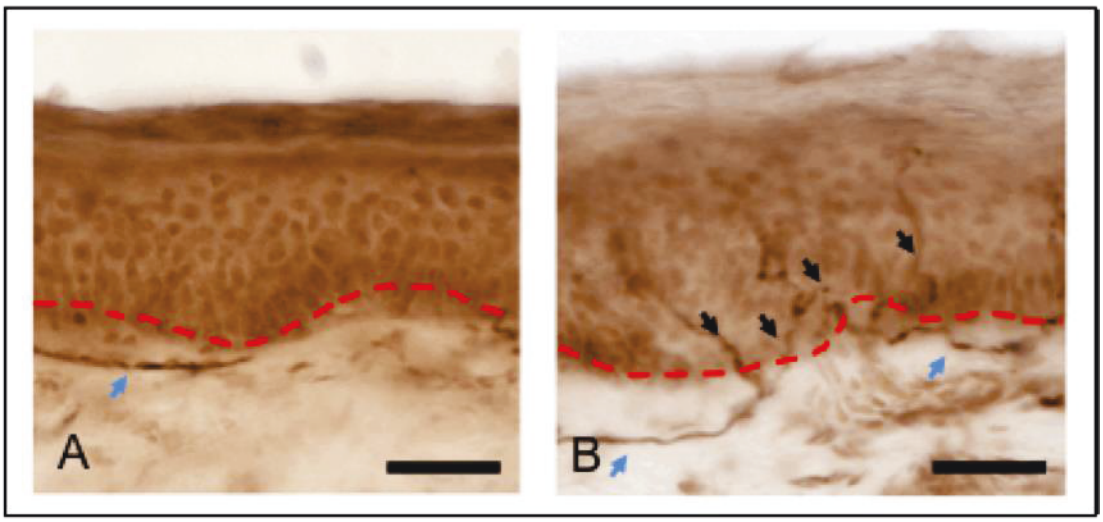


Table 3.1. Comparison of participants' demographics, characteristics and co-morbidities between HIV-No SN and HIV-SN groups enrolled in the study. *Continuous data if normally distributed were analysed with Student t test. Means and SD values are shown. ** No participants with Type I Diabetes. †Categorical data were analysed using ^{2} association. Values and percentages shown.

	HIV-No SN n=38			**HIV-SN n=28**		**p value**
Mean age yrs (SD)	47.69	(8.87)		51.32	(8.36)	0.097*
Male (%)	32	(84.21)		25	(89.28)	0.553§
Height cm (SD)	175.08	(8.82)		177.14	(7.76)	0.321*
Weight kg (SD)	77.11	(15.08)		80.50	(12.19)	0.334*
Years since HIV diagnosis (SD)	14.71	(7.79)		17.79	(7.02)	0.094*
Ethnicity						
White European (%)	33	(86.84)		24	(85.71)	0.553†
African Origin (%)	4	(10.53)		3	(10.71)	0.553†
Asian (%)	1	(2.63)		0	(0)	0.553†
Mixed ethnicity (%)	0	(0)		1	(3.57)	0.553†
Co-morbidities						
Type II Diabetes**	3	(7.89)		5	(17.86)	0.220†
Hepatitis C infection	9	(23.68)		6	(21.43)	0.829†
Hepatitis B infection	3	(7.89)		5	(17.86)	0.220†
Syphilis	6	(15.79)		4	(14.29)	0.497†
Chemotherapy exposure	1	(2.63)		3	(10.71)	0.174†

Table 3.2. Comparison of antiretroviral drug therapy use between HIV-No SN and HIV-SN groups. ARV - Antiretroviral drugs, dNRTIs - deoxy-Nucleoside Reverse Transcriptase Inhibitors, NNRTI – Non Nucleoside Reverse-transcriptase Inhibitor. * All NRTIs including 'd' NRTIs **Continuous data if normally distributed were analysed with Student *t* test. Means and SDs shown. §Continuous data not normally distributed were analysed using Mann-Whitney Rank Sum test. †Categorical data were analysed using ^{2} test of association. Values with percentages shown.

	HIV-No SN n=38			**HIV-SN n=28**		**p value**
On ARV therapy (%)	36	(94.74)		28	(100.00)	0.218†
Mean yrs on ARV (SD)	9.90	(6.43)		11.77	(5.90)	0.361§
'd' NRTIs drugs						
Ever used d NRTIs (%)	20	(52.63)		20	(71.43)	0.122†
Mean yrs on 'd' NRTIs (SD)	12.89	(2.32)		12.63	(3.72)	0.989§
Ever used zalcitabine (ddC) (%)	4	(10.53)		6	(21.43)	0.222†
Mean yrs on zalcitabine (ddC) (SD)	14.90	(1.68)		16.54	(1.50)	0.142**
Ever used stavudine (d4T) (%)	18	(47.37)		17	(60.71)	0.283†
Mean yrs on stavudine (d4T) (SD)	12.02	(1.40)		12.31	(1.77)	0.577**
Ever used didanosine (ddI) (%)	12	(31.58)		14	(50.00)	0.130†
Mean yrs on didanosine (ddI) (SD)	11.49	(2.84)		10.81	(3.35)	0.579**
NRTIs*						
Ever used NRTIs(%)	36	(94.74)		27	(96.43)	0.744†
Mean yrs on NRTIs (SD)	9.15	(6.45)		11.99	(4.99)	0.053**
NNRTIs						
Ever used NNRTIs (%)	28	(73.68)		25	(92.59)	0.115†
Mean yrs on NNRTIs (SD)	8.17	(6.53)		10.53	(6.53)	0.192**
Protease Inhibitor drugs						
Ever used PI drug (%)	21	(55.26)		21	(75.00)	0.099†
Mean yrs on protease inhibitor drugs (SD)	21.56	(14.02)		28.55	(21.10)	0.687§
Ever used indinavir (IDV) (%)	5	(13.16)		9	(32.14)	0.062†
Mean yrs on indinavir (IDV) (SD)	13.28	(0.68)		12.64	(1.15)	0.280**

Table 3.3. Mean values of QST parameters after z-score transformation for the three groups. Dynamic mechanical allodynia (DMA) and paradoxical heat sensations (PHS) are presented as mean number of events. * z-score transformed values outside 95% CI range of the DFNS normal values, percentages in brackets. ‡ comparison of number of participants HIV-No SN and HIV-SN using Chi-squared.

	Healthy controls n=36					**HIV-No SN n=38**						**HIV-SN n=28**				
			Number of participants with:					Number of participants with:					Number of participants with:			
	mean	SD	Loss	Gain		mean	SD	Loss	Gain		mean	SD	Loss	Gain	p-value‡	
			< -1.96 CI*	>1.96 CI*				< -1.96 CI*	>1.96 CI*				< -1.96 CI*	>1.96 CI*	Loss	Gain
CDT	0.02	0.83	1(2.8)	1 (2.8)		-0.68	0.75	2(5.3)	0		-1.34	0.93	8(28.6)	0	-	-
WDT	-0.01	0.75	0	0		-0.79	0.69	1(2.6)	0		-1.31	0.56	4(14.3)	0	0.009	-
TSL	-0.26	0.75	1(2.8)	0		-0.86	0.54	1(2.6)	0		-1.29	0.67	4(14.3)	0	0.080	-
CPT	-0.69	0.75	0	1(2.8)		0.24	0.71	0	0		-0.18	0.37	0	0	-	-
HPT	0.41	0.88	0	2(5.6)		-0.59	1.01	3(7.9)	1(2.6)		-1.47	1.05	9(32.1)	0	0.01	0.393
PPT	-0.38	1.04	2(5.6)	0		-1.09	0.87	7(13.2)	0		-1.29	1.13	7(25)	1(3.6)	0.518	0.240
MPT	0.33	0.71	0	1(2.8)		0.42	0.88	0	1(2.6)		-0.01	0.99	1(3.6)	0	0.240	0.387
MPS	0.86	1.17	0	7(19.4)		0.36	1.21	0	5(13.2)		-0.32	1.06	0	1(3.6)	-	0.385
WUR	-0.08	0.84	0	2(5.6)		-0.13	1.12	1(2.6)	2(5.3)		-0.21	1.66	2(7.1)	4(14.3)	0.385	0.208
MDT	-0.67	0.85	2(5.6)	0		-0.77	0.77	2(5.3)	0		-2.17	1.06	14(50)	0	<0.01	-
VDT	-0.06	0.81	2(5.6)	0		-0.84	1.01	4(10.5)	0		-2.24	1.62	14(50)	0	<0.01	-
DMA	Not present	-	-	-		Not present	-	-	-		Not present		-	-	-	
PHS events	0.07	0.24	NA	NA		0.62	0.92	NA	NA		0.67	0.87	NA	NA		

Table 3.4. Frequency of different patterns of sensory loss and gain in the HIV-SN group. Loss 0 = no loss of detection, Loss 1 = Loss of only thermal loss, Loss 2= Loss of only mechanical, Loss 3 = Loss of both thermal and mechanical. Gain 0 = No gain of thermal, Gain 1 = Gain of only thermal, Gain 2 = Gain of only mechanical, Gain 3 = Gain of thermal and mechanical.

HIV-SN n=28	Gain 0(no)	Gain 1 (thermal)	Gain 2 (mechanical)	Gain 3 (both)	Gain All
Loss 0 (no)	4 (14.3%)	0	0	0	4 (14.3%)
Loss 1(thermal)	0	0	0	0	0
Loss 2 (mechanical)	6(21.4%)	0	4(14.3%)	0	10(35.7%)
Loss 3 (both)	12(42.9%)	0	2(7.1%)	0	14(50%)
Loss All	22(78.6%)	0	6(21.4%)	0	28(100%)

Table 3.5. Correlation of IENFD and individual thermal and mechanical QST parameters. Pearson's correlation r values

	Thermal QST parameters						**Mechanical QST parameters**					
	CDT	**WDT**	**TSL**	**CPT**	**HPT**		**MDT**	**MPT**	**MPS**	**WUR**	**VDT**	**PPT**
r	-0.22	<0.01	-0.43	-0.21	-0.13		0.10	0.00	0.59	<0.01	-0.10	-0.05
p	0.29	0.99	<0.05	0.33	0.55		0.63	0.98	<0.05	0.70	0.67	0.83

Table 3.6. Comparison of participant reported symptoms between HIV-SN and HIV-No SN groups. *Continuous data if normally distributed were analysed with Student *t* test. Mean values and SDs shown.§ Continuous data not normally distributed were analysed using Mann-Whitney Rank Sum test. Mean values and SDs shown. †Categorical data were analysed using Chi squared test of association.

Reported Symptom	**HIV-No SN n=38**			**HIV-SN n=28**		**p value**
Any pain in hands and/or feet.	11	(28.95)		21	(75.00)	<0.001†
If experiencing pain: 7-day pain diary NRS (0-10)	2.8	(2.34)		5.65	(1.76)	<0.001*
Pain onset yrs after HIV diagnosis	12.29	(5.94)		9.5	(7.59)	0.358*
Pain duration years	9.5	(7.59)		8.25	(7.23)	0.811*
'Pins and needles' in feet and/or hands	19	(50.00)		17	(60.71)	0.388†
'Numbness' in feet and/or hands	14	(36.84)		21	(75.00)	<0.02†
Perceived 'Weakness' in upper or lower limbs	10	(26.32)		10	(35.71)	0.412†
Postural hypotension	9	(24.68)		14	(50.00)	<0.02†
Urinary dysfunction	6	(15.79)		12	(42.86)	<0.02†
Erectile dysfunction	12	(31.58)		14	(50.00)	0.152†
Nocturnal diarrhoea	10	(26.32)		8	(28.57)	0.839†

Table 3.7. Psychological and Sleep instruments questionnaire returned and completed per group. Numbers completed or returned with percentages in brackets.

	HIV-No SN n=38	**HIV-SN no pain n=7**	**HIV-SN with pain n=21**
Questionnaire booklets returned	32 (84.2)	6 (85.7)	19 (90.5)
ISI	31 (81.6)	6 (85.7)	19 (90.5)
DAPOS	29 (76.3)	6 (85.7)	19 (90.5)
PASS-20	29 (76.3)	6 (85.7)	19 (90.5)
PCS	31 (81.6)	6 (85.7)	19 (90.5)
BPI	30 (79.0)	6 (85.7)	19 (90.5)
SF-36	32 (84.2)	6 (85.7)	19 (90.5)

Table 3.8. Summary of Sleep and Psychological instruments results comparing HIV-No SN (n=38) and painful HIV-SN (n=21). *Statistical significance taken as $p \leq 0.05$.

	HIV-No SN			HIV-SN with pain		
Instrument	**Mean**	**SD**		**Mean**	**SD**	**p-value**
ISI						
Mean ISI total score (/28)	10.16	7.86		14.05	8.86	0.112
Participants with Clinical insomnia (ISI ≥15) (%)	7 (22.6%)			13 (68.4%)		<0.001**
Participants with Severe insomnia (ISI ≥22) (%)	3 (9.7%)			7 (22.6%)		<0.001**
DAPOS						
DAPOS depression (/25)	8.38	4.10		11.21	4.22	<0.05*
DAPOS anxiety (/15)	5.45	2.89		7.47	2.97	<0.05*
PASS-20						
PASS cognitive (/25)	8.45	5.28		14.95	5.69	<0.001**
PASS escape-avoidance (/25)	9.07	7.15		11.84	6.38	0.177
PASS fear (/25)	6.97	7.11		11.79	6.75	<0.05*
PASS psychological anxiety (/25)	4.83	6.45		9.21	7.54	<0.05*
PASS total (/100)	35.84	29.84		47.79	21.94	0.136*
PCS						
PCS rumination /16	5.68	4.85		8.84	5.11	0.330
PCS magnification /12	3.97	3.66		5.63	3.39	0.115
PCS helplessness /24	6.03	5.52		11.16	6.131	<0.02*
PCS total /52	14.13	11.81		23.74	12.64	<0.02*
BPI						
BPI interference /70	15.20	16.22		46.11	13.69	<0.001**
SF-36						
Physical Functioning (PF)	74.03	24.65		34.47	21.74	<0.001**
Role Physical (RP)	50.78	43.76		11.84	28.10	<0.001**
Bodily Pain (BP)	69.43	24.47		29.42	18.06	<0.001**
General Health (GH)	43.78	26.96		26.58	19.88	<0.05*
Vitality (VT)	49.53	24.08		25.79	24.45	<0.001**
Social Functioning (SF)	67.98	27.31		32.24	24.41	<0.001**
Role Emotional (RE)	54.17	46.18		15.79	32.14	<0.05*
Mental Health (MH)	63.25	21.25		47.79	17.05	<0.05*

Table 3.9. Comparison of metabolic factors: random plasma lipid profiles, glucose, BMI, waist-hip ratio and statin use for HIV-No SN and HIV-SN groups. Plasma lipid and glucose concentrations are displayed as mmol/l. Number of participants taking statins displayed with percentages in brackets. *Continuous data if normally distributed were analysed with Student *t* test. Means and SDs shown. §Continuous data not normally distributed were analysed using Mann-Whitney Rank Sum test. Means and SDs shown.†Categorical data were analysed using χ^2 test of association. Values and percentages shown. TRG - Triglycerides, HDL - High Density Lipoprotein, LDL - Low Density Lipoprotein, BMI - Body Mass Index.

	HIV-No SN n=38			**HIV-SN n=28**		**p value**
Total cholesterol	4.56	(1.08)		5.01	(1.07)	0.100*
Triglyceride	1.61	(0.77)		2.18	(1.09)	<0.020§
HDL	1.06	(0.36)		1.24	(0.44)	0.069*
LDL	2.75	(0.93)		2.71	(0.95)	0.860*
Cholesterol : HDL ratio	4.53	(1.57)		4.42	(1.27)	0.769 *
Random glucose	5.31	(1.20)		5.31	(1.07)	0.863§
BMI $kg.m^{-2}$	25.28	(5.34)		25.68	(3.69)	0.223§
Waist-hip circumference ratio (SD)	0.98	(0.09)		0.96	(0.12)	0.739§
Current statin use (%)	9	(23.70)		9	(32.14)	0.446†

Table 3.10. Comparison of using different combinations of the triumvirate criteria for the diagnosis of HIV-SN. The case definition used in the study required the presence of ≥2 out of the triumvirate: clinical signs of distal sensory neuropathy, ≥2 abnormal QST parameters or IENFD of ≤7.63 fibres/mm (1st column). Each column shows the consequences of changing the case definition. Data are displayed as absolute numbers with percentages of the groups in brackets. QST - Quantitative Sensory Testing criteria, SNE - Structured Neurological Examination, IENFD - Intra-Epidermal Nerve Fibre density criteria. *Sensitivity and specificity compared to using triumvirate of QST + CNE + IENFD criteria for the diagnosis of HIV-SN.

	QST + SNE + IENFD	QST + SNE	QST + IENFD	SNE + IENFD	QST	SNE	IENFD
Changed diagnosis							
'HIV-No SN' changed to 'HIV-SN' (%)	0 (0)	0 (0)	0 (0)	0 (0)	7 (18.42)	10 (26.32)	8 (21.05)
'HIV-SN' changed to 'HIV-No SN' (%)	0 (0)	6 (21.43)	17 (60.07)	8 (28.57)	6 (21.43)	0 (0)	11 (39.29)
Number Changed (% of total participants)	0 (0)	6 (9.09)	17 (25.76)	8 (12.12)	13 (19.70)	10 (15.15)	19 (28.79)
Sensitivity*	1.000	0.786	0.393	0.714	0.786	1.000	0.607
Specificity*	1.000	1.000	1.000	1.000	0.816	0.740	0.790

Original PINS criteria														
		CDT	**WDT**	**TSL**	**CPT**	**HPT**	**MDT**	**MPT**	**MPS**	**WUR**	**VDT**	**PPT**	**PHS**	**DMA**
HIV-No SN *n=38*	Mean (SD)	-0.68 (0.75)	-0.79 (0.69)	-0.86 (0.54)	0.24 (0.71)	-0.59 (1.01)	-0.77 (0.77)	0.42 (0.88)	0.36 (1.21)	-0.13 (1.12)	-0.84 (1.01)	-1.09 (0.87)	0.62 (0.92)	0
	Loss	2	1	1	0	3	2	0	0	1	4	7	0	0
	Gain	0	0	0	0	1	0	1	5	2	0	0	0	0
HIV-SN *n=28*	Mean (SD)	-1.34 (0.93)	-1.31 (0.56)	-1.29 (0.67)	-0.18 (0.37)	-1.47 (1.05)	-2.17 (1.06)	-0.01 (0.99)	-0.32 (1.06)	-0.21 (1.66)	-2.24 (1.62)	-1.29 (1.13)	0.67 (0.87)	0
	Loss	8	4	4	0	9	14	1	0	2	14	7	0	0
	Gain	0	0	0	0	0	0	0	1	4	0	1	0	0
BPNS as criteria														
		CDT	**WDT**	**TSL**	**CPT**	**HPT**	**MDT**	**MPT**	**MPS**	**WUR**	**VDT**	**PPT**	**PHS**	**DMA**
HIV-No SN *n=37*	Mean (SD)	-0.84 (0.82)	-0.85 (0.73)	-0.95 (0.62)	0.17 (0.68)	-0.88 (0.94)	-0.94 (1.06)	0.39 (0.90)	-0.26 (1.17)	-0.26 (1.09)	-0.98 (1.35)	-1.11 (0.79)	0.57 (0.84)	0
	Loss	4	2	2	0	6	4	0	0	2	3	4	0	0
	Gain	0	0	0	0	0	0	1	5	0	0	0	4	0
HIV-SN *n=29*	Mean (SD)	-0.84 (0.78)	-0.85 (0.89)	-0.9 (1.00)	0.16 (0.98)	-0.87 (1.14)	-0.94 (1.22)	0.39 (0.67)	0.30 (1.03)	0.26 (1.27)	-0.98 (0.69)	-1.12 (0.88)	0.57 (0.95)	0
	Loss	4	3	3	0	6	12	1	0	1	15	9	0	0
	Gain	0	0	0	0	1	0	0	1	5	0	1	5	0
Structured Neurological Exam (SNE) as criteria														
		CDT	**WDT**	**TSL**	**CPT**	**HPT**	**MDT**	**MPT**	**MPS**	**WUR**	**VDT**	**PPT**	**PHS**	**DMA**
HIV-No SN *n=28*	Mean (SD)	-0.78 (0.75)	-0.76 (0.43)	-0.90 (0.41)	-0.26 (0.73)	-0.82 (1.19)	-0.94 (1.19)	0.48 (0.72)	0.35 (1.17)	-0.02 (1.50)	-1.46 (1.61)	-1.03 (0.94)	0.54 (0.78)	0
	Loss	1	0	0	0	4	2	0	0	2	7	3	0	0
	Gain	0	0	0	0	1	0	0	3	2	0	1	2	0
HIV-SN *n=38*	Mean (SD)	-1.09 (0.75)	-1.19 (0.43)	-1.14 (0.41)	-0.07 (0.73)	-1.08 (1.19)	-1.73 (1.19)	0.04 (1.19)	-0.15 (0.71)	-0.25 (1.17)	-1.44 (1.50)	-1.28 (1.61)	0.77 (0.95)	0
	Loss	7	5	5	0	8	14	1	0	1	11	10	0	0
	Gain	0	0	0	0	0	0	1	1	1	0	0	6	0

Table 3.11. Comparisons of using original PINS study criteria, Brief Peripheral Neuropathy Screen (BPNS) and the Structured Neurological Examination (SNE) as HIV-SN criteria for QST z-score results; mean values (SD),'Loss' and 'Gain' of function: number of participant z scores ≤ -1.96, and ≥ 1.96 respectfully.

Paraesthesia and/ or numbness as criteria														
		CDT	**WDT**	**TSL**	**CPT**	**HPT**	**MDT**	**MPT**	**MPS**	**WUR**	**VDT**	**PPT**	**PHS**	**DMA**
HIV-No SN *n=23*	Mean (SD)	-0.82 (.079)	-0.78 (0.47)	-0.93 (0.44)	0.35 (0.80)	-0.71 (1.18)	-0.74 (0.87)	0.26 (1.00)	0.25 (1.36)	-0.30 (1.40)	-0.26 (1.12)	-1.14 (0.97)	-1.35 (0.85)	0
	Loss	1	0	0	0	3	1	1	0	2	4	5	0	0
	Gain	0	0	0	0	1	0	0	4	1	0	0	2	0
HIV-SN *n=43*	Mean (SD)	-1.05 (0.93)	-1.14 (0.75)	-1.10 (0.71)	-0.08 (0.46)	-1.10 (1.05)	-1.76 (1.12)	0.22 (0.93)	-0.12 (1.08)	-0.10 (1.35)	-1.62 (1.63)	-1.08 (1.00)	0.75 (0.94)	0
	Loss	7	5	5	0	9	15	0	0	1	14	8	0	0
	Gain	0	0	0	0	0	0	1	2	4	0	1	7	0
Pain Symptoms as criteria														
		CDT	**WDT**	**TSL**	**CPT**	**HPT**	**MDT**	**MPT**	**MPS**	**WUR**	**VDT**	**PPT**	**PHS**	**DMA**
HIV-No SN *n=38*	Mean (SD)	-0.63 (0.77)	-0.73 (0.62)	-0.80 (0.55)	0.32 (0.69)	-0.59 (1.08)	-0.82 (0.81)	0.25 (0.92)	0.32 (1.22)	-0.29 (1.21)	-1.16 (1.11)	-1.15 (0.97)	0.55 (0.89)	0
	Loss	1	0	0	0	4	2	1	0	2	6	6	0	0
	Gain	0	0	0	0	1	0	0	5	1	0	0	4	0
HIV-SN *n=28*	Mean (SD)	-1.42 (0.83)	-1.38 (0.58)	-1.37 (0.60)	-0.22 (0.38)	-1.46 (0.94)	-2.14 (1.09)	0.20 (1.06)	-0.29 (1.55)	0.01 (1.80)	-1.81 (1.02)	-1.2 (0.90)	0.85 (0.90)	0
	Loss	7	5	5	0	8	14	0	0	1	12	7	0	0
	Gain	0	0	0	0	0	0	1	1	4	0	1	5	0

Table 3.12. Comparisons of using symptoms of paraesthesia and/or numbness, and the presence of pain alone as HIV-SN criteria for QST z-score results; mean values (SD),'Loss' and 'Gain' of function: number of participant z scores ≤ -1.96, and ≥1.96 respectfully.

Chapter 4

Summary

Summary

Chapter 2: Findings and implications of the systematic review of pharmacological agents in the treatment of painful HIV-SN Chapter 2.

The systematic review and meta-analysis presented in Chapter 2 demonstrates that the pharmacological management of the painful symptoms of HIV-SN is at present unsatisfactory. There is currently no evidence of efficacy for many agents commonly used to treat other neuropathic pain states in the treatment of painful HIV-SN: amitriptyline, topical capsaicin (0.075%), gabapentin or lamotrigine all failed to show efficacy greater than placebo in the doses tested in the included RCTs.

The conclusion of the systematic review is that only recombinant human NGF (a commercially unavailable agent) and smoked cannabis are better than placebo in the management of pain in HIV-SN. An NNT for smoked cannabis was calculated as 3.38 with a 95% CI (2.19 – 7.50). This apparent efficacy should be tempered by the high proportion of inadvertent unblinding that occurred in at least one trial (92% of participants correctly guessing treatment allocation) (Ellis et al., 2009), and the potential for this to have occurred in the other included cannabis RCT, where previous recreational exposure to cannabis was an inclusion criteria for participants.

Limitations of this study and future work

The systematic review used the modified Jadad scale to assess the quality of RCTs entered into it. The Jadad scale has been previously criticised as being over-simplistic, showing low consistency between different raters (Hartling et al., 2009) and it places too much emphasis on blinding (V. W. Berger, 2006). Furthermore, it does not take into account allocation concealment.

Quality scales and resulting scores are not an appropriate way to appraise clinical trials. They tend to combine assessments of aspects of the quality of reporting with aspects of trial conduct, and to assign weights to different items in ways that are often difficult to justify (J. P. T. Higgins et al., 2011). If I was repeat the systematic review I would use the Cochrane Collaborations Risk of Bias Tool (CCRNT) for the above reasons *(J. Higgins & Altman, 2008)*. Whilst no assessment tool is infallible, the

CCRNT has taken into consideration advances in the understanding risk of bias in RCT and appears to be currently the most complete instrument available.

The systematic review in Chapter 2 also underlines the need for disease specific analgesic efficacy RCTs, as guidelines drawn up to treat neuropathic pain *per se,* based on RCTs for other conditions, do not reflect the response of HIV-SN to conventional analgesics or neuropathic pain adjuvants.

Cannabinoids have been extensively investigated and whilst useful in certain neuropathic pain states, their use is limited by the significant psychotropic effects of cannabinoid compounds (A. S. C. Rice, 2008). A notable exception is multiple sclerosis (MS) where cannabinoids have been shown to be useful in the treatment of MS associated spasticity and pain in at least a subset of patients (P. F. Smith, 2007). The apparent efficacy of smoked cannabis in the treatment of pain in HIV-SN would suggest that the use of cannabis extracts and synthetic compounds would be worthy of further investigation.

Unfortunately it is unlikely if there will be further work using recombinant NGF in the treatment of painful HIV-SN. Recombinant NGF is commercially unavailable after it proved disappointing in the treatment of painful diabetic neuropathy at Phase III trials and was so abandoned by the pharmaceutical company investigating it (Apfel, 2002).

Whilst high dose 8% capsaicin patches initially showed promise in the treatment of pain associated with HIV-SN, a subsequently published larger RCT showed no superiority of high dose capsaicin patches to placebo (D. B. Clifford et al., 2012).

If painful HIV-SN was an occasional complication of a rare condition, painful HIV-SN might remain an academic oddity. However as argued in Chapter 1, HIV-SN represents probably the largest cause of pain morbidity worldwide, potentially affecting more than a third of the 34 million persons infected with HIV. The conclusions of the systematic review underline the urgent need to seek out novel agents to treat this pain.

The systematic review was hampered by the paucity of good quality RCTs particularly for older neuropathic pain adjuvant drugs which are off-patent. Modern high quality RCTs suitable to assess the efficacy of pharmacological agents, are

complex and expensive to conduct. Unfortunately, the majority of these high quality RCTs are currently conducted by pharmaceutical companies who have the financial and logistical resources to undertake them. Consequently, this biases newer, on-patent agents where there is a financial incentive to find efficacy. It has been suggested that it should be the role of governmental research agencies, such as the National Institute of Health Research (NIHR) in both the US and UK to fund modern RCTs examining older agents against placebo, each other (head-to-head) and in combination therapies in a clinically meaningful way.

New evidence related to the use of high dose capsaicin patches has been published whilst this thesis was in preparation (D. B. Clifford et al., 2012). As suggested in an abstract assessed in the systematic review, the outcome of these trials demonstrates that high dose capsaicin patch is not superior to placebo in the treatment of painful HIV-SN. A new systematic review should be conducted and this high dose capsaicin study included formally assessed and integrated into the systematic review.

Chapter 3: **Patterns of sensory, psychological and metabolic dysfunction in HIV positive patients with and without HIV-associated peripheral neuropathy.** ***Findings, implications and future work***

One of the key findings of the phenotyping study is that the sensory dysfunction seen in HIV-SN is heterogeneous. Comparing the HIV-SN population to the HIV-No SN group the most profound difference is that of sensory loss across all modalities measured. Most frequently, this was seen in the large fibre modalities of MDT and VDT. Whilst differences were seen in small fibre function the picture across the group was quite mixed, with no discernable patterns evident. This highlights the fact that HIV-SN is not a pure small fibre neuropathy (as it has been widely considered) and potentially opens a new line of research in the mechanisms underlying HIV-SN. Moreover, this finding would support the use of MDT and VDT in low resource settings in preference to expensive thermal parameter QST equipment. In addition, the absence of gain of function phenomena is consistent with QST studies of other painful peripheral neuropathies. Nevertheless, a small sub-group with wind-up phenomenon was seen in 14% of the HIV-SN group patients (n=4) – whilst this is a small number, it is a comparable proportion of HIV-SN participants seen to have mechanical hyperalgesia in previous studies. However, as this thesis used the standardised DFNS

protocol, it is difficult to draw direct conclusions with this specific study (D M Simpson, Schifitto, et al., 2010). A future phenotyping study should actively look for this phenomenon, especially as the sub-group seen in the pregabalin RCT work of Simpson and collaborators in 2010 had a differential response to analgesics. The presence of high levels of catastrophising in this high WUR is another interesting phenomenon as would be useful to explore further.

This thesis showed that patients with HIV-SN had significantly higher triglyceride levels than patients without HIV-SN. This is a finding that further supports recent work in both HIV-SN and Diabetic neuropathy which suggests lipid dysfunction is an important factor in the development of painful peripheral neuropathies(Banerjee et al., 2011; Vincent et al., 2009; Wiggin et al., 2009). This opens an interesting question about the role of lipid lowering agents such as statins to act as disease modifying agents to prevent or slow the development of HIV-SN.

Strengths of this study

This comprehensive phenotyping of HIV-SN is important for the future development of animal models.

Although the phenotyping study presented in Chapter 3 produced a wealth of information, it will take a lot more time to fully explore its implications. Further analysis of the diagnostic utility of individual components tested in this study should be undertaken to yield a composite refined diagnostic protocol using the best of the sensory testing, screening tools and specific symptom questioning to produce a robust tool for screening and early detection of HIV-SN. This had been part of the original aims of the thesis but could not be completed due to time restrictions. Colleagues who intend to build on the work of this thesis have already started this work.

As part of this thesis a blood and tissue bank has been established. This is an important resource as these tissue samples have extensive phenotyping information associated with them. The hope is that this will be potentially useful in future genetic analysis studies examining painful HIV-SN.

Limitations of this study

One of the weaknesses of the phenotyping study was that the relatively small number of HIV infected participants recruited (n=66) into the study meant that a useful comparison between participants with non-painful (n=7) and painful HIV-SN (n=21) was not possible to do with meaning full statistical significance. Another potential consequence of small recruitment numbers was that many of the established risk factors for the development of HIV-SN were not confirmed in this study – most notably age and height. As argued in Chapter 3 some of this failure to see established risk factors can be accounted for in the population. Compared to many larger epidemiological studies, the population we recruited was quite homogeneous: male, white, tall and middle aged. Unfortunately, the labour intensive nature of phenotyping individuals over three to four hours each, meant that recruiting greater numbers was not practical. A future phenotyping study could utilise the work here and use a smaller number of components to reduce both participant and investigator work loads to allow a greater number of individuals to be recruited.

The use of a composite definition for HIV-SN, using abnormal QST (2 or more abnormal modalities), reduced IENFD (<7.63 fibres/mm) and a structured clinical examination meant that comparing these measures between the two groups might risk a circular argument. Reasons for this to be unlikely are outlined in the discussion of Chapter 3, however if a more robust definition for HIV-SN can be produced as described as above, future phenotyping studies might be free from this potential criticism.

There is also a need for ease to use, robust screening tools for the identification of HIV-SN that can be translated for use into resource poor settings. Part of the phenotyping study examined the efficacy of a number of neuropathy screening tools (and their constituent components), some of which have not been previously validated in the context of HIV-SN. This study will hopefully form the basis for future work directed at producing a composite screening tool for use in resource poor settings.

As part of this study a blood and tissue bank for each highly phenotyped individual with HIV-SN was established; this valuable resource will inform future work exploring genetic risk factors for the development of HIV-SN and painful HIV-SN.

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SUPLEMMENTAL DOCUMENTS

Supplemental Document 1:

Instrument To Measure The Likelihood Of Bias And Insufficient Statistical "Power" In Pain Research Reports (modified Jadad scale)

This is not the same as being asked to review a paper. It should not take more than 10 minutes to score a report and there are no right or wrong answers.

Please read the article and try to answer the following questions (see attached instructions):

1. Was the study described as randomised (this includes the use of words such as randomly, random, and randomisation)?
2. Was the study described as double blind?
3. Was there a description of withdrawals and dropouts?
4. Was there a description of a power calculation?

Scoring the items:

Either give a score of 1 point for each "yes" or 0 points for each "no". There are no in-between marks.

Give 1 additional point if: For question 1, the method is to generate the sequence of randomisation was described and it was **appropriate** (table of random numbers, computer generated, etc).

And/or: If for question 2 the method of double blinding was described and it was **appropriate** (identical placebo, active placebo, dummy etc).

And/or: If for question 4 the method and assumptions for power calculation was described and it was **appropriate** and the number of patients who were randomised and

analysed on an intention to treat basis was in concordance with this calculation.

Deduct 1 point if:- the

For question 1, the study was described as randomised

method of randomisation was **inappropriate** (patients were allocated

alternately, or according to date of birth, hospital number, etc) or the description was **unclear**.

And/or blind but the

For question 2, the study was described as double

method of blinding was **inappropriate** (e.g., comparison of tablet vs. injection with no double dummy) or the description **unclear.**

And/or:

If for question 4 the method and assumptions for power calculation was described and it was **inappropriate** or the number of patients who were randomised and analysed on an intention to treat basis did not reached the level required by the power calculation.

Guidelines for Assessment

1. Randomization

A method used to generate the sequence of randomization will be regarded as appropriate if it allowed each study participant to have the same chance of receiving each intervention and the investigators could not predict which treatment was next. Methods of allocation using date of birth, date of admission, hospital numbers, or alternation should not be regarded as appropriate.

2. Double blinding

A study must be regarded as double blind if the word "double blind" is used. The method will be regarded as appropriate if it is stated that neither the person doing the assessments nor the study participant could identify the intervention being assessed, or if in the absence of such a statement the use of active placebos, identical placebos, or dummies is mentioned.

3. Withdrawals and dropouts

Participants who were included in the study but did not complete the observation period or who were not included in the analysis must be described. The number **and** the reasons for withdrawal in each group must be stated. If there were no withdrawals, it should be stated in the article. If there is no statement on withdrawals, this item must be given no points.

4. Power calculations

The method for calculating the statistical power must be described and the assumptions regarding effect size, the alpha value (min. 0.05) and the power level (min. 0.8). The group sizes in the intention to treat analysis must be a minimum of that indicted by the power calculation.

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	Diagnosis	Date of Diagnosis
Other Past Medical History	1.	
	2.	
	3.	
	4.	
	5.	
	6.	
	7.	
	8.	
	9.	
	10.	
	11.	
	12.	
	13.	
	14.	
	15.	
	16.	
	17.	
	18.	
	19.	
	20.	

Current AV drugs	Dose/day	Date Commenced
1.		
2.		
3.		
4.		
5.		

Past AV drugs	Dose/day	Date Commenced	Date Finished
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

Current Drug treatment	Dose/day	Date Commenced
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8		
9.		
10.		
11.		
12.		
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Previous Drug treatment	Dose/day	Date Commenced	Date Finished
1.			
2.			
3.			
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20.			

Neuropathy Symptoms?		Date of onset	Comments
Paraesthesia	Y/N		
Numbness	Y/N		
Weakness	Y/N		
Postural hypotension	Y/N		
Urinary dysfunction	Y/N		
Erectile dysfunction	Y/N		
Nocturnal diarrhoea	Y/N		
Pain	Y/N		

Family history of neuropathy?	Family member	Diagnosis
Y/N	1.	
	2.	
	3.	
	4.	

Please rate the severity of each of the following symptoms on a scale of 1(mild) to 10 (severe):			
	(i) Pain, aching, burning in feet, legs (BPNS/TCSS)		0-10 or Always been normal (AN)
	(ii) "Pins and needles" in feet, legs (BPNS)		0-10 or Always been normal (AN)
	(iii) Numbness (lack of feeling) in feet, legs. (BPNS/TCSS)		0-10 or Always been normal (AN)
	(iv) Tingling in feet (TCSS)	Y/N	
	(v) Weakness in feet (TCSS)	Y/N	

Physician's Neuropathy Differential Diagnosis
1.
2.
3.
4.
5.

Supplemental Document 1: PINS-HIV Study CRF

Medical Notes:

		Value	Date of test
HIV	Current CD4+ count (450-1660)		
	Current CD4+ % (30-65%)		
	Nadir CD4+ count (450-1660)		
	Nadir CD4+ % (30-65%)		
	Current HIV-1 RNA (viral load RNA/ml)		
	Peak HIV-1 RNA (viral load RNA/ml)		

		Concentration	Date of test
Thyroid function	T4 $_{free}$ (9.0-26.0 pmol/l)		
	TSH (0.3-4.2mU/l)		
	T3		

Lipids	Cholesterol (<5.0 mmol/l)		
	Triglycerides (0-2.0 mmol/l)		
	HDL (0.9-1.9 mmol/l)		
	LDL (2.0-5.0 mmol/l)		
	HDL: Cholesterol ratio (0-5.0)		

		Value	Date of test
FBC	Hb		
	WCC		
	Plts		

		Value	Date of test
Blood electrolytes	Na^+		
	K^+		
	Ur		
	Cr		

		Value	Date of test
Liver Function Enzymes	Bilirubin		
	Alanine Transaminase		
	Alkaline Phosphatase		
	Gamma-Glutamyl Transpeptidase		

		Value	Date of test
Blood Glucose	Random		
	Hb_{A1C}		

	Test	Value	Date of test
Vitamin B_{12} (160-800nq/l)	Y/N		
Syphilis VDRL	Y/N	Neg/Pos	
Syphilis TPPA	Y/N	Neg/Pos	

Hepatitis B core antigen	Y/N		Neg/Pos
Hepatitis C RNA	Y/N	Neg/Pos	
Vitamin B_{12} (160-800nq/l)	Y/N		
Syphilis VDRL	Y/N	Neg/Pos	
Syphilis TPPA	Y/N	Neg/Pos	
Hepatitis B core antigen	Y/N	Neg/Pos	
Hepatitis C RNA	Y/N	Neg/Pos	

Neurophysiology						
	Median n.					
	Ulnar n.					
	Tibial n.					
	C. Peroneal n.					

Supplemental Document 1: PINS-HIV Study CRF

Questionnaires:

Questionnaire 1 7-day Pain Diary		Completed Questionnaire?	Y/N
		Completed Body map?	Y/N
	Score		
Day 1 am		0-10	
Day 1 pm		0-10	
Day 2 am		0-10	
Day 2 pm		0-10	
Day 3 am		0-10	
Day 3 pm		0-10	
Day 4 am		0-10	Total
Day 4 pm		0-10	
Day 5 am		0-10	
Day 5 pm		0-10	
Day 6 am		0-10	
Day 6 pm		0-10	
Day 7 am		0-10	
Day 7 pm		0-10	

Questionnaire 2 Neuropathic Pain Symptom Inventory (NPSI)		Completed Questionnaire?	Y/N
	Score		
Q1 Burning		0-10	
Q2 Squeezing		0-10	
Q3 Pressure		0-10	
Q4 Degree of Spontaneous ongoing pain		0-5	
Q5 Electric shocks		0-10	Total
Q6 Stabbing		0-10	
Q7 Degree of spontaneous paroxysmal pain		0-5	
Q8 Brush-evoked		0-10	
Q9 Pressure evoked		0-10	
Q10 Cold-evoked		0-10	
Q11 Pins and Needles		0-10	
Q12 Tingling		0-10	

Questionnaire 3: Depression Anxiety Positive Outlook Scale (DAPOS)	Score	Completed Questionnaire?	Y/N
Q1		0-5	
Q2		0-5	
Q3		0-5	
Q4		0-5	
Q5		0-5	Total
Q6		0-5	
Q7		0-5	
Q8		0-5	
Q9		0-5	
Q10		0-5	

Questionnaire 4: Pain Catastrophising Scale (PCS)	Score	Completed Questionnaire?	Y/N
Q1		0-4	
Q2		0-4	
Q3		0-4	
Q4		0-4	
Q5		0-4	
Q6		0-4	Total
Q7		0-4	
Q8		0-4	
Q9		0-4	
Q10		0-4	
Q11		0-4	
Q12		0-4	
Q13		0-4	

Questionnaire 5. Short-form Pain Anxiety Symptoms Scale (PASS-20)	Score	Completed Questionnaire?	Y/N
Q1		0-5	
Q2		0-5	
Q3		0-5	
Q4		0-5	
Q5		0-5	
Q6		0-5	
Q7		0-5	
Q8		0-5	Total
Q9		0-5	
Q10		0-5	

Q11		0-5
Q12		0-5
Q13		0-5
Q14		0-5
Q15		0-5
Q16		0-5
Q17		0-5
Q18		0-5
Q19		0-5
Q20		0-5

Questionnaire 6: Insomnia Severity Index (ISI)		Completed Questionnaire?	Y/N
	Score		
Q1a Initial		0-4	
Q1bMiddle		0-4	
Q1cTerminal		0-4	Total
Q2 Satisfaction		0-4	
Q3 Interference		0-4	
Q4 Noticeability		0-4	
Q5 Distress		0-4	

Questionnaire7: Brief Pain Inventory – 7-item pain interference subscore.		Completed Questionnaire?	Y/N
	Score		
Q1 General activities		0-10	
Q2 Mood		0-10	
Q3 Walking		0-10	Total
Q4 Work		0-10	
Q5 Relationships		0-10	
Q6 Sleep		0-10	
Q7 Enjoyment of Life		0-10	

Questionnaire 8: SF-36		Completed Questionnaire?	Y/N
	Sub-Scores		
SS1 Physical Functioning (PF)			
SS2 Role Limitations due to Physical Health (RP)			
SS3 Bodily Pain (BP)			
SS4 General Health (BP)			
SS5 Vitality (VT)			
SS6 Social Functioning (GH)			

SS7 Role Limitations due to Emotional problems (VT)	
SS8 Mental Health	

		Date
Blood taken?	Y/N	
Skin punch Bx taken?	Y/N	

IENFD result		

QST Data	Left	Right	
CDT			^{0}C
WDT			^{0}C
TSL			^{0}C
PHS			
CPT			^{0}C
HPT			^{0}C
MDT			mN
MPT			mN
MPS			log
DMA			log
WUR			
VDT			/8
PPT			kPa
CPT-NRS			/100 (CPT-NRS= Pain NRS on reaching CPT)
$ST_{VAS\ 50}$			^{0}C ($ST_{VAS\ 50}$ = temp where VAS is 50)
ST_{GRD}			ST_{GRD}= gradient of suprathreshold warm VAS curve

Level 1	Level 2	*Ethnicity Categories 2001 UK Census
White	White British Irish Other White background All white groups	
Mixed	White and Black Caribbean White and Black African White and Asian Other mixed background	
Asian or Asian British	Indian Pakistani Bangladeshi Other Asian background All Asian groups	
Black or Black British	Caribbean African Other Black background All Black groups	
Chinese or Other Ethnic Groups	Chinese Other ethnic group All Chinese or Other groups	
All ethnic groups	All ethnic groups	

Supplemental Document 3: QST Suprathreshold Heat Protocol

Suprathreshold testing was conducted after the completion of the DFNS QST protocol.

As with the DFNS QST protocol Surpathreshold thermal stimuli were generated using a SOMEDIC MSA100 thermal stimulator (SOMEDIC AB, Hörby, Sweden), which uses a fluid cooled Peltier element thermode measuring 25 x 50mm.

From a base line temperature of 32^0C, suprathreshold temperatures were reached by a ramped temperature increase of 1^0Cs^{-1}. The temperature sequence used was 46, 42, 40, 48, 50, 52, 44, 42, 48, 40, 44, 46, 50 and 52^0C. The supratheshold temperature being tested would then be held constant for 2 sec, during which the participant was requested to rate the greatest pain experienced for the temperature being tested using a 100mm electronic visual analogue scale (eVAS) transducer (SENSEbox™ 450-010, SOMEDIC AB, Sweden). VAS anchors were defined for the patient prior to testing, and were also written on the VAS scale: from left to right on eVAS: 0mm - No pain, and 100mm - Worst pain they could imagine.

QST DFNS/ PINS Protocol Data-sheet version 3.0

PINS No

Date	Tester ID	Room Temp	R Skin Temp	L Skin Temp	Participants DFNS ID
					4 4 2 0 8 0 1

Thermal Testing

Right S1

CDT	WDT	TSL	CPT	HPT
PHS			CPT NRS	

Number of paradoxical heat sensations

Left S1

CDT	WDT	TSL	CPT	HPT
PHS			CPT NRS	

Number of paradoxical heat sensations

MDT: Mechanical Detection Threshold (von Frey filaments)

Right S1		Left S1	
sensed	not sensed	sensed	not sensed
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN

MPT: Mechanical Pain Threshold (Pinprick set)

Right S1		Left S1	
blunt	sharp	blunt	sharp
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN
mN	mN	mN	mN

Stimulus Response Function: MPS (Mechanical Pain Sensitivity): Pinprick; ALL (Dynamic Mechanical Allodynia): Brush (BR), Q-tip (QT), Cotton-wool (CW).

Right S1

128	CW	32	256	BR
CW	256	128	8	32
32	128	BR	CW	16
256	8	CW	QT	128
BR	32	16	128	512
8	QT	256	64	CW
16	BR	512	32	64
QT	64	8	512	256
512	16	64	BR	QT
64	512	QT	16	8

Left S1

8	16	QT	512	64
QT	BR	64	16	512
256	512	8	64	QT
64	32	512	BR	16
CW	64	256	QT	8
512	128	16	32	BR
128	QT	CW	8	256
16	CW	BR	128	32
32	8	128	256	CW
BR	256	32	CW	128

QST DFNS/ PINS Protocol Data-sheet version 3.0

PINS No

Date

Participants DFNS ID

							4	4	2	0	8	0	1

WUR: Wind-Up Ratio pin-prick 256 (series of single-stimulus and 10 stims 1s^{-1})

Right		Left	
Single-stim	After 10 stims	Single-stim	After 10 stims
/100	/100	/100	/100
/100	/100	/100	/100
/100	/100	/100	/100
/100	/100	/100	/100
/100	/100	/100	/100

VDT: Vibration Detection Threshold: 64Hz 8/8scale medial malleolus

Right	Left
/8	/8
/8	/8
/8	/8

PPT: Pressure Pain Threshold

Right	Left
kPa	kPa
kPa	kPa
kPa	kPa
kPa	kPa
kPa	kPa

PINS Additional Protocol

Warm Suprathreshold

Right S1		Left S1	
Temp ^{0}C	VAS	Temp ^{0}C	VAS
46^{0}C	/100	46^{0}C	/100
42^{0}C	/100	42^{0}C	/100
40^{0}C	/100	40^{0}C	/100
48^{0}C	/100	48^{0}C	/100
50^{0}C	/100	50^{0}C	/100
52^{0}C	/100	52^{0}C	/100
44^{0}C	/100	44^{0}C	/100
42^{0}C	/100	42^{0}C	/100
48^{0}C	/100	48^{0}C	/100
40^{0}C	/100	40^{0}C	/100
44^{0}C	/100	44^{0}C	/100
46^{0}C	/100	46^{0}C	/100
50^{0}C	/100	50^{0}C	/100
52^{0}C	/100	52^{0}C	/100

Supplemental Document 5: Structured Neurological Examination (SNE)

The structured neurological examination of the upper and lower limb was designed by an experienced neurologist (DB), to exam in detail, clinical neurological signs which might be present in a peripheral neuropathy.

Upper and Lower Limb Examination

Inspection

The presence of any muscle wasting is noted, with special attention given to the First Dorsal Interosseous muscle in the upper limb and Extensor Digitorium Brevis in the lower limb. The presence of ulcerations and deformities is also noted.

Motor

Motor strength is assessed using the expanded MRC grading system (see below table for details) for shoulder abduction, elbow flexion/ extension and wrist extension in the upper limb. In the lower limb Hip flexion, Knee extension and Extensor Hallucis Longus power are assessed.

Sensory

Joint position proprioception – The subject's index finger at the level of the distal-interphalangeal joint is placed in the 'up' and 'down' position, with the examiner explaining to the subject the position the joint is in when the subject's eyes are open. The procedure is repeated with the subject's eyes closed for 3 different joint positions. If the subject fails to recognize 3 out of 3 positions, the examiner repeats the examination on the next proximal joint and so on, until a level is reached where the subject demonstrates normal joint proprioception. This level is then recorded.

Vibration perception – the examiner applies a maximally struck 128Hz tuning fork to the distal inter-phalangeal joint of the subject's index finger in the upper limb, and the distal interphalangeal joint in the first toe in the lower limb. The examiner asks the subject to indicate if they can feel the vibration. If they are able to detect vibration, the examiner asks if it feels the same as when applied to the subject's sternum. If the subject indicates that it does not feel the same, the assessment is repeated at the next most proximal joint until the subject indicates that the vibration sensation felt is the same when applied distally as when applied to the sternum. This level is then recorded.

Light touch – The examiner touches the palmer aspect of fingers 1 to 5 with a cotton wisp and asks the subject to point to the finger being touched with their eyes closed. The light touch stimulus is demonstrated first to the subject on their sternum with their eyes open.

Pin-prick perception – The examiner uses a Neurotip™ pin (Owen Mumford, Oxford U.K.) asking the subject to identify if the blunt or sharp part of the Neurotip pin is

being applied to the palmer aspect of the subjects index fingers. If the subject is not successful on 3 out of 3 applications, the examiner would repeat the assessment at the next most proximal joint. The level at which normal pin-prick sensation is recorded. The pin-prick examination is demonstrated first to the subject on their sternum with their eyes open

Temperature perception - The examiner uses cold and warm water filled test tubes, asking the subject to indicate if the test tube applied is warm or cold when applied to first the subjects palmer aspect of their finger tips with their eyes are closed. If the subject is unable to correctly identify the temperature, the assessment is repeated at the next most proximal joint. The examiner first demonstrates the test on the subjects sternum with their eyes open.

Reflexes

Using a standard 40 cm 'Queen Square' style reflex hammer. Reflexes assessed: biceps, triceps and brachioradialis in the upper limb; patella, Achilles' and plantar in the lower limb. The examiner records if the reflex was normal, absent, brisk or only present with a reinforcement maneuver. Plantar reflexes are recorded as up or down.