Clinical Phenotyping and Genotyping of HIV-Associated Sensory Neuropathy : The HIV-POGO study

NCT02555930

Study Protocol Version 7.0 23/02/2017

1. Introduction

HIV associated sensory neuropathy (HIV-SN) is a frequent complication of HIV infection and its treatment, affecting between 20% and 57% of infected individuals [1]. It is a length dependent, sensory distal symmetrical polyneuropathy which causes altered sensation, numbness and pain. Despite a reduction in the use of the neurotoxic 'd' class of NRTI anti-retroviral medication, the prevalence of HIV-SN has remained at around 40% in the developed world [2, 3], and of those affected by HIV-SN, 38% to 90% experience pain [3, 4]. HIV-SN is a worldwide problem; a recent study in India showed an incidence of 40% in ART naive patients [5] and, in Malawi, a prevalence of 56% has been reported in those taking stavudine-based antiretroviral protocols [6].

The introduction of newer combined antiretroviral treatment (cART) has changed the morbidity associated with HIV infection; mortality has decreased dramatically since that of the mid-1990s and HIV has become a chronic disease [7]. This has meant that co-morbidities associated with long-term HIV infection, especially those that are age related such as HIV-SN, cardiovascular pathology and neurocognitive dysfunction, are increasing in prevalence and becoming an important disease burden [8].

1.1 Diagnosis and Classification

The treatment of pain related to HIV-SN remains difficult, despite growing knowledge about the pathophysiology and mechanisms involved in its development. This is due to three important factors. Firstly, the identification and classification of HIV-SN can be challenging, and there is no worldwide consensus on the criteria required for the diagnosis of HIV-SN. Detailed history, clinical examination, nerve conduction tests, quantitative sensory testing to assess small fibre function [9, 10] and skin biopsies [11] to assess intra-epidermal nerve fibre density, are all used to aid diagnosis. However, some of these tests are expensive, time-consuming and, currently, are not universally available. Skin biopsies are also invasive, which limits their practical use as a longitudinal method of assessment. In other sensory neuropathies, it has been shown that loss of small fibre density can be monitored over time using corneal confocal microscopy (CCM), and that this correlates with results from skin biopsy [12]. This technique could potentially also be used for the long-term follow up of patients with HIV-SN and is the focus of further investigation.

It is generally accepted that a combination of subjective symptoms and objective clinical signs are required for a screening tool in HIV-SN [13]. An easy, simple screening tool – 'CHANT' (Clinical HIV-Associated Neuropathy Tool) has been recently developed, based on the detailed phenotyping of a group of patients with painful HIV-SN, in the cART era [14]. It is a 4 item tool which has been validated in both European and African patient groups [15].

The second reason for low treatment efficacy in HIV-associated neuropathic pain is the potential heterogeneity of pain mechanisms in the HIV population. Patients with HIV-SN related pain may respond differently to certain therapies because of a varying predominance in underlying pain 'driver' for that population. Pain drivers that have been shown to be important in other neuropathic pain conditions, such as conditioned pain modulation and temporal summation, could potentially be involved in HIV-SN related pain [16, 17]. Identification of a predominant pain driver in the individual, could lead to a more targeted trial of therapy.

1.2 Genetic Risk Factors

Thirdly, little is known about the genetic influences that may predispose an individual with HIV to develop a painful sensory neuropathy. So far, no genome-wide association studies have been published on HIV-SN. Instead, genetic investigation has focused on identifying 'candidate genes' that have been highlighted as being important in the pathophysiology of the disease. Research has focused on genes involved in mitochondrial function, as a potential mechanism for the neurotoxicity of NRTIs, and on genes involved in the inflammatory response, as a mechanism for direct viral-induced neuropathy.

A certain haplotype, involved in the electron transfer chain, has been identified in mitochondrial DNA as being a risk factor for the development of HIV-SN in Europeans on cART [18, 19], but only limited studies have been conducted in African ethnic groups. Some studies have also shown a link between polymorphisms of nuclear DNA, involved in the transcription of mitochondrial DNA [20]. Polymorphisms in the promoter region of the TNFα gene and the untranslated region of the IL12β gene have also been linked to an altered risk of developing HIV-SN in Europeans [21]. In general, very little is known about any genetic associations in the African and Asian populations.

Even less is known about what genetically predisposes patients with HIV-SN to develop neuropathic pain. Currently only the GTP cyclohydrolase 1 (GCH1) gene, involved in the production of pronociceptiove molecules, has been shown to have an association with HIV-SN related pain [22]. Therefore, more work remains to be done to identify the genetic and epigenetic influences in the cART era and in different ethnic groups.

1.3 HIV associated Neurocognitive Disorder and Neuropathic Pain

HIV associated Neurocognitive Disorder (HAND) is now recognised as an important and frequent complication of HIV infection. The prevalence has been reported as high as 69% when asymptomatic HAND is included [23] and a large population study in Canada recently showed that 50% of patients with one neurological complication of HIV had at least one other neurological complication [24]. It also showed that the two most common pathologies were HAND and HIV-SN [24]. The co-existence of these conditions means that one may have an impact on the identification and treatment of the other, and that there could be a shared mechanism or group of risk factors, for example gp120 induced neurotoxicity.

HAND is a spectrum of neurocognitive impairment including asymptomatic functional impairment, mild symptomatic dysfunction and severe HIV-associated dementia. [25] Milder forms of HAND are characterised by cortical features, such as impaired retrospective and prospective memory, and executive functioning. [26]This milder end of the spectrum is very different to HIV-associated dementia, where subcortical features predominate (such as the rapid, progressive, loss of concentration, impaired motor function and behavioural change [8]). All types of neurocognitive impairment could affect how a patient perceives and reports pain, how they perform with QST and how they respond to therapeutic intervention. Mild cognitive impairment may influence the ability to use certain pain scales, to effectively describe pain symptoms [27] and associated motor impairment may influence the ability to respond to stimuli in QST.

Interestingly, patients with dementia exhibit different responses to placebo due to a loss of expectation [28], however, this has not been explored in mild cognitive impairment. It has also been shown that patients with HIV-SN show a greater response to placebo than other neuropathic pain conditions [29]. This may mean that it is the altered response to placebo that is responsible for the heterogeneity in HIV-SN and that this may be due to differences in cognitive ability.

Patients with subclinical or early HAND may show a predisposition to HIV-SN and vice versa. It is thought that both conditions occur as a result of chronic neuroinflammation. The HIV retrovirus infects macrophages and dendritic cells, and can remain in the brain, which acts as a reservoir for the disease. [30] From simian models, the virus has been shown to enter the nervous system early in the disease. These models have shown that the chronic immune activation associated with HIV disease progression, causes dysregulation of macrophage function and an alteration in the production of cytokines and chemokines (for review [25]). This change occurs within both the central and peripheral nervous systems and has been shown to be crucial in the development of both HAND and HIV-SN. [31]

The toxic effects of viral gene products, such as the viral coat glycoprotein 120(gp120), have been identified in the development of HAND [32, 33] and HIV-SN [34]. Rodent models of HIV-SN have been studied with perineural administration of HIV-gp120. The inflammatory reaction results in spinal gliosis, macrophage infiltration and upregulation of various chemokines in the dorsal root ganglion [35, 36]. It also results in the reduction in intraepidermal nerve fibre density and mechanical hypersensitivity, which correlates to what is observed in human HIV infection [35]. Similar genetic targets, such as polymorphisms in the promoter region of TNFα [21, 37], have also been highlighted in both conditions, and this could indicate a joint genetic predisposition (for review [4, 38]).

2. Study Objectives

a) To recruit a cohort of patients with HIV with and without HIV-SN and to identify genetic factors associated with sensory neuropathy and neuropathic pain. This will be used to compare these factors with other neuropathic pain aetiologies recruited within the NeuroPain collaboration.

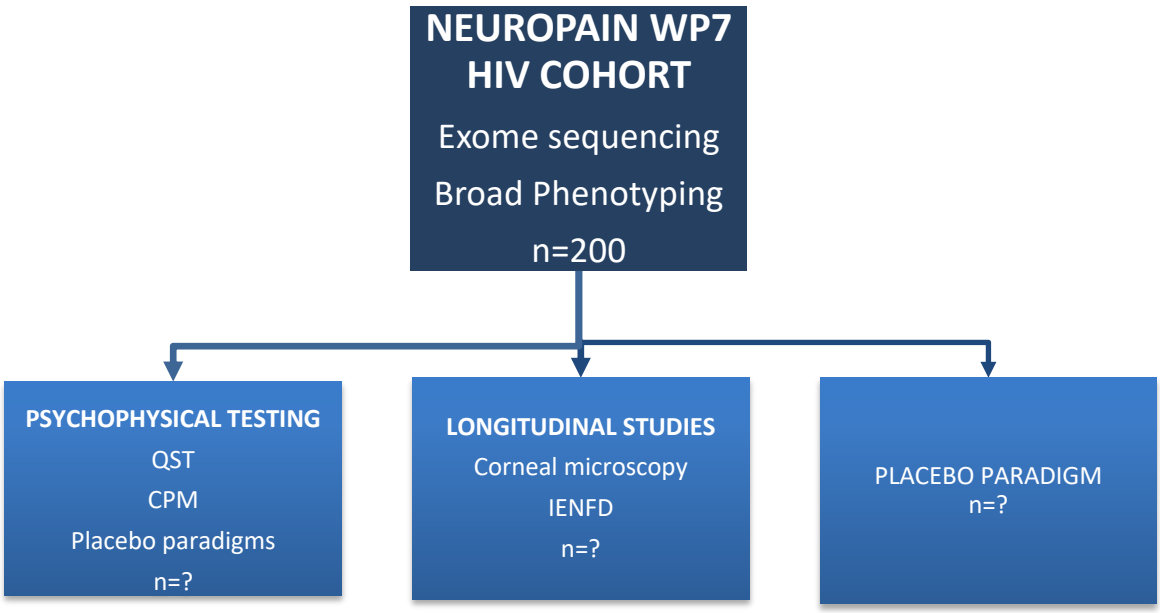
b) To further phenotype their symptoms with the use of well-validated questionnaires and sensory testing.

c) To perform a neurocognitive assessment on these patients in order to identify any correlation between HIV-SN and HAND.

d) To obtain blood samples for genetic analysis to further elucidate genetic risk factors for the development of HIV-SN and related pain syndromes. Analysis will be performed by collaboration with deCODE. This study represents a replication cohort for testing genetic hypotheses developed as a result of analysis of a much larger population.

e) To provide a database resource from which to recruit patients for three imbedded studies (see diagram below- details to be confirmed). The smaller secondary studies aim to more highly

phenotype patients with HIV neuropathy using conditioned pain modulation paradigms, corneal microscopy and placebo protocols.



The NeuroPain research group

This study is part of the NeuroPain Study Group investigating neuropathic pain and endogenous analgesic mechanisms through a broad range of animal and human studies. Imperial College London is Neuropain Partner 11, and this protocol is part of Work Package 7 in this study. Work Package 7 incorporates four partner groups. One group in Helsinki will be co-ordinating a phenotyping programme for patients following mastectomy that are at risk of chronic post-surgical pain. Another group, based in Berlin, will be conducting a similar protocol with another cohort of HIV patients. All three centres will send blood for exome wide sequencing to deCODE in Iceland and there is consensus between the groups for the core phenotyping of patients.

All three European patient cohorts are to be incorporated as 'replication cohorts'; this is because deCODE already has large amounts of genetic information from previous work on pain syndromes and other disease states. From this information they can apply targeted analysis of the NeuroPain patient cohorts. This also means that the large population numbers usually required for genetic studies are not required.

2.1 Justification for the study

To our knowledge, this will be the first large, prospective observational cross sectional cohort study to have been conducted with HIV patients, using detailed genetic analysis targeted at sensory neuropathy and neuropathic pain in the UK population. It will be the first prospective study to examine for any co-existence of HAND and sensory neuropathy and the effect on pain reporting and perception. Results from the HIV-POGO study will be examined in conjunction with patient cohorts with other neuropathic pain syndromes within the NeuroPain study.

3. Study Design

The Clinical Phenotyping and Genotyping of HIV-associated Sensory Neuropathy Study (HIV-POGO) is an observational, single-cohort, cross sectional study conducted at St Stephens Centre and Chelsea and Westminster Hospital NHS Trust, London.

3.1 Recruitment

Subjects (n=200) will be recruited from the St Stephens Centre at Chelsea and Westminster Hospital and associated clinics via HIV and chronic pain clinic lists, and from the local HIV population. They will undergo a formal consent process.

Patients already recruited to other studies by SSAT or those identified by the clinical team as having sensory neuropathy will be contacted by letter and telephone to ask whether they would be prepared to participate in the study and will be sent a patient information leaflet.

It is intended that 200 subjects with HIV will be recruited over 36 months. Despite the genetic nature of this study, only 200 subjects will be required as our cohort represents a 'replication cohort' to enable genetic hypothesis testing devised from results of a much larger neuropathy cohort in Iceland.

Patients will be identified as eligible from HIV and chronic pain clinic during a screening interview (see below for inclusion/exclusion criteria); they will be invited to attend a clinical appointment and at the end of the appointment will be given a booklet of questionnaires to be filled in at home which they will be asked to post back to the department. If they are able to fill the questionnaires in on their own, for any reason, they will be completed with the researcher during the appointment.

3.2 Questionnaire booklet

Patients will be asked to complete a series of questionnaires given to them at their appointment. They will be asked to post them back to the research group within 2 weeks and will be sent a reminder by text if the questionnaires are not received after 3 weeks. The majority of these scales are part of the 'core phenotyping', which has been agreed across different pain cohorts within the NeuroPain study, and with deCODE. These are described as 'core' phenotyping in the protocol. Some are specific to this HIV cohort and are described as 'specific' phenotyping. Questionnaires are expected to take approximately 45 minutes to complete. The questionnaires to be used have been summarised below.

Questionnaire	Time taken to complete (minutes)	Core or specific phenotyping
DAPOS : Depression Anxiety Positive Outlook Scale	3	Specific
HADS : Hospital Anxiety and Depression Scale	5	Core
BPI : Brief Pain Inventory	5	Core
NPSI: Neuropathic Pain Symptom Inventory	5	Specific
SF-36 : Quality of life index	10	Core
ISI : Insomnia Severity Inventory	3	Specific

TIPI : Ten Item Personality Inventory	3	Specific
PCS : Pain Catastrophizing Scale	5	Core
EMQ: Everyday Memory Questionnaire	5	Specific

Table 1. Summary of post-appointment questionnaires

a) Basic Psychological Assessment

- **The Depression Anxiety Positive Outlook Scale (DAPOS) (specific)** will be conducted as the confounding effect of psychological conditions are well recognised in neuropathic pain [39]. This scale has been validated in chronic pain populations and incorporates positive, as well as negative features, which are commonly associated with pain syndromes. Its use will retain backwards compatibility with the data from the HIV-PINS study, samples from which will be also genotyped by deCode within NeuroPain. It is important to recognise depression, as it may be that patients who are depressed are more likely to have pain, or that those with pain become depressed as a consequence of it [40, 41]. It may also be that patients with HAND have higher rates of depression.
- **The Hospital Anxiety and Depression Scale (HADS) (core)** will be used as another measure of anxiety and depression [42]. This is to enable coherence with previously collected data by deCODE and will also allow a direct comparison with DAPOS data in HIV patients.

b) Quality of Life

- **SF-36 (core)** This is a generic quality of life questionnaire which incorporates the impact of pain [43] and has been shown to be reliable, and valid, across a wide range of populations and conditions. Normative data for a variety of patient populations is also available. Despite being generic, it is thought to be a more precise and functional assessment of quality of life than those targeted specifically at patients with HIV [44].
- **Insomnia Severity Index (ISI) (specific)**. This is a subjective 7 item questionnaire [45] which has been shown to be reliable for pain patients with insomnia when compared to polysomnography and sleep diaries [46].

c) Pain Characterisation

- **Brief Pain Inventory (BPI) (core)** Although originally developed for assessing the interference of function in cancer pain [47], the BPI has been validated to measure the effect of other types of pain on functional interference across seven domains; general activity, mood, walking ability, relations with others, sleep and enjoyment of life.
- **Neuropathic Pain Symptom Inventory (NPSI) (specific)** This questionnaire gives further information about positive neuropathic symptoms and has been validated in chronic pain patients [48]. It has also been used to subgroup patients and has shown some correlation with QST results [49].

d) Temperament assessment

- **The Ten Item Temperament Inventory (specific)** [50] is a modified, shortened version of the NEO-Five Factor Inventory. Such temperament inventories examine the 'Big Five'

personality traits; neuroticism, openness, extraversion, agreeableness and conscientiousness. Neuroticism has been shown to correlate with higher pain vigilance and fear related pain behaviours in chronic pain patients [51]. Temperament domains have not been rigorously tested in patients with HIV sensory neuropathy in the past.

- **The Pain Catastrophising Scale (PCS) (core)** will be used to identify rumination, magnification and helplessness. It is a five minute, 13 item instrument, which has been validated in neuropathic pain [52, 53]. Catastrophizing has also recently been shown to impact on adherence to treatment in HIV patients [54].

3.3 The Clinical Appointment

Patients will be asked to attend a clinical appointment at Chelsea and Westminster Hospital, which will last approximately one hour and 45 minutes. At the clinical appointment a number tests will be performed. These are summarised in the table below.

Clinical Parameter	**Process**	**Time taken to complete (minutes)**	**Core or specific**
Collection of patient demographics	Analysis of patient notes and history taking	5	Core and specific
Structured neurological examination including blood pressure	Examination	10	Specific
Quantitative Sensory Testing (QST)	Psychophysical testing	30	Specific
CHANT : Clinical HIV-Associated Neuropathy Tool	History and examination	5	Core
QUESTOR body map	Body map representation of pain	2	Core
DN4-i : Doleur Neuropathique 4 Interview	Interview	3	Core
'CogState' cognitive function testing	Computer Based test	45	Specific
Peripheral Blood Sample	Blood Sample	5	Core

Table 2. Summary of tasks to be performed at clinical appointment

a) Gathering of patient information

Information will be collected from the patient interview, and from prior analysis of paper and electronic medical records. The following will be documented:

- Gender, age, ethnicity, sexual orientation
- Hand dominance
- Medical history including other potential causes for neuropathy and neurocognitive impairment
- Date of HIV diagnosis, complications, drug history including previous and current ART regimens
- Drug, smoking and alcohol history – both chronic and within the last 48 hours
- Educational level, occupation and socioeconomic status

- Results of clinical investigations previously performed:
  - Nerve conduction studies, QST and skin biopsy (where available)
  - Most recent CD4 count and viral load and CD4 nadir
  - LFTs/TFTs/B12/triglyceride levels/HepB/HepC/syphilis
  - CT/MRI/PET imaging of the brain (where available)

b) Basic examination

- Height
- Weight

c) *Structured Neurological Examination*

An upper and lower limb neurological examination will be performed to detect for clinical neuropathy. This will include an assessment of light touch and pinprick sensation, joint position and vibration sensation, deep tendon reflexes, muscle wasting and motor power, as described by the NeuPSIG guidelines [55].

A lying and standing blood pressure will be measured as an indicator of autonomic dysfunction. A lying non-invasive blood pressure will be recorded after lying for 5 minutes. The patient will be asked to stand and their blood pressure recorded at 1 and 3 minutes. A diagnosis of orthostatic hypotension will be made if there is a fall in systolic pressure of at least 20mm Hg or diastolic pressure of at least 10mm Hg [56]

d) *Case Definition: Diagnosis of HIV-SN*

All patients will be screened for HIV-SN based on the CHANT (Clinical HIV-associated Neuropathy Tool) screening tool [15]

This is a simple 4-item tool comprised of two subjective questions (self report of foot pain and of foot numbness), and two objective measures on clinical examination (loss of vibration sense and loss of ankle reflex). If the patient is positive for one symptom and one sign in a bilateral distribution they will be defined as having sensory neuropathy.

e) *Diagnosis of HIV-SN related neuropathic pain (HIV-SNP)*

Patients will be given a body map to illustrate any sites of pain, and the 7-item DN4-i (Douleur Neuropathique 4-interview) interview will be conducted to identify the probability of the patient having neuropathic pain. A score of ≥3 out of 7 from the patient reported outcome section of the DN4-i will be used as a positive diagnosis of neuropathic pain [57] as this cut-off has been shown to have sensitivity of above 80% in neuropathic pain conditions[58].

Patients will also be asked to indicate the areas that they experience pain. Symptoms must also be in a neuroanatomically plausible distribution, which could be attributable to HIV-SN; for example in a distal, symmetrical distribution. A QUESTOR legend for body areas will be used for this. Both a score of ≥3 on DN4-i and a plausible anatomical distribution will be required for definition of HIV-SNP.

f) Neurocognitive Assessment

- Although full neuropsychological testing is the gold standard for the diagnosis of HAND [60], it is very time consuming and requires specialist delivery which is not universally available. It is also used for the diagnosis of HAND rather than the measurement of it,

and such quantification of the disorder is important for this study, and for longitudinal follow up of patients. One computerised battery of tests (CogState) has been studied in HAND and has been shown to have a positive predictive value of 81%[61]. It also appears to be sensitive enough to monitor changes in ARV therapy and mirrors structural changes identified on MRS[62]. It includes eight tasks conducted with a computer screen and keyboard. There is a 15 minute 'training session' and the test takes approximately 20 minutes. It assesses eight attention, memory and executive function domains **(specific phenotyping).**

- A baseline level of intelligence quotient, such as the National Reading Test has not been included as it is not a longitudinal study and would not accurately assess IQ in such a multicultural population where English is frequently not the first language.
- A subjective measure of cognitive function will also be performed using the Everyday Memory Questionnaire (EMQ). This provides a patient reported measure of memory and attention to compare with the objective computerised measure.

g) Quantitative Sensory Testing **(specific phenotyping)**

Quantitative Sensory Testing (QST) will be conducted the German Research Network on Neuropathic Pain QST protocol [10]. This is a battery of somatosensory testing which uses mechanical, cold, warm and pinprick stimuli in 13 domains; detection thresholds (cold detection threshold (CDT), warm detection threshold (WDT), paradoxical heat sensation (PHS), thermal sensory lime (TSL), mechanical detection threshold (MDT) and vibration detection threshold (VDT)), pain thresholds (cold pain threshold (CPT), heat pain threshold (HPT), mechanical pain threshold (MPT) and pressure pain threshold (PPT)), and stimulus-response functions (mechanical pain sensitivity (MPS), wind-up ration (WUR) and dynamic mechanical allodynia (DMA)).

Full QST testing has been performed on patients with HIV-SN in a previous study [14]. This study showed that although 86% of HIV-SN patients showed a loss of function in at least one modality, no single sensory parameter was had a useful diagnostic utility. This study showed that there were no significant differences in CPT, DMA, MPT and MPS between those with HIV-SN and healthy controls, and between those who reported pain and those that did not. Other studies have however, highlighted MPT and MPS as useful parameters [49] in HIV-SN. Therefore, in this study, the full DFNS QST protocol will be performed. The testing will be performed at the site of maximum pain or, in those with no pain, on the dorsum of their right foot, in the S1 dermatome.

QST results have been shown to differ in populations with cognitive impairment, such as Alzheimer's disease [63], but have not been assessed in comparison to mild cognitive impairment in HIV. It has however, been shown that there is good test-retest correlation when conducting QST in mild to moderate Alzheimer's, indicating that despite the requirement for short term memory and other executive functions, QST can still be performed in a reliable manner [63].

h) Blood Testing

A peripheral blood sample (30ml) will be taken from each patient.

10ml will be processed and stored as per protocol, from deCODE, and will be used for genetic analysis. Patients will be consented for using the blood for genetic analysis and that further genetic

or biochemical tests may be performed in the future on the sample to investigate potential links with pain, neuropathy or HAND. A material transfer agreement (MTA) will be in place between Imperial College London and deCODE in Iceland outlining the responsibilities of each party in the transfer of the blood samples. The remainder of the blood will be stored at Chelsea and Wesminster Hospital until the end of the study. It will then be transfer to a secure tissue bank after processing at Chelsea and Westminster Hospital. This is for independent genetic or biochemical analysis in the future, for which patients will be fully consented.

i) Conditioned Pain Modulation **(Specific phenotyping)**

Conditioned Pain Modulation (CPM) is a method of testing a subjects intrinsic descending pain modulation. It is a way to measure the phenomenon that pain at one site can inhibit the sensation of pain at a distant site. It is it thought that deficiencies in CPM exist in a number of chronic pain conditions such as fibromyalgia, diabetic polyneuropathy and osteoarthritis. The protocol for CPM has now been established in our laboratory. It involves using a warm thermode which is placed on the patients forearm; the thermode it heated until the patient describes a pain score of 60 out of 100. This stimulus lasts for a maximum of 30 seconds. An alternative method is to use a pressure algometer to measure pain pressure threshold three times as a pressure test stimuli at the dominant upper arm (on the deltoid muscle). Either the thermal OR the pressure stimuli will be used, not both. Only patients unable to tolerate the pressure threshold will undergo thermal testing. The patient then is asked to place their hand in a bath of cold water (12°C) for one minute before the warm or pressure stimulus is repeated. The score the subject gives for the test stimulus before the cold stimulus is then subtracted for the score they give during the cold stimulus to give a value for 'CPM effect'. It is hypothesised that patients with pain will have a smaller CPM effect. To identify any placebo or nocebo effects from the cool water, the experiment will be repeated using room temperature water as a 'sham'.

Since subjects recruited up to the 30th October 2015 underwent the paradigm with the thermal test stimuli and the pressure pain threshold stimuli has been shown to be more reliable, patients tested between 1st December 2014 and 30th October 2015 will be invited to return to undergo the paradigm again, using the pressure pain threshold. Only patients who have indicated on their written consent form that they are happy to be contacted about future studies will be approached. They will be contacted by email or telephone and sent the additional patient information leaflet (PIL additional information version 1.0). At the clinical appointment they will undergo only CPM testing after signing a Consent Form Addendum (version 1.0). Reasonable travel expenses will be reimbursed.

j) Hand Held Nerve Conduction Studies **(Specific phenotyping)**

Formal nerve conduction studies (NCS) are used clinically to aid the diagnosis of small fibre neuropathy and are commonly used in the HIV population. However, formal NCS takes a considerable amount of time and must be performed at by a neurophysiologist at a specialist centre. Two new devices have been developed to identify sural nerve conduction abnormalities in a user-friendly way. These devices, the ADVANCE NCS System and the NC-Stat DPNCheck (both manufactured by Neurometrix) take only a couple of minutes and can be used by the non-expert. It involves passing an electric current through the ankle and measuring the speed and size of the nerve conduction of the sural nerve. These devices have been validated and are used clinically in the

diabetic population to detect a similar small fibre neuropathy to that seen in HIV patients. We intend to validate the use of these devices in HIV patients

k) Lower leg skin biopsy **(Specific phenotyping but OPTIONAL for a small proportion of patients)**.

Skin biopsy together with clinical examination is the gold standard for diagnosing HIV-SN in the clinical setting. This study attempts to use non-invasive means for defining HIV-SN based on examination findings and patient history, using a validated tool. Skin biopsies in a small proportion of patients with extreme phenotypes would allow for comparison with other measures obtained in this study such as NCS, genetic testing and cognitive function which have not been assessed in the context of skin biopsy previously. Biopsies will be taken, fixed and analysed using immunohistochemistry as per the European Federation of Neurological Societies guidelines and biochemical analysis may also be performed. Biopsies will be stored at Chelsea and Westminster Hospital until the end of the study, after which they will be transferred to an HTA approved Tissue Bank.

It is anticipated that upto 30 patients out of the cohort of 200 will undergo skin biopsy and this will be an optional section on the consent form. Patients will be excluded if they are known to be coagulaopathic or if they are taking anti-coagulant medication or if they have an allergy to local anaesthetic. They will be given an information leaflet and contact details should there be a problem with bleeding or infection.

3.4 Study Outcome Measures

The aim of the HIV-POGO study is to phenotype a cohort of HIV positive patients with and without HIV-SN and to identify factors associated with neuropathy and, in patients with neuropathy, factors associated with neuropathic pain. It is anticipated that genetic analysis will show differences between the phenotyped groups. We also aim to elucidate any neurocognitive impairment in the cohort and to examine whether there is any correlation with the HIV-SN phenotype or with how pain is reported.

4. Participant Entry

4.1 Pre-registration evaluations

All adult patients aged 18 years or older, attending HIV or chronic pain clinics at St Stephens Centre and Chelsea and Westminster hospital, or partnered clinics, will be invited to participate. Their eligibility for inclusion in the trial will be assessed with a brief questionnaire and those eligible will be offered patient information on the study.

4.2 Inclusion criteria

- Aged 18 years or older
- HIV positive

4.3 Exclusion criteria

- Pregnancy
- Refusal to consent, including for genetic analysis

- Pain ≥4 on NRS due to a pathology other than HIV-SN
- Co-incident severe CNS disease (as determined by the investigator) including a diagnosis of dementia
- Co-incident major psychiatric condition (DSM V criteria[64]: Depression 296.30-36 and Anxiety 300.0-3/21-23/29)
- Limited English language skills as unable to complete questionnaires

4.4 Withdrawal criteria

If a patient wishes to withdraw from the study, including their data and blood, they can do so at any stage during the protocol. This will not have any effect on their treatment or inclusion in future studies.

5. Adverse Events

This is an observational study and we are not expecting any adverse events (AE). The only potential anticipated AE would arise from the complications of phlebotomy. Any AEs will be recorded in a AE log and securely stored. Any serious AEs will be reported to the Chief Investigator (AR) within 24 hours. If the SAE was related to the study or occurred unexpectedly, it will be reported to the Sponsor and REC within 15 days.

6. Assessment and Follow up

Patients will be given the contact details for the Chief Investigator and the investigator conducting the appointment if they have any concerns in the period following the study.

The study will be complete one year after the 200th patient has completed their clinical appointment. This is to allow for further contact with the patient for clarification of information during the analysis phase.

7. Data storage and Data Analysis

7.1 Data Management

All data will initially be collected on paper case report forms (CRF). Subjects will be given a study specific identification number that will be used on the CRFs. Therefore no patient identifiable data will be entered on the CRFs. An anonymization log will be stored in the trial master file (TMF) as well as the original completed CRFs; the TMF will be kept in a locked room at Chelsea and Westminster Hospital.

Study information from the paper CRFs will be transcribed onto a password-protected database. Any patient identifiable data will be stored on NHS computers at Chelsea and Westminster Hospital. Such computers require password access and are only accessible to staff. Only anonymized data will be stored on a secure Imperial College computer at Chelsea and Westminster Hospital. Only the research team for this study will have access to this computer.

Relevant data will be shared with deCODE as collaborators, using a secure, encrypted database

7.2 Statistical Analysis

Statistical advice was sought from deCODE genetics statisticians with regards to sample size and analysis.

Genetic Analysis

Single nucleotide polymorphisms (SNPs) that show significant association to defined neuropathic pain phenotypes after genome-wide adjustment will be validated in several independent case-control groups. The validation of the associated signals identified in the GWA study is essential, as successful validation in additional groups would substantially increase our confidence in the associations observed. It will also allow us to determine if an allelic frequency is significantly different between cases and controls, since the familial correction factor will be much less than in the discovery cohort. These single-marker association studies will be analyzed, including adjustment for any familiality in patients or control groups. The p-values in each cohort will be adjusted by Bonferroni correction for number of markers tested or by randomization of phenotype procedure.

In those regions where we observe genome-wide significant associations to neuropathic pain phenotypes that are validated in a second set of independent samples, we intend to select additional markers for genotyping in order to refine the observed association signal and search for possible functional variants.

Re-sequencing will be done to screen for the actual mutation and/or to identify haplotypes that are rarer and have higher risk (for diagnostic purposes). The sensitivity of this method may allow us to detect different frequencies of rare variants such as 3% vs 10% in the general population between patient and control pools. Variants that show association to the phenotypes selected will be converted to a SNP assay and rerun on the entire sample set of the selected phenotype and controls for confirmation of the signal.

Power Calculations

The study design should ensure that large enough sample sizes will be typed in order to detect variants of moderate risk in the range of 1.4-2.0. Power calculations are used to estimate the minimum risk and frequencies that can be expected to detect given the sample size proposed. An example of 1100 subjects and 5000 controls in a GWA-study is presented. For example, we have over 90% power to detect a risk variant in 15% allelic frequency or higher with a risk ratio of 1.6, and more than 98% power to detect a risk variant in 20% allelic frequency or higher with a risk ratio of 1.6. The power is computed for a SNP surrogate of the mutation, which may or may not be a SNP, with R^2 between the SNP and the mutation assumed to be 0.8. A multiplicative model for risk is assumed (Falk & Rubinstein, 1987), and relative risk (RR) is defined as the risk of the mutation relative to the wild type.

The wider collaboration and use of replication cohorts

DeCODE will pursue 2 parallel approaches to replication of variants that they find in their discovery cohorts in Iceland. Firstly they will attempt cross-replication of variants found to have significant association in one type of chronic pain, after correction for multiple testing, to other types of chronic neuropathic pain.

They will test variants found in Iceland in well-phenotyped chronic pain cohorts from the US,

Canada, UK, and Israel, totaling over 12,000 patients. For all variants found in Iceland they will also look for association to other chronic pain phenotypes not represented in the discovery cohorts: HIV painful neuropathy from this group (200 patients) and 2 limb amputation cohorts collected by Dr. Seltzer from Israel and Cambodia (500 and 5500 patients, respectively).

8. Regulatory Issues

8.1 Ethics Approval

The Chief Investigator has obtained approval from the >>> Research Ethics Committee. The Chief Investigator will have a letter of approval from the Trust R&D department before the recruitment process can start. The study will be conducted in accordance with the recommendations adopted by the 18th World Medical Assembly, in Helsinki 1964.

8.2 Local Approval and NIHR portfolio adoption

Local approval will be sought from Chelsea and Westminster Hospital research and development department. The study will not start until permission has been granted.

The project is eligible for inclusion in the NIHR Clinical Research Network and this will be applied for via the IRAS system.

8.3 Consent

Consent to enter the study will be sought for each participant after full explanation has been given, a patient information leaflet offered and time allowed for consideration. The right of the patient to refuse to participate without giving a reason will be respected. All participants are free to withdraw at any time without giving reason and without prejudicing further treatment. The anonymisation log will include the bar code used on the blood sample; this means that an individuals sample can be traced should they wish to withdraw consent.

8.4 Confidentiality and Data Protection

The Chief Investigator and his team will preserve the confidentiality of participants. Access to data will only be granted in accordance with the Data Protection Act 1998. It will be the responsibility of the chief investigator to make sure that data stored in accurate. All subjects have the right to withdraw permission for the processing or storage of their data at any time.

8.5 The Human Tissue Act

Blood samples will be stored in accordance with the Human Tissue Authority.

The sample for analysis by deCODE will be labelled using their own barcode sticker system, but the barcode will be documented in the anonymisation log.

The samples stored for future analysis will be stored in a secure tissue bank. Subjects will be offered the patient information leaflet 'Donating Biological Samples for Research – Information for Donors' and will be asked to sign the Donor Consent Form. Each sample will be given a unique identification

number and the storage database will be maintained by Tissue Bank staff in accordance with the HTA.

Patients have the right to withdrawn permission for the storage or analysis of their blood sample at any time and this will be possible using the anonymization log.

8.6 Indemnity and Sponsorship

Sponsorship has been confirmed with Imperial College. As part of the approval an insurance assessment has been conducted and certification provided.

8.7 Funding

This study is funded by the NeuroPain FP7 Grant, awarded by the European Union (Code: WSSA P44758).

8.8 Audit and Inspection

The study may be subject to inspection and audit by Imperial College under their remit as Sponsor, to ensure adherence to GCP.

9. Study Management

The trial will be managed and conducted in accordance with GCP guidance. The Chief Investigator will be responsible for the development, management and security of the trial master file (TMF).

All trial documents will be archived in a secure, environmentally controlled location at Chelsea and Westminster Hospital for 10 years following termination of the study. Access to the archive will be limited to the Chief Investigator, the Sponsor and regulatory authorities.

10. Publication policy

Findings from this project will be primarily disseminated using the conventional format of peer review publication, in association with conference presentations.

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