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HIV neuropathy: an in vivo confocal microscopic study

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Abstract Several approaches exist for quantitative assessment of human immunodeficiency virus (HIV)-associated distal sensory polyneuropathy (DSP). While useful, each has some limitations. This study evaluated non-invasive, in vivo reflectance confocal microscopy (RCM) of Meissner corpuscles (MCs) as a measure of HIV-DSP. Forty-eight adults (29 HIV-infected, 19 controls) underwent RCM of MC density (MCs/mm^2) at the arch, fingertip, and thenar eminence (TE); ankle skin biopsy to measure epidermal nerve fiber density (ENFD); electrophysiologic studies; and tactile, vibration, and thermal threshold testing. HIV+ subjects were clinically categorized as having DSP signs or no signs. MC densities were lower in HIV+ subjects with DSP signs than in controls (arch, $p=0.0003$; fingertip, $p<0.0001$; TE, $p=0.0002$). Tactile thresholds in the TE and foot were worse in HIV-DSP than in controls, but in this mild DSP cohort, sural amplitudes, ENFD, and vibration and thermal thresholds did not differ significantly from controls. Fingertip MC densities and tactile thresholds at the foot were also lower in HIV+ subjects without DSP signs than in controls. Other sensory measures were not significantly different in HIV+ subjects without DSP signs than in controls. MC density correlated inversely with tactile thresholds at each imaging location. The results suggest that RCM of MC density complements existing sensory DSP measures and discriminates mild HIV-DSP from controls at a stage when sural amplitudes do not. Further studies are required to determine whether RCM of MC density can establish quantitative changes in DSP, in response to treatment or disease progression.

Keywords In vivo confocal reflectance microscopy · Meissner's corpuscle · Peripheral neuropathy · Skin biopsy · Outcome measure

Introduction

Sensory nerve terminals are involved early or preferentially in many forms of peripheral neuropathy, yet non-invasive microscopic approaches for their in vivo imaging in humans have been lacking. In vivo reflectance confocal microscopy (RCM) is a painless, non-invasive technique that is increasingly being used for the imaging of human skin lesions, including cancer (Rajadhyaksha et al. 1999; Selkin et al. 2001). The approach involves application of a low-level laser to illuminate a point within the skin. Due to naturally occurring variations in the refractive index in the skin, a portion of the light is reflected off the tissue and through a pinhole aperture onto a detector. RCM does not require the use of contrast agents or fluorophores and has a lateral resolution of 0.5–1 μm (Rajadhyaksha et al. 1999). We recently described the feasibility of RCM of Meissner corpuscles (MCs), the main touch-pressure sensation receptors in glabrous skin (hands, feet, digits) (Bolton et al. 1996; Dyck et al.

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1966; Herrmann et al. 2007; Almodovar et al. 2011). In order to explore whether MC imaging might yield a novel measure of distal sensory neuropathy, we selected as a model HIV-associated distal sensory polyneuropathy (DSP), the most common neurological complication of HIV infection (Herrmann et al. 2004; Schifitto et al. 2002; So et al. 1988; Tyor et al. 1995; Cornblath and McArthur 1988; Tagliati et al. 1999; Shy et al. 2003; Holland et al. 1997). Several methods (nerve conduction studies (NCS), quantitative sensory testing (QST), and skin biopsy from hairy skin for assessment of epidermal nerve fiber density (ENFD)) have been developed for monitoring of the small- and large-fiber sensory components of DSP (Holland et al. 1997; Polydefkis et al. 2002; Zhou et al. 2007; Herrmann et al. 1999; Cherry et al. 2005). While useful, each has limitations with respect to detection and objective monitoring of DSP (Shy et al. 2003; Polydefkis et al. 2002; Zhou et al. 2007; Herrmann et al. 1999; Cherry et al. 2005). Non-invasive approaches toward early detection and monitoring of HIV-DSP are needed to complement these measures and help establish quantitative changes in DSP, below the clinical threshold of detection, in response to treatment or disease progression.

The aims of the present study were to: 1. compare MC density (MC/mm^2) by in vivo RCM at the hand and foot between HIV-infected subjects with clinically defined DSP and healthy control subjects; 2. compare MC density by in vivo RCM at the medial sole and hand (digit 5 and the thenar eminence) between HIV-infected subjects without clinical DSP and healthy control subjects; and 3. determine relationships among in vivo RCM of MC density at the hand and foot and standard peripheral sensory measures.

Patients and methods

Forty-eight subjects (29 with HIV infection and 19 healthy controls) between the age of 18 and 65 years were recruited following written informed consent under an RSRB approved, NINDS supported protocol. HIV seropositivity was based on self-report and confirmed by ELISA and Western blot or viral load.

The inclusion/exclusion criteria for the HIV group were: age 18–65 years; no history of alcohol abuse; no B12 deficiency, hypothyroidism, or diabetes mellitus, based on screening serum B12 levels, thyroid function studies, and fasting blood glucose or hemoglobin A1C; no active opportunistic CNS infection or other chronic neurologic disorders; no familial neuropathy or other systemic disease or neurotoxin exposure (other than antiretroviral therapy) known to predispose to peripheral neuropathy; no signs or symptoms of a compression mononeuropathy; and no signs or symptoms of a myelopathy.

The inclusion/exclusion criteria for normal control subjects were: age 18–65 years; no history of diabetes mellitus, HIV infection or other conditions known to predispose to neuropathy; no clinical signs or symptoms of polyneuropathy or compression mononeuropathy; no history of neurotoxin exposure or alcohol abuse; no foot deformities suggestive of a hereditary neuropathy or history of a familial polyneuropathy; and no abnormality of fasting glucose, renal, liver, or thyroid function, serum vitamin B_{12}, HIV seropositivity, serum protein electrophoresis, or immunofixation. MC density, touch-pressure, and vibration thresholds at the hand for the control group have been described in part (Almodovar et al. 2011).

Subjects recruited to the HIV cohort were classified as having either (1) HIV infection with signs of DSP or (2) HIV infection with no DSP signs, whether they were symptomatic or not. Signs of DSP were defined by presence of one or more of the following clinical criteria: (1) bilaterally absent ankle reflexes, (2) reduced vibration sense at the toes, (3) abnormal proprioception at the toes, or (4) a distal stocking or stocking and glove reduction in pinprick perception. Symptoms of DSP included length-dependent complaints of pain, burning, dysesthesia, or paresthesia.

In vivo RCM of MC density

In vivo RCM of MCs (VivaScope 1500, Lucid. Inc., Rochester, NY) was performed over the volar surface of left hand digit 5, the left thenar eminence, and arch of the sole in all subjects. A single trained microscopist performed MC imaging at each site within a 6×6-mm area from the basal layer of the epidermis through the depth of the dermal papillae as previously described (Herrmann et al. 2007; Almodovar et al. 2011). A 6×6-mm mosaic image of individual adjacent 500×500-μm imaging fields was acquired within in a horizontal plane near the basal layer of the epidermis, so that the most superficial MCs were visible. Following the initial imaging sequence, seven mosaic images were captured at successive 20 μm depths within this 6×6-mm region, so that the volume of tissue was sampled through the depth of the dermal papillae. Systematic random sampling was used to count MCs in a blinded manner, within an 18-mm^2 area within each mosaic image. Mosaic images at successive depths were compared to avoid double counting of MCs. MC density was expressed as MCs per millimeter (Dyck et al. 1966; Herrmann et al. 2007; Almodovar et al. 2011).

Quantitative sensory testing

Quantitative sensory testing of vibration, cooling, and heat-pain (HP) thresholds was performed using the Case IVB system (WR Medical Electronics Company, Rochester, MN)

at the great toe/foot and digit V/hand. Cooling and vibration detection thresholds were assessed using the 4-2-1 algorithm and results expressed as a normal deviate (ND) based on age, height, and gender matched normative data (Dyck et al. 1993a, b). Heat-pain thresholds were assessed using the non-repeating ascending with null stimuli (NRA-NS) algorithm. Both a threshold (HP 0.5) and an intermediate response of pain from graded heating pulses (HP 5.0) were assessed using a visual analog scale and results expressed in just noticeable differences and converted to ND based on age, height, and gender matched normative data (Dyck et al. 1993b).

Touch pressure sensory thresholds were assessed at each RCM imaging site using a series of nine monofilaments of different bending forces. A 4-2-1 testing algorithm was applied including variable null stimuli. The touch pressure threshold was defined as that stimulus that was correctly identified at least 50 % of the time (Almodovar et al. 2011).

Electrophysiologic studies

Each subject underwent left sural nerve, medial plantar nerve, and ulnar nerve (digit V) sensory testing using standard antidromic surface recording and stimulation techniques for the sural and ulnar sensory nerves, and orthodromic methods for the medial plantar nerve. Limb temperature was maintained above 32 °C during electrophysiologic testing.

Skin biopsy (epidermal nerve fiber density)

All subjects underwent a 3-mm distal leg skin biopsy, 10 cm proximal to the left lateral malleolus. Skin biopsies were processed and immunostained with polyclonal antibodies to the panaxonal marker, protein gene product 9.5, according to previously published methods (Herrmann et al. 1999, 2004). A blinded observer assessed ENFD for each biopsy from four 50-μm sections according to established methodology.

Statistical methods

Pair-wise group comparisons of demographic variables among healthy controls and HIV-infected subjects with (HIV-DSP) and without DSP signs were performed using either Wilcoxon rank sum tests or Fisher's exact tests, as appropriate. Analysis of covariance based on ranks was used in the primary analyses to compare MC density between -DSP subjects and healthy control subjects at each of the three imaging sites (Conover and Iman 1982). The models for digit V adjusted for age and hand area; those for thenar eminence adjusted for age; and those for medial sole adjusted for age and height. A Bonferroni-adjusted significance level of 1.7 % (two-tailed) was used for each imaging site. Secondary analyses involved similar comparisons between HIV+ subjects without DSP signs and healthy control subjects. Conventional sensory measures, including monofilament thresholds, QST thresholds for vibration, cooling, and heat-pain, distal leg ENFD and sural, medial plantar and ulnar sensory NCS amplitudes, were compared among the groups (HIV-DSP and HIV without DSP signs versus control subjects) using analysis of covariance adjusting for age and gender.

Relationships between in vivo RCM of MC density and conventional sensory measures were examined at each imaging site in the overall study cohort using Spearman's rank correlation coefficients. A significance level of 1 % was used for analyses involving correlation coefficients.

Results

Clinical and demographic features

Clinical and demographic features for the 48 participants are shown in Table 1. Control and HIV-infected subjects were similar in age. More men were recruited in the HIV-infected groups (72 %) than in the control group (42 %). The mean duration of known HIV infection was 11±6 years. Thirteen HIV+ patients had signs of DSP and 16 did not. Among subjects with DSP signs, 6/13 (46 %) had two or more DSP signs, 8/13 (62 %) had bilaterally absent ankle reflexes, 10/13 (77 %) had abnormal vibration sensation at the toes, and 5/13 (38 %) had absent or reduced pin sensation in the feet. Eight of 13 subjects with DSP signs had DSP symptoms, while seven of 16 HIV-infected individuals without DSP signs had sensory symptoms or pain. Among HIV+ subjects, mean (±standard deviation) CD4 cell count was 425.3±277.9 cells/ml. Viral load was undetectable in 16 out of 29 HIV+ subjects, was less than 10,000 copies/ml in 8/29 subjects and was greater than 10,000 copies/ml in 5/29 subjects. Seventy-nine percent of HIV+ subjects were on highly active antiretroviral therapy at the time of the study, while 10 % of HIV-infected subjects had a known history of prior exposure to dideoxynucleoside agents (Table 1).

Comparisons of MC densities in HIV-infected subjects with and without DSP signs versus those in controls

MC densities were lower in HIV+ subjects with DSP signs than in controls at all three imaging sites, after controlling for relevant covariates (foot: $p=0.0003$; fingertip: $p<0.0001$; and thenar eminence: $p=0.0002$; Table 2 and 3, Figs. 1 and 2). Among HIV+ subjects without DSP signs, MC densities at digit V were lower than in controls ($p=0.002$), but those at the foot and thenar eminence did not differ significantly from those in controls. MC densities were lower at the foot ($p=0.01$) and thenar eminence ($p=0.01$) in HIV-infected subjects with DSP signs than those without DSP signs.

Table 1 Demographic and clinical characteristics of study participants

Variable	Controls (n=19)	HIV-NDSP (n=16)	HIV-DSP (n=13)
Age (years)	43.7 (8.1)	45.2 (10.3) (p=0.68)	45.6 (9.8) (p=0.55)
Gender (number M/F)	8/11	11/5 (p=0.12)	10/3 (p=0.051)
Height (cm)	168.5 (8.9)	170.0 (9.8) (p=0.55)	173.3 (6.4) (p=0.071)
Weight (kg)	77.1 (16.4)	79.4 (16.5) (p=0.67)	76.5 (22.4) (p=0.58)
CD4+ cell count (cells/ml)		369.4 (227.4)	494.1 (326.0)
Viral load (%)			
Undetectable		54 %	56 %
<10,000 copies/ml		31 %	25 %
≥10,000 copies/ml		15 %	19 %
HAART (% currently taking)		81 %	75 %
Known dideoxynucleoside drug exposure (%)		19 %	0 %

Values are mean (standard deviation) unless otherwise indicated. p values indicate comparisons between each of the HIV groups and the control group

HIV-NDSP HIV without signs of DSP, *HIV-DSP* HIV with signs of DSP, *HAART* highly active antiretroviral therapy

Comparison of epidermal nerve fiber density among HIV-infected subjects and controls

Epidermal nerve fiber density at the ankle tended to be lower in the HIV+ groups than in the control group (14.3± 4.9 fibers/mm), but these differences did not reach significance after controlling for age and gender. The DSP signs group had a mean ENFD of 10.3±3.8 fibers/mm (p=0.08) and those without DSP signs had a mean ENFD of 10.1±3.4 (p=0.027; Table 3).

Comparison of other sensory measures among HIV-infected subjects and controls

Touch-pressure thresholds at the sites of MC imaging were higher in HIV+ subjects with DSP signs than in controls at the foot (p=0.0009) and thenar eminence (p=0.01), but not at the fingertip (p=0.25), after controlling for age and gender (Tables 2 and 3). Touch-pressure thresholds in HIV+ subjects without DSP signs were higher at the foot than in controls (p=0.006). Vibration, cooling, and heat-pain thresholds did not differ significantly between the HIV-infected and control groups, although there were trends toward higher vibration (p=0.03) and lower (hyperalgesic) HP 0.5 thresholds at the toe/foot (p=0.05) in HIV-infected subjects with DSP signs compared to controls (Tables 2 and 3).

Medial plantar nerve action potential (NAP) amplitudes were lower among HIV+ subjects with DSP signs (6.0±2.4 μV) than in controls (10.6±4.0 μV, p=0.008), while those in HIV+ subjects without DSP signs did not differ significantly from those in the control group. Sural sensory nerve action potential (SNAP) amplitudes tended to be lower in HIV+ subjects with DSP signs than in controls (14.3±7.9 μV vs. 24.1±9.9 μV, p=0.06). Ulnar SNAP amplitudes were similar among the three groups (Table 2).

Table 2 Neuropathy outcomes (upper limb)

Variable	Controls (n=19)	HIV-NDSP (n=16)	HIV-DSP (n=13)
MC density, digit V (MCs/mm^2); median (IQR)	6.89 (3.11, 9.39)	1.64 (0.31, 4.73) (p=0.002)	1.34 (0.33, 1.67) (p<0.0001)
MC density, thenar eminence (MCs/mm^2); median (IQR)	2.00 (1.17, 3.67)	2.25 (1.34, 3.59) (p=0.90)	0.67 (0.33, 1.39) (p=0.0002)
Ulnar SNAP amplitude (μV)	35.1 (11.3)	32.8 (17.7) (p=0.66)	30.7 (14.3) (p=0.28)
Monofilament threshold, digit V; median (IQR)	0.07 (0.02, 0.07)	0.07 (0.07, 0.16) (p=0.05)	0.07 (0.07, 0.16) (p=0.25)
Monofilament threshold, thenar eminence; median (IQR)	0.07 (0.07, 0.16)	0.12 (0.07, 0.16) (p=0.09)	0.16 (0.12, 0.28) (p=0.01)
QST vibration, finger (ND)	0.62 (0.82)	0.54 (0.92) (p=0.66)	0.84 (1.53) (p=0.77)
QST cooling, hand (ND)	1.03 (1.32)	1.86 (1.24) (p=0.18)	1.94 (1.56) (p=0.17)
QST HP 0.5, hand (ND)	−0.91 (1.77)	−1.02 (1.79) (p=0.79)	−0.71 (1.53) (p=0.95)
QST HP 5.0, hand (ND)	−1.38 (1.87)	−1.15 (2.11) (p=0.89)	−1.01 (1.81) (p=0.98)
QST HP 0.5–5.0, hand (ND)	−0.20 (0.60)	0.06 (1.41) (p=0.71)	0.41 (0.91) (p=0.10)

Values are mean (standard deviation) unless otherwise indicated. Monofilament thresholds are measured as log of bending force of the monofilament in tenths of a milligram. p values indicate comparisons between each of the HIV groups and the control group

HIV-NDSP HIV without signs of DSP, *HIV-DSP* HIV with signs of DSP, *IQR* interquartile range (25th and 75th percentiles), *MC* Meissner corpuscle, *SNAP* sensory nerve action potential, *QST* quantitative sensory testing, *ND* normal deviate, *HP* heat pain

Table 3 Neuropathy outcomes (lower limb)

Variable	Controls (n=19)	HIV-NDSP (n=16)	HIV-DSP (n=13)
MC density, foot (MCs/mm^2); median (IQR)	1.39 (0.61, 2.50)	0.83 (0.22, 1.67) (p=0.32)	0.09 (0.00, 0.33) (p=0.0003)
ENF density, ankle (fibers/mm)	14.3 (4.9)	10.1 (3.4) (p=0.027)	10.3 (3.8) (p=0.08)
Sural SNAP amplitude (μV)	24.1 (9.9)	19.2 (8.7) (p=0.37)	14.3 (7.9) (p=0.06)
Plantar SNAP amplitude (μV)	10.6 (4.0)	8.8 (5.2) (p=0.65)	6.0 (2.4) (p=0.008)
Monofilament threshold, foot; median (IQR)	0.16 (0.07, 0.16)	0.40 (0.16, 1.00) (p=0.006)	0.70 (0.28, 1.00) (p=0.0009)
QST vibration, toe (ND)	0.66 (1.05)	0.57 (1.33) (p=0.79)	1.82 (1.47) (p=0.03)
QST cooling, foot (ND)	0.64 (1.67)	1.17 (1.35) (p=0.67)	2.03 (1.52) (p=0.11)
QST HP 0.5, foot (ND)	−0.62 (1.21)	−0.75 (2.14) (p=0.76)	−0.02 (1.85) (p=0.56)
QST HP 5.0, foot (ND)	−0.42 (1.79)	−0.35 (2.06) (p=0.83)	−0.42 (2.00) (p=0.62)
QST HP 0.5–5.0, foot (ND)	0.43 (1.21)	0.29 (1.45) (p=0.25)	−0.30 (0.90) (p=0.05)

Values are mean (standard deviation) unless otherwise indicated. Monofilament thresholds are measured as log of bending force of the monofilament in tenths of a milligram. p values indicate comparisons between each of the HIV groups and the control group

HIV-NDSP HIV without signs of DSP, *HIV-DSP* HIV with signs of DSP, *IQR* interquartile range (25th and 75th percentiles), *MC* Meissner corpuscle, *ENF* epidermal nerve fiber, *SNAP* sensory nerve action potential, *QST* quantitative sensory testing, *ND* normal deviate, *HP* heat pain

Associations between MC densities and other sensory measures

MC density at the foot was associated with MC density at the fingertip (r=0.47, p=0.002) and thenar eminence (r=0.48, p=0.0009), and was inversely associated with touch-pressure threshold at the arch (r=−0.41, p=0.006). There were weaker associations between MC density at the foot and sural SNAP amplitude (r=0.32, p=0.03) and ENFD (r=0.34, p=0.02). There was no significant association between MC density at the foot and medial plantar NAP amplitude, or with vibration, cooling, or HP thresholds at the foot (Table 4).

MC density at the fingertip was associated with MC density at the thenar eminence (r=0.42, p=0.004), the ulnar SNAP amplitude (r=0.44, p=0.005), and was inversely associated with touch-pressure threshold at the fingertip (r=−0.38, p=0.01). There was a weaker inverse relationship between MC density at the fingertip and cooling threshold at the hand (r=−0.41, p=0.02). There were no significant associations

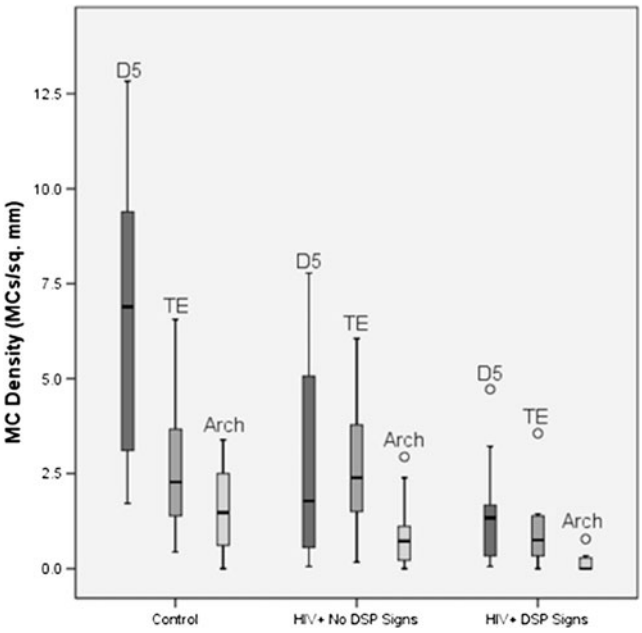

Fig. 1 Meissner corpuscle (*MC*) densities for control subjects and HIV-infected subjects with and without DSP signs. For each group, *boxplots* (median, quartiles, and extreme values (denoted by *open circles*)) of MC densities at the fingertip (*D5*), thenar eminence (*TE*), and arch are shown from *left* to *right*

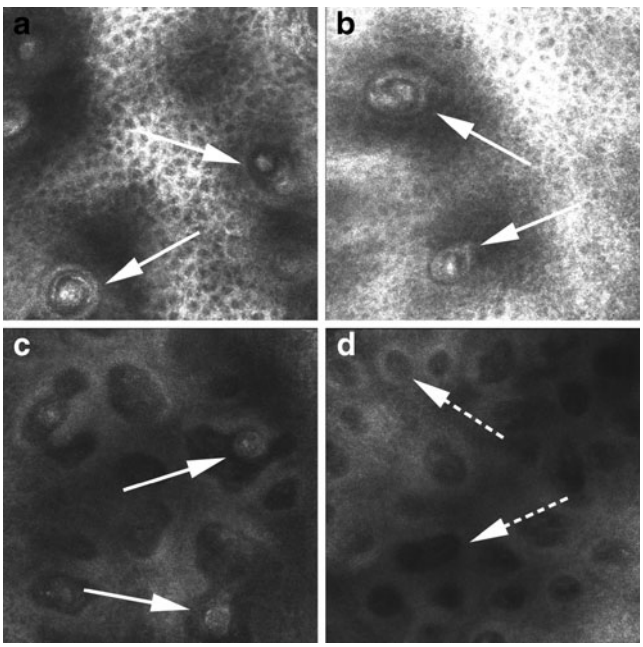

Fig. 2 RCM of Meissner corpuscles in a control subject (*arrows*, **a, b**—high power and **c**—lower power). **d** shows multiple dermal papillae without MCs (*broken arrow*) in a subject with HIV-DSP with marked depletion of MC density

between MC density at the fingertip and vibration or HP thresholds at the hand (Table 4).

MC density at the thenar eminence was inversely associated with touch-pressure threshold at the site of imaging (r=−0.46, p=0.001). There were weaker associations between MCD density at the thenar eminence and cooling threshold at the finger (r=−0.36, p=0.02) and HP 5.0–0.5 at the hand (r=−0.41, p=0.01).

Among HIV+ subjects, MC densities at the fingertip, thenar eminence, and foot were not associated with CD4+ lymphocyte count.

Discussion

We undertook a prospective study of in vivo imaging of MCs in HIV neuropathy. This study enrolled groups of HIV-infected subjects with or without signs of DSP as well as a prospective control group. HIV+ subjects with DSP signs had, as a group, very mild DSP as reflected by conventional measures of sensory neuropathy, including the sural SNAP amplitude and ankle ENFD.

In the present study, MC densities by in vivo RCM were reduced at the hand and foot in HIV+ subjects with DSP signs compared to controls at a time when sural and ulnar SNAP amplitudes did not differ significantly from those in control subjects and medial plantar NAP amplitudes were only mildly reduced relative to controls. These data indicate that mechanoreceptor imaging in the skin can provide evidence of peripheral sensory pathology in HIV infection when conventional electrophysiologic assessments of large myelinated sensory nerve fiber function show minimal changes.

There has been uncertainty regarding the relative involvement of small diameter unmyelinated sensory fibers versus large myelinated sensory fibers in HIV neuropathy. Both populations of sensory fibers are known to be involved during the course of HIV neuropathy, but some studies have suggested that small-fiber involvement may predominate, given that sural SNAPs are not uncommonly normal in HIV-DSP (Zhou et al. 2007). Contrary to the concept of HIV-DSP as a predominantly small-fiber neuropathy, however, is the clinical observation that ankle reflexes and vibratory sensation at the toes (mediated via myelinated fiber pathways) are commonly abnormal in individuals diagnosed with HIV-DSP, whether or not sensory electrophysiologic abnormalities are present (Cherry et al. 2005). The lack of an easily applied, localizing assessment of myelinated sensory nerve terminals has hitherto limited study of patterns of distal sensory fiber involvement in HIV-DSP. This study suggests that the nerve terminals of large-diameter sensory fibers are affected in HIV infection. HIV neuropathy in its mildest clinical form appears to be a small- and large-fiber sensory neuropathy, with preferential involvement of nerve terminals, rather than a pure small-fiber neuropathy.

This study incorporated a range of QST assessments. Vibration and thermal thresholds has been employed in longitudinal studies and therapeutic trials in HIV-DSP (Berger et al. 1993; Herrmann et al. 2006; McArthur et al. 2000). In the current sample, while vibration and thermal thresholds showed normal deviates that were in the direction of worse sensory function in HIV infection with DSP signs, significant differences from controls were not observed although the small sample sizes may have contributed to this. We also included assessment of touch-pressure thresholds. Touch-pressure thresholds have not been systematically studied in HIV neuropathy. Among all QST measures, touch-pressure thresholds best distinguished HIV+ subjects with DSP signs from controls. Monofilament thresholds were significantly

Table 4 Correlations between MC densities and conventional sensory measures

A. MC density, foot								
ENF density, ankle	Sural SNAP amplitude	Plantar SNAP amplitude	MF threshold, foot	QST vibration, toe	QST cooling, foot	QST HP 0.5, foot	QST HP 5.0, foot	QST HP 0.5–5.0, foot
r=0.34	r=0.32	r=0.26	r=−0.41	r=−0.003	r=−0.05	r=−0.11	r=0.03	r=0.15
p=0.02	p=0.03	p=0.10	p=0.006	p=0.99	p=0.76	p=0.54	p=0.89	p=0.39
B. MC density, digit V								
Ulnar SNAP amplitude	MF threshold, digit V	QST vibration, finger	QST cooling, hand	QST HP 0.5, hand	QST HP 5.0, hand	QST HP 0.5–5.0, hand		
r=0.44	r=−0.38	r=−0.19	r=−0.41	r=−0.11	r=−0.08	r=−0.21		
p=0.005	p=0.01	p=0.28	p=0.02	p=0.55	p=0.65	p=0.25		
C. MC density, thenar eminence								
Ulnar SNAP amplitude	MF threshold, thenar eminence	QST vibration, finger	QST cooling, hand	QST HP 0.5, hand	QST HP 5.0, hand	QST HP 0.5–5.0, hand		
r=0.29	r=−0.46	r=−0.24	r=−0.36	r=−0.02	r=−0.09	r=−0.41		
p=0.06	p=0.001	p=0.14	p=0.02	p=0.89	p=0.61	p=0.01		

MC Meissner corpuscle, *ENF* epidermal nerve fiber, *SNAP* sensory nerve action potential, *MF* monofilament, *QST* quantitative sensory testing, *HP* heat pain

elevated at the foot and thenar eminence in HIV+ subjects with DSP signs. Touch-pressure is the main sensory modality transduced by MCs (Baba et al. 2001). Touch-pressure thresholds provide information on the functional integrity of MCs and associated peripheral and central sensory pathways. Touch-pressure thresholds can be inexpensively assessed and as such may prove a useful adjunct in the clinical and research monitoring of HIV-DSP, particularly in resource constrained settings. However, touch-pressure thresholds are not localizing to either the peripheral or central nervous system and are a psychophysical modality that can be impacted by cognitive impairment in HIV infection. In vivo RCM, by contrast, provides non-invasive, quantitative, localizing, and more objective information regarding the sensory peripheral nervous system (PNS). For clinical and research questions where rigorous, quantitative assessment of different components of the sensory PNS is desirable, addition of MC imaging has advantages over assessment of touch-pressure thresholds alone.

HIV-infected subjects without DSP signs were less distinct from control subjects with respect to their peripheral sensory structure and function than were subjects with DSP signs. In vivo RCM of MC density among the HIV+ group without DSP signs was reduced at the fingertip as compared to controls, but not at the sole or palm. Medial plantar and sural SNAP amplitudes in HIV subjects without DSP signs did not differ significantly from those in controls. Touch-pressure sensation thresholds were worse at the foot in HIV+ subjects without DSP signs relative to controls. The occurrence of subclinical abnormalities of peripheral sensory structure and function in HIV+ subjects without DSP signs is consistent with previous investigations (Herrmann et al. 2004; McCarthy et al. 1995). In vivo RCM of MC density extends observations with regard to the spectrum of sub-clinical peripheral sensory involvement in HIV infection.

This study incorporated extensive evaluations of large- and small-fiber sensory structure and function so as to develop a detailed understanding of relationships between in vivo RCM of MC density and standard sensory neuropathy measures. The most consistent structure–function correlations were observed between MC densities and touch-pressure thresholds. Specifically, MC densities were inversely associated with touch-pressure thresholds at the site of imaging at all three locations. As MCs primarily subserve touch pressure sensation, these inverse associations make biological and clinical sense and support the functional meaningfulness of RCM of MC density (Baba et al. 2001). By contrast, weaker or insignificant associations were seen between MC densities and other measures of sensory perception including vibration and thermal thresholds as well as ENFD. This indicates that MC density assessments are complementary in the evaluation of the sensory PNS, providing quantitative information that is distinct from other sensory measures.

In this study, a clinical, rather than a neurophysiologic or pathologic approach was used to categorize HIV+ subjects. This was done in order to analyze relationships between RCM of MC density and standard neuropathy measures in a cohort of HIV-infected individuals as they present clinically.

Investigations of neuropathy measures in HIV infection have variously categorized subjects according to whether or not they had "at least one clinical sign of DSP" in some studies versus "presence of two or more DSP signs" in other studies (Simpson et al. 2006; Ellis et al. 2010). A threshold of "one clinical sign" can result in over-diagnosis of DSP, while the criterion of two or more signs under-diagnoses DSP (Simpson et al. 2006; Ellis et al. 2010). In our study the presence of "one or more DSP signs" was used to categorize subjects. This could potentially have resulted in misclassification of some individuals with regard to DSP. The impact however on the findings of this study was likely small, as approximately half of subjects in the DSP signs group had two or more signs, and the overall inference from this study that MC density might serve as a potential marker of early peripheral sensory involvement in HIV infection (whether clinical or sub-clinical) would not have been influenced by the method of categorization of subjects.

A further potential limitation of this study is that the HIV+ cohort did not contain subjects with advanced DSP. In part this was by design, in order to evaluate whether MC assessments could serve as a marker of early peripheral sensory involvement in HIV infection. The paucity of subjects with advanced DSP also reflects the changing spectrum of disease seen in our medical center in the era of HAART. Future studies will need to assess changes in MC structure and function across the full severity spectrum of HIV-DSP.

In summary, structural assessment of myelinated sensory terminals with in vivo RCM of MC density coupled with functional assessment of MCs (touch-pressure thresholds) appear to identify sensory PNS involvement in HIV infection at a time when changes in sensory NCS and conventional QST are sparse. RCM of MC density thus provides a structural marker of early sensory neuropathy in HIV infection and, in combination with standard measures of neuropathy, a more complete profile of the sensory involvement in HIV infection.

Additional longitudinal studies are required and to determine whether MC density assessment coupled with touch-pressure thresholds can be used reliably to follow progression or improvement of HIV neuropathy through the course of disease and therapy.

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