HIV neuropathy natural history cohort study

Assessment measures and risk factors

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Abstract—Background: Distal sensory polyneuropathy (DSP) is the most common neurologic complication of human immunodeficiency virus (HIV) infection. Risk factors for DSP have not been adequately defined in the era of highly active antiretroviral therapy. **Methods:** The authors evaluated 101 subjects with advanced HIV infection over 48 weeks. Assessments included a brief peripheral neuropathy (PN) screen (BPNS), neurologic examination, nerve conduction studies, quantitative sensory testing (QST), and skin biopsies with quantitation of epidermal nerve fiber density. Data were summed into a Total Neuropathy Score (TNS). The presence, severity, and progression of DSP were related to clinical and laboratory results. **Results:** The mean TNS (range 0 to 36) was 8.9, with 38% of subjects classified as PN-free, 10% classified as having asymptomatic DSP, and 52% classified as having symptomatic DSP. Progression in TNS from baseline to week 48 occurred only in the PN-free group at baseline (mean TNS change = 1.16 ± 2.76 , $p = 0.03$). Factors associated with progression in TNS were lower current TNS, distal epidermal denervation, and white race. As compared with the TNS diagnosis of PN at baseline, the BPNS had a sensitivity of 34.9% and a specificity of 89.5%. **Conclusions:** In this cohort of advanced human immunodeficiency virus (HIV)-infected subjects, distal sensory polyneuropathy was common and relatively stable over 48 weeks. Previously established risk factors, including CD4 cell count, plasma HIV RNA, and use of dideoxynucleoside antiretrovirals were not predictive of the progression of distal sensory polyneuropathy (DSP). Distal epidermal denervation was associated with worsening of DSP. As compared with the Total Neuropathy Score, the brief peripheral neuropathy screen had relatively low sensitivity and high specificity for the diagnosis of DSP.

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Distal sensory polyneuropathy (DSP) is the most common neurologic complication associated with human immunodeficiency virus (HIV) infection.¹ Pre-highly active antiretroviral therapy (HAART) era studies showed that advanced immunosuppression, as reflected by reduced CD4⁺ lymphocyte cell count and increased plasma HIV viral load, increased the risk and severity of DSP.^{2,3} However, risk factors for HIV-associated neurologic disease, including demen-

tia and peripheral neuropathy (PN), seem to be changing in the current HAART era.⁴⁻⁷

Although effective suppression of HIV may have a beneficial effect on peripheral nerve function,⁸ DSP still remains common, including in patients receiving HAART.^{4,7} Dideoxynucleoside antiretrovirals (d-drug ARV) are neurotoxic and have been demonstrated to increase the risk of PN.⁹⁻¹¹ However, other studies have shown that d-drug ARV do not increase the risk or severity of DSP.^{6,7,12} It is not clear whether mitochondrial mechanisms are responsible for d-drug ARV neurotoxicity.¹³⁻¹⁵

There is considerable interest in the development of a reliable measure to establish the diagnosis and track the severity of DSP, particularly given the lack

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of an accepted standard.¹⁶ However, detailed batteries, including quantitative studies¹⁷ and skin biopsy,^{18,19} are not practical in most settings. Although brief DSP screening tests are often used,²⁰ their utility has not been validated in multicenter studies of HIV infection.

The primary objective of this study was to determine risk factors for prevalent and incident DSP and progression of established DSP in subjects with advanced HIV disease. Other objectives included characterization of PN with novel clinical and quantitative batteries, comparison of brief and comprehensive screens for PN, and the investigation of a linkage between neurotoxic PN and mitochondrial dysfunction.

Methods. *Study design.* Adult AIDS Clinical Trials Group (ACTG) Protocol A5117 was a multicenter, prospective study of PN in HIV infection, performed at member sites of the ACTG and Neurologic AIDS Research Consortium (NARC) with neurologic expertise. All subjects signed informed consent, and institutional review boards of all participating institutions approved the study. Subjects were accrued between October 2001 and June 2002.

Subject selection. Eligibility criteria included age of 13 years or older and advanced HIV infection, as evidenced by a CD4⁺ lymphocyte cell count of less than 300 cells/mm³ and previous ARV exposure of 15 or more consecutive weeks. Exclusion criteria included conditions other than HIV or neurotoxic ARV therapy that might confound the diagnosis of PN. These include diabetes mellitus necessitating oral hypoglycemic or insulin therapy; vitamin B₁₂ level of less than 200 pg/mL; alcoholism, defined by any alcohol-related medical complication within 6 months of study entry; and treatment with neurotoxins other than ARV, or potential neurotrophic agents, such as human growth hormone or acetyl carnitine derivatives.

Study procedures. *Clinical measures.* Nonphysician clinicians (generally study nurses) performed a brief peripheral neuropathy screen (BPNS), adapted from previous studies,^{20,21} and within 14 days of the baseline visit. The BPNS instrument consists of brief questions regarding symptoms of DSP, including the following: 1) pain, aching, or burning in feet/legs; 2) pins and needles sensation in feet/legs; and 3) numbness in feet/legs. Subjects graded each of these symptoms bilaterally from 00 (absent) to 10 (severe). The summary BPNS symptom score grade was based on the highest symptom value as follows: Gr 0 = 00; Gr 1 = 01 to 03; Gr 2 = 04 to 06; Gr 3 = 07 to 10. An examination was performed of distal lower extremity vibration (Gr 0 = perception of a maximally struck 128-Hz tuning fork at the great toe for more than 10 seconds; Gr 1 = 6 to 10 seconds; Gr 2 = less than 5 seconds; Gr 3 = no feeling) and ankle reflexes (Gr 0 = absent; Gr 1 = hypoactive; Gr 2 = normal; Gr 3 = hyperactive; Gr 4 = clonus). To facilitate staff training, an instruction manual, video, and posttraining competency self-test were provided, and individual instruction was provided to the study clinicians by neurologist investigators.

Neurologists, experienced in the care of HIV-infected subjects, performed a standardized neurologic examination with focus on peripheral nerve function at study entry and at weeks 24 and 48. Quantitative sensory testing (QST) was performed using the Computerized Assisted Sensory Evaluator (CASE-IV, Stillwater, MN), which included functional assessments of vibration and cooling of the right arm and leg, and heat pain of the right leg, with methods as previously described.^{22,23} Nerve conduction studies (NCSs) included right peroneal motor nerve and antidromic sural sensory nerve testing, with standardized anatomic landmarks, and limb temperature maintained above 32°C. Before testing of HIV-infected subjects, two HIV-uninfected subjects were studied to test each site's proficiency with QST and NCS. Control NCS waveforms required approval by the Core Laboratory at Washington University, and QST studies required approval by the A5117 statistician.

The Total Neuropathy Score (TNS), adapted from previous studies,¹⁷ included the following evaluations: sensory and motor

symptoms, sensibility to pin and vibration, tendon reflexes, motor function, QST, and NCS (sural sensory and peroneal motor nerve amplitudes). Each of the nine components was graded from 0 (normal) to 4 (most abnormal), yielding a maximum abnormality of 36. A modified TNS, excluding the sensory symptoms component, was established to determine the correlation of pain (which is included in the TNS sensory symptoms component) with the remainder of the components of the TNS.

Pain measures. Two measures of pain were recorded daily by subjects, in the morning and evening, for the 7 days before each scheduled visit. The first measure was the visual analog scale (VAS), measured on a horizontal scale from 0 to 10 cm, with higher scores indicating more pain, without calibration except at the endpoints—"no pain" or "pain as bad as it could be." Ratings were obtained at 9:00 AM and 9:00 PM, specifically for neuropathic pain, and estimated as averages over a 12-hour period. Diaries were reviewed with subjects for verification of the procedure. Poles of the scale were alternated to decrease response bias from prior ratings.²⁴ The second measure was the Gracely pain scale (GPS), measured on a verbal differential descriptor scale from 0 to 20, with higher scores indicating more pain.²⁵ At each time point (baseline and weeks 24 and 48), the median GPS and VAS over a 7-day period before the clinic visit were computed for each subject and used as summary measures for a particular time point.

Skin biopsy. Three-millimeter punch skin biopsies were performed at the proximal thigh (PT) and distal leg (DL), at baseline and week 48, with methods as previously described.²⁶ Standard procedures were used for fixation, storage, and shipping. All biopsies were processed centrally in the Cutaneous Nerve Laboratory at Johns Hopkins University and stained for the panaxonal marker anti-PGP9.5.²⁷ Epidermal nerve fiber densities (ENFDs) were quantified by one observer using prespecified counting rules.^{27,28} We determined in a previous study that the intrarater reliability for ENFD density measures is 0.91.¹⁸

Venous lactate. Blood for venous lactate was collected, with methods as previously described (<http://aactg.s-3.com/members/psmet.htm>).²⁹ Briefly, subjects were instructed not to participate in vigorous exercise for at least 24 hours before sample collection and to sit relaxed for 5 minutes before venipuncture. A tourniquet was not used. Blood was collected in a chilled 5-mL gray-top tube (sodium fluoride-potassium oxalate) and placed on ice. The specimen was then centrifuged for 10 minutes at 800g at 4°C, and plasma was separated and assayed at local clinical laboratories.

Analysis of mtDNA. Blood was collected in a 10-mL acid citrate dextrose tube (Becton Dickinson, San Jose, CA), and lymphocytes were isolated by Ficoll gradients. Dry pellets of 1×10^7 lymphocytes were stored at -70 °C. Total DNA was isolated using QIAamp DNA Blood Mini kit and DNeasy Tissue kit; both are from Qiagen Inc. (Valencia, CA). DNA integrity was examined by agarose gel electrophoresis. Intact DNA was assayed for mitochondrial DNA (mtDNA) copies per cell using mt primers that amplify a region of mtDNA (90 bp) that encodes for NADH dehydrogenase subunit 2 and genomic primers specific for the region of the genome encoding the Fas ligand (98 bp) by real-time PCR.³⁰ The PCR reactions were assayed with the Lightcycler FastStart DNA Master SYBR Green I mix in the LightCycler real-time instrument (Roche Diagnostic Corporation, Indianapolis, IN). Each sample and standard was run in duplicate (20 µL reaction volume) containing 1X SYBR Green master mix, 0.5 µM of each primer, and a 10 ng sample of DNA. PCR cycling conditions were as follows: denaturation for 1 cycle, 95 °C for 10 minutes; amplification for 40 cycles, 95 °C for 10 seconds, 58 °C for 5 seconds, and 72 °C for 5 seconds. A melt curve was performed from 65 °C at a 0.3 °C/second ramp rate with continuous acquisition. The results were then analyzed with Lightcycler Version 4.0 software. Mitochondrial DNA copies per cell were calculated using the formula (mtDNA copies/genomic DNA copies) $\times$ 2 = mtDNA copies per cell.³⁰

Definitions of DSP. **TNS.** The reference for defining PN in this study is derived from the following five key components of the TNS: sensory symptoms, sensory function, tendon reflexes, QST, and sural nerve amplitude. Because the TNS is focused on findings relevant to neuropathy, rather than CNS disease, tendon reflexes were graded from normal/brisk (Grade 0) to completely absent (Grade 4). The diagnosis of DSP required at least three of the five key components to be abnormal. A subject was considered to have asymptomatic DSP (ADSP) if the sensory symptoms com-

ponent was normal and at least three of four remaining key components (sensory function, tendon reflexes, sural amplitude, and QST) were abnormal, with at least one of the abnormal components being either sural amplitude or QST. A subject was defined as having symptomatic DSP (SDSP) if the sensory symptoms component score was abnormal and at least two of four remaining key components (sensory function, tendon reflexes, sural amplitude, and QST) were abnormal, with at least one of the abnormal components being either sural amplitude or QST.

BPNS. Subjects were classified as PN positive on the BPNS if they met two criteria: 1) at least mild loss of vibratory sensation in both great toes and 2) both ankle reflexes absent or hypoactive relative to the knees. Otherwise, they were PN negative. Subjects were categorized with SDSP if they were classified as PN positive and had a severity score of 1 or greater (at least mild) on the subjective PN grade (described above). PN-positive subjects who did not meet this requirement had ADSP.

Revised PN definitions. Because our originally established definitions of PN on the BPNS provided low sensitivity in this study when compared with the TNS (see Results), and the TNS established fewer cases of ADSP as compared with several other studies,^{7,20} revised PN definitions were established for additional post hoc analyses.

According to the revised TNS definition, a subject had ADSP if the sensory symptoms component was normal and at least two of four remaining key components (NCS, QST, sensory function, and tendon reflexes) of the TNS were abnormal, with at least one of the abnormal components being either sural amplitude or QST. The remainder of the TNS definition is unchanged.

For the revised BPNS, subjects were PN positive if they met either of the following criteria: 1) at least mild loss of vibratory sensation in both great toes or 2) both ankle reflexes absent or hypoactive relative to the knees. The remainder of the BPNS definition is unchanged.

Statistical analysis. Descriptive statistics were used to describe the study sample and change in variables over time. Population parameters were estimated with confidence intervals. Analyses were both cross-sectional and longitudinal in nature. Signed rank tests were used to evaluate within-group changes for continuous variables, and Kruskal-Wallis tests were used to evaluate between-group differences. The performance of the BPNS was described with sensitivity, specificity, and predictive values. Spearman rank order correlation coefficients and scatter plots were used to examine correlations. Univariate and multivariate risk factors for progression of neuropathy were identified with linear and logistic generalized estimating equations models. Shift tables were used to describe group transitions over time. No adjustment was made for multiple comparisons. All *p* values are two sided. A significance level of 0.10 was used in univariate analyses, whereas 0.15 was used to assess significance in multivariate modeling.

Results. Cohort demographics. Of 101 subjects that enrolled in the study, 83 completed the full 48-week observation period. The characteristics of the subjects are shown in table 1. Reasons for noncompletion included 3 deaths, 6 withdrawals, and 9 lost to follow-up. Baseline PN status in the noncompleter subjects was as follows: no PN, 9; asymptomatic PN, 1; symptomatic PN, 8. This distribution of PN status is similar to that of subjects who completed the study. The study sample was 90% male and 60% white, with a median age of 45 years. Median baseline CD4⁺ cell count was 169 cells/mm³, and median log HIV-1 RNA was 2.95 copies/mL (41 viral load measures were censored at or below 2.52 log₁₀ copies/mL). Forty-nine percent of subjects were receiving d-drug ARV at entry, including zalcitabine (ddC), stavudine (d4T), or didanosine (ddI).

Neuropathy baseline and progression. The TNS score and change from baseline to each follow-up visit are summarized by baseline PN status in table 2. The mean TNS score at baseline was 8.9, with 38% of subjects classified as PN-free, 10% with ADSP, and 52% with SDSP. There was an increase in TNS at week 48 only for the PN-free group

Table 1 Cohort characteristics

Characteristic	Total, n = 101
Age, y	
Mean	44.48
SD	6.93
Median	45
Sex, n (%)	
Male	91 (90)
Female	10 (10)
Race/ethnicity, n (%)	
White non-Hispanic	61 (60)
Black non-Hispanic	22 (22)
Hispanic	12 (12)
Asian, Pacific Islander	4 (4)
American Indian, Alaskan	2 (2)
IV drug abuse history, n (%)	
Never	81 (80)
Previously	20 (20)
Total alcohol use, n (%)	
Does not drink	39 (39)
Drinks some alcohol	45 (45)
Drinks frequently	17 (17)
ddI/d4T/ddC use at entry, n (%)	
No therapy	52 (51)
Single d-drug ARV agent	41 (41)
Two d-drug ARV agents	8 (8)
CD4 count, cells/mm ³	
Mean	171.14
SD	94.19
Median	169
Log HIV-1 RNA (copies/mL)	
Mean	3.51
SD	1.21
Median	2.95

d-drug ARV = dideoxynucleoside antiretroviral; ddI = didanosine; d4T = stavudine; ddC = zalcitabine; HIV = human immunodeficiency virus.

(mean TNS change = 1.16; *p* = 0.03). Transition within the trichotomous neuropathy states (PN-free, ADSP, and SDSP) is shown in table 3. At 48 weeks, of the 28 subjects that were PN-free at baseline, 4 transitioned to ADSP, and 6 transitioned to SDSP. Of 8 subjects with baseline ADSP who were followed to 48 weeks, none developed SDSP, and 2 reverted to PN-free. In the 45 subjects with baseline SDSP who were followed to 48 weeks, 2 improved to ADSP, and 6 improved to PN-free.

Cross-sectional univariate analysis at baseline revealed that variables associated with higher TNS included older age, white race, and DL ENFD (table E-1 on the *Neurology* Web site at www.neurology.org). There was no correlation between sex, CD4 cell count, log HIV RNA, or alcohol use and baseline TNS. Notably, use of d-drug ARV was associ-

Table 2 Total Neuropathy Score

	Total	No PN	ADSP	SDSP
TNS (0–36), week 0				
n	100	37	10	53
Mean	8.9	4.17	7.4	12.49
SD	5.56	2.56	2.01	4.91
Median	8	4	8	12
TNS change,* weeks 0–24				
n	91	32	10	49
Mean	0.57	0.87	0.1	0.47
SD	2.83	2.8	1.45	3.07
Median	0	0.5	–0.5	0
TNS change, weeks 0–48				
n	82	28	9	45
Mean	0.47	1.16	–0.9	0.32
SD	3.55	2.76	1.79	4.14
Median	0.5	1.19	–1	0

* Positive change indicates increase in Total Neuropathy Score (TNS), i.e., worsening of peripheral neuropathy (PN) severity (see text).

ADSP = asymptomatic distal sensory polyneuropathy; SDSP = symptomatic distal sensory polyneuropathy.

ated with lower baseline TNS (estimate = –3.90; $p = 0.01$). This finding was most notable for d4T use (estimate = –2.67; $p = 0.02$). Specifically, 68% of subjects without PN had received d-drug ARV, compared with 32% of subjects who had not received d-drug ARV. In contrast,

Table 3 Transition in neuropathy status

	Week 0			
	No PN	ADSP	SDSP	Total
Week 24*				
No PN	22 (24.2)‡	2 (2.2)	2 (2.2)	26 (28.6)
ADSP	4 (4.4)	7 (7.7)‡	0	11 (12.1)
SDSP	6 (6.6)	1 (1.1)	47 (51.6)‡	54 (59.3)
Total	32 (35.2)	10 (11.0)	49 (53.9)	91 (100)
Week 48†				
No PN	18 (22.2)‡	2 (2.5)	6 (7.4)	26 (32.1)
ADSP	4 (4.9)	6 (7.4)‡	2 (2.5)	12 (14.8)
SDSP	6 (7.4)	0	37 (45.7)‡	43 (53.1)
Total	28 (34.6)	8 (9.9)	45 (55.6)	81 (100)

Values represent n (%).

* Ten subjects' week 24 neuropathy status could not be determined.

† Twenty subjects' week 48 neuropathy status could not be determined.

‡ No change in neuropathy state.

PN = peripheral neuropathy; ADSP = asymptomatic distal sensory polyneuropathy; SDSP = symptomatic distal sensory polyneuropathy.

of subjects with PN, 43% had received d-drugs, whereas 57% did not. In multivariate modeling, predictors of baseline TNS were age ($p = 0.03$) and cumulative years of ddI exposure ($p = 0.05$). Use of d-drug ARV at baseline was associated with lower baseline TNS ($p = 0.02$).

Generalized estimating equations models were used to model 24-week change in TNS and examine potential risk factors for progression of PN. These analyses resulted in two observations per subject with the 24-week change (i.e., 0 to 24 and 24 to 48) as the dependent variable. Because this population was relatively stable in their PN status, the ability to detect risk factors for progression was limited. Initial univariate results (table E-1) were used as a first cut in determining risk factors. Univariate predictors of TNS change were higher baseline plasma HIV RNA ($p = 0.09$) and absence of PN at baseline ($p = 0.08$). In multivariate analysis, predictors of TNS worsening included current TNS (as current TNS increases, TNS change decreases; $p = 0.01$), thigh/distal leg ENFD ratio (as ratio increases, TNS change increases; $p = 0.11$), and white race (increased TNS change in whites; $p = 0.09$). With the exception of ddC, used only in a single subject, the use of d-drug ARV, at entry, cumulative years of exposure, or change in exposure over the course of the study, was not a predictive factor in progression of the TNS.

In addition to an analysis of baseline factors in the risk of progression of PN, we examined the impact of change in key variables over the 48-week course of the study. Plasma HIV RNA was used as a measure of virological control, and CD4 count as a measure of immunologic status, due to either change in ARV regimens or natural history of disease. Increased plasma HIV RNA over 48 weeks was associated with increased pain, as measured by the GPS ($r = 0.29$, $p = 0.01$). However change in viral load was not associated with change in pain as measured on the VAS ($r = 0.17$, $p = 0.15$) or in the TNS ($r = 0.05$, $p = 0.68$). Reduction in CD4 count was associated with increase in the TNS ($r = 0.25$, $p = 0.02$), although not with change in pain on GPS ($r = 0.08$, $p = 0.48$) or VAS ($r = 0.04$, $p = 0.76$). Given the relatively weak correlations and risk inherent with multiple testing, these results should be interpreted with caution.

Subjects were also classified as having transitioned across neuropathy categories (no PN→ADSP→SDSP). Worsening for patients who maintained SDSP was defined as an increase in TNS. In univariate analysis, predictors for transition/progression included higher baseline thigh/distal leg biopsy ENFD ratio ($p = 0.07$), higher cumulative years of d4T ($p = 0.02$), drinking liquor ($p = 0.08$), and baseline HIV RNA greater than 5,000 copies/mL ($p = 0.03$). Multivariate analysis revealed that risk factors for worsening of PN category were current TNS (as current TNS increases, odds of transition decrease; $p = 0.14$), DL ENFD (as the density increases, odds of transition decrease; $p = 0.11$), and d4T use (increases odds of transition; $p = 0.11$).

BPNS vs TNS. Each subject's PN status was determined with the BPNS in comparison with results of the a priori defined TNS. In the determination of PN at baseline (table E-2A), the BPNS sensitivity was low (34.9%) and specificity was high (89.5%). Predictive values were calculated assuming that the prevalence of neuropathy in the general population is similar to that observed in this

study. The BPNS had a high positive predictive value (84.6%) but a low negative predictive value (45.3%). In determining PN status (PN-free, ADSP, or SDSP), the correct classification rate of the BPNS was only 54.5% (table E-2C).

Because of the low sensitivity of the BPNS, as compared with the TNS, and the unexpectedly low rate of ADSP, as determined by the TNS, we examined whether modifying the criteria for PN by the BPNS and the TNS improved sensitivity and specificity of the BPNS (see Methods). As shown in table E-2, B and D, the revised TNS yielded a lower percentage of PN-free subjects (21.8%), and a higher percentage of baseline ADSP (25.7%) as compared with categorization using the original TNS (PN-free: 37.6%; ADSP: 9.9%), whereas the percentage of SDSP was unchanged. That is, the revised criteria shifted the distribution of neuropathy states to include more ADSP and fewer PN-free. In comparison with the original TNS in the determination of whether subjects had PN, the revised BPNS had an improved sensitivity of 76.2% and a reduced specificity of 55.3% (data not shown). An optimal balance was achieved with a comparison between the revised BPNS and the revised TNS. Using this analysis, in the determination of whether subjects had PN or were PN-free, the revised BPNS had a sensitivity of 73.4%, a specificity of 68.2%, a positive predictive value of 89.2%, and a negative predictive value of 41.7% (table E-2B). In determining whether subjects had no PN, ADSP, or SDSP, the correct classification rate of the BPNS was 64.4% (table E-2D).

Nonphysician clinicians (usually study nurses) were trained in the performance of a focused PN examination. However, they frequently misclassified ankle reflexes (table E-3A) and distal vibratory sensation (table E-3B), as compared with the neurologists' findings, in the same limbs of the same patients. For examination of ankle reflexes, because the grading scales for nonphysicians and neurologists were different, we compared their reflex examination findings as follows (nonphysician/neurologist grades): 0 = 2; 1 = 1; 2/3/4 = 0. The nonphysicians' correct classification rate of ankle reflexes was 53 out of 101 (52%).

Pain measures. Pain measures, including VAS and GPS measured at baseline and weeks 24 and 48, correlated with the modified TNS (which excluded the sensory symptoms component, as discussed in Methods; $p < 0.01$). Pain measures also correlated at baseline with ENFD of the DL ($p < 0.01$). There was no significant change in pain measures over time. There was no correlation between pain change and change in modified TNS.

Skin biopsy. The figure displays the mean and 95% CI of the ENFD at each week. At baseline, ENFD/mm (mean $\pm$ SD) was 9.58 ± 4.96 at the PT site, 6.08 ± 3.35 at the DL, 7.82 ± 3.79 for the average of PT and DL, and 2.19 ± 2.64 for the ratio of PT to DL. Overall, the mean densities at both sites were within normal limits; however, 51/99 of subjects at baseline had densities in the abnormal ranges of less than 8/mm at PT or less than 5/mm at DL. Two subjects had a missing PT and a DL of 5/mm or greater. At baseline, there were relatively weak inverse correlations between DL ENFD and the TNS ($r = -0.262$, $p < 0.01$), and neuropathic pain (Gracely: $r = -0.250$, $p = 0.01$; VAS: $r = -0.248$, $p < 0.01$). At week 48, lower DL ENFD was correlated with higher VAS ($p = 0.03$) and TNS

Figure. Mean epidermal nerve fiber density (ENFD), obtained by skin biopsy, at the distal leg and proximal thigh, at baseline and 48 weeks. Vertical bars represent 95% CIs. PN = peripheral neuropathy.

($p < 0.01$). Higher average ENFD was associated with lower baseline VAS ($r = -0.254$, $p = 0.01$). Determinations of ENFD at the PT and DL in each of the PN groups revealed no significant change over 48 weeks. There was no association between change of TNS or measures of neuropathic pain and change of ENFD. Further details of the baseline ENFD results and morphologic features will be reported separately.

Mitochondrial analyses. Baseline plasma lactate was 1.49 ± 0.77 mmol/L, and peripheral blood mononuclear cell (PBMC) mtDNA copies/cell was 529 ± 466 for subjects overall. At baseline, mtDNA copies/cell was 607 ± 839 in PN-free patients and 510 ± 320 in patients with PN ($p = 0.65$). There was a decrease in mtDNA over the duration of the study (median decrease = 112.5 copies/cell; $p < 0.01$). Lactate levels did not change significantly over the 48 weeks. Baseline mtDNA copies/cell did not differ across the four PN/d-drug ARV status groups ($p = 0.59$; table 4). Baseline and week 0 through 48 changes in mtDNA content did not correlate with lactate values, TNS, history of alcohol use, VAS, GPS or ENFD. Baseline lactate levels did not correlate with the TNS.

Discussion. Highly active antiretroviral therapy has resulted in a dramatic impact on morbidity and mortality in patients with HIV infection. However, its impact on neurologic disease is less clear. Epidemiologic studies have demonstrated a marked decline in the incidence rates of neurologic

Table 4 mtDNA by neuropathy and d-drug status at baseline

	mtDNA, copies/cell			
	PN ⁺ /D ⁺	PN ⁺ /D ⁻	PN ⁻ /D ⁺	PN ⁻ /D ⁻
n	34	43	12	7
Mean	466.88	543.3	445.92	884.29
SD	324.94	315.19	201.06	1,377.21
Median	353.5	434	411.5	257

mtDNA = mitochondrial DNA; PN = peripheral neuropathy;
D = dideoxynucleoside (d-drug) antiretroviral exposure.

opportunistic infections, such as cryptococcal meningitis and cerebral toxoplasmosis.³¹ However, primary neurologic diseases, such as HIV-associated cognitive disorders and PN, remain prevalent despite HAART.^{5,31} The current study demonstrates that PN was present in 62% of a well-characterized cohort of subjects. Of subjects entering without DSP, 34.6% (10/28) developed either ADSP or SDSP during the study. However, given the relatively small sample size, inclusion criteria selecting subjects with advanced HIV infection, and possible ascertainment bias, it is difficult to extrapolate these figures to the overall HIV population.

Data from pre-HAART era cohorts indicate that the risk of PN is most common in subjects with advanced immunosuppression and high plasma HIV set points.² We have shown in a previous study that the severity of PN is also associated with high plasma HIV RNA levels.¹² Therefore, in the current study, we selected subjects with advanced HIV disease, and who were ARV experienced, with the assumption that these subjects were at highest risk for progressive PN, or for transitioning from a neuropathy-free state to incident PN. However, multivariate analysis revealed that neither CD4 cell count nor plasma HIV viral load was correlated with the presence or progression of PN. These data are consistent with similar results reported by another recent study examining predictors of neuropathy in the Northeast AIDS Dementia Consortium.⁷ That study, which enrolled a similarly immunosuppressed cohort, with high exposure to HAART, found that PN (defined clinically without physiologic testing, and combining ADSP and SDSP) was present in 67% of their subjects. There was no difference in immunologic or virologic markers between subjects with or without DSP, and these variables did not predict progression to SDSP.

Several explanations are possible for the lack of associations observed in the current study between CD4 cell count or plasma HIV RNA with PN. Because entry criteria limited enrollment to subjects with a CD4 count of less than 300 cells/mL, this may have selected a group of patients already at higher risk for neuropathy, despite more than 3 months of ARV use. This may have blunted the influence of the CD4 level on observed rates of PN. HAART usually results in suppression of HIV viral load, and some

degree of immune reconstitution. Therefore, median plasma HIV viral load in our cohort was 2.95 copies/mL, despite relatively low CD4 cell counts. It is possible that damage to the peripheral nervous system of patients receiving HAART had occurred at earlier periods of their illness, with less effective ARV, and associated higher viral load and lower CD4 cell counts. This concept is supported by a recent study reporting that CD4 nadir, rather than absolute CD4 count measured cross-sectionally, is a predictor of HIV PN.⁵ Similarly, in the era of HAART, previously established correlations with markers of HIV state are no longer relevant for PN or cognitive dysfunction.^{2,32} Therefore, relationships between CD4 cell count, viral load in plasma or CSF, and HIV-associated cognitive motor disorder and dementia are not evident in HAART-treated cohorts.³³

Relatively few risk factors were associated with the presence or progression of PN, as defined by the TNS, in our cohort. These include white race, as shown in other studies.^{5,7,34} Advancing age, which has proven to be an important risk factor for PN in several studies,^{6,34} was associated with the presence of PN at baseline but did not correlate with progression of PN in this study. It is possible that this trial may have been too short (48 weeks), with subjects too stable in their PN status to robustly reveal risk factors for progression. Notably, the only group in this study that progressed significantly on the TNS was that group that was PN-free at baseline. It is possible that PN may have progressed to a point of plateau in those with PN at entry.

Early studies of d-drug ARV indicated that these agents might cause or exacerbate PN.⁹⁻¹¹ However, more recent trials have shown that d-drug ARV did not add further risk to the occurrence of HIV-associated PN.^{5-7,12} Therefore, it is not clear how best to predict which patients are at highest risk for the development of DSP while being treated with d-drug ARV regimens. Although the use of d-drug ARV is declining in the United States because of the availability of alternate ARV agents with less toxicity, they remain an important component of first-line ARV regimens in the developing world.

Although 49% of our subjects were receiving d-drug ARV at entry (including 8% receiving dual d-drug ARV), neither d-drug ARV use at baseline nor their cumulative use was associated with significant worsening of the TNS. Notably, using the revised TNS definition, exposure to d-drug ARV regimens was associated with a lower risk of PN at baseline. While this seems paradoxical, similar results were reported in another recently reported cohort.⁵ In that study, although use of d-drug ARV was associated with incident PN in the first year of their use, increasing duration of d-drug ARV was associated with a decreased incidence of PN. Another study also found that use of d-drug ARV was not associated with incident SDSP.⁷ These data and our results are consistent with the possibility that in patients most susceptible to the development of PN due to d-drug

ARV, this usually happens early in the course of their use, often resulting in discontinuation of d-drugs. Recent studies have provided genetic evidence that HIV-infected patients with certain mitochondrial haplotypes have a higher risk of d-drug neurotoxic neuropathy.³⁵ It is possible that such predisposed patients with early development of neurotoxic neuropathy may be those most likely to discontinue d-drug ARV and be “weeded out” from subsequent cohort studies. In contradistinction, those patients who are able to remain on d-drug ARV are those less likely to have developed significant or disabling PN. This results in an apparent “neuroprotective” effect of subjects on long-term d-drug ARV regimens and may simply represent a form of survival bias. Furthermore, the benefits of ARV on virological control, regardless of class, may have a salutary effect on PN and counterbalance their neurotoxicity.⁸

A major limitation in any study of PN is the lack of an accepted standard definition for PN diagnosis. There are wide differences in the diagnostic criteria of PN among studies. A meta-analysis of PN trials in which methodologic criteria for the diagnosis of PN were specified found that the most robust measures for a validated PN diagnosis include a combination of positive symptoms, signs, and electrodiagnostic study results.¹⁶ Although it is difficult to combine disparate clinical and quantitative measures into a single measure of PN, investigators developed the TNS for the study of diabetic PN.¹⁷ This measure, which combines components of neurologic symptoms and signs, NCS, and QST, performed well when validated with the Neuropathy Symptom Score and Neuropathy Impairment Score. We adapted the TNS, incorporating components most relevant to the findings expected in HIV-associated DSP. It is reassuring that in the current study, measures of pain and ENFD obtained on skin biopsy correlated with TNS values.

Our original definition of PN, based on components of the TNS, yielded relatively few subjects with ADSP (10%), in contrast to several other studies, which reported a considerably higher frequency of ADSP.^{7,20} Because our original criteria for the TNS diagnosis of ADSP may have been excessively stringent, we established a relaxed and more inclusive PN definition, which shifted the categorization and yielded a higher percentage of baseline PN (78.2%), due to an increase in the diagnosis of ADSP (25.7%), as compared with the original PN definition. Further studies are required to determine which criteria yield the best balance of sensitivity and specificity for the diagnosis of PN. It is also necessary to determine to what extent quantitative studies, which are time-consuming, costly, and potentially noxious, contribute to the reliability of the diagnosis of PN. Further analyses of the current study are under way to address these questions.

In many large studies of infectious or metabolic diseases, with the primary focus on endpoints such

as plasma HIV viral load with ARV therapy, or glucose control in diabetes mellitus, it is not possible to incorporate sophisticated testing for PN. In general, neither examination by neurologists nor laboratory measures, such as NCV, QST, or skin biopsy, is possible. Therefore, it is critical to have a diagnostic measure of PN that may be performed relatively simply and reliably by nonneurologic personnel. We have reported the high rate of inaccuracy of PN diagnoses in a primary ARV study, as executed by experienced HIV investigators, but without predefined diagnostic criteria for PN.³⁶ Conversely, another study reported a good correlation between the diagnoses of PN as established by trained nonphysician clinicians and those of an experienced neurologist.²⁰

The current study, using the BPNS, found high specificity (89.5%) but relatively poor sensitivity (34.9%) of the BPNS as compared with the TNS in the diagnosis of PN. Similar to the TNS, it is possible that our BPNS diagnostic criteria for PN were too stringent. With the modification of the BPNS PN criteria from the requirement of abnormal vibration *and* depressed reflexes to abnormal vibration *or* depressed reflexes, the sensitivity of the BPNS increased to 73.4%, with decline of specificity to 68.2%. A recent study found good correlation between the diagnosis of PN, using similar criteria for the BPNS, and quantitative measures, including QST and ENFD.³⁷

It is also possible that the BPNS as performed in this study was too limited to provide an accurate assessment of peripheral nerve function. For example, nonphysician clinicians examined only ankle reflexes, whereas study neurologists performed all tendon reflexes. The comparison of ankle with knee reflexes provides a more reliable assessment of depression of ankle reflexes, particularly when there is coexistent peripheral and CNS disease.

Another possible explanation for the discrepancy between the results obtained in other studies and our results in the performance of the BPNS²⁰ relates to the difficulties in quality control in a multicenter study as opposed to a single site. Both studies of HIV neuropathy conducted in single sites reported good performance of the BPNS in establishing a reliable diagnosis of PN.^{20,37} We attempted to optimize study nonphysician clinicians' training in the performance of a focused neurologic examination with the use of training videos and certification of nursing competence by a supervising neurologist. Despite these efforts, nonphysician examiners in our study frequently reported vibratory and reflex findings that differed from a neurologist's examination of the same patient and limb. This has implications for the use of the BPNS or its components to identify PN in clinical trials. As a screening instrument, higher sensitivity would be desirable, but the relatively low specificity would indicate a need for confirmation of identified cases of PN by more comprehensive expert

evaluations. These data suggest that use of rigorous diagnostic measures of PN, such as those used in the TNS, would be ideal in certain studies requiring accurate diagnosis of neuropathy. Because this is not practical in many large-scale epidemiologic cohort studies, future trials should explore other means of training and supervision to overcome this potentially serious impediment to the performance of nonneurologic personnel in diagnosing PN.

In contrast to some previous studies, our study demonstrated no correlation between HIV-associated PN and plasma lactate levels or PBMC mtDNA content. The lack of association with lactate differ from findings in a previous cohort of subjects with PN related to d-drug ARV use³⁸ but are similar to results from a cross-sectional study.³⁹ The absence of change in PBMC mtDNA content in relation to PN in this study is concordant with findings of a longitudinal study evaluating PBMCs in patients taking several nucleoside ARV and exhibiting PN.⁴⁰ Mitochondrial deficiencies may be tissue specific, and the measurement of mtDNA in PBMCs may not reflect mitochondrial function in peripheral nerve or other tissues. For example, in several series, mtDNA content in PBMCs did not correlate with the presence of lipodystrophy in HAART-treated patients.^{41,42} It is also possible that our results may be due in part to platelet contamination of PBMCs. Platelets have mitochondria but no nucleus. In these studies, PBMCs were isolated using Ficoll gradients that did not remove all the platelets. Platelet contamination of up to 20% may change the amount of mtDNA measured in PBMCs from HIV-seropositive and -negative patients.⁴³ These results suggest that further studies are necessary to definitively evaluate the role of mitochondria in PN.

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NeuroImages

Figure. On the source images from the brain magnetic resonance angiography study, a long segment of the right anterior inferior cerebellar artery (AICA) is noted from the basilar artery extending far laterally into the internal auditory canal. After a sharp “hairpin” loop, the AICA reverses course and exits the internal auditory canal (arrows).

Pulsatile tinnitus from a redundant arterial loop

Richard Libman, MD, FRCP(C); and Alan Johnson, MD, New Hyde Park, NY

A 21-year-old woman presented with a 5-month history of right-sided pulsatile tinnitus. A general medical and neurologic evaluation revealed no abnormalities and no cervical or cranial bruits. She perceived the pulsatile tinnitus during the examination, and it corresponded to her pulse. Studies with normal results included carotid Doppler, transcranial Doppler, MRI and angiography of brain, magnetic resonance venography, and CT of

temporal bones with contrast. On the axial source images of the three-dimensional time of flight brain magnetic resonance angiography, a long redundant loop of the right anterior inferior cerebellar artery was noted entering and exiting the right internal auditory canal (figure).

Pulsatile tinnitus has many causes, including arteriovenous malformations, intracranial aneurysms, vascular tumors of the skull base, occlusive arterial disease, arterial ectasia, aberrant course of the carotid artery, venous bruits, and increased intracranial pressure.¹ Previous reports have postulated that “redundant arterial loops” that compress the cochlear nerve in the internal auditory canal or at the root entry zone can cause pulsatile tinnitus.²

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