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Higher Levels of Cerebrospinal Fluid and Plasma Neurofilament Light in Human Immunodeficiency Virus-Associated Distal Sensory Polyneuropathy

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Background. Neurofilament light (NFL) chain concentrations, reflecting axonal damage, are seen in several polyneuropathies but have not been studied in human immunodeficiency virus (HIV) distal sensory polyneuropathy (DSP). We evaluated NFL in cerebrospinal fluid (CSF) and plasma in relation to DSP in people with HIV (PWH) from 2 independent cohorts and in people without HIV (PWoH).

Methods. Cohort 1 consisted of PWH from the CHARTER Study. Cohort 2 consisted of PWH and PWoH from the HIV Neurobehavioral Research Center (HNRC). We evaluated DSP signs and symptoms in both cohorts. Immunoassays measured NFL in CSF for all and for plasma as well in Cohort 2.

Results. Cohort 1 consisted of 111 PWH, mean ± SD age 56.8 ± 8.32 years, 15.3% female, 38.7% Black, 49.6% White, current CD4+ T-cells (median, interquartile range [IQR]) 532/μL (295, 785), 83.5% with plasma HIV RNA ≤50 copies/mL. Cohort 2 consisted of 233 PWH of similar demographics to PWH in Cohort 1 but also 51 PWoH, together age 58.4 ± 6.68 years, 41.2% female, 18.0% Black, Hispanic, non-Hispanic White 52.0%, 6.00% White. In both cohorts of PWH, CSF and plasma NFL were significantly higher in both PWH with DSP signs. Findings were similar, albeit not significant, for PWoH. The observed relationships were not explained by confounds.

Conclusions. Both plasma and CSF NFL were elevated in PWH and PWoH with DSP. The convergence of our findings with others demonstrates that NFL is a reliable biomarker reflecting peripheral nerve injury. Biomarkers such as NFL might provide, validate, and optimize clinical trials of neuroregenerative strategies in HIV DSP.

Keywords. HIV; polyneuropathy; biomarker; cerebrospinal fluid; neurofilament light.

We and others have demonstrated that distal sensory polyneuropathy (DSP) is a disabling chronic condition in people with human immunodeficiency virus (HIV, PWH),—even with viral suppression on antiretroviral therapy (ART) [1]. Consequences of DSP include disability [2, 3], neuropathic pain [1], and poor balance [4, 5]. Neuroregenerative strategies are becoming available [6]. A reliable surrogate marker for peripheral nerve regeneration might make clinical trials of neuroregenerative strategies more efficient and provide biological validation [7, 8]. Cerebrospinal fluid (CSF) neurofilament light (NFL) might be a useful marker [9–13], as CSF ensheaths the proximal nerve root sleeve [14], and thus its molecular constituents, including biomarkers of axonal injury, likely reflect dorsal root ganglia (DRG) health [15]. HIV-associated DSP is recognized as a DRG sensory neuronopathy, likely caused by the infiltration of activated macrophages into DRG [16–18] [19]. Cerebrospinal fluid (CSF) and serum NFL levels also are increased in a variety of peripheral neuropathies [11–13, 15, 20–22], but this has not been systemically evaluated in HIV DSP. We tested the hypothesis that participants with DSP would have higher CSF and plasma NFL levels than those without DSP.

METHODS

Study Participants

We studied 2 independent, prospective, community-based cohorts of PWH. Cohort 1 was a multicenter (CNS HIV AntiRetroviral Effects Research [CHARTER] [1], March 2016 to January 2020) and Cohort 2 was a single-center (San Diego HIV Neurobehavioral Research Center [HNRC] [23],

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October 1999 to March 2019). All participants in Cohort 1 were HIV seropositive; Cohort 2 included both HIV seropositive and HIV seronegative individuals. Inclusion criteria were HIV seropositive or HIV seronegative and having had a systematic evaluation for DSP signs and symptoms. Exclusions were individuals with a history of diabetes mellitus preceding HIV, neurological illnesses unrelated to HIV that might confound the study assessments, and inability to comply with the study evaluations. Both studies were approved by local Institutional Review Boards; the experiments were undertaken with the understanding and written consent of each subject; the study conformed with the World Medical Association Declaration of Helsinki.

DSP Evaluations

Participants in both cohorts were evaluated by trained clinicians using standardized clinical examinations for findings of DSP and interviews for symptoms of DSP. DSP signs were bilateral, distal vibration, sharp, and ankle reflex loss. Participants were also categorized as having 0, 1, or ≥2 DSP signs. Symptoms were neuropathic pain, paresthesias, and loss of sensation. To avoid the inclusion of mononeuropathies and radiculopathies, in the case of both signs and symptoms, we required symmetrical, bilateral, distal abnormalities.

Other Clinical Evaluations

A trained clinical examiner interviewed and examined participants to collect information such as ART regimens, nadir CD4+ T-cell counts, and history of diabetes mellitus. Although this study was not focused on the relationship of NFL to neurocognitive impairment, we nevertheless evaluated the latter as a potential confound. Neurocognition was measured using a comprehensive neuropsychological battery covering seven domains as specified by the Frascati criteria [24]. The battery is described in detail in a previous publication and included tests of executive function, learning, memory, attention, working memory, psychomotor speed, and speed of information processing [25]. Raw test scores were converted to standardized T-scores (mean 50, standard deviation 10) corrected for age, education, sex, and race/ethnicity, and overall performance was indexed by the global deficit score (GDS) method [26].

Clinical Laboratory Evaluations

All participants provided blood, and in Cohort 2, we collect CSF via lumbar puncture. HIV infection was diagnosed using an enzyme-linked immunosorbent assay (ELISA) with Western blot confirmation. HIV RNA was measured using commercial assays and considered undetectable at a lower limit of quantification (LLQ) of 50 copies/mL. CD4+ T cells were measured by flow cytometry and nadir CD4+ T-cell count by self-report.

NFL Assays

In Cohort 1, NFL was measured in duplicate in CSF only using a commercially available ELISA (TECAN Life Sciences; lifesciences.tecan.com). In Cohort 2, NFL was quantified in plasma and CSF using the ultrasensitive single molecule array (Simoa) platform using the Quanterix NF-light Advantage kit (no. 103186) at 1:4 dilution for plasma and 1:100 dilution for CSF, as recommended by the manufacturer. The intra-assay variability of the duplicate measurements was <5%.

Statistical Analyses

Demographic and clinical characteristics were summarized using means and standard deviations (SD), medians, and interquartile ranges (IQR) or percentages, as appropriate. NFL concentrations were $\log_{10}$-transformed to improve their skewed distribution. NFL levels in CSF and plasma were compared in participants with and without DSP signs and symptoms using analysis of variance (ANOVA) when the distribution of the outcome variable was not significantly different from normal. Non-parametric analysis was applied when variable distributions significantly deviated from normal. Pairwise comparisons were corrected using Tukey HSD test. To limit potential for Type 1 error, primary analyses were done for the relationship between number of DSP signs and NFL levels; all other analyses were secondary. Multivariable regression assessed the associations between NFL levels in CSF and plasma and DSP signs and symptoms after adjusting for relevant covariates and to test interaction effects between NFL and significant covariates. Because previous studies have shown that older nucleoside reverse transcriptase inhibitor antiretrovirals (so-called "d-drugs") have substantial peripheral neurotoxicity, we evaluated this as another potential confounding condition. Analyses were conducted using JMP Pro version 15.0.0 (SAS Institute, Cary, North Carolina, USA, 2018).

RESULTS

Cohort 1 (PWH Only)

Demographics and HIV Disease Characteristics

Participants in Cohort 1 were 111 PWH, age mean ± SD 56.8 ± 8.32 years, 15.3% female, 38.7% Black, 10.8% Hispanic, 49.6% non-Hispanic White, estimated duration of HIV (median, IQR) 22.4 (16.9, 27.7) years, current CD4+ T-cells (median, IQR) 532/μL (295, 785), nadir CD4+ T-cells 95 (15, 199), 98.2% on ART, 83.5% with plasma HIV RNA ≤50 copies/mL, 90.9% with CSF HIV RNA ≤50 copies/mL and d-drug exposure median (IQR) 18.3 (0, 67.0) months. Participants took 51 different ART regimens, with the most common being dolutegravir/abacavir/lamivudine (12.8%), EFV/FTC/TFV (11.0%), COBI/EVG/FTC/TAF (9.17%), and DTG/FTC/TAF (6.42%). The most common regimen types were: integrase inhibitor + 2 nucleoside reverse transcriptase inhibitors (NRTIs) (38.5%),

protease inhibitor + non-nucleoside RTI + 2 NNRTIs (20.2%), and 2 NRTI + NNRTI (19.3%).

NFL Levels According to DSP Signs, Cohort 1

Figure 1 shows that higher $\log_{10}$ CSF NFL was related to increasing numbers of DSP signs (ANOVA $P = .0108$); ≥2 DSP signs (N = 53, 3.15 ± 0.174), intermediate with 1 sign (N = 29, 3.13 ± 0.249), and lowest with 0 signs (N = 29, 3.01 ± 0.190). CSF NFL was significantly related to reduced vibratory sensation (3.14 ± 0.168 vs 3.054 ± 0.221, $P = .016$) and reflexes (3.17 ± 0.203 vs 3.02 ± 0.183, $P = .0001$), but not to reduced sharp sensation (3.11 ± 0.190 vs 3.09 ± 0.205, $P = .619$). CSF NFL was not related to DSP symptoms (neuropathic pain, paresthesias, loss of sensation; ps >0.50).

Potential Confounding Conditions, Cohort 1

Older age correlated with higher CSF NFL (r = 0.423, $P = 3.64 \times 10^{-6}$). Additionally, there was a stepwise increase in age according to the number of DSP signs: for ≥2 signs versus 1 sign versus 0 signs, age in years was 60.3 ± 7.44, 54.7 ± 6.97, and 52.4 ± 8.52, respectively ($P = 2.68e{-}6$). We therefore assessed a multivariable regression model that included age, DSP and their interaction as predictors of CSF NFL. Since the interaction was not significant ($P = .975$), it was removed from the model. We found the significant association between DSP signs and CSF NFL became non-significant ($P = .165$) after controlling for age ($P = .00007$). ART status (on vs off) was not related to log CSF NFL levels ($P = .731$) or to DSP signs ($P = .495$). D-drug exposure was not related to CSF NFL levels ($P = .288$) or to DSP signs ($P = .678$).

CSF NFL was highest in PWH of "Other" ethnicities (N = 1, 3.70) compared to non-Hispanic White, (3.09 ± 0.184) and Hispanic (3.06 ± 0.147) ($P = .0187$). In a multivariable regression, both the number of DSP signs and race/ethnicity, but not their interaction, were significantly associated with higher CSF NFL (ps = 0.00779 and .0134, respectively). Neurocognitive performance (GDS) was not significantly related to CSF NFL levels (r = −0.153, $P = .108$). CSF NFL levels were similar in participants with detectable versus undetectable plasma HIV RNA (3.12 ± 0.137 vs 3.11 ± 0.218, $P = .758$). CSF NFL was not related to CSF HIV RNA (r = 0.0305, $P = .401$) or to nadir (r = −0.124, $P = .193$) or current CD4+ T-cell count (r = −0.0731, $P = .448$), CSF NFL was not associated with diabetes mellitus (22.1% of participants), hepatitis C virus positive serology (42.3%), lifetime alcohol use disorder (55.0%), body mass index or serum creatinine (ps > 0.15). Total duration of past exposure to d-drugs (median 2.0, IQR 0, 36.9 months) was not related to CSF NFL (r = 0.092, $P = .335$) or to DSP signs (ANOVA $P = .617$). Currently taking ART also was not related to CSF NFL ($P = .731$) or to DSP signs ($P = .495$). ART regimen and regimen type were not related to CSF NFL or to DSP signs (ps > 0.15).

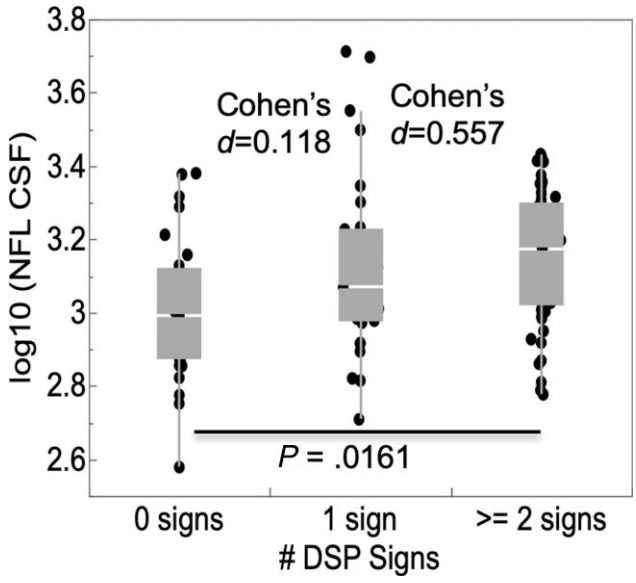

Figure 1. Box plots showing CSF NFL levels in Cohort 1 according to the number of DSP signs. Boxes represent interquartile ranges, whiskers represent 5th (lower) and 95th (upper) percentiles. Cohen's *d*, effect size. Abbreviations: CSF, cerebrospinal fluid; DSP, distal sensory polyneuropathy; NFL, neurofilament light.

Cohort 2 (PWH and PWoH)

Demographics and disease characteristics for Cohort 2 are listed in Table 1. All PWH had undetectable plasma HIV RNA. PWH were much more likely to have ≥2 signs of DSP than PWoH (53.0% vs 6.0%, $P = 1.01e{-}10$). Among PWH, 33% had been exposed to d-drugs in the past.

NFL Levels According to HIV Serostatus and DSP Signs, Cohort 2

Plasma NFL was significantly higher in PWH than PWoH (1.12 ± 0.275 vs 1.03 ± 0.208, $P = .0321$), and CSF NFL was numerically higher in PWH than PWoH (2.89 ± 0.297 vs 2.82 ± 0.244, $P = .118$). NFL was on average higher in CSF than plasma in both PWH and PWoH (for PWH, 2.7-fold higher, 2.89 ± 0.297 vs 1.12 ± 0.275; for PWoH, 2.6-fold 2.82 ± 0.244 vs 1.03 ± 0.208). CSF and plasma NFL levels were highly correlated in both PWH (r = 0.469, $P = 2.77e{-}12$) and PWoH (r = 0.527, $P = 8.40e{-}5$). Figure 2 shows that both CSF and plasma NFL levels were higher in PWH with abnormal DSP signs (for CSF, ≥2 signs 2.94 ± 0.302, 1 sign 2.82 ± 0.291, 0 signs 2.94 ± 0.302, ANOVA $P = .0303$; for plasma, >2 signs 1.17 ± 0.296, 1 sign 1.09 ± 0.229, 0 signs 1.05 ± 0.258, ANOVA $P = .0309$). Table 2 shows that among the three individual DSP exam findings, vibratory sensation was related to higher CSF NFL, and both vibratory sensation and reflexes were related to higher plasma NFL levels in PWH. Among PWoH, more DSP signs were associated with higher plasma NFL (≥2 signs 1.28 ± 0.0682 vs 1 sign 1.11 ± 0.177 vs 0 signs 0.970 ± 0.202,

Table 1. Comparison of Demographics and HIV Disease Characteristics in Cohort 2

	All	PWH	PWoH	*P*
N	249	199	50	…
Age in y—mean (SD)	57.5 6.70	57.3 6.68	58.3 6.74	.732
Sex female—N (%)	55 (22.1%)	34 (17.1%)	21 (42.0%)	**.0015**
Race/ethnicity Black—N (%)	44 (17.8%)	35 (17.8%)	9 (18.4%)	.629*
Race/ethnicity Hispanic—N (%)	17 (6.91%)	14 (17.1%)	3 (6.1%)	…
Race/ethnicity non-Hispanic white—N (%)	137 (55.7%)	111 (56.3%)	26 (53.1%)	…
Race/ethnicity other—N (%)	48 (19.5%)	37 (18.7%)	11 (22.5%)	…
Nadir CD4—median (IQR)	…	54 (13, 200)	…	…
Current CD4—median (IQR)	…	453 (286, 668)	…	…
Undetectable plasma HIV RNA—N (%)	…	199 (100%)	…	…
Est. duration of HIV in y—median (IQR)	…	17.1 (11.7, 23.5)	…	…
On ART—N (%)	…	199 (100%)	…	…
Duration of d-drug use—median (IQR)	…	0 (0, 31.1)	…	…

Bold values are statistically significant.
Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; PWH, people with HIV; PWoH, people without HIV; SD, standard deviation.
**P* value for difference between PWH and PWoH across all ethnicities.

$P = .0061$), but not CSF NFL (≥2 signs 3.00 ± 0.204 vs 1 sign 2.82 ± 0.300 vs 0 signs 2.81 ± 0.216, $P = .415$).

Potential Confounds, Cohort 2

Among PWH, the number of abnormal DSP signs increased with increasing age (ANOVA $P < .0013$). CSF and plasma NFL levels also were higher in older participants ($r = 0.300$, $P = 1.85e{-}5$ and $r = 0.291$, $P = 3.40e{-}5$, respectively). In a multivariable model including the number of DSP signs, age, and their interaction as predictors of CSF NFL, age was significant ($P = .00058$), but DSP signs ($P = .137$) and the interaction of age with DSP signs ($P = .930$) were not significant. Similarly, in a multivariable model including the number of DSP signs, age, and their interaction as predictors of plasma NFL, age was significant ($P = .0109$); DSP signs ($P = .174$) and the interaction were not ($P = .425$). To further characterize these interrelationships, we evaluated collinearity. The variance inflation factor (VIF) for the collinearity of age and DSP signs was 1.07, indicating no collinearity.

Males and females did not differ with respect to CSF NFL (2.90 ± 0.291 vs 2.82 ± 0.326, $P = .154$) or plasma NFL (1.13 ± 0.276 vs 1.08 ± 0.279, $P = .405$). Among PWH, ethnicity was not significantly related to NFL in CSF or plasma (*P*-values 0.337 and 0.314, respectively). CSF NFL was not related to nadir ($r = 0.0670$, $P = .350$) or current ($r = 0.00388$, $P = .958$) CD4+ T lymphocytes. Similarly, plasma NFL was not related to nadir ($r = -0.0551$, $P = .442$) or current ($r = -0.128$, $P = .0790$) CD4 count. Duration of d-drug exposure was not related to DSP signs or CSF or plasma NFL levels (ps > 0.20). PWH with diabetes had more signs of DSP (no signs 14.6%, 1 sign 17.1%, ≥2 signs 68.3%)

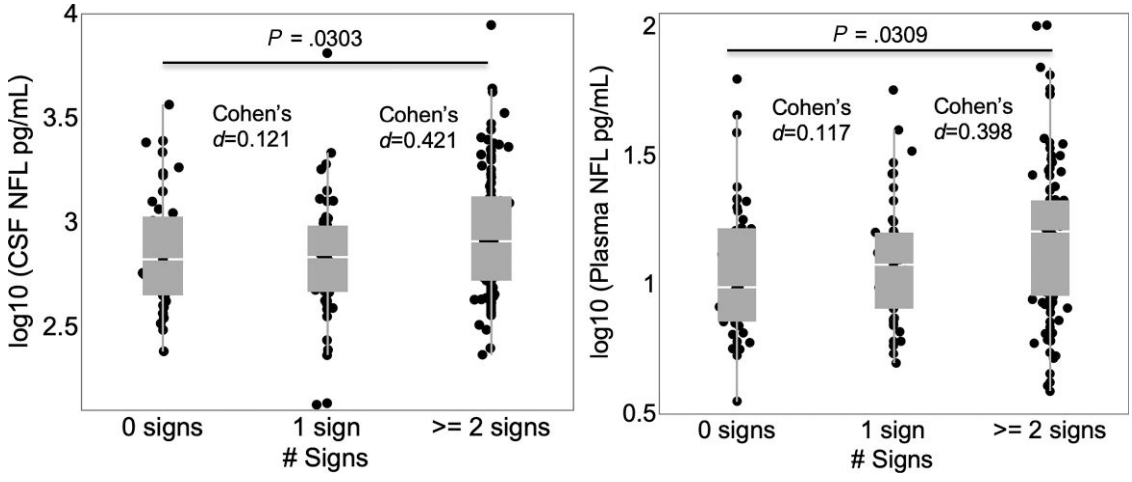

Figure 2. CSF and plasma NFL levels according to the number of DSP signs for PWH in Cohort 2. Boxes represent interquartile ranges, whiskers represent 5th (lower) and 95th (upper) percentiles. Cohen's *d*, effect size. Abbreviations: CSF, cerebrospinal fluid; DSP, distal sensory polyneuropathy; NFL, neurofilament light; PWH, people with human immunodeficiency virus.

Table 2. CSF and Plama NFL Levels According to Each Sign of DSP for PWH (Cohort 2)

	CSF NFL			Plasma NFL		
Symptom/Finding	Normal	Abnormal	*P*	Normal	Abnormal	*P*
Vibratory sensation	2.83 ± 0.0316	2.94 ± 0.0273	**.0057**	1.05 ± 0.254	1.18 ± 0.280	**.0018**
Reflexes	2.87 ± 0.264	2.91 ± 0.317	.448	1.07 ± 0.248	1.16 ± 0.288	**.0360**
Sharp sensation	2.88 ± 0.303	2.91 ± 0.284	.574	1.12 ± 0.263	1.12 ± 0.301	.988

Bold values are statistically significant.
Abbreviations: CSF, cerebrospinal fluid; DSP, distal sensory polyneuropathy; NFL, neurofilament light; PWH, people with human immunodeficiency virus,

compared to those without diabetes (no signs 25.3%, 1 sign 28.6%, ≥2 signs 46.1%; overall $P = .0386$). Those with diabetes had similar levels of CSF NFL and plasma NFL (ps > 0.15) to those without. In PWH, neurocognitive performance (GDS) was not related to CSF or plasma NFL (ps > 0.10). There were no interactions between neurocognitive performance and HIV status for either CSF or plasma NFL (ps > 0.20).

Rates of the following comorbid conditions did not differ between PWH and PWoH: diabetes (21.0% vs 18.0%, $P = .632$); hepatitis C virus positive serology (28.2% vs 28.0%, $P = .979$). Higher $\log_{10}$ serum creatinine levels were associated with higher CSF NFL ($r = 0.411$, $P = 2.13e{-}11$) and plasma NFL ($r = 0.528$, $P = 5.73e{-}19$). In a multivariable model for CSF NFL, DSP signs remained significant ($P = .0142$), while creatinine and the interaction were not. In a multivariable model for plasma NFL, the interaction and the main effect of DSP signs were not significant, while creatinine was ($P = 1.67e{-}16$). Hepatitis C virus serostatus was not significantly associated with DSP ($P = .879$).

DISCUSSION

In 2 independent cohorts, PWH with abnormal signs of DSP had higher NFL levels in CSF than those without DSP, consistent with axonal injury in DSP. In Cohort 2, plasma NFL also was measured and showed higher levels with DSP than without. The observed relationships between DSP and NFL were robust to consideration of potential confounds including viral suppression, nadir and current CD4, diabetes mellitus, and use of neurotoxic d-drugs and ART. Although the relationship of NFL to CNS disease (cognitive impairment) was not the focus of these analyses, we found that cognitive impairment did not significantly confound the relationship between NFL and DSP. In both cohorts, the association of DSP with CSF NFL was no longer significant after adjustment for age. This finding suggests that aging may drive both neuropathy and elevated NFL. Elevated plasma NFL in those with DSP was not specific to PWH, but present also in PWoH, suggesting a more general mechanism of neuropathogenesis, rather than one particular to HIV DSP. NFL levels were not associated with DSP symptoms, suggesting the symptoms depend on factors other than neurodegeneration.

The frequency of DSP, defined as at least two neuropathy signs, was very high despite viral suppression. This, along with prior evidence that DSP continues to develop de novo and worsen in the modern era, particularly in older PWH [1], is consistent with an active peripheral neurodegenerative process. Among the potential sources of ongoing peripheral neurodegeneration are persistent neuroinflammation [27, 28], neurotoxicity of ART [29] and comorbidities [30], though the comorbidities evaluated here were not associated with DSP.

In both cohorts, higher NFL levels were seen with exam findings of larger fiber (vibration, reflexes), rather than small (sharp sensation) DSP. HIV DSP is a mixed large and small fiber axonal neuropathy [31]; our findings suggest that large fibers contribute to axonal injury to a greater extent than small fibers. This is plausible since the larger the caliber of the axons, the more microtubules—which are stabilized by neurofilament proteins—are available to degenerate, releasing NFL into the extracellular space, where it can be measured.

These findings, in conjunction with elevated CSF NFL in other peripheral neuropathies [20–22], raise the question of why peripheral nerve injury leads to elevated NFL in CSF as well as plasma. Levels in CSF were approximately 1000-fold higher than plasma, making it implausible that NFL passively diffuses from blood into CSF. We suspect that elevated CSF NFL in the context of peripheral neuropathies is related to the presence of CSF in nerve root sleeves surrounding dorsal root ganglia (DRG) [14], with elevated CSF NFL levels reflecting injury to DRG sensory neurons. Indeed, HIV DSP is recognized as a DRG sensory neuronopathy, likely caused by the infiltration of activated macrophages into DRG [16–18]. Consistent with this, in vitro, DRG neurons exposed to supernatants from HIV-infected macrophages showed axonal retraction without neuronal cell death [19].

Although HIV is known to cause DSP independent of other factors, HIV DSP is typically multifactorial; d-drug exposure and HIV-associated diabetes are particularly common in HIV and are believed to be important contributors. We did not exclude participants in whom these contributors existed. In fact, the inclusion of such cases enhances the generalizability of our findings to other cohorts. The neurotoxicity of older nucleoside reverse transcriptase inhibitor antiretrovirals (so-called d-drugs) is well-described, and indeed, although none of our

participants took d-drugs at the time of assessment, many had been exposed to d-drugs in the past. However, neither diabetes nor d-drugs were related to NFL levels or DSP.

Strengths of this study include the replication across two independent cohorts that differed substantially in demographics, emphasizing the robustness and generalizability of our findings. Additionally, although we used different assay methods for NFL in the two cohorts, findings were similar for both assays.

Limitations of this study include its cross-sectional nature, precluding causal inference. Unobserved variables may have confounded our results. Reverse causation—elevated NFL causing peripheral neuropathy—is implausible. Neuropathy diagnoses were based on clinical exam findings; we did not perform electromyography and nerve conduction studies to further evaluate this. However, we have previously published on the predictive value of the clinical diagnosis using the approach described in the current manuscript compared to diagnoses using QST and NCV measures. In 1 study [32], the specificity of the clinical diagnosis of DSP was high (89.5%), and the positive predictive value was 84.6%. In a second study using similar methods [33], the clinical DSP correct classification rate relative to an electrophysiological approach was 78%, with a specificity of 88%. Thus, we are confident that our clinical diagnoses represent a high probability of DSP. Some neuropathy etiologies were not systematically evaluated here and could have been excluded with blood tests, strengthening the study. It was not practical to perform an exhaustive search for the myriad alternative causes, and this would have been cost-prohibitive. The number of female participants was relatively small, although no sex interactions were observed for the relationship between NFL and DSP. NFL levels in those with and without DSP substantially overlapped, limiting the usefulness of NFL for diagnostic purposes. Because NFL levels overlapped between the DSP and non-DSP groups, and because this study was not longitudinal, we cannot claim NFL as a diagnostic or prognostic tool, although the latter might be evaluated in future longitudinal studies.

The convergence of our findings in PWH with those in other polyneuropathies provides convincing evidence that NFL is a reliable correlate of peripheral nerve injury [11–13, 15, 20]. Notably, new neuroregenerative interventions have become available to treat DSP [6]. This is important because biomarkers such as NFL might be used as a surrogate marker of treatment response with neuroregenerative treatments, potentially increasing the efficiency of clinical trials [7, 8]. An additional future direction is considering whether elevated NFL levels might presage new onset DSP, offering possibilities for prevention if effective neuroprotective strategies become available.

Notes

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Author Contributions. R. J. E.: Conceptualization, Funding acquisition, Formal Analysis, Writing—original draft

A. C., Y. L., D. C., J. W., B. T., C. M. M., L. H. R., D. B. C., J. A. M., B. B. G., J, R. P., C. J. P.: Investigation, Writing—review and editing

S. L, L.: Funding acquisition, Investigation, Writing—review and editing

Data sharing. Data will be made available on request to the corresponding author.

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