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RESEARCH REPORT

Neurofilament Light Chain Levels in a Large Idiopathic Peripheral Neuropathy Cohort

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ABSTRACT

Background: Neurofilament light chain (Nf-L) has been identified as a biomarker of neurodegeneration in many neuromuscular conditions, including several subtypes of polyneuropathies. The purpose of this research was to investigate whether Nf-L is also a promising biomarker for idiopathic peripheral neuropathy (IPN), the second most common subtype of axonal polyneuropathy.

Methods: Nf-L levels were quantified using an ultrasensitive digital immunoassay SiMoA in plasma samples from 294 subjects. Participant inclusion required a diagnosis of IPN confirmed by electrodiagnostic testing, intraepidermal nerve fiber density (IENFD), and/or neuromuscular examination. Laboratory testing recommended by the American Academy of Neurology for the evaluation of polyneuropathy was normal in all subjects.

Results: In our cohort, the majority of participants (78.1%, $N = 228$) had Nf-L levels in the age-adjusted normal range. Those with elevated Nf-L levels had higher scores on two different neuropathy severity scores and were more likely to have abnormal electrodiagnostic testing, including reduced action potential amplitude in peroneal motor and sural sensory nerves. No differences in blood Nf-L levels were observed in those participants with a short duration (≤ 1.5 years) versus long duration (≥ 5 years) of disease. Nf-L levels were also not correlated with the presence of neuropathic pain, nor the location of paresthesia. Nf-L expression had the strongest correlation with age.

Conclusions: In this cohort with IPN, Nf-L levels correlated with disease severity as assessed by clinical examination and electrophysiology. However, given that Nf-L was in the normal range for the majority of subjects in our cohort, its use as a biomarker for clinical trials evaluating new treatments for IPN will be limited.

1 | Background

Peripheral neuropathies (PN) are one of the most common neurological disorders and affect approximately 11.8% of the US adult population aged over 40 [1]. For about 30% of all patients diagnosed with PN, no underlying etiology can be identified despite extensive laboratory and electrodiagnostic testing [2]; these patients are classified as having idiopathic peripheral polyneuropathy (IPN).

Abbreviations: CMAP, compound muscle action potential; FDR, false discovery rate; IENFD, intraepidermal nerve fiber density; IPN, idiopathic peripheral neuropathy; LFN, large fiber neuropathy; MNCV, motor nerve conduction velocity; Nf-L, neurofilament light chain; PN, peripheral polyneuropathy; PNRR, Peripheral Neuropathy Research Registry; SFN, small fiber neuropathy; SiMoA, single molecule assay; SNAP, sensory nerve action potential; SNCV, sensory nerve conduction velocity; TNSr, Total Neuropathy Score reduced; UENS, Utah Early Neuropathy Scale.

The first two authors contributed equally to this work.

Neurofilament (NF) levels have recently emerged as an important blood-based biomarker for multiple neurological diseases. First discovered by Gabriel Valentin in 1836, NFs are key structural components of the neuronal cytoskeleton [3–6] and are found in both the central and peripheral nervous systems [7]. NFs are important for supporting radial expansion of myelinated axons and for promoting faster nerve conduction velocities [8]; they are therefore more abundant in larger, myelinated axons [9].

The recent development of ultrasensitive digital immunoassay technology can detect neurofilament light chain (Nf-L) levels at concentrations as low as single-digit picograms per milliliter [10]. This allows Nf-L concentrations to be measured directly from blood plasma [11]. Since these assays have become available, Nf-L expression has been confirmed as a reliable clinical biomarker for disease activity for many neurological conditions, including amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and Parkinson's disease [11]. Nf-L levels were also confirmed to be elevated in various types of peripheral polyneuropathies, such as diabetic sensory polyneuropathy (DSPN) [9, 12], chemotherapy-induced PN (CIPN) [13, 14], chronic inflammatory demyelinating polyneuropathy (CIDP) [7], amyloid PN [15], HIV-associated PN [16], and pyridoxine-induced sensory neuropathy [17].

Disease progression in IPN is often slow and predominantly affects small unmyelinated fibers in early disease stages [18]. Novel biomarkers are needed to monitor outcomes during interventional trials in IPN, but there are limited data defining baseline Nf-L levels in this disease population. In this retrospective cohort study, we aimed to investigate the role of Nf-L as a potential biomarker for IPN disease activity as well as disease severity.

2 | Methods

2.1 | Participant Cohort

The Peripheral Neuropathy Research Registry (PNRR) is a large, multicenter database and biorepository of participants with a diagnosis of diabetic or idiopathic PN, sponsored by the Foundation for Peripheral Neuropathy. The collected data set includes a neuromuscular examination, electrodiagnostic testing, laboratory testing results as recommended by the American Academy of Neurology (AAN) for the most common causes of PN [19]; as well as an evaluation for the presence and severity of metabolic syndrome. In addition, all PNRR participants were also asked to complete a questionnaire that discusses the presence and severity of the most common symptoms associated with PN, PN duration, as well as their medical history [20]. All PNRR participants provided written consent, authorizing this research, and ethical approval was obtained from the Johns Hopkins University School of Medicine Institutional Review Board.

2.2 | Inclusion Criteria

PNRR participants with a diagnosis of IPN enrolled at Johns Hopkins Hospital were included in this study. The diagnosis of PN was confirmed by one of the following three criteria: (i) abnormal NCS findings, (ii) abnormal skin biopsy, or (iii) abnormal neurological examination confirming small fiber neuropathy (SFN) through bilaterally reduced pinprick or vibration sense at the hallux [21]. In addition, the participants needed to have both the seven-item reduced Total Neuropathy Score (TNSr) and the Utah Early Neuropathy Scale (UENS) information on record.

2.3 | Nf-L Assay

We utilized the ultrasensitive SiMoA NF-light digital immunoassay from Quanterix for the quantitative determination of Nf-L at concentrations as low as single-digit picograms per milliliter [10]. The SiMoA immunoassay uses two noncompeting monoclonal antibodies that are specific to Nf-L (Uman Diagnostics, Umeå Sweden) for quantification [22, 23]. All samples used in this analysis were processed on the same day on four pallets of 74 samples each (296 samples overall). Established age-adjusted reference ranges for SiMoA assay were utilized to identify participants with elevated Nf-L levels beyond the 95th percentile (Figure S1). A flow chart of the study design is provided as (Figure S2).

2.4 | Outcome Measures

The primary outcome measures were TNSr and UENS. Both are neuropathy severity scores, whereby the TNSr was a modification of the original TNS to evaluate predominantly sensory PN [24, 25]. The maximum score of the TNSr is 28, and the seven sub-items include area of paresthesia, pinprick, vibration sense, muscular weakness, deep tendon reflexes, peroneal compound nerve action potential (CMAP), and sural sensory nerve action potential (SNAP) (Figure S3a). The UENS was developed specifically for the evaluation of early onset diabetic neuropathy, and most assessments are done only at the toe level, including muscular weakness, vibration sense, proprioception, allodynia, and Achilles tendon reflex. However, pinprick is evaluated separately for six different sections of the legs, accounting for up to 24 points out of the maximal score of 42 [26] (Figure S3b). As only 64 Hz Rydel-Seiffert tuning fork values were captured as part of the PNRR data set, age-adjusted Rydel-Seiffert tuning fork values were used to evaluate vibration sense instead of a regular 128 Hz tuning fork.

Secondary outcome measures were (i) neurological examination findings, (ii) nerve conduction study (NCS) parameters, (iii) neuropathy onset, and (iv) pain intensity and location. Given that the reference sural SNAPs are age dependent [27], a normalized sural SNAP was calculated for each participant using the normative value established by the Johns Hopkins EMG-laboratory: <65 years of age $>9\,\mu V$, and ≥ 65 years $>5\,\mu V$.

The pain intensity reported by each participant as part of the patient questionnaire, in the form of a 0–10 numerical rating pain scale, was used to determine if Nf-L levels were correlated to pain intensity. Also, the reported pain locations were utilized to evaluate if Nf-L levels were correlated to the area of paresthesia,

converting the information from the patient questionnaire into a 0–4 scale: 0 = *no pain*, 1 = *pain in feet only*, 2 = *feet and legs*, 3 = *feet, legs and hands*, 4 = *both upper and lower extremities plus other areas*.

Finally, subgroup analysis was conducted between patients with small- (SFN) and large- (LFN) fiber IPN, for both clinical and electrodiagnostic features. LFN was defined as having abnormal electrodiagnostic testing on record, usually in the form of an abnormal age-adjusted sural sensory nerve action potential (sural SNAP), and SFN was defined as having normal electrodiagnostic evaluation in combination with either abnormal skin biopsy or sensory examination. Patients within each of these two subgroups were further subcategorized into normal range versus elevated Nf-L levels.

2.5 | Statistical Analysis

ANOVA was used to describe the cohorts of patients with normal versus elevated Nf-L levels. Chi-square and two-sample *t*-tests were utilized to assess differences between the two patient cohorts.

Raw Nf-L levels showed significant deviation from normality (Skewness–Kurtosis test: $\chi^2(2) = 280.16$, $p < 0.0001$) and logarithmic transformation of Nf-L substantially improved normality ($\chi^2(2) = 5.46$, $p = 0.0653$), particularly addressing skewness ($p = 0.3238$) (Figure S4). We conducted regression analysis to assess the correlation between log(Nf-L level) and the primary and secondary outcome measures. Given that age and BMI were significant confounds in the univariate linear

TABLE 1 | Demographics and laboratory testing results.

	Entire cohort	Normal Nf-L levels	Elevated Nf-L levels	*p*	Statistical method
Cohort size in % (*N*)	100 (292)	78.1 (228)	21.9 (64)	—	—
Pure SFN in % (*N*)	38.7 (113)	44.7 (102)	17.2 (11)	**0.0001**	1
Painful PN in % (*N*)	71.2 (208)	72.4 (165)	67.2 (43)	0.4185	1
Mean Nf-L level in pg/mL ± SD (range)	21.2 ± 20.3	15.8 ± 8.0 (2.7, 43.6)	40.5 ± 34.4 (13.6, 249.9)	**< 0.0001**	2
Male:female ratio	1:0.55	1:0.52	1:0.68	0.3437	1
Race—percent Caucasian	95.9	94.7	100.0	0.4570	1
Mean duration of PN symptoms in years ± SD (range)	5.8 ± 6.2	5.7 ± 6.3 (0.3, 39)	6.2 ± 5.8 (0.3, 26)	0.2266	2
Median age in years ± SD (range)	64.5 ± 14.6	65.0 ± 14.8 (22–88)	62.5 ± 13.6 (21–91)	0.6048	3
Median weight in kg ± SD (range)	88.7 ± 19.6	90.3 ± 19.7 (52.6, 183.0)	86.4 ± 18.0 (46.2, 125.6)	**0.0061**	3
Median height in cm ± SD (range)	177.8 ± 10.1	177.8 ± 9.9 (152.4, 205.7)	177.8 ± 10.6 (154.9, 208.2)	0.3600	3
Median body mass index ± SD (range)	28.0 ± 5.3	28.4 ± 5.5 (14.6, 49.1)	26.5 ± 4.4 (16.4, 37.6)	**0.0062**	3
Mean HbA1c in % ± SD (range)	5.30 ± 0.28	5.31 ± 0.27 (4.5, 5.9)	5.25 ± 0.31 (4.1, 5.7)	0.1468	3
Mean vitamin B12 in pg/mL ± SD (range)	782.7 ± 467.3	779.6 ± 469.5 (205, 2000)	793.4 ± 463.1 (241, 2000)	0.8365	3
Mean creatinine in mg/dL ± SD (range)	1.00 ± 0.96	1.01 ± 1.07 (0.45, 16.7)	0.97 ± 0.28 (0.5, 1.8)	0.9193	2
Mean thyroid stimulating hormone (TSH) in IU/mL (range)	2.17 ± 2.18	2.21 ± 2.40 (0.01, 29.74)	2.03 ± 1.02 (0.26, 5.58)	0.9237	2
Mean triglyceride in mg/dL ± SD (range)	126.8 ± 113.7	125.9 ± 112.9 (32, 1402)	130.1 ± 73.2 (47, 339)	0.2667	2
Mean high density lipid in mg/dL ± SD (range)	55.4 ± 17.2	54.5 ± 16.6 (22, 118)	58.5 ± 19.0 (31, 106)	0.1736	3

Note: The statistical tests were conducted between normal Nf-L level and elevated Nf-L level groups. Statistical tests used: 1 = chi-square for categorical data, 2 = Wilcoxon rank-sum test (Mann–Whitney *U*) for non-normal data, and 3 = Student's *t*-test for normal data. Bold indicates statistically significant >0.05.

regression analysis ($r = 0.6555$, $p < 0.00005$ for age, $r = -0.1754$, $p = 0.0032$ for BMI), multivariate linear regression analysis was conducted with age and BMI as the primary variables. Partial correlation analysis was then utilized to assess the strength and direction of the linear relationship between Nf-L levels and the variables of interest while controlling for the confounding effect of age and BMI.

Distribution analysis was conducted to assess if Nf-L levels were correlated with recent onset. There was no clear definition for "recent onset," so we proposed the definition, "recent onset: ≤1.5 years since onset" and "non-recent onset: ≥5 years since onset" based on clinical experience. In these sub-cohorts, age adjustment for Nf-L level was conducted, as patients with longer durations of PN were also older. The non-parametric two-sample Wilcoxon rank-sum (Mann–Whitney) *U* test was conducted given that the distribution of Nf-L within these groups were non-normally distributed. To minimize the risk of false discovery rates (FDR), the *p* value was corrected for multiple comparisons using the Benjamini–Hochberg (BH) method.

3 | Results

Nf-L levels were assayed in a total of 296 plasma samples. No values were obtained from the SiMoA assay for two of the samples, reducing the number of samples included in this analysis to 294. In 21.9% ($N = 64$) of the samples, the Nf-L levels were determined to be elevated beyond the 95th percentile, while in 78.1% ($N = 228$) of the samples, it was considered within the age-adjusted normal range.

TABLE 2 | Neuropathy Severity Scores.

	Entire cohort	Normal Nf-L levels	Elevated Nf-L levels	*p*	BH-adjusted *p* value
Utah Early Neuropathy Scale (UENS)					
UENS (total scale) ± SD (range) (out of 42)	12.0 ± 9.2	11.0 ± 8.9 (0, 40)	15.5 ± 9.7 (0, 38)	**0.0008**	—
Muscular weakness (out of 4)	0.95 ± 1.66	0.82 ± 1.57	1.41 ± 1.91	**0.0264**	**0.0370**
Pinprick score ± SD (range) (out of 24)	6.81 ± 5.88 (0, 12)	6.42 ± 5.65 (0, 10)	8.19 ± 6.50 (2, 12)	0.0548	0.0639
Large fiber (vibration and proprioception) (out of 8)	3.45 ± 2.70	3.12 ± 2.64	4.59 ± 2.60	**0.0001**	**0.0004**
Vibration sense (out of 4)	2.50 ± 1.74	2.36 ± 1.77	3.02 ± 1.50	**0.0094**	**0.0165**
Proprioception (out of 4)	0.94 ± 1.41	0.76 ± 1.32	1.58 ± 1.55	**< 0.0001**	**< 0.0001**
Achilles tendon reflex (out of 4)	2.04 ± 1.85	1.87 ± 1.84	2.64 ± 1.79	**0.0034**	**0.0079**
Allodynia (out of 2)	0.09 ± 0.42	0.11 ± 0.45	0.03 ± 0.25	0.2956	0.2956
Total Neuropathy Score reduced (TNSr)					
TNSr (total score) ± SD (range) (out of 28)	9.0 ± 6.2	8.0 ± 5.9 (0, 26)	15.0 ± 6.0 (2, 24)	**< 0.0001**	—
Symptom location (out of 4)	2.33 ± 1.15	2.30 ± 1.19	2.45 ± 0.97	0.3197	0.3197
Pinprick (out of 4)	1.73 ± 1.31	1.64 ± 1.30	2.02 ± 1.30	**0.0358**	**0.0418**
Vibration sense (out of 4)	1.27 ± 1.17	1.16 ± 1.13	1.66 ± 1.25	**0.0358**	**0.0418**
Muscular weakness (out of 4)	0.61 ± 1.06	0.52 ± 0.96	0.94 ± 1.30	**0.0256**	**0.0418**
Reflexes (out of 4)	1.38 ± 1.38	1.22 ± 1.33	1.92 ± 1.41	**0.0004**	**0.0014**
Peroneal CMAP (out of 4)	1.33 ± 1.76	1.19 ± 1.72	1.86 ± 1.79	**0.0052**	**0.0121**
Sural SNAP (out of 4)	1.89 ± 1.85	1.62 ± 1.81	2.82 ± 1.69	**< 0.0001**	**0.0007**

Note: UENS and TNSr total score and sub-scores. The statistical tests were conducted between normal Nf-L level and elevated Nf-L level groups. Wilcoxon rank-sum test, with and without Benjamini–Hochberg (BH) correction was applied to each item (sub-score) of UENS and TNSr. Bold indicates statistically significant >0.05.

3.1 | Patient Demographics

The median age of all participants was 64.5 ± 14.6 years; two-thirds of the patients were male, and 95.9% ($N = 280$) of the participants indicated that they were Caucasian. Median duration of PN was 5.8 ± 6.2 years (range: 0.3–39 years) and the majority of patients (71.2%, $N = 208$) reported neuropathic pain. There were no statistically significant differences in demographics between the normal range versus elevated Nf-L level cohorts for most of the parameters; however, the normal range cohort had a higher median weight and BMI (26.5 ± 4.4 vs. 28.4 ± 5.5 $p = 0.0062$). The proportion of participants with a diagnosis of pure SFN was 44.7% ($N = 102$) versus 17.2% ($N = 11$) in the cohort with elevated Nf-L levels ($p = 0.0001$) (Table 1).

3.2 | Neuropathy Severity Scales

The UENS and TNSr scores were the two primary outcome measures for this analysis. Overall, the median UENS was 12.0 ± 9.2, whereby 12 corresponds to 28.6% of the maximum score of 42. Participants with elevated Nf-L levels had a higher UENS of 15.5 ± 9.7 (36.9% of max) while it was 11.0 ± 8.9 (26.2% of max) in the cohort with normal range Nf-L levels ($p = 0.0008$). Each of the sub-components of the UENS scored higher in the cohort with elevated Nf-L levels compared to the normal range; the only exception was allodynia (Table 2).

The median TNSr for the overall cohort was 9.0 ± 6.2, which corresponded to 32.1% of the maximum score of 28. For the cohort with elevated Nf-L levels, the median TNSr was significantly higher at 15.0 ± 6.0 (53.6% of max) versus the normal Nf-L cohort, where it was 8.0 ± 5.9 (28.6% of max) ($p < 0.0001$). When comparing each scale item separately, the cohort with elevated Nf-L levels scored higher for each item except for symptom location.

Multivariate linear regression analysis (Table 3) indicated a strong correlation between age and log(Nf-L) levels ($r = 0.6555$, $p < 0.0001$), and between BMI and log(Nf-L) levels ($r = -0.1754$, $p = 0.0032$). After accounting for the effects of age and BMI, and controlled for using the Benjamini–Hochberg procedure, both UENS ($p = 0.0074$, Figure 1a) and TNSr ($p < 0.0001$, Figure 1b) remained significantly correlated with log(Nf-L) levels, with TNSr having a slightly greater correlation than UENS. These observations were confirmed in the partial correlation analysis, though they were small (Table 4).

3.3 | Nerve Conduction Studies

NCS data was available for 94.9% ($N = 277$) of the entire cohort and was considered abnormal in 61.7% ($N = 171$) of them. The proportion of participants with abnormal NCS results was significantly higher in the elevated Nf-L level cohort, with 82.3%

TABLE 3 | Primary outcome measures versus log(Nf-L).

Comparison	Correlation coefficient	Correlation coefficient (adjusted for age and BMI)	Univariate *p* value	Univariate BH-adjusted *p* value	Multivariate *p* value (adjusted for age and BMI)	Multivariate BH-adjusted *p* value (adjusted for age and BMI)
log(Nf-L) vs. age	0.6555	—	< 0.0001	< 0.0001	—	—
log(Nf-L) vs. BMI	−0.1754	—	0.0026	0.0032	—	—
log(Nf-L) vs. PN onset	0.081	−0.0203	0.1677	0.1677	0.7301	0.8090
log(Nf-L) vs. UENS	0.3339	0.2125	< 0.0001	< 0.0001	0.0003	**0.0009**
log(Nf-L) vs. TNSr	0.4543	0.3135	< 0.0001	< 0.0001	< 0.0001	**< 0.0001**
log(Nf-L) vs. pain intensity	−0.2073	0.0143	0.0004	0.0005	0.8090	0.8090
log(Nf-L) vs. pain location	−0.2642	0.0155	< 0.0001	< 0.0001	0.7927	0.8090
log(Nf-L) vs. peroneal MNCV	−0.372	−0.1828	< 0.0001	< 0.0001	0.0067	**0.0121**
log(Nf-L) vs. peroneal CMAP	−0.4425	−0.1652	< 0.0001	< 0.0001	0.0059	**0.0121**
log(Nf-L) vs. sural SNCV	−0.1828	−0.1733	0.0177	0.0195	0.1322	0.1983
log(Nf-L) vs. sural SNAP	−0.5182	−0.2542	< 0.0001	< 0.0001	< 0.0001	**< 0.0001**

Note: Univariate and multivariate linear regression analysis between log(Nf-L) level and various neurophysiological parameters, both unadjusted (univariate) and adjusted (multivariate) for age and BMI. All raw *p* values were adjusted by the Benjamini–Hochberg (BH) procedure to control FDR; the resulting values are reported as BH-adjusted *p* values. Bold indicates statistically significant >0.05.

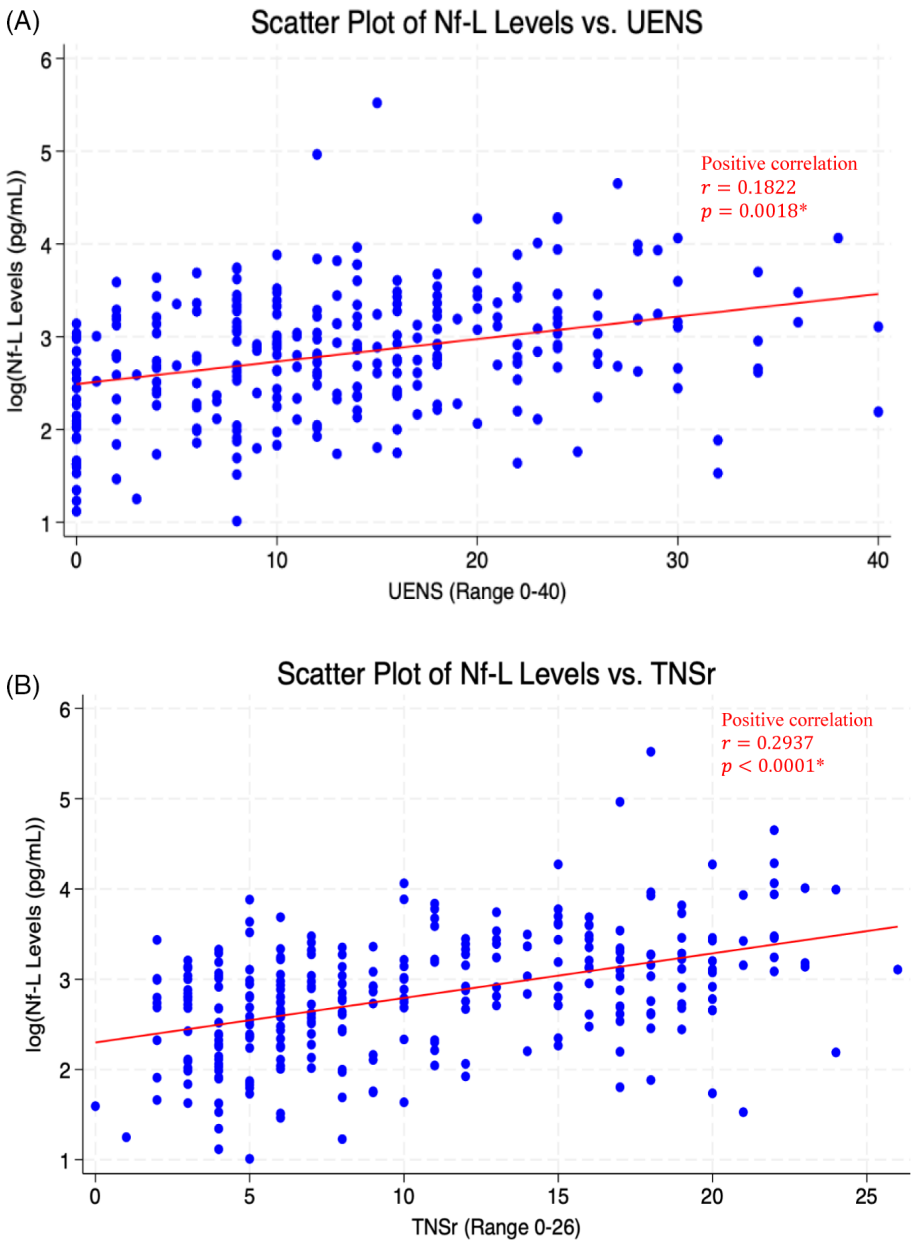

FIGURE 1 | (a) Scatter plot of log(Nf-L) levels versus UENS scores. Positive correlation: $r = 0.1822$, $p = 0.0018$*. (b) Scatter plot of log(Nf-L) versus TNSr scores. Positive correlation: $r = 0.2937$, $p < 0.0001$*.

TABLE 4 | Partial correlation analysis.

Variable	Partial correlation after adjusting for age and BMI (variance in Nf-L explained by this variable)	*p*
UENS total scale	0.0246	**0.0074**
TNSr total score	0.0832	**<0.0001**
Peroneal MNCV	0.0259	**0.0171**
Peroneal CMAP	0.0275	**0.0057**
Sural SNAP	0.0431	**0.0005**

Note: Partial correlation of variance of Nf-L levels with the individual significant variables identified in the multivariate linear regression analysis following age and BMI adjustment. Squared partial correlation represents the unique variance explained by each predictor after controlling for other variables. Bold indicates statistically significant >0.05.

($N = 51$) compared to 55.8% ($N = 120$) in the normal range group ($p = 0.0003$). For those with abnormal NCS testing, the NCS interpretation was predominantly axonal and affected both sensory and motor nerves (Table 5).

In the initial analysis, the percentage of participants with nonresponsive peroneal motor and sural sensory nerves was higher in the elevated Nf-L cohort ($p = 0.0290$ and $p = 0.0001$, respectively), there were no significant differences detected for conduction velocity and action potential amplitudes. Only the age-adjusted normative values for sural SNAP were significantly lower in those with elevated Nf-L level cohort. When repeating the analysis with log(Nf-L) (Table 3) there was a negative correlation between Nf-L levels and peroneal CMAP and sural SNAP ($p = 0.0170$ and $p = 0.005$, respectively) (Figure 2a,b). Partial correlation (Table 4) showed a modest contribution of these variables to the variance in Nf-L levels, with sural SNAP

TABLE 5 | Nerve conduction studies.

	Entire cohort	Normal Nf-L levels	Elevated Nf-L levels	*p*	Statistical method
Participants with NCS data (% of cohort)	277 (94.9)	215 (94.3)	62 (96.9)	—	—
Abnormal NCS in %	61.7	55.8	82.3	**0.0003**	1
Predominantly axonal in %	80.3	82.5	72.7	0.1773	1
Mixed (axonal and demyelinating) in %	20.1	16.7	28.3	0.0760	1
Predominantly sensory in %	35.2	37.3	30.2	0.3630	1
Sensorimotor in %	64.5	62.7	70.9	0.3840	1
Peroneal motor nerve					
Nonresponsive peroneal nerve in %	19.1	16.3	28.6	**0.0290**	1
Mean peroneal MNCV ± SD (range) in m/s	41.9 ± 6.3 (28, 56)	42.5 ± 5.9 (29, 64)	39.9 ± 6.3 (28, 56)	0.1322	2
Mean peroneal CMAP ± SD (range) in mV	2.78 ± 2.5 (0, 10.7)	3.03 ± 2.5 (0, 10.7)	1.90 ± 1.9 (0, 7.6)	0.0988	2
Sural sensory nerve					
Nonresponsive sural nerve in %	38.4	32.2	59.7	**0.0001**	1
Mean sural SNCV ± SD (range) in m/s	46.4 ± 6.5 (32, 71)	46.3 ± 6.6 (32, 71)	46.7 ± 6.3 (36.7–64)	0.9225	2
Mean sural SNAP ± SD (range) in μV	7.5 ± 7.8 (0, 49)	8.5 ± 7.1 (0, 49)	4.1 ± 7.5 (0, 39.3)	0.9839	2
Age-adjusted normative values for sural SNAP: < 65 years ≥ 9 μV and ≥ 65 years ≥ 5 μV					
Mean normalized sural SNAP ± SD (range)	1.01 ± 1.13 (0, 5.44)	1.15 ± 1.16 (0, 5.44)	0.50 ± 0.88 (0, 4.37)	**0.00005**	2

Note: The statistical tests were conducted between normal Nf-L level and elevated Nf-L level groups. Statistical tests used: 1 = chi-square for categorical data and 2 = Wilcoxon rank-sum test (Mann–Whitney *U*) for non-normal data. Bold indicates statistically significant >0.05.

accounting for the most out of the NCS parameters at 3.36% ($p = 0.0022$).

3.4 | Onset of PN Symptoms

When evaluating any correlation between Nf-L expression and time elapsed since onset of PN symptoms, no significant difference in Nf-L levels was identified after correcting for age and BMI, as the cohort with longer PN duration was also significantly older (Table 6).

3.5 | Pain Intensity and Location

There was no significant difference between the two cohorts regarding the prevalence of neuropathic pain. The pain intensity was initially negatively correlated to Nf-L expression (Table 1), but no correlation was identified when adjusted for age and BMI in the multivariate linear regression analysis (Table 3 and Figure S5a).

When correlating Nf-L levels to pain location, the results were also not statistically significant, neither for the analysis using absolute Nf-L values nor when using log(Nf-L) (Table 3a and Figure S5b).

3.6 | Small Versus Large Fiber Neuropathy Analysis

As the percentage of participants with SFN was significantly higher in the normal Nf-L level group (Table 1), the analysis was repeated by dividing the cohort into SFN-only and large fiber neuropathy (LFN) subgroups. The LFN subgroup also contains participants with mixed, large, and small fiber neuropathy. More

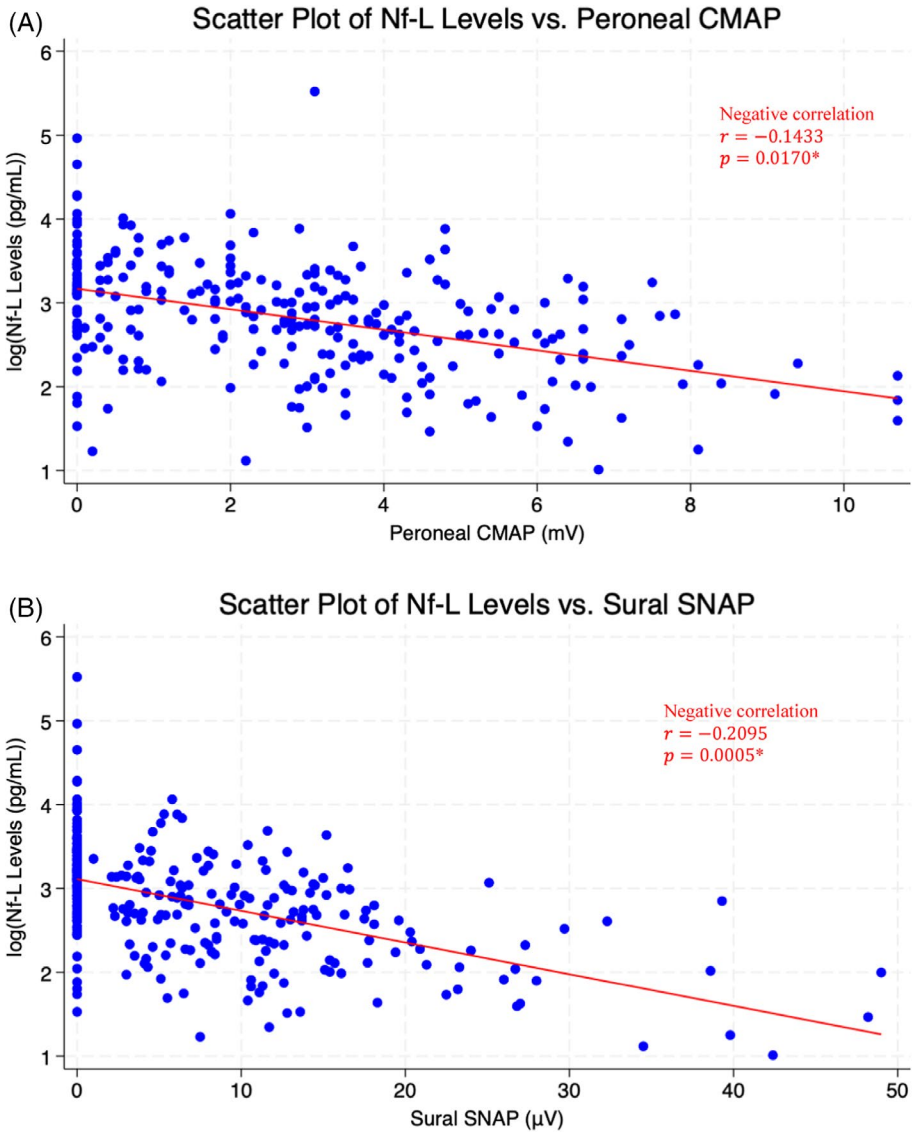

FIGURE 2 | (a) Scatter plot of log(Nf-L) versus peroneal compound muscle action potential. Negative correlation: $r = -0.1433$, $p = 0.0170$*. (b) Scatter plot of log(Nf-L) versus sural sensory nerve action potential. Negative correlation: $r = -0.2095$, $p = 0.0005$*.

patients (61.3%, $N = 179$) were included in the LFN subgroup compared with SFN-only (38.7%, $N = 113$). The demographics were similar, except that the LFN cohort was significantly older, with a median age of 68 years versus 55 years in the SFN-only cohort ($p < 0.0001$); and only 9.7% ($N = 11$) of patients with SFN-only had elevated Nf-L levels, compared with 29.6% ($N = 53$) of patients with LFN ($p < 0.0001$) (Figure S6a).

When comparing the primary and secondary outcome measures in the subset of participants with SFN or LFN, only TNSr showed a significant difference between those with normal range versus elevated Nf-L levels (Figures S6b and S7).

4 | Discussion

Nf-L expression has recently been confirmed as a reliable clinical biomarker for disease activity for many neurological conditions, including diabetic neuropathy, CIDP, amyloid neuropathy, and CIPN (see Table S8). The primary objective of this research was to evaluate if Nf-L expression is a potential biomarker for disease activity for IPN in future intervention trials; the secondary objectives were to investigate if Nf-L levels are an indicator for symptom severity or neuropathic pain.

Given that 78.1% ($N = 228$) of our cohort had plasma Nf-L levels in the age-adjusted normal range, the use of Nf-L as a general biomarker for disease activity in IPN will be limited; but our findings indicate that Nf-L plasma levels are an indicator for disease severity. As Nf-L is mostly expressed in the larger myelinated nerve fibers of the peripheral nervous system [11], participants with elevated Nf-L levels had a higher incidence of abnormal electrodiagnostic testing results ($p = 0.0003$), as well as nonresponsive peroneal motor and sural sensory nerves ($p = 0.0290$ and $p = 0.0001$, respectively). After normality was improved by transforming Nf-L into log(Nf-L), both peroneal CMAP and sural SNAP were significantly lower in those with elevated Nf-L plasma levels ($p = 0.0170$ and $p = 0.0005$, respectively).

TABLE 6 | Nf-L expression in relation to onset of PN symptoms.

Grouping	*N*	Mean onset of PN in years ± SD (range)	Mean Nf-L ± SD (range)	Mean age ± SD (range)	Mean BMI ± SD (range)	*p*	
						Unadjusted	Adjusted for age and BMI
Recent onset ≤ 1.5 years	71	0.92 ± 0.36 (0.3, 1.5)	20.8 ± 29.6 (2.7, 249.9)	57.8 ± 15.2 (21, 82)	27.8 ± 5.5 (14.6, 49.1)	0.0173	0.2339
Non-recent onset ≥ 5 years	121	11.07 ± 6.53 (5, 39)	23.7 ± 19.2 (3.1, 143.3)	65.5 ± 12.8 (26, 91)	28.9 ± 5.4 (17.5, 46.1)		

Note: Wilcoxon rank-sum test (Mann–Whitney *U*) is conducted for statistical analysis. The statistical tests were conducted on Nf-L levels between the recent-onset group and the non-recent-onset group.

Nf-L expression as an indicator for disease severity was further confirmed by the correlation between Nf-L expression and both neuropathy severity scores ($p < 0.0001$). When dividing both the TNSr and UENS into their sub-items, Nf-L expression was correlated to all scores evaluating large nerve fiber function; in particular, both proprioception and vibration sense were negatively correlated. In contrast, the sub-items evaluating primarily small nerve fiber function, such as pinprick and presence of allodynia, did not show a statistically significant correlation to Nf-L expression in UENS ($p = 0.0548$, $p = 0.2956$, respectively) and only a weak correlation for pinprick in TNSr ($p = 0.0358$).

The strongest correlation for Nf-L expression was with age ($r = 0.6555$); this is similar to previous research, which indicated a correlation coefficient of $r = 0.61$ for age in a cohort of participants with diabetic sensory polyneuropathy [9]. As our cohort also included younger participants (range 21, 91), it made it necessary to correct for age, and all variables demonstrated an age effect, with weakened *p* values after age adjustment, confirming that Nf-L level interpretation must be done in context to age (see Figure S9a–d).

To evaluate if Nf-L is a useful biomarker in those with LFN-IPN, we repeated our analysis by sorting the participants into SFN and LFN cohorts and then dividing them further into the normal versus elevated Nf-L level subcategories. TNSr remained statistically significant in both cohorts, and proprioception and sural SNAP remained statistically significant in the LFN cohort, but all other outcome measures were no longer statistically significant. This suggests that Nf-L levels are not a useful biomarker for disease severity in SFN, and its sensitivity appears also to be limited to more advanced stages of LFN. These observations in regard to SFN are in concurrence with a recent article published by Zohar et al., who reported elevated Nf-L levels in only 10% in a cohort of participants with SFN [28]. Similar to their findings, the majority of the participants in our SFN cohort with abnormal Nf-L levels also had a medical history of comorbidities that could be contributing factors (Table S10).

4.1 | Limitations

Several limitations of our study should be considered. First, the majority of participants were Caucasian (95.9%, $N = 280$), and 59.9% ($N = 175$) were male, which may limit the generalizability of our findings to more diverse populations. Second, all participants were enrolled from a single center, which could introduce site-specific biases. Third, Nf-L levels were assessed at a single time point, precluding analysis of temporal trends or assessment of Nf-L as a dynamic biomarker for disease progression or treatment response in IPN.

5 | Conclusions

In our cohort, the Nf-L levels were in the age-adjusted normal range for 78.1% ($N = 228$); this will limit its use in any upcoming intervention trial for new therapeutics for IPN. However, it is a biomarker for disease severity, as our research demonstrates that Nf-L levels are correlated to both neuropathy severity scores

(clinical features) as well as peroneal CMAP and sural SNAP (electrodiagnostic features). Therefore, Nf-L may be a useful adjunct to clinical evaluations for disease severity and overall prognosis.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1.**