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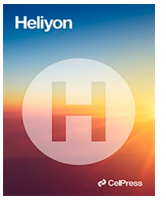

Research article

Novel strategy: Identifying new markers for demyelination in diabetic distal symmetrical polyneuropathy

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ABSTRACT

Objective: To develop a novel strategy for identifying acquired demyelination in diabetic distal symmetrical polyneuropathy (DSP).
Background: Motor nerve conduction velocity (CV) slowing in diabetic DSP exceeds expectations for pure axonal loss thus implicating superimposed acquired demyelination.
Methods: After establishing demyelination confidence intervals by regression analysis of nerve conduction data from chronic inflammatory demyelinating polyneuropathy (CIDP), we prospectively studied CV slowing in 90 diabetic DSP patients with and without at least one motor nerve exhibiting CV slowing (groups A and B) into the demyelination range by American Academy of Neurology (AAN) criteria respectively and 95 amyotrophic lateral sclerosis (ALS) patients. Simultaneously, secretory phospholipase A2 (sPLA2) activity was assessed in both diabetic groups and 46 healthy controls.
Results: No ALS patient exhibited CV slowing in more than two motor nerves based on AAN criteria or the confidence intervals. Group A demonstrated a significantly higher percentage of patients as compared to group B fulfilling the above criteria, with an additional criterion of at least one motor nerve exhibiting CV slowing in the demyelinating range and a corresponding F response in the demyelinating range by AAN criteria (70.3 % *vs.* 1.9 %; $p < 0.0001$). Urine sPLA2 activity was increased significantly in diabetic groups as compared to healthy controls (942.9 ± 978.0 *vs.* 591.6 ± 390.2 pmol/min/ml, $p < 0.05$), and in group A compared to Group B (1328.3 ± 1274.2 *vs.* 673.8 ± 576.9 pmol/min/ml, $p < 0.01$). More patients with elevated sPLA2 activity and more than 2 motor nerves with CV slowing in the AAN or the confidence intervals were identified in group A as compared to group B (35.1 % *vs.* 5.7 %, $p < 0.001$). Furthermore, 13.5 % of patients in diabetic DSP Group A, and no patients in diabetic DSP Group B, fulfilled an additional criterion of more than one motor nerve with CV slowing into the demyelinating range with its corresponding F response into the demyelinating range by AAN criteria.
Conclusion: A combination of regression analysis of electrodiagnostic data and a urine biological marker of systemic inflammation identifies a subgroup of diabetic DSP with superimposed acquired demyelination that may respond favorably to immunomodulatory therapy.

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1. Introduction

Distal Symmetrical Polyneuropathy (DSP) is the most prevalent manifestation of diabetic neuropathy-a common chronic complication of diabetes mellitus that increases the risk of complications including lower limb amputations and ulcers [1–3].

The first pathological studies implied that diabetic DSP is an irreversible axonal neuropathy with axonal degeneration, regeneration, and to a lesser degree, of secondary demyelination [4–6]. However, it is increasingly recognized that the conduction velocity (CV) slowing in diabetic DSP exceeds that observed in non-diabetic distal axonal neuropathy [7–9] and this pathology may be reversible [10–12]. Therefore, it has been suggested that motor nerve conduction slowing in DSP may be due to superimposed acquired inflammatory demyelination rather than a simple loss of the fastest conducting large myelinated fibers [8].

Early axonal loss and delay of immunotherapy initiation in chronic inflammatory demyelinating polyneuropathy (CIDP) has been associated with long-term nerve fiber loss and disability [13]. Macrophage-associated demyelination has been reported in CIDP and diabetic neuropathy [14–16]. Notably, the presence of macrophages within the endoneurium has been identified in a subset of patients with CIDP. In addition to a macrophage induced inflammatory reaction, the induction of autoantibodies to myelin components provoking complement cascades and subsequent phagocytosis by macrophages has also been proposed to be the mechanism underlying demyelination in CIDP [17]. A multicenter study demonstrated that muscle atrophy with a decreased compound muscle action potential (CMAP) amplitude was significantly more pronounced in non-responders to intravenous immunoglobulin (IVIG) in patients with diabetic neuropathy. Moreover, conduction block became less frequent after IVIG in those responders [18]. In a prospective study, IVIG significantly relieved pain in painful DSP [19]. These findings imply that peripheral demyelination is potentially reversible when diagnosed at an early stage before the occurrence of irreversible axonal loss resulting in muscle atrophy and severe disability. Therefore, a biomarker that indicates this early demyelinating activity would be especially useful.

Despite ongoing debates about the mechanism of conduction slowing in diabetic DSP, neuroinflammation is gaining recognition as a significant contributor to its pathogenesis [20–23]. Emerging evidence implicates immune and inflammatory processes, especially in acquired demyelinating polyneuropathies associated with diabetes [11,12,22].

Of particular interest in the context of neuroinflammation in diabetic neuropathy is secretory phospholipase A2 (sPLA2). Elevated glucose levels enhance sPLA2 activity, with increased expression noted in Schwann cells. Notably, sPLA2 activity is significantly higher in type II diabetic patients with clinical cardiovascular disease, correlating with low-grade inflammation and endothelial activation [24,25]. The downstream component of sPLA2, cyclooxygenase activity, has been implicated in the pathogenesis of experimental diabetic neuropathy [26,27].

Previously, we compared sPLA2 activity of the urine and plasma of rodents and humans [28–31]. These studies suggested that both measurements reflect changes in systemic sPLA2 activity, depending on the timing of the measurement relative to the inflammatory stimulus. However, plasma measurements are only dependable in a restricted window following such a stimulus due to the feedback mechanisms of sPLA2 products [29]. In relapsing/remitting multiple sclerosis (MS) patients and in the rodent models of MS, elevations in urinary sPLA2 measurements follow a predictable schedule relative to immunization and relapse symptoms [30]. We therefore measured urine sPLA2 activity in this study as a marker of inflammation.

In order to address the unmet clinical need for early identification of inflammatory demyelination in diabetic neuropathy, we proposed a novel diagnostic strategy that combines regression analysis of motor nerve conduction velocity [32] with urine sPLA2 as a biomarker of systemic inflammation. This innovative approach aims to identify potential demyelination when conduction slowing coincides with axonal loss in diabetic DSP, thus enabling early intervention and tailored therapeutic strategies.

2. Materials and methods

2.1. Subjects

Ninety consecutive adult subjects diagnosed with diabetic DSP and 46 controls were recruited from the Neuromuscular Division of the Department of Neurology at New Jersey Medical School. The study received approval from the Institutional Review Board of New Jersey Medical School, and informed consent was obtained from all enrolled subjects (IRB No. Pro2021001392). All adult participants providing voluntary informed consent and meeting the inclusion criteria were invited to participate.

The diagnosis of diabetic DSP was based on a documented history of diabetes mellitus with abnormal hemoglobin A1c (HbA1c), the presence of symmetric signs indicating distal sensory dysfunction with or without distal limb weakness, and the absence or reduction of deep tendon reflexes. Subjects exhibiting proximal weakness indicative of CIDP, the presence or coexistence of other known causes of neuropathy (e.g., B12 deficiency, exposure to neurotoxic agents), use of anti-inflammatory drugs, presence of malignancy, or other causes of systemic inflammation and active infection were excluded from the study.

Clinical, neurophysiological data, and urine samples were collected from both diabetic DSP patients and controls. Electrodiagnostic testing was conducted in a quiet room, maintaining limb temperature at or above 32 °C, using standard methods and distances [33]. Parameters for nerve conduction studies, including CMAP, distal latency (DL), CV, and F wave minimum latency, were measured for the median, ulnar, tibial, and fibular nerves bilaterally. The Inflammatory Neuropathy Cause and Treatment (INCAT) disability score, which quantitates upper and lower limb function, was assessed in all patients for comparison purposes.

Ninety consecutive diabetic patients exhibiting abnormal nerve conduction studies were enrolled in the study and categorized into two groups: (1) Group A comprised 37 diabetic subjects with DSP, demonstrating at least one motor nerve with CV slowing falling within the demyelinating range defined by the American Academy of Neurology (AAN) [28] though not meeting the AAN criteria for

CIDP; (2) Group B comprised 53 diabetic patients with DSP but lacking nerve CV slowing within the AAN demyelinating range.

2.2. Regression analysis of electrodiagnostic parameters

The regression analysis equations, originally derived from CIDP patients, were intended for the identification of conduction slowing in ALS patients [32] and in diabetic DSP (our current focus). This is particularly relevant when the observed conduction slowing exceeds what would be anticipated from an exclusive axonopathy. Moreover, although many different criteria have been proposed, (e.g., EFNS/PNS), the AAN criteria were chosen as they have the highest specificity. This ensured specificity over sensitivity. Our primary objective is to exclude patients with CV slowing solely due to axonal loss. The establishment of CIDP criteria with high specificity is crucial, as it minimizes the inclusion of false-positives and reduces the likelihood of improperly categorizing conduction slowing from axonal neuropathy as demyelination. This approach enhances confidence in interpreting conduction slowing as being compatible with demyelination. Consistent with our study protocol, wherein 5-8 motor nerves were analyzed, it is noteworthy that the EFNS/PNS specificity criteria ranged from 59.6 % to 62.9 %, while the AAN specificity criteria reached 100 % [34].

As previously outlined [32], we retrospectively identified an initial group of 76 patients with CIDP using the AAN criteria for primary acquired demyelination [35]. Motor nerve CVs from this CIDP group were utilized to formulate regression equations that determine, for each CMAP amplitude, the expected range of slowing in the context of a primary demyelinating polyneuropathy. Abnormal motor CV data were collected from the median, ulnar, tibial, and fibular nerves, with measurements taken for both CMAP and CV for each motor nerve. The obtained data were then converted to a percentage of the lower limit of normal using our established laboratory values. Through linear regression analysis, we developed equations linking CV to the distal CMAP amplitude of the median, ulnar, fibular, and tibial nerves in CIDP patients. The resulting values for each motor nerve attribute were expressed as square root transformations, fourth root transformations, or log10 transformations to achieve the optimal linear relationship between CMAP amplitude and CV.

The developed and validated equations assessing the range of slowing in CIDP patients were subsequently applied to analyze CV slowing in a cohort of 95 patients diagnosed with amyotrophic lateral sclerosis (ALS) who fulfilled the El Escorial revised criteria of the World Federation of Neurology [36]. The ALS group served as a negative control, which determined the maximum number of motor nerves falling outside the confidence intervals of the regression analysis for each patient, beyond which CV slowing would be indicative of acquired demyelination.

Regression analysis was then employed to investigate motor CV slowing in a prospectively recruited cohort of 90 diabetic DSP patients who were assessed for urine sPLA2 activity.

2.3. Urine sPLA2 enzyme activity

We performed urine sPLA2 activity assays utilizing the methodology previously described in our work [29], using random urine samples obtained from 90 diabetic patients and 46 controls. The assay underwent optimization using an excess amount of substrate. Validation procedures involved confirming linear product formation in the presence of excess substrate over a 40-min measurement period, thereby ensuring that the measured reaction rate correlates proportionally with the urinary concentration of active sPLA2 [30]. Following optimization and validation, we proceeded to compare urine sPLA2 activity in healthy controls with that of diabetic patients experiencing DSP.

2.4. Statistical analysis

Basic data summary statistics were conducted using MS Excel 2016. For advanced statistical analysis, SAS Software (version 9.4) was employed. Categorical variables were summarized by their counts and percentages, with group distributions compared using Fisher's exact test or chi-square test as appropriate. Descriptive summaries for continuous variables included means and standard deviations, and differences between group means were assessed using the non-parametric Mann-Whitney *U* test.

Raw electrophysiological data, specifically median, ulnar, fibular, and tibial CMAP amplitudes, underwent transformation to establish a more linear relationship between CMAP amplitude and velocity and latency measures, following previously described methods [32].

Pearson's correlation coefficient (r) was employed to assess the correlation between CMAP amplitude and CV, DL, and F latency. The interpretation of correlation coefficients was based on Cohen's conventions, categorizing them as follows: small/weak (0.1–0.3), medium/moderate (0.3–0.5), large/strong (>0.5) correlation.

The associations between transformed CMAP amplitude data (independent variable) and transformed CV, DL, and F latency data (dependent variables) were examined within each group and across groups using scatterplots, multiple linear regression analysis, and analysis of residuals. Multiple linear regression models were crafted to compare the Y-intercepts (representing CV, DL, or F) and the slopes of the regression lines (indicating the relationship between CMAP amplitude and CV, DL, or F) between the groups for both upper and lower extremities. All tests for statistical significance were two-sided, with a significance level set at 0.05.

3. Results

The demographic, clinical, and electrodiagnostic summary statistics for 37 patients in diabetic DSP group A and 53 patients in group B are presented in Table 1.

No significant differences were observed in age and gender between the two diabetic DSP groups (Table 1). However, the mean INCAT score was significantly higher in group A as compared to group B (2.3 vs. 1.2, $p < 0.05$). Additionally, mean CMAP amplitude and CV were significantly lower in all studied motor nerves in group A compared to group B (Table 1).

3.1. Regression analysis

Fig. 1 illustrates regression plots for converted normalized CV and converted normalized CMAP amplitude in the median, ulnar, tibial, and fibular motor nerves of diabetic DSP patients. The regression equations were formulated to establish optimal linear relationships between CMAP amplitude and corresponding CV. The resulting confidence intervals, while excluding instances of severe conduction slowing, it is noteworthy that all severe CV slowing outside these intervals fell within the demyelinating range defined by the AAN criteria. Therefore, in this study, the regression confidence intervals were utilized in conjunction with the AAN criteria for primary demyelination to identify instances of conduction slowing not severe enough to meet the AAN demyelination criteria.

In the ALS group, no patient exhibited CV slowing in more than two motor nerves based on the AAN criteria, or the confidence intervals derived from regression equations (Fig. 1). Hence, the identification of more than two motor nerves displaying CV slowing, according to the AAN criteria or regression equation ranges, implies the presence of CV slowing beyond what would be expected from a pure axonal loss, potentially indicating an associated demyelinating process.

In diabetic patients, the percentage of patients with more than twomotor nerves exhibiting CV slowing in the demyelinating range, as defined by the AAN or regression equation criteria, was significantly higher in Group A compared to Group B (83.8 % vs. 28.3 %, $p < 0.0001$; Fig. 1). Similarly, the percentage of patients with more than two motor nerves manifesting CV slowing, based on the AAN or regression equation criteria for demyelination, along with an additional criterion of at least one motor nerve exhibiting CV slowing in the demyelinating range and a corresponding F response in the demyelinating range by AAN criteria, was also significantly higher in Group A as compared to Group B (70.3 % vs. 1.9 %; $p < 0.0001$). The likelihood of having more than two motor nerves with CV slowing in the demyelinating range was significantly higher in diabetic DSP Group A as compared to diabetic DSP Group B (0.84 vs. 0.28, $p < 0.0001$).

We examined the correlation between CMAP amplitude, treated as an independent variable, and CV (DL and F), considered as dependent variables, for both diabetic groups and the ALS group. Pearson correlation coefficients indicate a significant correlation between CMAP amplitude and CV across all studied groups (Table 2).

Multiple linear regression was used to compare the coefficients for the slopes and Y-intercepts of CMAP amplitude versus CV and DL regressions in both the diabetic groups and the ALS group (Fig. 1 and Table 2). In the ulnar nerve (Fig. 1), the slopes of the regression lines for CMAP amplitude (independent variable) versus CV (dependent variable) were significantly different among the three studied groups (diabetic DSP group A vs. diabetic DSP group B vs. ALS group; $p < 0.05$). However, no significant difference was observed in the slopes of the regression lines for the fibular, tibial, and median nerves across the three groups.

Examining the Y-intercept regression coefficients (with CV as the dependent variable and CMAP amplitude as the independent variable), significant differences were noted. Specifically, the Y-intercept coefficients were significantly lower in diabetic group A compared to diabetic group B and ALS for the tibial, fibular, and median nerves (Fig. 1; Table 2). These findings imply the existence of CMAP-independent CV slowing in intermediate motor nerve segments in diabetic DSP, indicating a potential demyelinating contribution superimposed on axonal loss. Furthermore, the analysis revealed a notably higher proportion of motor nerves with CV slowing in the AAN or regression analysis ranges in group A compared to group B, suggesting the presence of a more diffuse demyelinating process in group A that, while not severe enough to meet the AAN criteria for primary demyelination, is still significant. While regression analysis proved effective in characterizing CV slowing in diabetic DSP and identifying instances beyond axonal loss that

Table 1
Demographic and electrodiagnostic characteristics of diabetic DSP patients.

	Group A (n = 37)	Group B (n = 53)	p-value
Age (years)	57.7 ± 10.84	57.5 ± 11.26	NS
Sex			
Male %	45.9 %	26.4 %	
Female %	54.1 %	73.6 %	NS
Mean INCAT score	2.3 ± 2.09	1.2 ± 1.11	<0.01
Urine sPLA2 activity (pmol/min/ml)	1328.3 ± 1274.21	673.8 ± 576.93	<0.01
Number of patients with increased urine sPLA2 (>mean of controls ± 2SD, >1371.93 pmol/min/ml)	15 (40.5 %)	8 (15.1 %)	<0.01
CMAP distal amplitude (mV)			
Tibial nerve	2.9 ± 2.13	5.9 ± 3.16	<0.01
Fibular nerve	3.0 ± 2.08	4.1 ± 2.13	<0.01
Median nerve	5.2 ± 2.41	7.4 ± 2.75	<0.01
Ulnar nerve	5.8 ± 3.22	8.1 ± 2.33	<0.01
Conduction Velocity (m/s)			
Tibial nerve	32.6 ± 6.81	40.6 ± 5.49	<0.01
Fibular nerve	34.8 ± 8.25	43.2 ± 5.36	<0.01
Median nerve	38.6 ± 6.54	49.9 ± 5.75	<0.01
Ulnar nerve	43.0 ± 8.16	52.5 ± 7.54	<0.01

NS = not significant at $p < 0.05$.

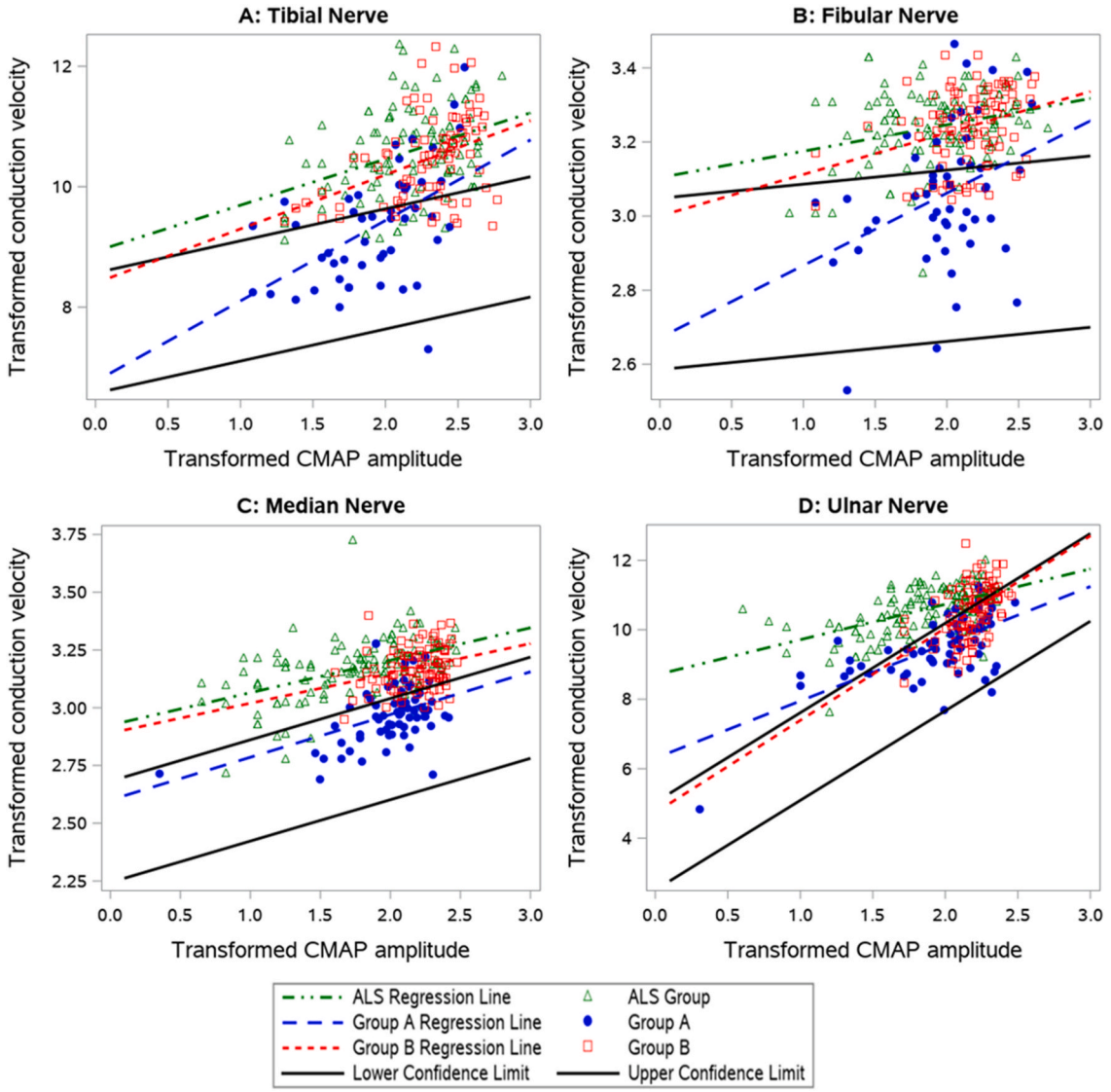

Fig. 1. Regression plots of converted normalized conduction velocity (CV) (y-axis, dependent variable) and converted normalized compound muscle action potential (CMAP) amplitude (x axis, independent variable) in tibial nerve (A), fibular nerve (B), median nerve (C), and ulnar nerve (D) were illustrated for amyotrophic lateral sclerosis (ALS) patients, Group A and Group B. All raw CMAP amplitudes and CV data were expressed as fraction of lower limit of normal and then transformed in the studied nerves to achieve a more linear relationship between CMAP amplitude and CV measurements. ALS group data points are depicted by hollow triangles, diabetic Group A data points by filled circles and diabetic Group B data points by hollow squares. Regression lines for ALS, diabetic Group A and diabetic Group B are depicted by a dash-double-dot line, a dash line, and a short-dashed line, respectively.

conventional electrodiagnostic tests failed to detect, there persisted an overlap of CV slowing between axonal loss and mild demyelination.

3.2. Urine sPLA2 activity

The average sPLA2 activity was elevated significantly in diabetic patients as compared to healthy controls (942.9 ± 977.97 pmol/min/ml vs. 591.6 ± 390.15, $p < 0.05$). Furthermore, a noticeable increase in mean urine sPLA2 activity was observed in diabetic DSP group A as compared to group B (1328.3 ± 1274.21 vs. 673.8 ± 576.93, $p = 0.0014$). However, no significant difference in sPLA2 activity was found between group B and the control group. Importantly, there was no correlation identified between urine sPLA2 activity and the INCAT score.

To visually depict the heightened sPLA2 activity in individual patients, we established an increased sPLA2 activity threshold above the cut-off level of 1371.93 pool/min/ml. This threshold corresponds to the mean plus two standard deviations (Mean + 2SD) of sPLA2

Table 2
Linear regression analysis of amplitude-dependent variation in DL, CV, and F response.

	Change in DL	Change in CV	Change in F		Change in DL	Change in CV	Change in F
Group A							
Median nerve (n)	69	67	59	**Tibial nerve (n)**	48	48	39
Intercept	2.227	2.970	2.058	Intercept	90.655	6.768	2.049
Slope	−0.095	3.801	−0.001	Slope	−0.037	1.336	−0.002
r[a]	−0.249	0.439	−0.016	r[a]	−0.136	0.518	−0.097
P-value	<0.05	<0.01	NS	*P*-value	NS	<0.01	NS
Ulnar nerve (n)	67	67	62	**Fibular nerve (n)**	52	52	33
Intercept	3.232	6.302	−1.525	Intercept	3.166	2.672	2.297
Slope	−0.069	1.648	5.360	Slope	−0.101	0.195	−0.153
r[a]	−0.118	0.651	0.542	r[a]	−0.177	0.352	−0.467
P-value	NS	<0.01	<0.01	*P*-value	NS	<0.05	<0.01
Group B							
Median nerve (n)	99	99	92	**Tibial nerve (n)**	86	86	80
Intercept	2.552	2.891	2.100	Intercept	93.132	8.400	2.041
Slope	−0.256	0.129	−0.006	Slope	−0.078	0.899	−0.005
r[a]	−0.445	0.269	−0.305	r[a]	−0.443	0.398	−0.332
P-value	<0.01	<0.01	<0.01	*P*-value	<0.01	<0.01	<0.01
Ulnar nerve (n)	104	103	99	**Fibular nerve (n)**	89	89	61
Intercept	4.104	4.736	10.568	Intercept	3.163	3.001	2.038
Slope	−0.526	2.656	−0.379	Slope	−0.141	0.112	−0.042
r[a]	−0.507	0.507	−0.037	r[a]	−0.264	0.302	−0.122
P-value	<0.01	<0.01	NS	*P*-value	<0.05	<0.01	NS
ALS Group							
Median nerve (n)	122	121	62	**Tibial nerve (n)**	135	104	88
Intercept	2.175	2.925	2.102	Intercept	97.573	8.926	2.020
Slope	−0.133	0.141	−0.008	Slope	−0.071	0.767	−0.004
r[a]	−0.601	0.494	−0.357	r[a]	−0.352	0.369	−0.362
P-value	<0.01	<0.01	<0.01	*P*-value	<0.01	<0.01	<0.01
Ulnar nerve (n)	133	128	74	**Fibular nerve (n)**	119	112	59
Intercept	3.705	8.697	10.881	Intercept	3.342	3.104	2.038
Slope	−0.368	1.019	−0.529	Slope	−0.196	0.071	−0.043
r[a]	−0.709	0.550	−0.287	r[a]	−0.398	0.272	−0.235
P-value	<0.01	<0.01	<0.05	*P*-value	<0.01	<0.01	NS

CV, conduction velocity; DL, distal latency. n: number of motor nerves.
NS = not significant at $p < 0.05$.
[a] Pearson correlation coefficient.

activity in 46 controls.

The incidence of patients exhibiting elevated sPLA2 activity and having more than two motor nerves with CV slowing within the AAN or regression equations ranges was significantly higher in group A when compared to group B (35.1 % vs. 5.7 %, $p = 0.0005$). Furthermore, 13.5 % of patients in diabetic DSP group A met an additional criterion, demonstrating at least one motor nerve with CV slowing in the demyelinating range according to AAN or regression criteria, along with the corresponding F response falling within the demyelinating range by AAN criteria. Notably, no patient in diabetic DSP group B fulfilled this additional criterion.

4. Discussion

This study endeavored to enhance the sensitivity of conventional electrophysiologic criteria to distinguish primary demyelinating from primary axonal neuropathies by utilizing the CMAP as an independent variable and CV as a dependent variable. This relationship allows for the differentiation between CMAP amplitude-dependent conduction slowing, (i.e., slowing related to the loss of fast-conducting axons), and CMAP amplitude-independent conduction slowing. Previous studies have reported amplitude-independent CV slowing in diabetic DSP, implying a contribution of superimposed demyelination [7,8].

In our study, we initially attempted to plot the square root transformation of raw data for CMAP and conduction parameters, similar to previous studies [8,37,38]. However, a more optimal linear relationship between CMAP amplitude and CV was achieved through regression analysis using a combination of square root, fourth root, or log10 transformations. The conduction data utilized to formulate the regression equations were obtained from patients diagnosed with CIDP in our study. Establishing a regression analysis from CIDP patients, who manifest random areas of peripheral demyelination, has the advantage of detecting lesser degrees of demyelination that are often overlooked by conventional electrodiagnostic methods.

Our study uncovered a significantly higher percentage of patients with more than two motor nerves exhibiting motor CV slowing in the demyelinating range, as defined by either the AAN criteria or regression analysis criteria, in diabetic DSP group A as compared to DSP group B. In this group, each patient had at least one motor nerve with CV slowing according to the AAN criteria. This contrasted with diabetic DSP group B, wherein no patient demonstrated motor demyelinating range nerve CV slowing by AAN criteria. The overlap between the two groups was significantly reduced when an additional criterion was applied—the presence or absence of prolonged F response into the AAN demyelinating range. This finding implies more diffuse demyelination, particularly involving the

proximal part of the motor nerve. Moreover, the likelihood of observing more than two motor nerves with CV slowing in the demyelinating range was significantly higher in diabetic DSP group A as compared to diabetic DSP group B. These findings not only suggest that CV slowing extends beyond what would be expected exclusively from axonal loss but also indicate that the presence of one motor nerve with CV slowing in the demyelinating range by AAN criteria in diabetic DSP implies a generalized mild or moderate demyelinating process. A similar finding was reported by Herrmann et al., who observed significantly lower regression coefficients for the Y-intercept of CMAP amplitude as the independent variable and CV as the dependent variable in diabetic DSP group in all extremities compared to the ALS group, supporting the presence of a demyelinating component in conduction slowing within intermediate motor nerve segments [8].

Compared to diabetic DSP group B, patients in DSP group A exhibited higher INCAT scores and lower CMAP amplitudes in all motor nerves evaluated. These findings suggest a more severe diabetic neuropathy in group A. Similar results were reported by Dunnigan et al. when investigating the clinical and electrodiagnostic classification of nerve injury in 173 diabetic patients as part of an ongoing longitudinal cohort study [39].

In the multiple linear regression analysis of CMAP amplitude versus CV in diabetic DSP, CMAP-independent CV slowing was identified indicative of a myelinopathy. Although it reduced the overlap of electrodiagnostic data obtained from patients with diabetic DSP, CIDP, ALS, as well as the overlap between group A and B in our study, it did not exclusively distinguish primary mild demyelination from primary mild axonal loss [7–9].

Regarding the role of sPLA2s in diabetic neuropathy, our study revealed that the mean urine sPLA2 activity was increased significantly in diabetic patients as compared to healthy controls. Additionally, the mean level of urine sPLA2 was significantly higher in diabetic DSP group A as compared to group B.Moreover, 13.5 % of patients in diabetic DSP group A, and no patients in diabetic DSP group B, displayed increased sPLA2 activity along with more than two motor nerves exhibiting CV slowing and at least one corresponding F response in the demyelinating range.

In summary, our study illustrated that the combination of regression analysis of CV slowing in diabetic DSP and urine sPLA2 activity identified a subgroup of diabetic DSP with a significant contribution of acquired demyelination superimposed on the typical axonal loss in diabetic DSP. Any patient with greater than expected CV slowing and/or urine sPLA2 should be considered for immunomodulating therapy.

Urine sPLA2 acquisition is a feasible and non-invasive procedure. As an inflammatory marker, elevated sPLA2 may provide additional evidence of potentially reversible acquired demyelination. Although blood neurofilament levels are an known indicator of nerve injury, elevated levels indicate a greater degree of irreversible axons loss [40]. However, due to the limited number of patients' urines that were used for the assay of sPLA2, the conclusion concerning the ability of sPLA2 as a biomarker in diabetic neuropathy requires further investigation. Thus, at present, the confirmation of a direct correlation between sPLA2 activity and the nerve conduction slowing needs to be established. With the addition of more subjects, the specificity, sensitivity, and value-added benefit of using sPLA2 levels to detect acquired demyelination will be forthcoming.

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Data availability statement

The data associated with this study have not been deposited into any public repository. The data included supporting this article is available from the corresponding author upon reasonable and justified request.

CRediT authorship contribution statement

Nizar Souayah: Conceptualization. **Hongxin Chen:** Data curation. **Zhao Zhong Chong:** Writing – review & editing, Formal analysis. **Tejas Patel:** Formal analysis. **Ankit Pahwa:** Formal analysis. **Daniel L. Menkes:** Writing – review & editing, Investigation. **Timothy Cunningham:** Writing – review & editing, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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