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RESEARCH REPORT

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Clinical, histologic, and immunologic signatures of Small Fiber Neuropathy in Systemic Lupus Erythematosus

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Abstract

Background and Objectives: Systemic Lupus Erythematosus (SLE) often causes damage to small nerve fibers, leading to distressing painful and autonomic symptoms. Despite this, Small Fiber Neuropathy (SFN) remains an underrecognized complication for SLE patients. In this cross-sectional study, we aimed to assess SFN in patients with SLE and to explore its correlations with immunologic disease features and clinical manifestations.

Methods: We recruited 50 SLE patients (1 male to 12.5 females, aged 20–80 years) reporting painful disturbances. We conducted a comprehensive clinical and neurophysiological evaluation, using Nerve Conduction Studies and Quantitative Sensory Testing. Additionally, we carried out an extensive laboratory assessment of disease-related serological parameters. We also performed a thorough skin biopsy analysis, investigating somatic and autonomic innervation while detecting complement and inflammatory cell infiltrates within the skin.

Results: Out of 50 patients, 19 were diagnosed with SFN, primarily characterized by a non-length-dependent distribution; 7 had a mixed neuropathy, with both large and small fiber involvement. Patients with SFN were younger than patients with a mixed neuropathy ($p = .0143$); furthermore, they were more likely to have a history of hypocomplementemia ($p = .0058$) and to be treated with cyclosporine A ($p = .0053$) compared to patients without neuropathy. However, there were no significant differences in painful and autonomic symptoms between patients with and without SFN.

Discussion: This study highlights the relevant frequency of SFN with a non-length-dependent distribution among SLE patients experiencing painful symptoms.

Eleonora Galosi and Carmelo Pirone are contributed equally.

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Indeed, SFN emerges as an early manifestation of SLE-related neuropathy and is closely associated with hypocomplementemia, suggesting a potential pathogenic role of the complement system. Moreover, SFN may be influenced by disease-modifying therapies. However, the precise role of SFN in shaping painful and autonomic symptoms in patients with SLE remains to be fully elucidated.

KEYWORDS
cyclosporine A, hypocomplementemia, neuropathic pain, quantitative sensory testing, skin biopsy, Small Fiber Neuropathy, Systemic Lupus Erythematosus

1 | INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex autoimmune condition renowned for its wide-ranging neurological and psychiatric manifestations, collectively referred to as neuropsychiatric SLE.[1,2] Among the common neurological complications within neuropsychiatric SLE, peripheral neuropathy is supposed to be highly prevalent, manifesting in various phenotypes, such as chronic axonal sensory polyneuropathy, acute demyelinating polyneuropathy, mononeuritis multiplex, and cranial nerves neuropathy.[3,4]

Small Fiber Neuropathy (SFN), characterized by a selective damage of unmyelinated and thinly myelinated small nerve fibers, has been described in SLE patients by several studies, albeit with varying prevalence rates.[5–10] Patients with SLE commonly experience painful and autonomic disturbances, historically attributed to comorbid conditions such as arthritis, fibromyalgia, microcirculatory dysfunction, and mood disorders.[11,12] However, the role of SFN in these symptoms has only been partially explored, along with its associations with immunologic features, disease-related manifestations, and disease activity.[8,13]

Notably, SFN is not included in the American College of Rheumatology criteria for neuropsychiatric SLE case definitions,[14] which serve as a guideline for identifying neurological and psychiatric conditions related to SLE. Consequently, widely accepted diagnostic tools for SFN assessment, such as quantitative sensory testing and skin biopsy,[15] are infrequently used in the evaluation of SLE patients.

A deeper understanding of SFN diagnostic findings, underlying mechanisms, and clinical impact in SLE patients is crucial to facilitate clinicians in early detection, proper management, and effective treatment of this underrecognized SLE manifestation.

In this cross-sectional study, we aimed to assess the frequency of SFN in patients with SLE experiencing pain, and to evaluate how small fiber damage is associated with immunologic disease features and clinical symptoms. To achieve this, we enrolled patients with SLE who reported painful disturbances and conducted a comprehensive clinical, serologic, neurophysiological evaluation, using Nerve Conduction Studies and Quantitative Sensory Testing, and an extensive skin biopsy analysis, assessing both somatic and autonomic innervation and detecting complement and inflammatory cells infiltration within the skin.

2 | METHODS

2.1 | Study design and patient cohort

Between September 2020 and July 2022, we screened consecutive patients with SLE, diagnosed according to 2019 EULAR/ACR criteria.[16] The screening procedures took place at the Lupus Clinic of Sapienza University of Rome and consisted of a clinical interview focused on the presence of pain. Patients were asked to report the presence of pain symptoms, their onset, and their intensity on a 0–10 points Numeral Rating Scale (NRS).

Out of the initially screened patients with SLE, we therefore enrolled patients with SLE who reported painful symptoms with a NRS≥4 from at least 3 months, regardless of pain characteristics and distribution. These enrolled patients with SLE and painful symptoms underwent a comprehensive assessment, including clinical, neurophysiological, and skin biopsy evaluations, at the Peripheral Neuropathy and Neuropathic Pain Unit within the Department of Human Neuroscience at Sapienza University of Rome. They also underwent peripheral blood sample collection at the Lupus Clinic for determination of autoantibodies and the assessment of serum levels of C3 and C4.

Our exclusion criteria encompassed individuals below 18 years of age, those with cognitive disturbances, and those with major psychiatric disorders, which were assessed through clinical history and examination. In addition, we conducted a thorough clinical and laboratory assessment to rule out polyneuropathy etiologies other than SLE. This assessment included conditions such as diabetes, B12 deficiency, kidney failure, other autoimmune diseases, and a history of alcohol abuse.

SFN was diagnosed according to Besta criteria.[15] These criteria necessitate the presence of at least two abnormal findings out of three commonly used to assess small fiber damage: (i) at least two clinical signs of small fiber impairment (e.g., pinprick and thermal sensory loss, allodynia, hyperalgesia), (ii) abnormal warm and/or cold detection thresholds at the foot as assessed with Quantitative Sensory Testing (QST), and (iii) reduced intraepidermal nerve fiber density at the distal leg as assessed by skin biopsy.

We diagnosed a mixed fiber neuropathy, characterized by impairment in both large and small fibers, when patients displayed Nerve Conduction Studies abnormalities indicating involvement of large-

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myelinated fibers and met the Besta criteria for small fiber damage.[17,18] Neuropathic pain diagnosis was based on the widely agreed grading system.[19] The diagnosis of fibromyalgia was made according to ACR 2016 criteria.[20]

Each patient underwent a comprehensive clinical and diagnostic test investigation, which included clinical examination, questionnaires on small fiber-related symptoms and fibromyalgia symptoms, Nerve Conduction Study, Quantitative Sensory Testing, venous blood sampling, and skin biopsy at both distal and proximal sites. Data collection was performed using standardized protocols by designated staff members, each blinded to the results of the others.

We implemented rigorous measures to mitigate potential confounding factors. Our recruitment process exclusively targeted individuals diagnosed with SLE according to widely recognized criteria 16. The diagnosis of SFN relied on widely agreed recommendations.[15] Furthermore, we meticulously adhered to established protocols for NCS, QST, skin biopsies, and blood samples. This commitment to standardized methods aimed to ensure consistency in data collection and minimize variability associated with non-standardized sampling procedures.

2.2 | Clinical examination

We collected an extensive clinical history, encompassing details on demographic data, disease duration, disease-related manifestations with focus on neuropsychiatric events, comorbidities, and both prior and ongoing disease-modifying and analgesic treatments.

To assess disease activity, we used the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2k),[21] while chronic damage was evaluated using the Systemic Lupus International Collaborating Clinics (SLICC) damage index.[22]

A careful neurological examination was conducted, with particular attention to the assessment of sensory signs, utilizing bedside tools, in accordance with recommended practices.[23] We assessed patients for both negative symptoms and signs (e.g., tactile, vibration, pinprick, and thermal hypoesthesia) and positive symptoms and signs (such as spontaneous pain, allodynia, and pinprick hyperalgesia). During the clinical examination, patients were asked to mark the areas of pain distribution on a somatic map.[24] This graphical representation of pain allowed us to differentiate between patients with distally distributed pain and those with widespread pain.

Neuropathic pain diagnosis was based on the widely recognized grading system.[19] According to this widely agreed reference standard, neuropathic pain diagnosis is graded as "possible" in presence of a history of relevant neurological lesion or disease and if pain has an anatomically plausible distribution, "probable" if pain is associated with sensory signs in the same territory, "definite" if a diagnostic test confirms the neurological lesion explaining the pain.

Patients were administered the Neuropathic Pain Symptoms Inventory Questionnaire (NPSI) to dissect and quantify the different neuropathic pain qualities[25] and completed the Composite Autonomic Symptom Score (COMPASS-31) to assess autonomic symptoms.[26] To assess the presence of concomitant fibromyalgia syndrome according to the ACR 2016 criteria and determine the severity of its symptoms, we administered the Fibromyalgia Rapid Screening Tool (FIRST), Widespread Pain Index (WPI), and Severity Score (SS).[27,28]

The NPSI score served as the clinical outcome measure for neuropathic pain symptoms, while the COMPASS-31 score was used as the outcome measure for autonomic symptoms.

2.3 | Serological assessment of disease-related variables

Each participant underwent peripheral blood sample collection. Laboratory evaluation included the determination of autoantibodies and the assessment of serum levels of C3 and C4. Specifically, the following tests were conducted: (i) Antinuclear antibodies (ANA) were determined using indirect immunofluorescence (IIF) on HEp-2, with a titer of ≥1:160 or a rating of ++ or higher on a scale from + to ++++; (ii) anti-double-stranded DNA (anti-dsDNA) antibodies were assessed with IIF *Crithidia luciliae*, with a titer of ≥1:10; (iii) extractable nuclear antigen antibodies (ENA), which includes anti-Ro/SSA, anti-La/SSB, anti-Sm, and anti-RNP, were measured by ELISA assay with titers above the reference laboratory's cut-off; (iv) anti-cardiolipin (anti-CL) antibodies (IgG/IgM isotype) were tested by ELISA in serum or plasma, with medium or high titers (e.g., >40 GPL or MPL or above the 99th percentile); (v) anti-β2 glycoprotein-I (anti-β2GPI) antibodies (IgG/IgM isotype) were assessed by ELISA in serum (above the 99th percentile); (vi) lupus anticoagulant (LA) was determined following the guidelines of the International Society on Thrombosis and Hemostasis[29]; (vii) C3 and C4 serum concentrations measured by radial immunodiffusion.

2.4 | Nerve conduction study

Patients underwent a comprehensive Nerve Conduction Study, as previously described.[17] Our recording methods adhered to the recommendations of the International Federation of Clinical Neurophysiology.[30,31] Data were compared with age-adjusted normative ranges.[32]

The amplitude of the sural nerve sensory action potential (sural SNAP) was used as the primary outcome measure to define the presence of large-myelinated fiber involvement.

2.5 | Quantitative sensory testing

Quantitative Sensory Testing was performed on all patients by trained examiners, following the standardized protocol of the German Research Network on Neuropathic Pain.[33,34] We evaluated the dorsum of the right foot as the "test site," as this area is commonly the most painful in patients with neuropathies. *Z*-scores were calculated for each Quantitative Sensory Testing variable using log-transformed raw patient values and a widely accepted dataset of normative

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values,[33,35] with negative z-scores indicating a loss of perception and positive z-scores indicating a gain of perception. Abnormal values were defined as those exceeding ±1.96 standard deviations (SDs).

For assessing small thermo-nociceptive fibers, cold and warm detection thresholds were measured as major outcome parameters.

2.6 | Intraepidermal nerve fiber investigation

Patients underwent skin biopsies at two sites: the distal leg, 10 cm above the lateral malleolus, and the proximal thigh, 20 cm below the antero-superior iliac spine. These biopsies were performed using a 3-mm disposable circular punch after local lidocaine anesthesia under sterile conditions, without the need for sutures.[36] Following fixation for 24 h at 4°C in Zamboni's fixative, the samples were sectioned at 23°C with a cryostat (MEV, SLEE medical).

Intraepidermal nerve fiber density was assessed on 50-micron thick sections using indirect immunofluorescence, employing the pan-neuronal marker PGP9.5, as previously described.[37] Intraepidermal nerve fiber density was calculated by two blinded operators (EG and LT) according to the guidelines of the European Federation of Neurological Societies and Peripheral Nerve Society.[36] Normative values from a widely recognized dataset were employed.[38] The leg/thigh ratio of intraepidermal nerve fiber density was calculated using a reference value of 0.48 to discern whether axonal loss followed a distal gradient (values ≤0.48 consistent with distal axonopathy, and values >0.48 consistent with non-length-dependent peripheral nerve damage, i.e., ganglionopathy).[39]

Intraepidermal nerve fiber density served as the primary morphometric outcome variable for the assessment of small somatic thermo-nociceptive nerve fibers.

2.7 | Autonomic nerve fiber investigation

Autonomic innervation was evaluated on 50-micron thick skin sections through indirect immunofluorescence, utilizing the pan-neuronal marker PGP9.5. Piloerector muscle nerve fiber density was assessed for each patient across all the piloerector muscles available in the sections, as previously described,[37] and expressed as the number of fibers per millimeter. Sweat gland nerve fiber density was analyzed in a semiquantitative fashion for each patient based on all available sweat glands in the sections, as previously described.[37,40–42] Each patient received a score from 0 to 4, representing the average of densities from the three most innervated glands in their samples, with 0 indicating the absence of identifiable nerve fibers and 4 representing normal nerve fiber density. To ensure measurement consistency, two separate operators (EG and PF) performed the same quantification on patients. Piloerector muscle and sweat gland nerve fiber density values were compared with a dataset of normative values from 25 age- and sex-matched healthy subjects from our laboratory, serving as a control group.

Piloerector muscle and sweat gland nerve fiber density were considered as morphometric outcome measures for the assessment of autonomic small nerve fibers.

2.8 | C3 deposition and inflammatory cell infiltrates in the skin

To evaluate complement infiltration at the basal membrane in skin biopsy sections, direct immunofluorescence was used to visualize C3 fragment. 10-micron thick sections were incubated overnight with a mouse anti-human collagen IV monoclonal antibody (Millipore, 1:500). The following day, sections were incubated for 1 h with anti-mouse-488 secondary antibodies (Jakson, 1:300) and then with an anti-C3 direct polyclonal antibody (Dako, 1:20) for 1 h. Nuclei were contrasted with DAPI. C3 immunofluorescence was scored on a scale from 0 to 3+ by two blinded operators (ML and VdM).

The presence and distribution of inflammatory cell infiltrates in the cutaneous biopsies were investigated through immunohistochemical analysis, performed after the paraffin inclusion of the distal skin biopsy samples remaining from the previous analysis. Immunohistochemistry was conducted using an automated immuno-stainer (Leica-Bond Max, Leica Microsystems GmbH, Wetzlar, Germany) with the Bond Polymer Refine Detection kit (Leica-Bond Max, Leica Microsystems GmbH, Wetzlar, Germany), following the manufacturer's instructions. The sections were incubated with the following antibodies (ready to use): CD45 as a pan-leukocytic cellular marker (PA0042), CD3 as a marker of T-lymphocytes (LN10), and CD20 as a marker of B-lymphocytes (L26) (Dako, Glostrup, Denmark). Cellular infiltrates were examined in the dermis and were categorized as either perivascular or diffuse. Positive cells were counted by two pathologists (ML and VdM) in all section areas at ×40 magnification, under a light microscope (Zeiss). The total number of positive cells was calculated and reported as mean ± SD, median, and range, with a semiquantitative score (0–4) assigned to each patient, reflecting the density of cell infiltrate. In detail, the absence of skin cell infiltrate was rated as 0; 1 indicated the presence of a rare, 2 of a mild, 3 of a moderate, and 4 of a severe infiltrate.

The presence of C3 infiltrate and the semiquantitative density score of inflammatory cell infiltrates were considered as main skin biopsy outcome variables of immunologic activity.

2.9 | Standard protocol approvals, registrations, and patient consents

The study protocol was approved by the local ethics committee (0867/2020) of Policlinico Umberto I, Rome, Italy. It was carried out according to the principles of the 1964 Declaration of Helsinki. Each enrolled subject provided a written informed consent, and all study data were obtained and elaborated in accordance with our institutional ethical committee regulations on human experimentation.

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TABLE 1 Demographic and clinical variables in patients with SLE with MFN, SFN, and no neuropathy.

	SLE patients, $n = 50$	MFN, $n = 7$ (14%)	SFN, $n = 19$ (38%)	no-NP, $n = 24$ (48%)	p^*	p^{**}
Demographic variables						
Age, years, m (SD)	44.5 (17.6)	61.3 (12.9)	45.5 (13.6)	39.7 (18.8)	–	.0143
SLE duration, years, m (SD)	14.3 (10.1)	19.4 (14.1)	14.6 (9.1)	12.5 (9.3)	–	–
Female gender, *n* (%)	46 (92%)	5 (71%)	18 (95%)	23 (96%)	–	–
SLE clinical manifestations, *n* (%)						
Joint involvement	46 (92%)	7 (100%)	19 (100%)	21 (88%)	–	–
Skin involvement	36 (72%)	5 (71%)	14 (74%)	17 (71%)	–	–
Hematological	22 (44%)	0 (0%)	11 (58%)	12 (50%)	–	–
Kidney involvement	9 (18%)	2 (29%)	6 (32%)	2 (8%)	–	–
Serositis	8 (16%)	1 (14%)	4 (21%)	3 (12%)	–	–
SLE severity scores, m (SD)						
SLEDAI-2k	1.82 (3.45)	2.33 (5.71)	2.59 (3.86)	1.05 (2.06)	–	–
SLICC damage index	0.90 (1.34)	2.67 (2.50)	0.84 (1.01)	0.48 (0.73)	–	–
Previous SLE treatments, *n* (%)						
Glucocorticoids	50 (100%)	7 (100%)	19 (100%)	24 (100%)	–	–
Hydroxychloroquine	48 (96%)	6 (86%)	19 (100%)	22 (92%)	–	–
Cyclosporine A	11 (22%)	0 (0%)	9 (47%)	2 (8%)	.0053	–
Mycophenolate mofetil	13 (26%)	3 (43%)	7 (37%)	3 (12%)	–	–
Methotrexate	10 (20%)	0 (0%)	4 (21%)	6 (25%)	–	–
Azathioprine	10 (20%)	2 (29%)	4 (21%)	4 (17%)	–	–
Belimumab	7 (14%)	0 (0%)	4 (21%)	3 (12%)	–	–
Cyclophosphamide	7 (14%)	0 (0%)	5 (26%)	2 (8%)	–	–
Rituximab	2 (4%)	0 (0%)	0 (0%)	2 (8%)	–	–

Note: Continuous variables are expressed as mean (m) and standard deviation (SD); categorical variables are expressed as number of patients presenting the selected variable (*n*) and relative percentages (%). *T*-test or Mann-Whitney test were used to compare continuous variables, as appropriate depending on data normality distribution, and Fisher's exact test to compare categorical variables. *p* values <.05 are reported. p^*: comparisons between patients with SFN and without neuropathy. p^{**}: comparisons between patients with MFN and SFN.

Abbreviations: MFN, mixed fiber neuropathy; no-NP, patients without neuropathy; SFN, Small Fiber Neuropathy; SLE, Systemic Lupus Erythematosus; SLEDAI-2k, Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC damage index, Systemic Lupus International Coordinating Committee damage index.

2.10 | Statistical analysis

A preliminary univariate analysis was conducted to describe the key demographic, clinical, serological, and diagnostic test variables in patients with SLE. Continuous variables were presented as means ± SD, and categorical variables were expressed as percentage frequencies (Tables 1–3). Since the normal distribution was rejected for all continuous variables, as confirmed by the D'Agostino-Pearson omnibus normality test, non-parametric tests were used. Differences in the primary continuous outcome variables (clinical, serological, and diagnostic test variables) between groups were evaluated using the Mann-Whitney *U* test. Categorical variables were compared between groups using Fisher's exact test. No adjustment for multiple comparisons was applied since all comparisons were predetermined, and our primary aim was to test each variable independently.

Within the patients' group, a correlation matrix based on the Spearman test was used to initially assess the bivariate relationships between the principal non-Gaussian continuous clinical, diagnostic test, and laboratory outcome variables. Subsequently, simple linear regression was employed to evaluate linear relationships between selected correlated variables.

A multivariable logistic regression analysis, adjusted for main confounders, was performed with SFN as the dependent variable. The Hosmer-Lemeshow test was used to assess the goodness of fit of the logistic regression model.

The statistical analysis was conducted using Statistical Package for Social Sciences 25.0 (SPSS, Chicago, IL, USA) and GraphPad 8.0 (La Jolla, CA, USA). A *p*-value of <.05 was considered statistically significant.

3 | RESULTS

We consecutively screened for painful symptoms 235 patients with SLE. 102 patients were ruled out because they did not exhibit painful

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TABLE 2 Diagnostic test and serological variables in patients with SLE with MFN, SFN, and no neuropathy.

	SLE patients, $n = 50$	MFN, $n = 7$ (14%)	SFN, $n = 19$ (38%)	no-NP, $n = 24$ (48%)	p^*	p^{**}
Diagnostic test variables, mean (SD)						
Sural SAP (μV)	12.9 (6.4)	2.4 (1.7)	13.4 (4.8)	15.7 (5.0)	–	<.0001
Distal IENFD, fibers/mm	11.0 (5.0)	7.5 (4.7)	8.7 (3.2)	14.2 (4.7)	.0001	–
Proximal IENFD, fibers/mm	14.3 (4.9)	14.4 (5.9)	12.7 (3.9)	15.8 (5.3)	.0305	–
Distal PMNFD, fibers/mm	71.8 (27.4)	58.4 (16.2)	60.7 (29.6)	83.5 (22.2)	–	–
Proximal PMNFD, fibers/mm	80.1 (28.7)	64.0 (30.4)	87.7 (30.3)	80.5 (25.1)	–	–
Distal SGNFD, score (0–4)	2.8 (0.9)	2.3 (1.2)	2.8 (0.8)	2.9 (0.9)	–	–
Proximal SGNFD, score (0–4)	3.1 (0.7)	2.5 (0.9)	3.2 (0.8)	3.1 (0.7)	–	–
CDT (°C)	21.6 (7.9)	16.2 (9.6)	19.0 (8.1)	25.3 (5.6)	.0006	–
WDT (°C)	40.5 (4.9)	46.6 (3.5)	41.6 (4.1)	37.8 (4.1)	.0018	.0108
Serological variables, *n* (%)						
Anti-dsDNA	30 (60%)	3 (44%)	17 (67%)	13 (54%)	–	–
Hypocomplementemia	28 (56%)	4 (57%)	16 (84%)	10 (42%)	.0058	–
Anti-cardiolipin	17 (34%)	1 (14%)	7 (37%)	9 (37%)	–	–
Anti-SSA	14 (28%)	1 (14%)	7 (37%)	6 (25%)	–	–
Anti-phospholipids	10 (20%)	1 (14%)	5 (26%)	4 (21%)	–	–
Anti-B2 glycoprotein-I	8 (16%)	1 (14%)	3 (16%)	4 (21%)	–	–
Lupus anticoagulant	7 (14%)	0 (0%)	4 (17%)	2 (8%)	–	–
Anti-RNP	7 (14%)	0 (0%)	5 (26%)	2 (8%)	–	–
Anti-SSB	5 (10%)	2 (29%)	1 (5%)	2 (8%)	–	–
Anti-SM	5 (10%)	0 (0%)	2 (10%)	3 (12%)	–	–

Note: Continuous variables are expressed as mean (SD); categorical variables are expressed as number of patients/healthy subjects presenting the selected variable (*n*) and relative percentages (%). *T*-test or Mann-Whitney test were used to compare continuous variables, as appropriate depending on data normality distribution, and Fisher's exact test to compare categorical variables between patients with and without SFN. *p* values <.05 are reported. p^*: comparisons between patients with SFN and without neuropathy. p^{**}: comparisons between patients with MFN and SFN.
Abbreviations: CDT, cold detection threshold; Hypocomplementemia, reduction of C3 and/or C4 under the laboratory's reference values; IENFD, intraepidermal nerve fiber density of PGP9.5 immunoreactive fibers; MFN, mixed fiber neuropathy; no-NP, patients without neuropathy; PMNFD, piloerector muscle nerve fiber density of PGP9.5 immunoreactive fibers; SFN, Small Fiber Neuropathy; SGNFD, sweat gland nerve fiber density of PGP9.5 immunoreactive fibers; SLE, Systemic Lupus Erythematosus; Sural SAP, sural sensory action potential; WDT, warm detection threshold.

symptoms. Furthermore, 41 were excluded due to concomitant Sjogren's syndrome, eight for coexisting diabetes, six due to comorbid chronic kidney disease, and three for a B12 vitamin deficiency. Additional 25 patients with painful symptoms declined to participate in the study (Figure 1).

We therefore enrolled 50 patients with SLE and painful symptoms, which could manifest as either selectively distal or widespread in distribution. The gender distribution among the enrolled patients was 1 male to 12.5 females, and their ages ranged from 20 to 80 years.

Patients' demographic and clinical features are reported in Table 1. The main diagnostic test outcome measures, including Nerve Conduction Study, Quantitative Sensory Testing, skin biopsy variables, and serological parameters are shown in Table 2. Patients' painful and autonomic symptoms, as assessed by NPSI and COMPASS-31, are outlined in Table 3.

3.1 | Small Fiber Neuropathy findings and clinical symptoms

Out of the 50 enrolled SLE patients experiencing painful symptoms, 72% ($n = 36$) reported pain with a distal distribution. In particular, 32% ($n = 16$) exclusively experienced distal pain, 42% ($n = 21$) had widespread pain with additional distal involvement, whereas 26% ($n = 13$) complained of widespread pain without distal involvement (as shown in Figure 2); overall, out of 50 patients, 68% ($n = 34$) had widespread pain.

As shown in Table 3, 56% of patients ($n = 28$) reported neuropathic pain symptoms at the NPSI, with an average score of 22.17 (SD 11.26). The most commonly reported type of pain was pins and needles paresthesia (56%), followed by paroxysmal (46%) and burning pain (40%). No significant differences in the main demographic, clinical, diagnostic test, and serological variables were observed between

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TABLE 3 Pain related scores, neuropathic pain, and autonomic symptoms in patients with SLE with MFN, SFN, and no neuropathy.

	SLE patients, $n = 50$	MFN, $n = 7$ (14%)	SFN, $n = 19$ (38%)	no SFN, $n = 24$ (48%)	p^*	p^{**}
DN4 score (0–10), m (SD)	3.9 (2.2)	3.9 (2.8)	4.3 (2.0)	3.7 (2.2)	–	–
NPSI score (0–100), m (SD)	18.6 (11.4)	16.3 (14.5)	21.3 (12.0)	17.1 (9.9)	–	
SFN-SIQ (0–39), m (SD)	10.4 (4.9)	10.7 (6.2)	11.9 (5.2)	9.0 (4.0)	–	–
WPI (0–19), m (SD)	10.0 (4.5)	12.7 (3.9)	10.1 (3.7)	9.4 (5.1)	–	
SS (0–12), m (SD)	7.4 (2.5)	7.5 (1.9)	7.9 (2.4)	6.9 (2.6)	–	–
Definite neuropathic pain diagnosis (grading system)	17 (34%)	4 (57%)	13 (68%)	–		
Neuropathic pain symptoms at the NPSI, *n* (%)	28 (56%)	4 (57%)	13 (68%)	11 (46%)	–	–
Tingling paresthesia	34 (68%)	6 (86%)	14 (74%)	16 (67%)	–	–
Pins and needles paresthesia	28 (56%)	4 (57%)	11 (58%)	13 (54%)	–	–
Paroxysmal pain	23 (46%)	3 (43%)	10 (53%)	10 (42%)	–	–
Burning pain	20 (40%)	3 (43%)	8 (42%)	9 (37%)	–	–
Squeezing pain	18 (36%)	2 (29%)	8 (42%)	9 (37%)	–	–
Pressure pain	12 (24%)	2 (29%)	7 (37%)	5 (21%)	–	–
Stabbing pain	11 (22%)	2 (29%)	3 (16%)	6 (25%)	–	–
Dynamic mechanical allodynia	8 (16%)	0 (0%)	5 (26%)	3 (12%)	–	–
Pressure allodynia	3 (6%)	0 (0%)	2 (10%)	1 (4%)	–	–
Cold allodynia	3 (6%)	0 (0%)	1 (5%)	2 (8%)	–	–
Autonomic symptoms, *n* (%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	–	–
Orthostatic intolerance	35 (70%)	6 (86%)	14 (74%)	15 (62%)	–	–
Palpitations	32 (64%)	4 (57%)	13 (68%)	18 (75%)	–	–
Dry eyes	32 (64%)	5 (71%)	10 (53%)	17 (71%)	–	–
Dry mouth	29 (58%)	5 (71%)	9 (47%)	14 (58%)	–	–
Flushing	28 (56%)	4 (57%)	11 (58%)	14 (58%)	–	–
Urinary disfunction	27 (54%)	5 (71%)	10 (53%)	12 (50%)	–	–
Sudomotor disfunction	23 (46%)	3 (43%)	11 (58%)	11 (46%)	–	–
Stypsis	20 (40%)	2 (29%)	7 (37%)	11 (46%)	–	–
Diarrhea	15 (30%)	2 (29%)	6 (32%)	7 (29%)	–	–

Note: Continuous variables are expressed as mean (m) and standard deviation (SD); categorical variables are expressed as number of patients presenting the selected variable (*n*) and relative percentages (%). T-test or Mann-Whitney test were used to compare continuous variables, as appropriate depending on data normality distribution, and Fisher's exact test to compare categorical variables between patients with and without SFN. *p* values <.05 are reported. p^*: comparisons between patients with SFN and without neuropathy. p^{**}: comparisons between patients with MFN and SFN.

Abbreviations: DN4, Douleur Neuropatique en 4 questions; MFN, mixed fiber neuropathy; NPSI, Neuropathic Pain Symptoms Inventory score; SFN, Small Fiber Neuropathy; SFN-SIQ, Small Fiber Neuropathy Symptoms Inventory Questionnaire; SLE, Systemic Lupus Erythematosus; SS, Symptom Severity Scale; WPI, Widespread Pain Index.

patients with and without neuropathic pain symptoms at the NPSI. According to the grading system for neuropathic pain diagnosis, 17 patients (34%) finally received a diagnosis of definite neuropathic pain due to the presence of diagnostic tests abnormalities. Of these patients, 13 had a SFN and 4 a mixed fiber neuropathy.

All patients complained of at least one autonomic symptom as assessed by COMPASS-31, with orthostatic intolerance (70%) being the most frequently reported (Table 3).

Indeed, 26 patients (52%) had a peripheral neuropathy; among these, 19 received a diagnosis of SFN, while seven patients exhibited both Nerve Conduction Study and skin biopsy abnormalities, indicative of a mixed fiber neuropathy involving both large and small nerve fibers. Notably, none of the patients demonstrated isolated Nerve Conduction Study abnormalities without concurrent signs of small fiber involvement, suggesting the absence of pure large fiber neuropathy cases.

Patients with SFN showed a significant reduction of intraepidermal, piloerector muscle, and sweat gland nerve fiber density both at distal and proximal sites compared with healthy controls (distal intraepidermal nerve fiber density: $p = .0054$; proximal intraepidermal nerve fiber density: $p = .0406$; distal piloerector muscle nerve fiber density: $p = .0044$; proximal piloerector muscle nerve fiber density: $p = .0364$; distal sweat gland nerve fiber density: $p < .0001$; proximal sweat gland nerve fiber density: $p = .0052$).

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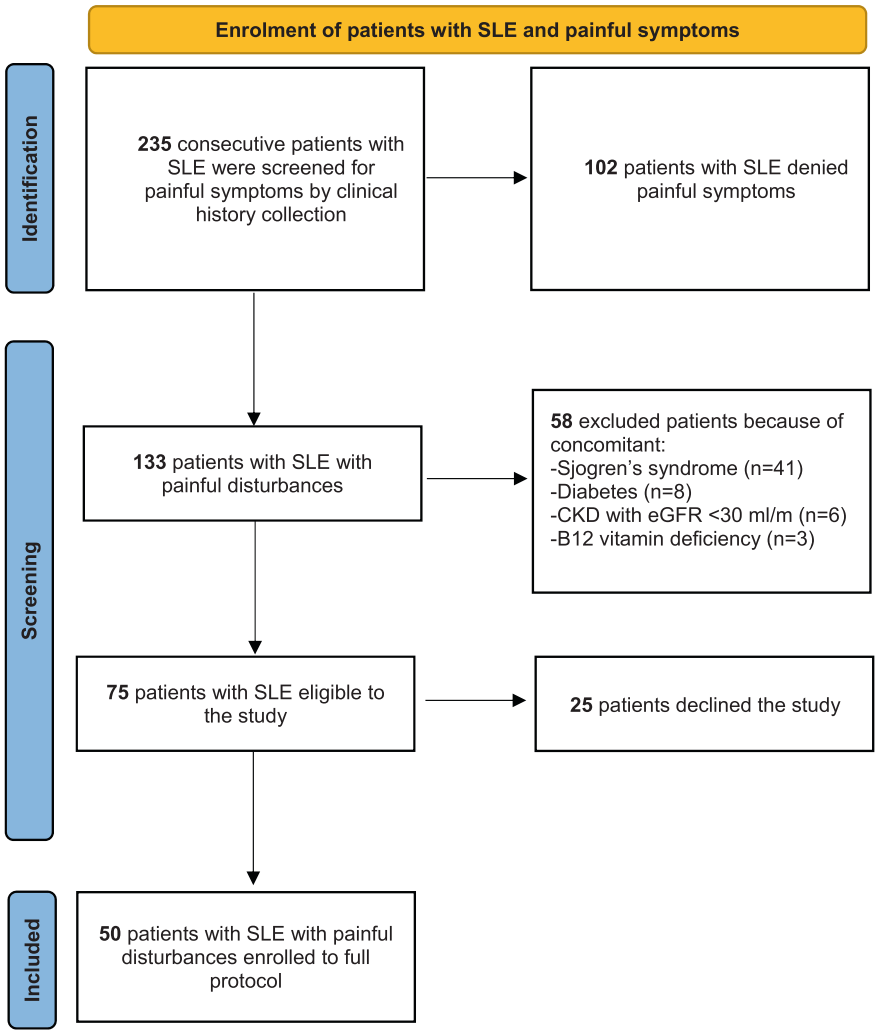

FIGURE 1 Flow chart of screening and enrolment of patients with Systemic Lupus Erythematosus. We screened for painful symptoms 235 patients with SLE by clinical history collection. We excluded 102 because they did not complain painful symptoms. Furthermore, 41 were excluded due to concomitant Sjogren's syndrome, 8 for coexisting diabetes, 6 due to comorbid chronic kidney disease, and 3 for a B12 vitamin deficiency. Additional 25 patients with painful symptoms declined to participate in the study. We therefore enrolled to the full study protocol 50 patients who reported painful symptoms.

Out of 19 patients with SFN, 89% ($n = 17$) showed a leg/thigh ratio >0.48, compatible with a non-length-dependent ganglionopathy-like fibers reduction pattern at skin biopsy (Figure 3). Furthermore, six out of seven patients with mixed fiber neuropathy showed the same non-length-dependent pattern.

No differences were found in painful and autonomic clinical symptoms, as assessed by NPSI and COMPASS-31, or in demographic clinical variables, between patients with and without SFN (Table 1 and 3).

Comparatively, patients with SFN were younger than those with mixed fiber neuropathy ($p = .0143$, SFN = 45.5 ± 13.6 years vs mixed = 61.3 ± 12.9 years) and displayed lower warm detection thresholds ($p = .0108$, SFN = 41.6 ± 4.1°C vs Mixed = 46.6 ± 3.5°C).

Of the 50 SLE patients with painful symptoms, 74% ($n = 37$) met the ACR 2016 criteria for fibromyalgia. Among these 37 patients with fibromyalgia, 46% ($n = 17$) had no neuropathy, 43% ($n = 16$) were diagnosed with SFN, and 11% ($n = 4$) had a mixed neuropathy. Ten fibromyalgia patients without a neuropathy diagnosis (27% of fibromyalgia patients) had isolated small fiber abnormalities at skin biopsy.

3.2 | Correlations between Small Fiber Neuropathy and immunologic variables

SFN patients showed a higher frequency of hypocomplementemia, as assessed by C3 and/or C4 reduction, in their disease history ($n = 16/19$, 84%) when compared to patients without neuropathy ($n = 10/24$, 42%) ($p = .0058$). Furthermore, the presence of low serological complement levels was associated with a decrease in intraepidermal nerve fiber density at the proximal site ($p = .006$; $r = 0.404$).

We observed the presence of C3 complement fragments at the level of skin basal membrane in 20 SLE patients (43%). In detail, 15 exhibited mild degrees of C3 infiltration, 4 had a moderate degree, and 1 patient showed severe infiltration (Figure 4).

Skin cell infiltrate analysis was conducted in 20 patients, who still had sufficient skin sections following previous analyses. Perivascular leukocyte infiltrates were observed in 75% of the examined patients ($n = 15$), in the absence of macroscopic signs of skin inflammation (Figure 4). The infiltrate severity was rated as mild in 9 patients, moderate in 1, and severe in 5. CD45-positive leukocytes were identified as CD3+ and CD20-negative, indicating T-lymphocytes.

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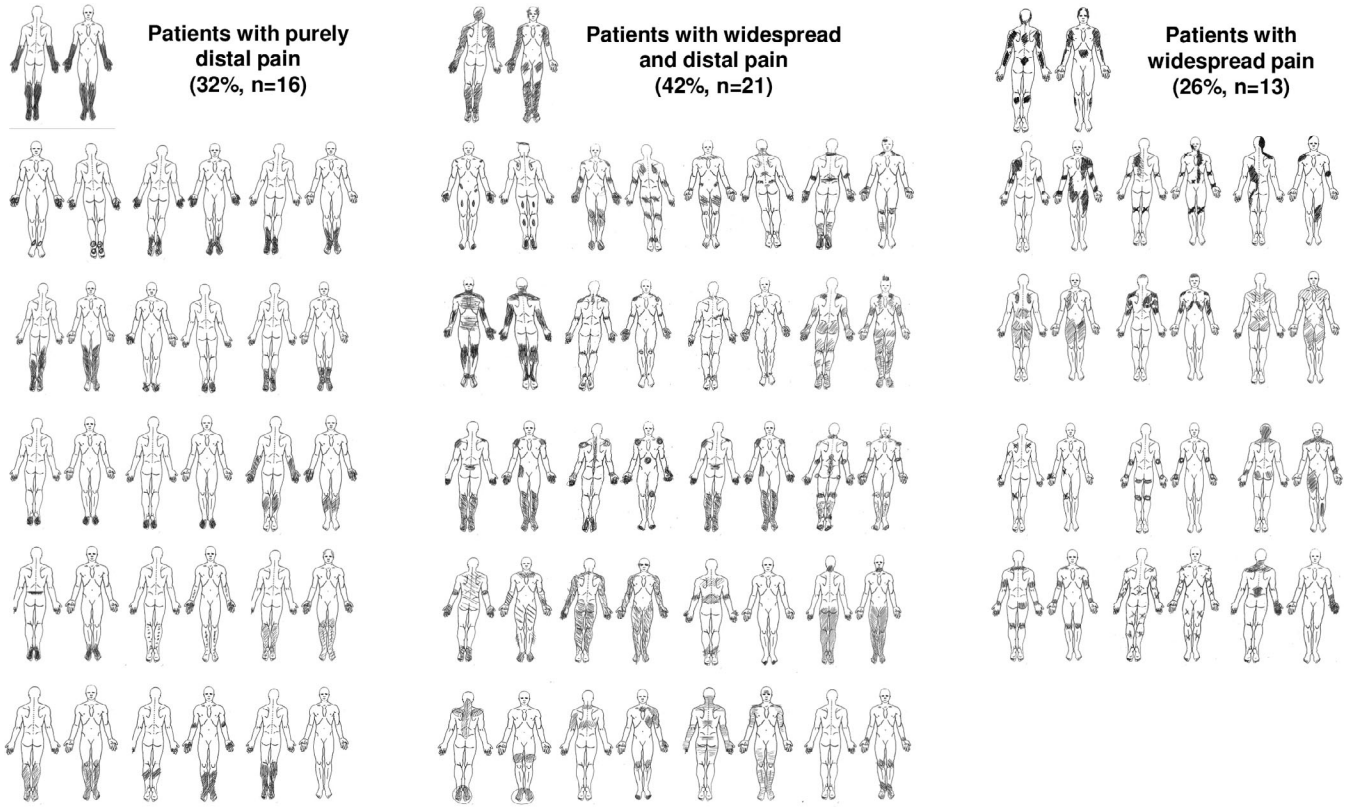

FIGURE 2 Pain distribution as drawn by patients on somatotopic maps. Patients were asked to draw their pain distribution on a somatotopic map. 32% of them ($n = 16$) referred an exclusively distally distributed pain, 42% ($n = 21$) reported distal and widespread pain, 26% ($n = 13$) had widespread pain without distal involvement.

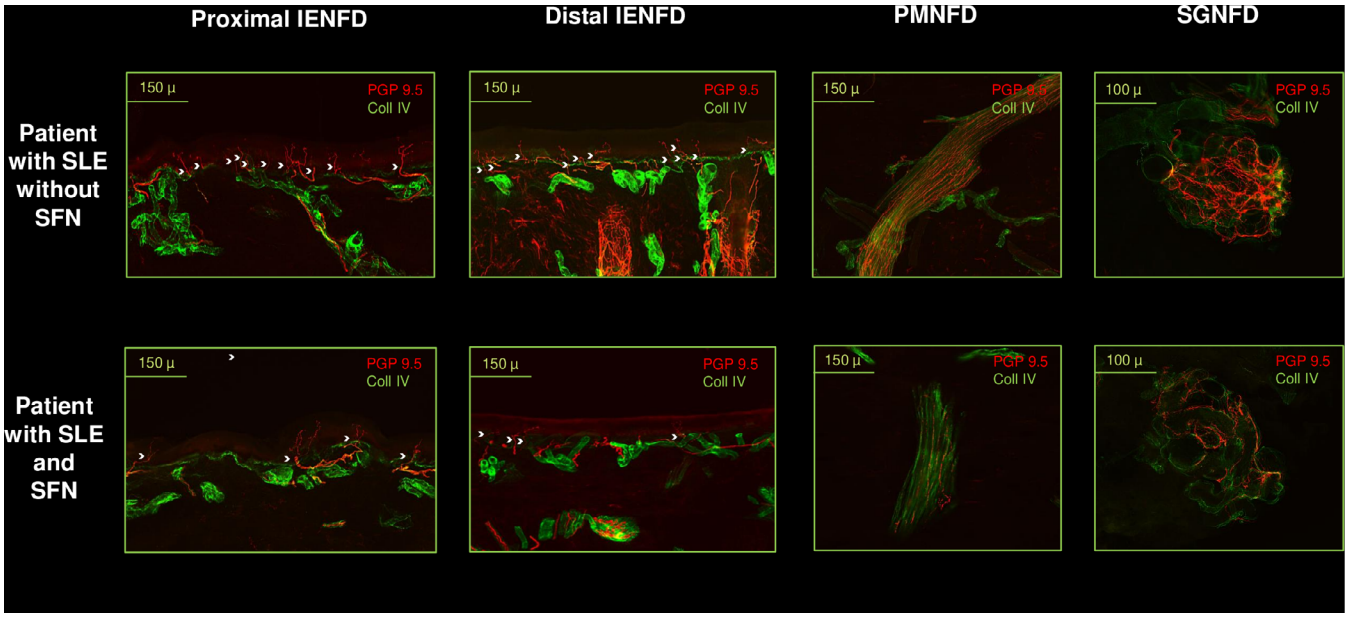

FIGURE 3 Skin biopsy innervation findings. Representative pictures from patients with SLE with and without Small Fiber Neuropathy (SFN), showing intraepidermal nerve fiber density (IENFD) from a proximal and distal site, and autonomic structures for piloerector muscle (PMNFD) and sweat gland (SGNFD) nerve fiber density calculation. Nerve fibers are marked in red by PGP9.5 immunostaining, whereas green staining corresponds to collagen IV. Both proximal and distal IENFD, PMNFD, and SGNFD were reduced in patients with SLE and SFN.

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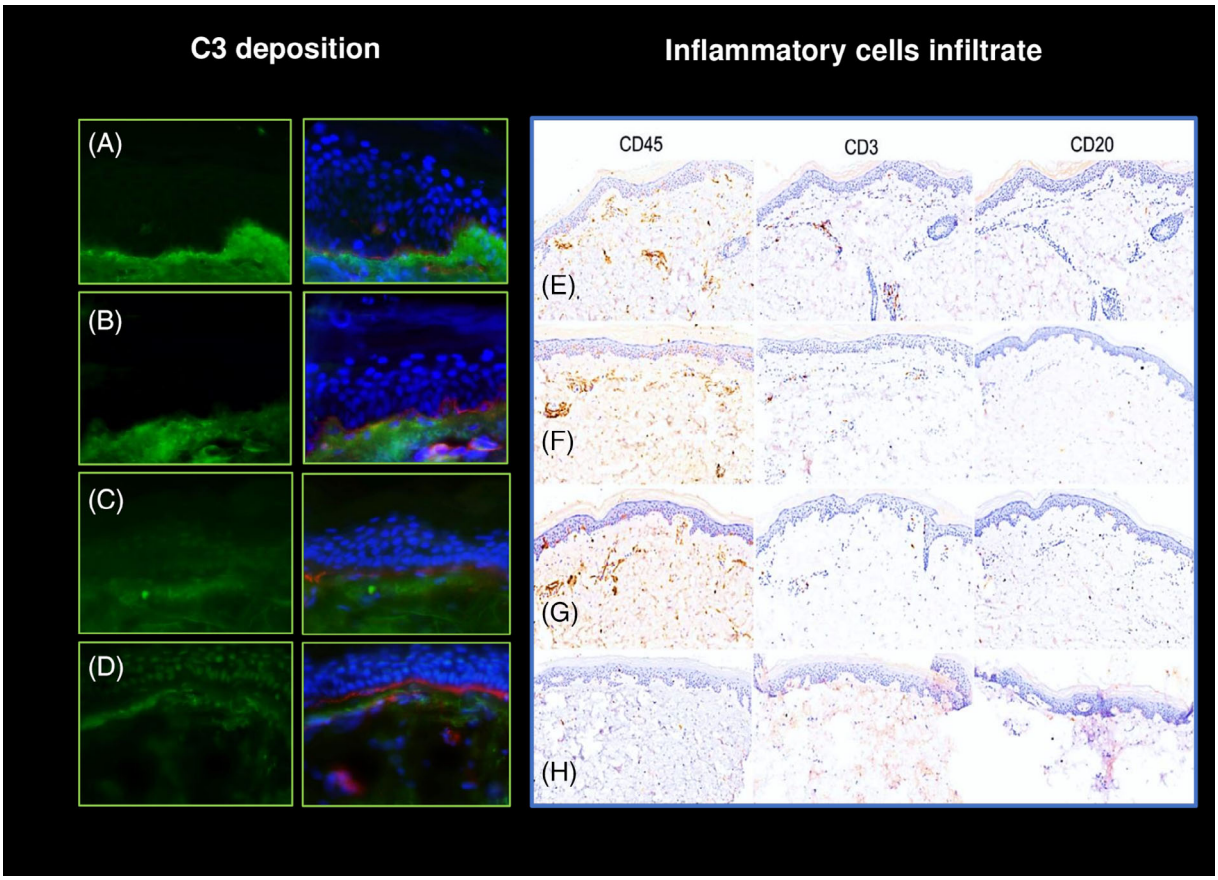

FIGURE 4 C3 deposition and inflammatory cells infiltrates in patients' skin. Exemplificative images of direct immunofluorescence from a patient with C3 deposition at the level of the skin basal membrane (A,B) and a negative control (C,D). C3 infiltrate is marked in green, basal membrane in red, epidermis cellular nuclei in blue (DAPI). Immunohistochemistry sections from patients with positive inflammatory cells infiltrates in the dermis (E–G) and a negative control (H). Inflammatory cells were marked through CD45, CD3, and CD20 and resulted positive to CD45 and CD3, consistently with T-lymphocytes. Perivascular leukocytes infiltrate was found in 75% of the examined patients ($n = 15$), in absence of macroscopic signs of skin inflammation. Nor C3 deposition or inflammatory cells infiltrate was associated with SFN in our study.

Notably, the frequency of C3 deposition and the presence of inflammatory cell infiltrates in the skin did not significantly differ between patients with ($n = 9/19$, 47%) and without SFN ($n = 10/24$, 42%).

Furthermore, we observed a higher prevalence of treatment with Cyclosporine A in the clinical history of patients with SFN ($n = 9/19$, 47%) when compared to patients without neuropathy ($n = 2/24$, 8%) ($p = .0053$).

To account for potential confounders, a multivariable logistic regression analysis was conducted, considering SFN as the dependent variable. The analysis was adjusted for key factors, including age, gender, disease activity, chronic damage, and major comorbidities (such as fibromyalgia, rheumatoid arthritis, and Hashimoto thyroiditis). Hypocomplementemia and prior treatment with Cyclosporine A were identified as independent risk factors for SFN (OR 7.5 with a 95% confidence interval of 1.7–33.6; OR 12.5 with a 95% confidence interval of 2.2–69.9, respectively). The Hosmer-Lemeshow's test yielded p values <.05, confirming the statistical model's validity.

4 | DISCUSSION

In our cross-sectional study, we found that a significant proportion of patients with SLE and painful disturbances, approximately 50%, had a peripheral neuropathy; among these patients, the majority (38%) presented with SFN, selectively involving small nerve fibers, whereas 14% exhibited a mixed neuropathy, with signs of both large and small fiber damage.

Notably, hypocomplementemia and previous treatment with Cyclosporine A were identified as independent risk factors for SFN in our patient cohort.

4.1 | Small Fiber Neuropathy findings

SFN has been previously reported in SLE, with varying prevalence rates, largely influenced by different study designs and diagnostic criteria.[5–10] In the largest-ever studied cohort including approximately 2000 patients with SLE, retrospectively enrolled, SFN was identified

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in 17% of cases.[4] Our cross-sectional study, the first one relying on widely recognized diagnostic criteria for SFN in patients with SLE, shows that this condition is highly prevalent in SLE patients experiencing painful symptoms. Our diagnostic approach was based on clinical assessment, Quantitative Sensory Testing, and skin biopsy findings, which are among the most widely agreed-upon tests for assessing small fiber damage. Our data strongly suggest the importance of actively investigating small fiber damage in SLE patients through dedicated small fiber testing.

Remarkably, in our study, 89% of SLE patients with SFN exhibited a leg/thigh ratio of IENFD >0.48, showing a non-length-dependent intraepidermal nerve fibers reduction pattern at skin biopsy. Previous studies have shown that values of leg/thigh ratio higher than 0.48 are significantly associated with ganglionopathies, that is, non-length-dependent neuropathies due to a pathologic involvement of dorsal root ganglia.[39] A non-length-dependent distribution for peripheral damage is usually considered a red flag for autoimmune diseases, paraneoplastic conditions, and other rare disorders.[43] Sjogren syndrome's associated neuropathy has traditionally been described as non-length dependent,[44] whereas insufficient evidence has been gathered regarding small fiber damage distribution in other autoimmune conditions. Our study showed that a striking majority of patients with SLE-associated neuropathy had a non-length-dependent reduction of intraepidermal nerve fiber density, with far greater frequency than it has been reported for Sjogren's syndrome,[45,46] thus suggesting that a ganglionic damage might underlie small fiber impairment in SLE patients. Consistently, piloerector muscle and sweat gland nerve fibers density, the two main autonomic fibers related outcome measures, were reduced in SLE patients with SFN both at distal and proximal skin biopsy sites compared with healthy subjects. These findings suggest that also dermal autonomic fibers could be involved in a non-length-dependent fashion in SLE-associated SFN, raising the possibility that autonomic ganglia might be parallelly involved.

In our study, patients with SFN showed a less severe impairment of warm detection threshold, one of the main small fiber-related functional variables, compared to patients with mixed neuropathy. Furthermore, patients with SFN were younger respect to those with mixed neuropathy. These findings suggest that SFN, characterized by a selective small fiber involvement, could be an earlier and milder manifestation of SLE-associated neuropathy in comparison with a mixed neuropathy, with both large and small fiber damage.

The ACR neuropsychiatric SLE case definitions, which serve as primary tool for recognizing neurological and psychiatric comorbidities associated with SLE, currently include peripheral neuropathies diagnosed by conventional methods such as Nerve Conduction Study 1. However, SFN is not yet included into neuropsychiatric SLE case definitions, as its diagnosis requires specific small fiber testing. Our data suggest that SFN might represent an early manifestation of a SLE-related neuropathy. This finding therefore underscores the importance of long-term follow-up for patients with SLE with SFN, due to their heightened risk of developing a mixed neuropathy, which is recognized as a major SLE complication and may necessitate a more aggressive therapeutic approach.

4.2 | Correlations between Small Fiber Neuropathy and immunologic variables

In our study, we observed a significant association between SFN and hypocomplementemia. SLE is a complex autoimmune disease characterized by complement activation, which can result in tissue injury. Reduced complement serum levels are considered a diagnostic marker at the disease's onset and a biomarker for disease activity; finally, hypocomplementemia is strongly linked to severe SLE manifestations, such as glomerulonephritis.[47]

The identified connection between the presence of SFN and hypocomplementemia suggests as complement-mediated damage may contribute to small fiber damage, potentially playing a role in the pathogenesis of SLE-related neuropathy. Additionally, this association indicates that SFN is more likely to occur in patients with a more active disease.

The connection between SFN and hypocomplementemia could have important therapeutic implications. It can be hypothesized that treatments capable of normalizing serum complement levels, like the biologic drugs belimumab and anifrolumab, might play a role in preventing or treating this specific disease manifestation.[48,49] However, further studies are needed to test the potential effectiveness of biologic drugs in SLE patients with SFN.

In our study, we did not find any correlation between small fiber damage and complement C3 fragment deposition at the dermo-epidermal junction. This finding may align with our observation of non-length-dependent small fiber loss in patients with SLE, suggesting that small fiber involvement and immunologic damage may occur at more proximal nerve regions, such as the dorsal root ganglia. It is worth noting that the absence of correlation we observed between small fiber damage and complement C3 fragment deposition contradicts previous research demonstrating complement deposits in various tissues in SLE patients, included the basal membrane of clinically normal skin.[50,51] Accordingly, we cannot exclude that the lack of correlation in our study might be attributed to the limited availability of biomarkers of complement activation in peripheral tissues like skin; notably, we have only analyzed C3 fragments.[52] Additionally, as previously reported,[10] we identified a perivascular infiltrate of T-lymphocytes in the skin of most of our patients (75%). However, we did not find any significant difference in the degree of inflammatory cell infiltration between patients with and without SFN.

Interestingly, we found that treatment with cyclosporine A was independently associated with the presence of SFN. This finding suggests that multiple factors, including disease-modifying therapy, might contribute to the onset of SFN in SLE patients. Neurotoxicity is a well-known side effect of calcineurin inhibitors; peripheral neuropathies have been reported in patients treated by cyclosporine A, although not frequently.[53,54] To the best of our knowledge, this is the first study to suggest a possible role of cyclosporine A in the development of SFN. Further studies specifically aimed at evaluating the neurotoxicity of cyclosporine A on small nerve fibers are needed to confirm this finding, especially in patients with pathological conditions not typically associated with SFN.

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4.3 | Correlations between Small Fiber Neuropathy and clinical symptoms

Among our consecutively enrolled patients with SLE and painful symptoms, only 32% had pain with a purely distal distribution to the lower limbs, which is traditionally considered the more common presentation of SFN, whereas 68% of patients had widespread pain. Pain distribution did not significantly differ between patients with and without SFN. Noticeably, more than 70% of SFN patients had widespread pain, a distribution possibly consistent with non-length-dependent small fiber damage.

In our cohort of patients, painful symptoms as assessed by the NPSI were unrelated to small fiber-related variables, as evaluated by skin biopsy and QST. This finding is in line with previous studies[52] and supports the view that neuropathic pain does not merely reflect axonal loss and that it may be associated with functional and ultrastructural changes, which conventional small fiber testing might not detect.

We did not find significant differences in the main demographic, clinical, and serological features between patients with and without SFN. It follows that the clinical impact of small fiber damage on painful symptoms often complained by patients with SLE remains uncertain, possibly due to coexisting disease complications contributing to painful disturbances. For instance, as shown in Table 2, 46 patients (92%) had joint involvement and suffered from arthralgias. Furthermore, more than 70% of our patients fulfilled the ACR 2016 criteria for fibromyalgia, thus suggesting that the presence of painful symptoms is frequently associated with fibromyalgia in SLE patients.

It is well-established that a considerable proportion of patients with fibromyalgia, often up to 50%, have isolated skin biopsy abnormalities commonly defined as "small fiber pathology." These abnormalities are of uncertain clinical significance and occur in absence of a clear evidence of functional small fiber impairment.[24,55] In our patients with associated fibromyalgia syndrome, 27% showed isolated skin biopsy abnormalities, fitting the characterization of small fiber pathology. By contrast, about 40% of patients with fibromyalgia met the Besta criteria for SFN. These findings indicate that a significant proportion of patients with SLE-related SFN also have comorbid fibromyalgia. As a result, the interaction and mutual contribution of SFN and fibromyalgia to painful symptoms in SLE patients become complex and challenging to decipher.

Among our 50 patients with SLE, 12% did not meet the criteria for SFN or fibromyalgia syndrome. This observation suggests that other independent factors may contribute to their painful symptoms.

All our patients complained of at least one autonomic symptom. Indeed, these symptoms are usually reported in patients with SFN, due to small autonomic fiber disfunction, and their presence has also been reported in fibromyalgia, with unclear relationship with small fiber pathology.[55,56] However, the COMPASS-31 score, the main outcome variable related to autonomic symptoms, did not differ between patients with and without SFN, thus showing that also the impact of SFN on autonomic symptoms remains elusive.

5 | LIMITATIONS

In our study, we selected patients with SLE based on their reports of painful symptoms, which may have led to an increased frequency of SFN in our cohort. Consequently, the generalizability of our findings concerning SFN frequency may be limited by referral bias.

The evaluation of autonomic small nerve fibers suffered from some limitations. We did not perform any autonomic function testing to assess small autonomic fibers,[57] and the use of morphometric skin biopsy parameters like piloerector muscle and sweat gland nerve fiber density as main outcome variables for autonomic small fiber assessment could be considered a potential limitation of our study. Indeed, whereas intraepidermal nerve fiber density is a widely accepted reference standard measure for SFN diagnosis, no standardized consensus exists regarding the quantitative assessment of autonomic fibers.[58] To enhance our data consistency, we evaluated piloerector muscle innervation through a quantitative procedure,[59] with high inter-operator agreement. We performed a semiquantitative analysis of sweat glands innervation and verified its consistency between two distinct operators.[42,60] However, although widely used due to its feasibility, this semiquantitative approach may have poor inter- and intra-reviewer reliability.[41]

6 | CONCLUSIONS

Our study shows that SFN with a non-length-dependent distribution is a frequent finding in patients with SLE experiencing painful symptoms and is an early manifestation of SLE-related neuropathy, thus suggesting that small fiber testing should be implemented in patients' evaluation and follow-up. Furthermore, SFN is associated with hypocomplementemia, reflecting disease immunologic activity, and may also be related to cyclosporine A treatment. However, its role in conditioning painful and autonomic symptoms in SLE patients remains uncertain.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest or financial interest related to the findings of this study.

DATA AVAILABILITY STATEMENT

Original supporting data are electronically stored at our Institution and are available on request. Please write an e-mail to the corresponding author (EG) to have full access to the original data.

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