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Subclinical Corneal Nerve Fiber Damage and Immune Cell Activation in Systemic Lupus Erythematosus: A Corneal Confocal Microscopy Study

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Purpose: The purpose of this study was to evaluate the utility of corneal confocal microscopy (CCM) in identifying small nerve fiber damage and immune cell activation in patients with systemic lupus erythematosus (SLE).

Methods: This cross-sectional comparative study included 39 consecutive patients with SLE and 30 healthy control participants. Central corneal sensitivity was assessed using a Cochet-Bonnet contact corneal esthesiometer and a laser scanning CCM (Heidelberg, Germany) was used to quantify corneal nerve fiber density (CNFD), nerve branch density (CNBD), nerve fiber length (CNFL), and Langerhans cell (LC) density.

Results: Age was comparable among patients with SLE (33.7 ± 12.7) and controls (35.0 ± 13.7 years, $P = 0.670$) and the median duration of disease was 3.0 years (2.0–10.0 years). CNBD ($P = 0.003$) and CNFL ($P = 0.019$) were lower and mature LC density ($P = 0.002$) was higher, but corneal sensitivity ($P = 0.178$) and CNFD ($P = 0.198$) were comparable in patients with SLE compared with controls. The SELENA-SLEDAI score correlated with CNFD ($\rho = -0.319$, $P = 0.048$) and CNFL ($\rho = -0.373$, $P = 0.019$), and the total and immature LC densities correlated with CNBD ($\rho = -0.319$. $P = 0.048$, and $\rho = -0.328$, $P = 0.041$, respectively). Immature LC density was higher ($P = 0.025$), but corneal sensitivity and nerve fiber parameters were comparable between patients with (33%) and without neuropsychiatric symptoms and SLE.

Conclusions: Corneal confocal microscopy identifies distal corneal nerve fiber loss and increased immune cell density in patients with SLE and corneal nerve loss was associated with disease activity.

Translational Relevance: Corneal confocal microscopy may enable the detection of subclinical corneal nerve loss and immune cell activation in SLE.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune connective tissue disease which affects multiple organ systems with a reported incidence varying between 0.3 and 23 per 100,000 person-years.[1] SLE is diagnosed by the presence of 4 out of 17 clinical and immunologic criteria based on involvement of the skin, blood, kidneys, joints, and nervous system. Although lupus nephritis is an established prognostic marker for increased mortality,[2] central and peripheral nervous system involvement is also associated with poorer outcomes and higher mortality.[3,4] Indeed, the American College of Rheumatology (ACR) has designated peripheral

and central nervous system involvement as neuropsychiatric systemic lupus erythematosus (NP-SLE).[5]

Overt peripheral neuropathy in patients with SLE has been associated with a higher disease activity score, increased frequency of fever, mucocutaneous involvement, and arthritis and immunologic abnormalities.[6] There is a growing body of evidence demonstrating a significant subclinical small fiber neuropathy in patients with SLE.[7–12] Small fiber neuropathy is typically diagnosed from neuropathic symptoms and signs alongside an objective measure of small fiber damage by evaluating warm or cold sensory thresholds or intraepidermal nerve fiber density (IENFD).[13] However, sensory threshold testing can be subjective and variable and skin biopsy is invasive, precluding their use for the diagnosis of small fiber neuropathy in SLE.

We have pioneered the rapid noninvasive technique of corneal confocal microscopy (CCM) to demonstrate corneal nerve fiber loss in a number of central[14–18] and peripheral[19–22] neurodegenerative diseases and increased Langerhans cells (LCs) in inflammatory and immune-mediated neuropathies[23–27] and long-coronavirus disease (COVID).[28] Moreover, we have shown that CCM has equivalent diagnostic utility to quantitative sensory testing and enhances the diagnosis of small fiber neuropathy.[29]

The aim of the present study was to assess whether CCM could identify subclinical small nerve fiber damage and immune activation in relation to disease activity and the presence of NP-SLE and lupus nephritis.

Methods

Thirty-nine patients with SLE and 30 healthy control participants were included in this cross-sectional comparative study in a tertiary referral university hospital. The study was reviewed and approved by the institutional research ethics committee and the principles of the Declaration of Helsinki were followed. Written informed consent was obtained from all participants.

Patients with SLE were recruited from the rheumatology department based on the revised ACR diagnostic criteria for SLE.[30] All consecutive patients who did not meet the exclusion criteria and were eligible for CCM examination were enrolled in the study. Exclusion criteria were a history of ocular surgery or trauma, any corneal pathology, contact lens use, diabetes mellitus, or a systemic disease that might cause neuropathy. Patients with a Schirmer's test (without topical anesthesia) score of $\leq$5 mm in 5 minutes were also excluded.

Disease activity was scored by an experienced rheumatology specialist using the Safety of Estrogens in Lupus Erythematosus National Assessment modification of the SLE Disease Activity Index (SELENA-SLEDAI), which consists of 24 items, grouped according to the most-frequently affected 9 organ systems.[31] Scores for the SELENA-SLEDAI range from 0 to 105, and disease activity was categorized as follows: no activity (score = 0), mild activity (score 1–5), moderate activity (score 6–10), high activity (score 11–19), very high activity (score $\geq$20).[32] Laboratory data, including complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), C3, C4, anti-nuclear antibody (ANA), anti-dsDNA, anti-extractable nuclear antigen (ENA) panel, anti-cardiolipin-IgG, lupus anticoagulant, complete urine analysis, and 24-hour urine protein were measured. The diagnoses of NP-SLE and lupus nephritis were established according to the ACR definitions.[5,33]

All participants underwent a complete ophthalmologic examination. Central corneal sensitivity threshold was assessed with a contact corneal esthesiometer (Cochet-Bonnet, Luneau, France) by applying low pressure perpendicular to the center of the cornea using a 0.12-mm diameter nylon monofilament. At the beginning of the assessment, the nylon filament was extended to the maximal length of 6.0 cm, corresponding to the lowest possible pressure, and was decreased gradually in 5-mm steps until a response was elicited and verified twice as an indicator of the threshold for corneal sensitivity.

Laser scanning CCM was performed using the Rostock Corneal Module/Heidelberg Retina Tomograph III (Heidelberg Engineering, Germany). The full thickness of the central cornea was scanned using the "section" mode, and digital images were obtained with an image size of 400 $\times$ 400 µm and a lateral digital resolution of 1 µm/pixel. A standardized image selection protocol was used,[34] and three high-quality sub-basal nerve plexus images were selected and analyzed from each subject. The images were analyzed with a validated manual image segmentation algorithm (CCMetrics, University of Manchester, Manchester, UK),[35] and three nerve plexus parameters were quantified: corneal nerve fiber density (CNFD; fibers/mm^2); corneal nerve branch density (CNBD; branches/mm^2); and corneal nerve fiber length (CNFL; mm/mm^2). The same image frames were used to quantify LC density (cells/mm^2). The total number of highly reflective cells were counted manually using the nerve branch density quantification feature of the CCMetrics software. Cells with dendritic structures were considered as

mature LCs and those without dendritic structures were considered as immature LCs, as per a previously described method.[36] The observer performing the quantitative analysis of the CCM images was masked regarding the severity of disease activity. Only the data obtained from the right eyes were included in the analyses.

Statistical analysis was performed using IBM SPSS Statistics version 21.0 software. Basic descriptive statistics were calculated and reported as the mean ± SD or median (interquartile range [IQR]). The Pearson χ^2 test was used to compare categorical variables. All continuous data were tested for normality using the Kolmogorov-Smirnov test. A pre-study power analysis was not performed to determine sample size as no previous data were available. However, a post hoc power analysis based on CNFL revealed a power of 66.5%. Independent samples *t*-test for normally distributed data and Mann-Whitney *U* test for non-normally distributed data were used to compare the parameters between the subjects with SLE and healthy control participants. The correlations among variables were analyzed using Pearson's or Spearman's correlation tests, as appropriate. For all evaluations, a two-sided *P* value of less than 0.05 was considered statistically significant.

Results

The clinical and immunologic characteristics of the patients with SLE and control participants are summarized in Table 1. There were no significant differences between patients with SLE and control subjects for age ($P = 0.670$) and sex ($P = 0.442$). The median (IQR) time from the initial diagnosis of SLE was 3.0 years (IQR = 2.0–10.0 years, range = 0.5–22 years). The median (IQR) SELENA-SLEDAI score was 4.0 (IQR = 2.0–6.0, range = 0–14). Fourteen of 39 (35.9%) patients had a SELENA-SLEDAI score equal to or greater than 6, indicative of moderate to high disease activity. At the time of examination, 32 (82%) patients were receiving hydroxychloroquine, 21 (54%) azathioprine, 19 (49%) corticosteroids, 9 (23%) mycophenolate mofetil, 7 (18%) rituximab, 2 (5%) cyclosporine, 2 (5%) cyclophosphamide, and 2 (5%) were receiving intravenous immunoglobulin infusion as monotherapy or in combination.

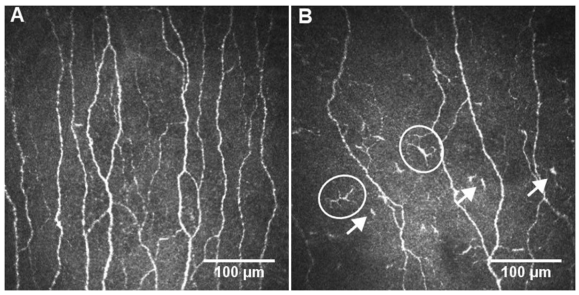

Figure 1. Corneal confocal microscopic images of the subepithelial nerve plexus in a healthy subject (**A**) and a patient with systemic lupus erythematosus (**B**), showing reduced nerve fibers and increased mature (*circles*) and immature (*arrows*) Langerhans cells.

Representative CCM images of the central corneal subepithelial nerve plexus in a healthy subject and a patient with SLE are shown in Figure 1. CNBD ($P = 0.003$) and CNFL ($P = 0.019$) were significantly lower, and the mature LC density ($P = 0.002$) was higher, but corneal sensitivity ($P = 0.178$), CNFD

Table 1. Baseline Characteristics of the Study Participants

	Control Subjects (*n* = 30)	Patients With SLE (*n* = 39)
Age, years, mean ± SD	35.0 ± 13.7	33.7 ± 12.7
Sex, F/M, *n*	26/4	36/3
Duration of disease, years, median (IQR)	–	3.0 (2.0–10.0)
SELENA-SLEDAI, median (IQR)	–	4.0 (2.0–6.0)
Erythrocyte sedimentation rate, mm/h, median (IQR)	–	16.0 (8.0–26.0)
C-reactive protein, mg/L, median (IQR)	–	3.0 (0.8–7.9)
Neuropsychiatric SLE, *n* (%)	–	13 (33.3)
Anti-dsDNA positivity, *n* (%)	–	30 (76.9)
Antiphospholipid syndrome, *n* (%)	–	6 (15.4)
C3 hypocomplementemia, *n* (%)	–	19 (48.7)
C4 hypocomplementemia, *n* (%)	–	12 (30.8)
Lupus nephritis, *n* (%)	–	11 (28.2)

Abbreviations: SELENA-SLEDAI, Safety of Estrogens in Lupus Erythematosus National Assessment modification of the Systemic Lupus Erythematosus Disease Activity Index; SLE, systemic lupus erythematosus.

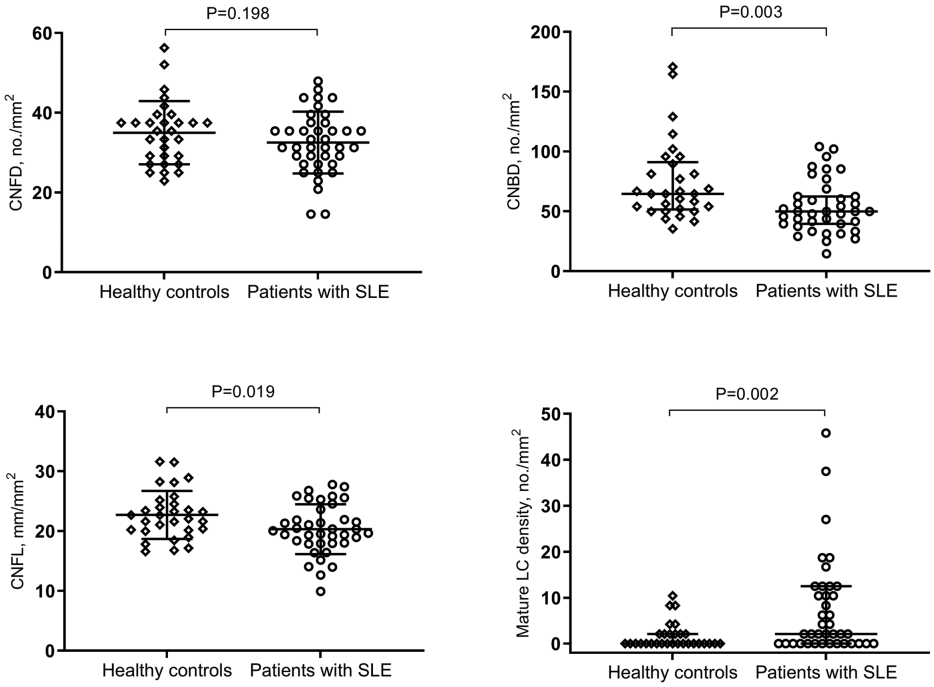

Figure 2. Corneal nerve fiber parameters and mature LC density presented as individual data points in patients with SLE and healthy control participants, showing a significant reduction in CNBD and CNFL, and an increase in mature LC density in patients with SLE. Error bars indicate mean (SD) for CNFD and CNFL, and median (IQR) for CNBD and mature LC density. Abbreviations: CNFD, corneal nerve fiber density; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber length; LC, Langerhans cell.

Table 2. Corneal Sensitivity and Corneal Confocal Microscopy Parameters in Patients With Systemic Lupus Erythematosus and Healthy Control Participants

	Control Subjects ($n = 30$)	Patients With SLE ($n = 39$)	*P* Value
Central corneal sensitivity, cm, median (IQR)	6.0 (6.0–6.0)	6.0 (5.5–6.0)	0.178[a]
CNFD, no./mm^2, mean ± SD	35.1 ± 8.0	32.6 ± 7.8	0.198[b]
CNBD, no./mm^2, median (IQR)	64.6 (51.6–95.8)	50.0 (39.6–68.7)	**0.003**[a]
CNFL, mm/mm^2, mean ± SD	22.8 ± 4.1	20.4 ± 4.2	**0.019**[b]
LC density, no./mm^2, median (IQR)	13.6 (0–33.3)	18.7 (4.2–47.9)	0.200[a]
Mature LC density, no./mm^2, median (IQR)	0 (0–2.1)	2.1 (0–12.5)	**0.002**[a]
Immature LC density, no./mm^2, median (IQR)	11.5 (0–29.2)	10.4 (2.1–43.7)	0.648[a]

Abbreviations: CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; LC, Langerhans cell.

[a]Mann-Whitney *U* test.

[b]Independent samples *t*-test.

The bold *P* values represent statistically significant differences.

($P = 0.198$), and total ($P = 0.200$) and immature ($P = 0.648$) LC densities were comparable in patients with SLE compared to control subjects (Fig. 2, Table 2). Of the patients with SLE, 13 of 39 (33%) had central nervous system involvement and fulfilled the criteria for NP-SLE. Immature LC density was higher with no difference in the corneal nerve or other LC parameters in patients with and without NP-SLE (Table 3). There was no significant difference in corneal sensitivity, corneal nerve, and LC parameters between subjects with ($n = 11$ [28%]) and without lupus nephritis ($P > 0.05$ for all; Supplementary Table S1).

The median (IQR) value of Schirmer's test was 16.0 mm (IQR = 10.0–21.0 mm) in 5 minutes in patients with SLE. No significant differences were observed in any of the study parameters between

Table 3. Corneal Sensitivity and CCM Parameters in Patients With and Without Neuropsychiatric Systemic Lupus Erythematosus (NP-SLE)

	NP-SLE ($n = 13$)	Non-NP-SLE ($n = 26$)	*P* Value
Central corneal sensitivity, cm, median (IQR)	6.0 (5.8–6.0)	6.0 (5.5–6.0)	0.418[a]
CNFD, no./mm^2, mean ± SD	31.9 ± 6.3	32.9 ± 8.5	0.698[b]
CNBD, no./mm^2, median (IQR)	52.1 (44.8–57.8)	46.9 (36.5–79.2)	0.895[a]
CNFL, mm/mm^2, mean ± SD	20.0 ± 3.5	20.6 ± 4.6	0.691[b]
LC density, no./mm^2, median (IQR)	43.8 (5.2–103.1)	13.6 (3.7–30.2)	0.081[a]
Mature LC density, no./mm^2, median (IQR)	2.1 (0–11.5)	2.1 (0–12.5)	0.735[a]
Immature LC density, no./mm^2, median (IQR)	43.8 (5.2–89.6)	9.4 (1.6–17.6)	**0.025**[a]

Abbreviations: CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; LC, Langerhans cell.

[a]Mann-Whitney *U* test.

[b]Independent samples *t*-test.

The bold *P* values represent statistically significant differences.

subgroups of patients with a Schirmer score of 6–10 mm ($n = 10$ [26%]) and those higher than 10 mm ($P > 0.05$ for all; Supplementary Table S2).

CNFD ($P = 0.008$), CNBD ($P = 0.005$), and CNFL ($P = 0.005$) were lower, with no difference in corneal sensitivity ($P = 0.675$) and total, mature, or immature LC densities ($P = 0.740$, $P = 0.740$, and $P = 0.573$, respectively) in patients with moderate to high disease activity (SELENA-SLEDAI ≥6, $n = 14$ [36%]) compared with patients with no or mild disease activity (SELENA-SLEDAI <6). The SELENA-SLEDAI scores correlated with CNFD ($\rho = -0.319$, $P = 0.048$) and CNFL ($\rho = -0.373$, $P = 0.019$), and total and immature LC densities correlated with CNBD ($\rho = -0.319$, $P = 0.048$, and $\rho = -0.328$, $P = 0.041$, respectively). Central corneal sensitivity correlated with CNBD ($\rho = 0.336$, $P = 0.037$) and CNFL ($\rho = 0.411$, $P = 0.009$). There were no significant correlations between CCM parameters and Schirmer test scores, 24-hour urine protein, spot urine protein, or the estimated glomerular filtration rate (eGFR; $P > 0.05$ for all).

Discussion

To our knowledge, this is the first study in patients with SLE showing corneal nerve loss associated with disease severity, and an increase in mature LC density. Neuropsychiatric SLE is a well-recognized entity in patients with SLE, and although most studies have focused on central nervous system disease, peripheral nerve involvement has a reported prevalence of 5.9% to 27.8%,[11,37,38] as acknowledged in the Systemic Lupus International Collaborating Clinics (SLICC) criteria and the ACR case definitions for NP-SLE.[5,39] It is associated with a reduced quality of life,[37] especially when patients develop small fiber neuropathy.[40] However, small fiber neuropathy is an underdiagnosed manifestation of SLE, as conventional electrophysiological tests only detect large fiber damage. Indeed, Oomatia et al.[11] showed that small fiber neuropathy was more frequent than mononeuritis multiplex, demyelinating neuropathies, and plexopathies in SLE. Similarly, Omdal et al.[7] reported a significant reduction in IENFD in 15 patients with SLE without symptoms or signs of neuropathy of whom only 2 patients had abnormalities in nerve conduction. In another cohort of 60 patients with SLE, of 8 patients with reduced IENFD, 6 patients (75%) had normal nerve conduction studies.[8] Tseng et al.[9] found reduced IENFD in 82.2%, compared to abnormal quantitative sensory tests in 33.3% and abnormal nerve conduction studies in 31.1% of patients with SLE. These studies indicate a large burden of subclinical small fiber disease in SLE, and our study confirms a lower CNFD, which was not significant and may be attributed to greater involvement of the more distal branches.

CCM has increasingly been used to identify nerve fiber damage in various peripheral and central neurodegenerative disorders.[17–21] We and others have shown comparable corneal nerve fiber and intra-epidermal nerve fiber loss in patients with diabetes and fibromyalgia,[41,42] and related it to disease severity in patients with Fabry disease.[21] However, the association between small fiber damage and the severity of disease activity in patients with SLE is controversial. Tseng et al.[9] reported an inverse correlation between IENFD and SLEDAI, whereas Omdal et al.[7] found no relationship between IENFD and SLEDAI or other clinical and immunologic markers of disease activity in SLE. In this study, we have found a significant correlation

between the SLEDAI and severity of corneal nerve loss, and all corneal nerve parameters were lower in patients with moderate to high disease compared to no or mild disease activity.

In a recent study, both the cutaneous silent period and IENFD did not differ between patients with and without NP-SLE.[12] We also show no difference in corneal nerve parameters between patients with and without NP-SLE or lupus nephritis. The lack of difference in corneal nerve parameters may indicate different mechanisms underlying these other major prognostic manifestations in patients with SLE.

In vivo corneal confocal microscopy has been used to evaluate the density of mature and immature LCs with a high degree of correlation with *in vitro* immunostaining.[43] We and others have shown increased LCs in several immune-mediated and inflammatory conditions, including chronic inflammatory demyelinating polyneuropathy (CIDP), Behçet's disease, and multiple sclerosis.[23,25–27,36] Recently, increased corneal LCs predicted clinical progression of CIDP with 100% sensitivity/specificity[44] and rituximab treatment of a patient with anti-neurofascin-155 neuropathy was associated with a reduction in corneal LCs, antibody titers, and an excellent clinical response.[45] Teunissen et al.[46] showed the development of dendritic processes and an increase in cell size in mature epidermal LCs and the presence of dendritic structures has been used to differentiate mature from immature corneal immune cells.[47,48] We have found an increase in mature LC density in patients with SLE and Resch et al.[49] also reported an increase in total and mature corneal LCs in patients with SLE. In a patient with SLE with symptomatic small fiber neuropathy, increased corneal LC density fell after treatment with systemic corticosteroids and returned into the normal range after commencing intravenous immunoglobulin and was associated with an improvement in symptoms of small fiber neuropathy.[50] Indeed, we show that total and immature LC density correlated inversely with corneal nerve branch density, suggesting a relationship between immune cells and small nerve fiber integrity. The overall lack of difference in total and immature LC densities between patients with SLE and control subjects in our study may be attributable to the high proportion of patients on immunosuppressive treatments.

Recent studies have identified corneal nerve loss and increased immune cells in patients with dry eye.[51,52] We have not undertaken comprehensive screening for dry eye using the Ocular Surface Disease Index questionnaire or an evaluation of the tear break-up time test or ocular surface staining. However, we excluded patients with a Schirmer's test of ≤5 mm to avoid confounding effects of dry eye and we also found no correlations between Schirmer's test and any of the study variables.

The cross-sectional design and small sample size preclude us from drawing causal inferences between changes in LCs and corneal nerves in SLE. A lack of neuropathic pain assessment and other measures of small fiber neuropathy, including IENFD and quantitative sensory testing limits conclusions regarding corneal nerve loss and small fiber neuropathy. Nevertheless, we have recently shown that CCM can enhance the ability to diagnose small fiber neuropathy.[29]

In conclusion, we demonstrate significant subclinical corneal nerve fiber loss which was related to disease severity in patients with SLE. Furthermore, quantifying mature LC density may allow an assessment of immune activation and response to therapy in SLE. Our findings provide the basis for larger longitudinal studies to evaluate the clinical utility of CCM as a rapid noninvasive ophthalmic imaging marker of small fiber damage and disease activity in patients with SLE.

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