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RESEARCH REPORT

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Reduced association between dendritic cells and corneal sub-basal nerve fibers in patients with fibromyalgia syndrome

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Abstract

In our study, we aimed at investigating corneal langerhans cells (LC) in patients with fibromyalgia syndrome (FMS) and small fiber neuropathy (SFN) as potential contributors to corneal small fiber pathology. We enrolled women with FMS (n = 134) and SFN (n = 41) who underwent neurological examination, neurophysiology, prostaglandin analysis in tear fluid, and corneal confocal microscopy (CCM). Data were compared with those of 60 age-matched female controls. After screening for dry eye disease, corneal LC were counted and sub-classified as dendritic (dLC) and non-dendritic (ndLC) cells with or without nerve fiber association. We further analyzed corneal nerve fiber density (CNFD), length (CNFL), and branch density (CNBD). Neurological examination indicated deficits of small fiber function in patients with SFN. Nerve conduction studies were normal in all participants. Dry eye disease was more prevalent in FMS (17%) and SFN (28%) patients than in controls (5%). Tear fluid prostaglandin levels did not differ between FMS patients and controls. While corneal LC density in FMS and SFN patients was not different from controls, there were fewer dLC in association with nerve fibers in FMS and SFN patients than in controls ($P < .01$ each). Compared to controls, CNFL was lower in FMS and SFN patients ($P < .05$ each), CNFD was lower only in FMS patients ($P < .05$), and CNBD was lower only in SFN patients ($P < .001$). There was no difference in any CCM parameter between patients with and without dry eyes. Our data indicate changes in corneal innervation and LC distribution in FMS and SFN, potentially based on altered LC signaling.

KEYWORDS

corneal confocal microscopy, fibromyalgia syndrome, Langerhans cells, pain, small fiber neuropathy

1 | INTRODUCTION

In recent studies, small nerve fiber pathology was found in subgroups of patients with fibromyalgia syndrome (FMS).[1-12] Applying corneal confocal microscopy (CCM), corneal denervation was also reported in FMS patients.[9,13,14]

Small fiber pathology is not specific for FMS but may rather be found in many painful and painless conditions which differ in clinical

presentation and pathophysiology.[15] FMS is distinct from small fiber neuropathy (SFN) and presents with widespread muscular pain that is regularly associated with depression, fatigue, and sleep disturbance.[16] SFN patients mostly report superficial acral pain without such additional symptoms. While nerve conduction studies are normal to marginally abnormal in FMS and SFN patients, both diseases also share pathological findings in small fiber tests. The impact of small nerve fiber impairment on FMS symptoms remains elusive. Reduction of corneal innervation was found in patients with painful (ie, herpes zoster ophthalmicus[17]) and painless (ie, inflammatory[18] and diabetic neuropathy,[19] multiple sclerosis[20]) neurological diseases paralleled by an increase in corneal Langerhans cell (LC) density and LC-nerve fiber contact. Integrity of the corneal sub-basal nerve plexus is regulated by interactions between immune cells and nerve fiber endings.[21,22] Their communication is crucial controlling the corneal immune status.[23,24] A disruption of these fine-tuned neuro-immune interactions may alter corneal innervation. Hence, they are promising targets for pathophysiological mechanisms in small fiber pathology.

In this prospective and controlled study, we investigated corneal immune cells of patients with FMS compared to SFN and healthy controls. We report a reduction of nerve fiber associated LC in patients with FMS compared to healthy controls and even more so in patients with SFN which may contribute to corneal small fiber pathology.

2 | MATERIALS AND METHODS

2.1 | Study participants

One hundred and thirty-four female patients with FMS and 60 age- and gender-matched healthy controls were examined between September 2014 and August 2018. We prospectively recruited study participants among patients who contacted us for enrolment. All patients were examined by a neurologist and FMS was confirmed applying current diagnostic criteria.[25-27] Additionally, we recruited 41 female patients with SFN treated as in- or out-patients at our department.[28]

All study participants fulfilled the following inclusion criteria: diagnosis of FMS or SFN[25-28] and age ≥ 18 years. Exclusion criteria were: history of diabetes mellitus, polyneuropathy, renal insufficiency, untreated thyroid dysfunction, acute inflammatory disease, malignancy ≤5 years, drug or alcohol misuse, severe psychiatric disorder requiring treatment, usage of hard contact lenses, eye diseases or surgery, pain of other cause and undistinguishable from FMS pain, pending compensation claims. Additionally, we documented any history of autoimmune diseases. All patients and controls gave written informed consent. Our study was approved by the ethics committee of the University of Würzburg Medical Faculty (121/14).

2.2 | Laboratory and electrophysiological assessment

Serum levels of electrolytes, vitamin B12, thyroid-stimulating hormone (TSH), hepatic and renal marker proteins, and HbA1c were measured. We performed an oral glucose tolerance test (OGTT). Conduction studies of the right sural and tibial nerves were performed in all patients to exclude polyneuropathy.[29]

2.3 | CCM image acquisition

All study participants underwent slit lamp examination by an ophthalmologist. Corneal sensitivity was tested using a Cochet-Bonnet esthesiometer (Luneau Ophtalmologie, Chartres Cedex, France). Both eyes were then anesthetized using Conjuncain EDO eye drops containing 0.4% oxybuprocaine hydrochloride (Bausch & Lomb GmbH, Berlin, Germany) and lubricated by a drop of Corneagel EDO (Bausch & Lomb GmbH, Berlin, Germany). A Heidelberg Retina Tomograph Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany) capped with a sterile TomoCap (Heidelberg Engineering GmbH, Heidelberg, Germany) was used to obtain approximately 70 images per eye using the section mode. By applying the fine focus of the microscope, the focal plane was set to Bowman's layer and then moved horizontally and vertically to capture central and pericentral images from the cornea. Image size was 384 × 384 pixels with a pixel size of 1.047 μm, thus representing a 400 × 400 μm^2 field of view. Time for image acquisition did not exceed 5 minutes in any case and no participant suffered from corneal or visual complications afterwards.[30] Per patient, six different images of the sub-basal nerve plexus from the central cornea, three from each eye, were chosen by an observer blinded to group allocation, based on image quality, overall contrast, correct focal plane, and absence of artifacts as previously recommended.[31,32] Coded images were assessed offline by a second investigator unaware of the study objectives.

2.4 | Image evaluation

We used purpose-written, proprietary image analysis software ACCMetrics and CCMetrics (M.A. Dabbah, Imaging Science, Manchester, UK) to determine the following parameters: corneal nerve fiber density (CNFD, that is, number of main nerve fibers [no./mm^2]), nerve fiber length (CNFL, that is, total length of nerve fibers [mm/mm^2]), nerve fiber width (CNFW, that is, the average axial diameter of all nerve fibers analyzed [mm]), nerve branch density (CNBD, that is, number of branches arising from the main nerves [no./mm^2]), and nerve fiber fractal dimension (CFracDim, that is, measure of spatial distribution and structure complexity of corneal nerve fibers). CNFD, CNFL, CNFW, and CFracDim were automatically determined by ACCMetrics software and CNBD was manually measured using CCMetrics software.

LC were counted manually on the same images used for nerve fiber quantification. We defined all hyperreflective structures showing a cell body as LC. The number of LC per mm^2 is referred to as the total number of cells (LC_{total}). Based on morphological appearance,[33] LC were subclassified as dendritic cells (dLC, that is, cells showing dendrite-like elongations in addition to their cell body, Figure 1A) and non-dendritic cells (ndLC, that is, cells only consisting of a cell body,

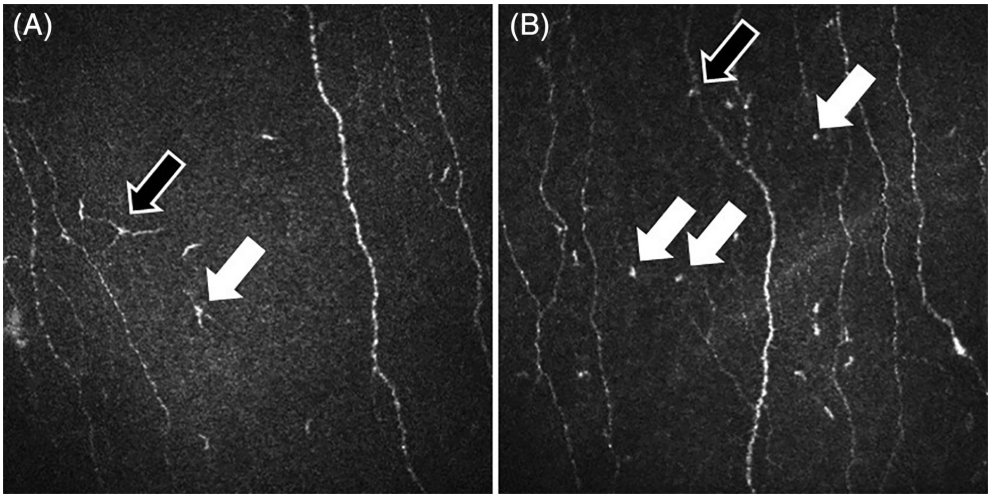

FIGURE 1 CCM images of FMS patients showing corneal nerves and LC. A, Two prominent dendritic LC. Black arrow: $dLC_{fiber\ assoc.}$. White arrow: $dLC_{no\ assoc.}$. B, Several LC showing no dendrites. Black arrow: $ndLC_{fiber\ assoc.}$. White arrow: $ndLC_{no\ assoc.}$. Abbreviations: $dLC_{fiber\ assoc.}$, dendritic cells with nerve fiber association; $dLC_{no\ assoc.}$, dendritic cells without nerve fiber association; FMS, fibromyalgia syndrome; $ndLC_{fiber\ assoc.}$, non-dendritic cells with nerve fiber association; $ndLC_{no\ assoc.}$, non-dendritic cells without nerve fiber association

TABLE 1 General characteristics of study cohort

	FMS (n = 134)	SFN (n = 41)	Healthy controls (n = 60)
Age (years)	51 (21-74)	55 (22-73)	50 (22-64)
BMI (kg/mm^2)	24 (16-42)	25 (19-42)	24 (17-42)
Disease duration (years)	12 (0.75-56)	4 (0-20)	N.A.
Current pain intensity (NRS)	5 (0-9)	4 (0–8)	N.A.
Reduced corneal sensitivity[b]	17/132 (13%)	4/40 (10%)	6/59 (10%)
Laboratory findings			
- HbA1c (ref.: ≤6.1%)	5.4 (4.7-6.4; 2 [1%])	5.6 (3.6-7.7; 5 [12%])	N.A.
- OGTT(2 hours) (ref.: ≤140 mg/dL)	121 (65-217; 18 [13%])	127 (79-284; 12 [29%])	
- TSH (ref.: 0.3-4.0 mIU/L)	1.8 (0-22; 18 [13%])	1.6 (0.2-9.2; 6 [14%])	
- Vitamin B12 (ref.: 197-866 pg/mL)	452 (183-2000; 9 [7%])	450 (215-2000; 5 [12%])	
Medication			
- Any	126 (94.0%)	39 (95.1%)	22 (36.7%)
- Analgesics	113 (84.3%)	32 (78.0%)	0
- Drugs with anticholinergic effect	42 (31.3%)	13 (31.7%)	1 (1.7%)
Possible etiology of SFN[a]			
- Idiopathic	N.A.	17 (41.5%)	N.A.
- Diabetes mellitus or IGT		9 (22.0%)	
- Thyroid dysfunction		6 (14.6%)	
- Genetic		8 (19.5%)	
- Parainfectious		3 (7.3%)	
- Autoimmune		6 (14.6%)	

Note: Data are given as median and range in brackets. For laboratory findings, the number and percentage of subjects with pathological values (ie, beyond our laboratory's normal values) are provided.

Abbreviations: BMI, body mass index; FMS, fibromyalgia syndrome; IGT, impaired glucose tolerance; N.A., not applicable; NRS, numeric rating scale; OGTT, oral glucose tolerance test; SFN, small fiber neuropathy; TSH, thyroid-stimulating hormone.

[a]In some SFN patients, more than one etiology was assumed, therefore percentages add up to >100%.

[b]Reduced corneal sensitivity was defined as Cochet-Bonnet esthesiometry <5 mm in at least one eye.

TABLE 2 Findings of corneal Langerhans cells in the corneal sub-basal nerve plexus

	FMS (n = 134)	SFN (n = 41)	Controls (n = 60)	*P* (overall[a])	*P* (FMS vs Controls[b])	*P* (SFN vs Controls[b])	*P* (FMS vs SFN[b])
$dLC_{fiber\ assoc.}$	2.1 (0-45.8)	1.6 (0-32.3)	4.2 (0-58.3)	<.01	<.05	<.01	>.05
$dLC_{no\ assoc.}$	8.3 (0-129.2)	5.2 (0-66.7)	9.9 (0-97.9)	>.05	N.A.	N.A.	N.A.
dLC_{total}	11.5 (0-160.4)	7.3 (0-99.0)	14.1 (0-147.9)	>.05	N.A.	N.A.	N.A.
$ndLC_{fiber\ assoc.}$	1.0 (0-37.5)	1.0 (0-8.3)	1.0 (0-9.4)	>.05	N.A.	N.A.	N.A.
$ndLC_{no\ assoc.}$	4.2 (0-85.4)	4.7 (0-65.6)	3.6 (0-17.7)	>.05	N.A.	N.A.	N.A.
$ndLC_{total}$	5.2 (0-105.2)	7.3 (0-67.7)	5.7 (0-20.8)	>.05	N.A.	N.A.	N.A.
$LC_{fiber\ assoc.}$	5.2 (0-53.1)	3.1 (0-37.5)	7.3 (0-61.5)	<.05	>.05	<.01	>.05
$LC_{no\ assoc.}$	12.5 (0-214.6)	10.4 (0-106.3)	15.6 (0-100)	>.05	N.A.	N.A.	N.A.
LC_{total}	19.8 (0-255.2)	13.5 (0-143.8)	22.9 (0-152.1)	>.05	N.A.	N.A.	N.A.

Note: Data are given as median and range in brackets. Unit of measurement is (cells/mm^2).
[a]Non-parametric Kruskal-Wallis test.
[b]Non-parametric Mann-Whitney-*U* test.
Abbreviations: FMS, fibromyalgia syndrome; SFN, small fiber neuropathy; $dLC_{fiber\ assoc.}$, dendritic cells with nerve fiber association; $dLC_{no\ assoc.}$, dendritic cells without nerve fiber association; dLC_{total}, all dendritic cells; $ndLC_{fiber\ assoc.}$, non-dendritic cells with nerve fiber association; $ndLC_{no\ assoc.}$, non-dendritic cells without nerve fiber association; $ndLC_{total}$, all non-dendritic cells; $LC_{fiber\ assoc.}$, total number of cells with nerve fiber association; $LC_{no\ assoc.}$, total number of cells without nerve fiber association; LC_{total}, total cell number; N.A., not applicable.

Figure 1B). For every LC, we determined whether it was in association with nerve fibers ($LC_{fiber\ assoc.}$/$LC_{no\ assoc.}$) as described previously.[18] Nerve fiber association was assumed when either the cell body or a dendrite touched a nerve fiber. To assess whether changes in $LC_{fiber\ assoc.}$ were due to an increased/ decreased chance of LC nerve fiber interaction resulting from changes in CNFL, we also calculated the $LC_{fiber\ assoc.}$/CNFL and $dLC_{fiber\ assoc.}$/CNFL ratio.

2.5 | Screening for dry eye disease

Study participants underwent a screening procedure for dry eye disease (DED) before CCM which consisted of a Schirmer's test (Haag-Streit UK Ltd, Harlow Essex, UK) without anesthesia and an interview using the German version of the Ocular Surface Disease Index (OSDI).[34] The cut-off value for a positive Schirmer's test was ≤5 mm wetting of the test strip within 5 minutes.[35] Results of the OSDI were classified as normal (0-12), mild (13-22), moderate (23-32) or severe (>32) DED.[36] We defined a positive screening result for DED as a positive Schirmer's test in ≥1 eye and an OSDI score > 12.

2.6 | Tear fluid analysis

Tear fluid samples were collected from 74 FMS patients and 39 healthy controls to investigate prostaglandin (PGE_2, PGD_2) concentrations as they have been shown to correlate with pain and symptom severity in patients with dry eyes.[37] The obtained fluid volume was sufficient for prostaglandin quantification in 52/74 FMS patients and 26/39 healthy controls.

For the analysis of PGE_2 and PGD_2 concentrations, 10 μL of tear fluid were investigated using nano liquid chromatography-tandem mass spectrometry (LC-MS/MS). To enhance tear flow, mint oil was applied on the subjects' cheeks (Pharma Aldenhoven GmbH & Co. KG, Aldenhoven, Germany). Tear samples were collected using cotton sponges (Lohmann & Rauscher International GmbH & Co. KG, Rengsdorf, Germany). Sponges were centrifuged at 4000 rpm for 5 min at room temperature yielding an average of 80 μL tear fluid per subject.

An Eksigent Nano LC system (ultra 2D, Sciex, Darmstadt, Germany) coupled to a mass spectrometer 5500 QTrap (Sciex, Darmstadt, Germany) equipped with a nanospray ion source operating in negative electrospray ionization mode was used. For data acquisition and quantification Analyst software V 1.6 and MultiQuant software V 3.0 (Sciex, Darmstadt, Germany) were used. Ratios of analyte peak area and internal standard area (*y*-axis) were plotted against concentration (*x*-axis) and calibration curves were calculated by least square regression with $1/x$ weighting. Calibration range was 0.04 to 8 ng/mL tear fluid for all analytes.

2.7 | Statistical analysis

We used SPSS 24.0 (IBM, Ehningen, Germany) for our analysis. Data are given as median and range. OSDI results, nerve fiber parameters, and LC data were compared using the non-parametric Kruskal-Wallis test with post hoc analysis by the non-parametric Mann-Whitney-*U* test. The χ^2 test was applied for categorical data. Correlation analysis was performed using the bivariate Spearman correlation coefficient with Bonferroni-Holm adjustment. Afterwards, linear univariate regression models with Bonferroni-Holm adjustment were applied to the data. *P*-values <.05 were considered statistically significant.

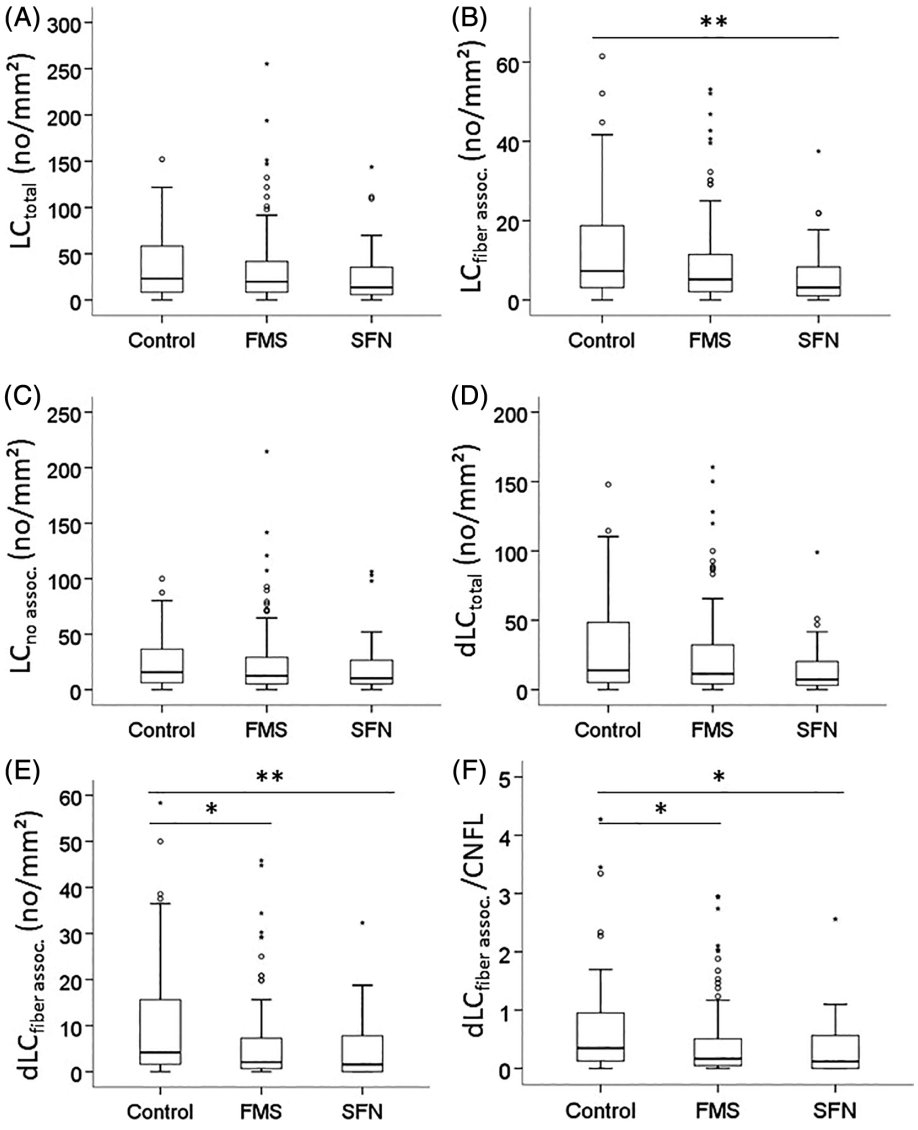

FIGURE 2 Density of corneal LC in FMS patients, SFN patients, and healthy controls. A, There was no intergroup difference in LC_{total}. B, A lower density of $LC_{fiber\ assoc.}$ was found in SFN ($P < .01$), when comparing patients with healthy controls. C, There was no intergroup difference in $LC_{no\ assoc.}$. D, There was no intergroup difference in dLC_{total}. E, A lower density of $dLC_{fiber\ assoc.}$ was found when comparing FMS ($P < .05$) and SFN ($P < .01$) patients with healthy controls. F, The ratio of $dLC_{fiber\ assoc.}$ was lower in FMS and SFN patients compared to controls ($P < .05$ each). Abbreviations: dLC_{total}, all dendritic cells; $dLC_{fiber\ assoc.}$, dendritic cells with nerve fiber association; $dLC_{no\ assoc.}$, dendritic cells without nerve fiber association; FMS, fibromyalgia syndrome; LC, Langerhans cells; LC_{total}, total cell number; $LC_{fiber\ assoc.}$, total number of cells with nerve fiber association; $LC_{no\ assoc.}$, total number of cells without fiber association; SFN, small fiber neuropathy. *$P < .05$, **$P < .01$

3 | RESULTS

3.1 | Study population, laboratory, and electrophysiological findings

Table 1 summarizes clinical data and medication use of the study participants. Neurological examination, blood cell count, and basic laboratory parameters were normal in all FMS patients. Nerve conduction studies excluded large fiber neuropathy. In SFN patients, neurological examination revealed tactile hypoesthesia in 9/41 (22%), thermal hypoesthesia in 9/41 (22%), impairment of vibration sense at the toes in 3/41 (7%), hyperalgesia in 7/41 (17%), hypoalgesia in 5/41 (12%), dysesthesias in 3/41 (7%), allodynia in 4/41 (10%), and pins and needles paresthesia in 1/41 patients (2%). When present, tactile hypoesthesia was mild and impairment of vibration sense was limited to the metatarsophalangeal joint, thus not fulfilling exclusion criteria of SFN.[38] There were no electrophysiological indicators of relevant large fiber impairment in any SFN patient.[39] There was no sign of acute inflammation in 37/41 cases (90%). In 4/41 (10%) patients, laboratory parameters were indicative of mild inflammation (ie, increased leucocyte count of 11.100/μL; 12.000/μL; 15.600/μL and increased C-reactive protein level of 2.5 mg/dL in one patient) but were not accompanied by clinical symptoms of acute inflammatory disease (eg, fever, headache).

3.2 | Lower density of dendritic LC in association with nerve fibers in FMS and SFN patients compared to controls

Table 2 summarizes findings for LC and the cell subclasses. There were no differences in LC_{total} between groups (Figure 2A). The number of $LC_{no\ assoc.}$ did not differ between groups (Figure 2B). However, the $LC_{fiber\ assoc.}$ count was lower in SFN patients compared to controls ($P < .01$) but did not differ between FMS patients and controls ($P > .05$; Figure 2C). dLC_{total} density was similar in FMS patients, SFN patients, and controls (Figure 2D). $dLC_{fiber\ assoc.}$

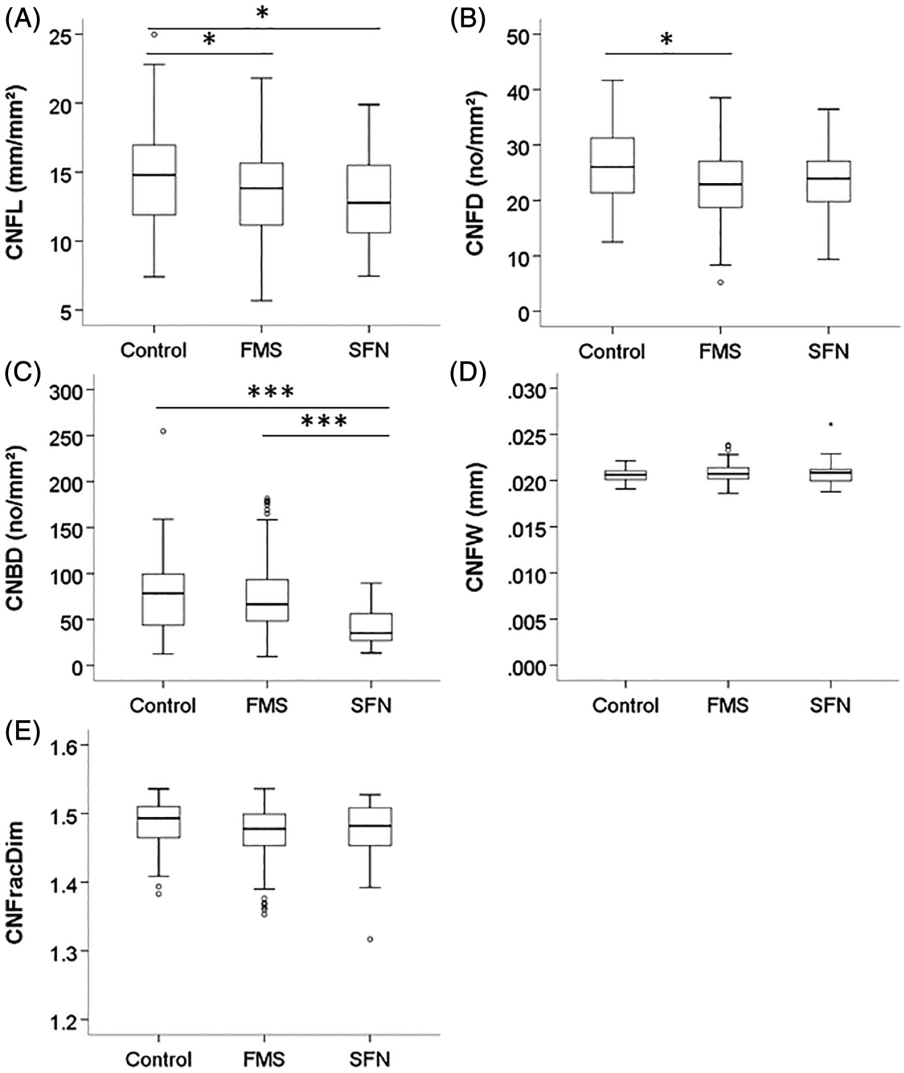

FIGURE 3 Corneal sub-basal nerve plexus characteristics in FMS patients, SFN patients, and healthy controls. A, In FMS and SFN patients CNFL was lower than in healthy controls ($P < .05$). B, CNFD in FMS patients, but not in SFN patients, was lower than in controls ($P < .05$). C, CNBD did not differ between FMS patients and controls but was lower in SFN patients than in FMS patients or healthy controls ($P < .001$ each). D, There was no intergroup difference for CNFW. E, There was no intergroup difference in CNFracDim. Abbreviations: CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; CNFracDim, corneal nerve fractal dimension; CNFW, corneal nerve fiber width; FMS, fibromyalgia syndrome; SFN, small fiber neuropathy. *$P < .05$, ***$P < .001$

were lower in both FMS and SFN patients compared to healthy controls ($P < .01$ each; Figure 2E). In FMS patients, the $LC_{fiber\ assoc.}$/CNFL ratio was as high as in controls. SFN patients presented with lower values than controls ($P < .01$, data not shown) while the $dLC_{fiber\ assoc.}$/CNFL ratio was lower in both patient groups compared to controls ($P < .05$ each, Figure 2F). There were no intergroup differences for $ndLC_{no\ assoc.}$, $ndLC_{fiber\ assoc.}$ or $dLC_{no\ assoc.}$ (data not shown).

3.3 | Lower CNBD distinguishes SFN from FMS patients

Figure 3 illustrates CCM fiber findings. CNFL was lower in FMS and SFN patients compared to controls ($P < .05$ each; Figure 3A). CNFD was lower in FMS patients than in controls ($P < .05$) but did not differ between SFN patients and controls (Figure 3B). In contrast, CNBD did not differ between FMS patients and controls but was lower in SFN patients than in controls ($P < .001$) or FMS patients ($P < .001$; Figure 3C). There were no intergroup differences for CNFW and CFracDim (Figure 3D,E).

3.4 | LC density does not correlate with age or CNFD

CCM parameters did not correlate with age or disease duration in patients and controls. In addition, $dLC_{fiber\ assoc.}$ did not correlate with CNFL, CNFD, or CNBD (Table S1). Disease duration was not predictive of CCM parameters in FMS and SFN patients. However, linear regression models predicted LC_{total} (F [1, 38] = 8.605, $P < .05$, $R^2 = .185$), $LC_{fiber\ assoc.}$ (F [1, 38] = 11.287, $P < .05$, $R^2 = .229$), dLC_{total} (F [1, 38] = 11.315, $P < .05$, $R^2 = .229$), $dLC_{fiber\ assoc.}$ (F [1, 38]=10.798, $P < .05$, $R^2 = .221$), and $dLC_{no\ assoc.}$ (F [1, 38] = 9.138, $P < .05$, $R^2 = .194$) by age in SFN patients. Age was not predictive of corneal innervation and LC in FMS patients and controls.

3.5 | Higher prevalence of DED in FMS and SFN patients does not influence LC counts or sub-basal nerve plexus characteristics

Schirmer's test was pathological in ≥1 eye of 33/134 FMS patients (25%), 17/40 SFN patients (43%), and 12/60 healthy controls (20%).

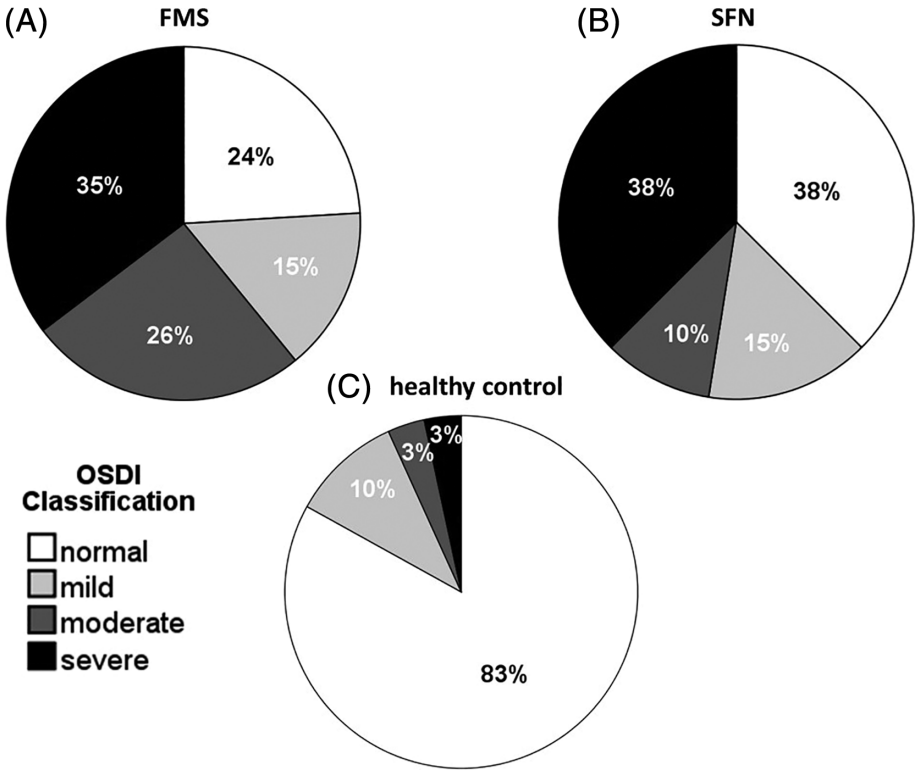

FIGURE 4 Distribution of OSDI severity among FMS patients, SFN patients, and healthy controls. Distribution of OSDI among healthy controls, A; FMS patients, B; and SFN patients, C. Dry eye disease symptoms assessed by OSDI score were either classified as normal (<13), mildly (13-22), moderately (23-32), or severely pathological (>32). Abbreviations: FMS, fibromyalgia syndrome; OSDI, ocular surface disease index; SFN, small fiber neuropathy

Pathological (>12) OSDI scores were found in 101/133 FMS patients (76%), 25/40 SFN patients (63%), and 10/59 controls (17%). Figure 4 shows the distribution of OSDI scores.

Screening for DED was positive in 22/131 (17%) FMS patients, 11/40 (28%) SFN patients, and 3/59 controls (5%). The prevalence of DED was higher in FMS (χ^2 [1] = 4.881, $P < .05$) and SFN patients compared to controls (χ^2 [1] = 9.865, $P < .01$), with no difference between both groups (χ^2 [1] = 2.255, $P > .05$). Each patient and control group was tested separately for differences between participants with and without DED. DED did not influence CNFL, CNFD, CNBD, CNFW, CFracDim, dLC_{total}, $dLC_{fiber\ assoc.}$, or $LC_{fiber\ assoc.}$ (Figure S1).

3.6 | Tear fluid prostaglandin concentrations do not differ between FMS patients and controls

The obtained volume of tear fluid was sufficient for prostaglandin analysis in 8/15 (53%) FMS patients and 1/2 (50%) controls with and 43/59 (73%) FMS patients and 24/37 (65%) controls without DED. There were no differences in PGD_2 and PGE_2 concentrations when comparing FMS patients (PGD_2: 0.15 [0.06-0.71] pg/μL, PGE_2: 0.20 [0.01-1.56] pg/μL) with controls (PGD_2: 0.13 [0.06-0.31] pg/μL, PDE2: 0.22 [0.02-1.09] pg/μL). There was no correlation between PGD_2 or PGE_2 and LC or corneal sub-basal nerve plexus parameters in FMS patients and controls (data not shown). Furthermore, there were no differences in prostaglandin concentrations comparing FMS patients with (PGD_2: 0.16 [0.06-0.38] pg/μL, PGE_2: 0.36 [0.03-1.05] pg/μL) and without DED (PGD_2: 0.13 [0.07-0.71] pg/μL, PGE_2: 0.20 [0.01-1.56] pg/μL) ($P > .05$ each).

3.7 | Comorbid autoimmune diseases in FMS and SFN patients with DED

As assessed by interview, 4/22 (23%) FMS patients (n = 3 Hashimoto thyroiditis, n = 1 lichen rubber) and 4/11 (36%) of SFN patients (n = 3 Hashimoto thyroiditis, n = 1 lupus erythematosus) with DED reported a history of autoimmune disease in contrast to none of the controls.

4 | DISCUSSION

We examined corneal LC and sub-basal nerve fibers in FMS and SFN patients compared to healthy controls and report on lower numbers of dLC in association with nerve fibers in FMS and SFN patients. We further show lower CNBD in patients with SFN compared to FMS independent of DED.

We assume that the immune cells observed via CCM are Langerhans cells since corneal epithelial dendritic cells exclusively resemble LC in terms of antigen expression and ultra-structural morphology in healthy human eyes.[40] There was no sign of corneal inflammation in our patients that might have caused migration of other immune cells into the cornea.

Lower CNFD and CNFL counts were reported in small cohorts of patients with FMS when investigated with CCM compared to healthy controls.[9,13,14] Also, reduced nerve fiber branching[9] and thinning of

corneal stromal nerve fibers was found in FMS patients compared to healthy controls.[13] We reproduced these findings for CNFD and CNFL, though did not observe a difference in nerve branching (CNBD) between FMS patients and healthy controls. One reason for this discrepancy may be that Oudejans et al. assessed CNBD applying ACCMetrics software and compared data with published CCMetrics values, whereas we exclusively used CCMetrics. We also observed no difference in CNFW in our cohort which does not contradict a thinning of stromal nerve fibers as only sub-basal nerves were used for our analysis. In line with other studies, low CNFD or CNFL was only present in a subgroup of FMS patients which may indicate heterogeneity in pathophysiological mechanisms underlying FMS symptoms.

Applying CCM, lower CNFD was found in previous studies comparing SFN patients with healthy controls.[41-43] Further findings in SFN patients were shorter CNFL,[41,43] less nerve fiber branching,[41] and higher corneal nerve tortuosity[43] than in healthy controls. LC counts did not differ between SFN patients and controls.[43] We showed lower CNFL and corneal nerve fiber branching in SFN patients than in controls, whereas CNFD did not differ. Furthermore, we observed a lower density of LC in association with nerve fibers in SFN patients than in healthy controls but did not detect any difference in total LC counts which matches previous findings.[43] These results may indicate that LC do not change in total number but reduce their interactions with nerve fibers in SFN.

Corneal innervation and immune cell density appear to be altered similarly in FMS and SFN patients as we found lower CNFL and lower counts of dLC with nerve fiber association also in FMS patients. A reduction of LC—nerve associations may indicate a lack of neurotrophic signaling and nerve growth. However, there was no direct correlation between any nerve fiber parameter and $dLC_{fiber\ assoc.}$ density. We cannot exclude an influence of low CNFL on the observed $LC_{fiber\ assoc.}$ and $dLC_{fiber\ assoc.}$ counts. If there are fewer nerve fibers per mm^2 there may simply be fewer chances for LC to contact a fiber. However, CNFL did not correlate with $LC_{fiber\ assoc.}$ and $dLC_{fiber\ assoc.}$ in our study. Also, the ratio of $dLCfiber_{assoc.}$/CNFL was lower in FMS and SFN patients than in controls. This means that low $dLCfiber_{assoc.}$ values are not generally found in patients with low CNFL and that there is fewer dLC-nerve fiber co-localizations per mm of nerve fiber found in patients. We conclude from this that $dLC_{fiber\ assoc.}$ is reduced independently of CNFL in FMS and SFN patients.

The co-occurrence of changes in corneal innervation and corneal immune cell density has previously been shown in patients with chronic inflammatory demyelinating polyneuropathy (CIDP) who had lower CNFD, CNFL, and CNBD compared to healthy controls. The number of immune cell infiltrates was also higher in CIDP patients compared to controls and decreased with disease duration; further, cell counts positively correlated with the degree of motor impairment.[18] In patients with multiple sclerosis, CNFD, CNFL, and CNBD were lower than in healthy controls, whereas the density of dendritic cells was higher in the patient group.[20] Examining patients with diabetes mellitus with and without neuropathy in comparison to healthy controls, lower CNFD, CNFL, and CNBD, and higher nerve fiber tortuosity and LC density were found.[19] These findings were in line with data from mouse models of type 1 diabetes in which an increase of corneal LC density was found to correlate with rising blood glucose levels after induction of diabetes and a gradual decrease in CNFD.[44]

Patients with DED of any etiology have a higher LC density in their central cornea than healthy controls, with the highest densities found in autoimmune-associated DED.[45-47] LC were larger and had more[46] or longer[21] dendrites in DED patients than in healthy controls. These findings were paralleled by a lower CNFD and higher corneal nerve tortuosity and corneal nerve beading in DED patients; surprisingly, LC area and dendrite length correlated positively with CNFD and negatively with beading.[21]

Elevated densities of LC combined with reduced CNFL, CNFD, and CNBD are a common finding in CIDP, multiple sclerosis, diabetic neuropathy, and DED which are all assumed to include at least some autoimmune involvement. In contrast, we did not find these combinations in our patients. LC density was not different from healthy controls, the subtype of dendritic LC in association with nerve fibers was even lower in both patient groups. Hence, autoimmune mechanisms may be less involved in corneal changes in FMS or SFN. Instead, alterations may be caused by a reduced release of neurotrophic factors by LC as these cells play a key role in maintaining corneal[21] and epidermal[48] innervation.

As CNFL and $dLC_{fiber\ assoc.}$ are altered similarly in FMS and SFN patients, our findings may hint toward common pathophysiological pathways determining corneal LC and nerve interactions in small fiber pathology of both diseases. However, CNBD was not different from healthy controls in FMS patients, whereas SFN patients showed a lower CNBD than controls or FMS patients. Also, while FMS patients had lower CNFD than healthy controls, SFN patients did not. This may indicate denervation at a more proximal part of nerves in FMS than in SFN which also fits the finding of reduced stromal nerve width in FMS patients[13] and parallel findings on unmyelinated dermal nerve fiber bundles to be thinner in FMS patients than in SFN patients as well as healthy controls.[8]

In SFN patients, LC density was predicted by age, in FMS patients it was not. Surprisingly, disease duration was not predictive of any CCM parameter in FMS and SFN patients. This may indicate that the observed corneal changes do not accumulate over time. In our cohort, FMS patients also had longer disease duration than SFN patients which may be due to underrepresentation of FMS patients with a short disease duration as the diagnostic process for FMS often takes more time than for SFN.[49]

Another common finding in both FMS and SFN patients are symptoms of DED. In a recent study, FMS patients scored higher in the OSDI questionnaire than healthy controls but did not differ in Schirmer's test results or tear film break up time.[50] Another study found self-reported eye pain and dry eyes as well as reduced vision related quality of life to be common in chronic musculoskeletal pain patients with and without FMS diagnosis.[51] In turn, autoimmune diseases, such as Sjogren's syndrome may cause SFN[52]; hence, DED may be more frequent among SFN patients.

Our data confirm that FMS and SFN patients do have dry eyes more frequently than healthy controls. We did not include a complete diagnostic workup for DED in our study[36] but assessed symptoms of dry eyes (OSDI questionnaire) and applied an objective test for tear fluid deficiency (Schirmer's test) which should ensure a robust approximation of the actual DED prevalence in each group. DED can influence corneal LC and nerve fibers per se and its high prevalence among our patients may cause a bias. However, we compared FMS and SFN patients with and without positive dry eye screening and found no differences in corneal LC or nerve fiber parameters. We assume that the observed differences in corneal LC and nerve fiber parameters between patients suffering from FMS or SFN and healthy controls were independent of the high frequency of dry eyes in FMS and SFN patients. Instead, dry eyes may be an additional feature of both diseases. In SFN, this is in line with other autonomic and trophic symptoms. Another possible explanation may be the more frequent use of anticholinergic drugs among FMS and SFN patients resulting in an increase of iatrogenic dry eye symptoms.[53]

Tear film concentration of PGE_2 were higher and of PGD_2 lower in patients with dry eyes correlating with symptom severity.[37] We did not find any differences in tear fluid prostaglandin concentrations between those patients with and without DED. This may be due to a selection bias since analysis was only possible in patients with a sufficient amount of tear fluid collected.

There are some limitations to our study: Our assessment included the examination of the corneal sensitivity using Chochet-Bonnet esthesiometry prior to CCM. Although we believe that this should be of minor influence, we cannot exclude a potential effect on corneal LC activation. Next, CCM can indicate contact between LC and nerves, but to prove an ultrastructural interaction, high-resolution ex vivo techniques are required. We can only assume that $dLC_{fiber\ assoc.}$ are interacting with associated nerve fibers. Finally, we enhanced tear flow for tear fluid collection by facial application of mint oil to the study participants, which may have influenced prostaglandin concentrations.

We demonstrate that CCM is a powerful tool to identify even subtle differences in small fiber pathology. Our finding of differences between FMS and SFN patients in CNBD indicates that precise examination of the corneal sub-basal nerve plexus may be instrumental to differentiate between both conditions. We also identify a reduced interaction between dendritic LC and corneal nerve fibers and speculate that reduced neurotrophic support from LC may contribute to nerve fiber loss in patients with FMS and SFN.

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CONFLICT OF INTEREST

R.A.M. has received honoraria for presentations from Novo Nordisk, Pfizer, and Merck and research support from Pfizer. C.S. has received consulting fees and speaker honoraria from Air Liquide, Alnylam, Astellas, CSL Behring, Grifols, LFB, Pfizer, Sanofi Genzyme, Shire, UCB. N.Ü. has received honoraria for presentations from Shire Corp.; she has received research support from Sanofi Genzyme and Shire Corp. The other authors report no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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