

# Small Nerve Fiber Damage and Langerhans Cells in Type 1 and Type 2 Diabetes and LADA Measured by Corneal Confocal Microscopy

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**PURPOSE.** Increased corneal and epidermal Langerhans cells (LCs) have been reported in patients with diabetic neuropathy. The aim of this study was to quantify the density of LCs in relation to corneal nerve morphology and the presence of diabetic neuropathy and to determine if this differed in patients with type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and latent autoimmune diabetes of adults (LADA).

**METHODS.** Patients with T1DM ( $n = 25$ ), T2DM ( $n = 36$ ), or LADA ( $n = 23$ ) and control subjects ( $n = 23$ ) underwent detailed assessment of peripheral neuropathy and corneal confocal microscopy. Corneal nerve fiber density (CNFD), branch density (CNBD), length (CNFL) and total, immature and mature LC densities were quantified.

**RESULTS.** Lower CNFD ( $P < 0.001$ ), CNBD ( $P < 0.0001$ ), and CNFL ( $P < 0.0001$ ) and higher LC density ( $P = 0.03$ ) were detected in patients with T1DM, T2DM, and LADA compared to controls. CNBD was inversely correlated with mature ( $r = -0.5$ ;  $P = 0.008$ ), immature ( $r = -0.4$ ;  $P = 0.02$ ) and total ( $r = -0.5$ ;  $P = 0.01$ ) LC density, and CNFL was inversely correlated with immature LC density ( $r = -0.4$ ;  $P = 0.03$ ) in patients with T1DM but not in patients with T2DM and LADA.

**CONCLUSIONS.** This study shows significant corneal nerve loss and an increase in LC density in patients with T1DM, T2DM, and LADA. Furthermore, increased LC density correlated with corneal nerve loss in patients with T1DM.

**Keywords:** Langerhans cells, corneal confocal microscopy, type 1 diabetes, type 2 diabetes, LADA

Diabetic peripheral neuropathy (DPN) is the most prevalent complication of diabetes mellitus.<sup>1</sup> The etiology of diabetic neuropathy is complex, and, although hyperglycemia and hyperlipidemia are major drivers, recent studies suggest a significant contribution from immune and inflammatory components.<sup>2</sup> Corneal confocal microscopy (CCM) is a non-invasive ophthalmic technique that has been used to demonstrate corneal nerve loss in patients with DPN.<sup>3</sup> CCM can also be used to quantify Langerhans cells (LCs) and stromal keratocytes<sup>4,5</sup> in images comparable with histochemical methods.<sup>6</sup> Corneal LCs are professional antigen-presenting cells of the cornea.<sup>7</sup> LCs characterized by a cell body and dendrites reside primarily in the basal epithelium or sub-basal layer of the cornea with an average cell density of 21 to 34 cells/mm<sup>2</sup> in the central cornea, and they lie in close proximity to corneal nerve fibers.<sup>8</sup>

In healthy subjects, the majority of LCs are mature with dendrites and are found in the peripheral cornea, whereas immature LCs without dendrites are found in the central cornea.<sup>9,10</sup> Trauma, infection, and cytokines and chemokines can lead to activation and maturation of LCs.<sup>11</sup>

Experimental and clinical studies support the role of inflammation in the pathogenesis of DPN.<sup>12</sup> Lauria et al.<sup>13</sup> reported an increase in the number of LCs and a reduction in the intraepidermal nerve fiber density in the footpad of streptozotocin diabetic rats. Increased epidermal LC density has been related to a loss of intraepidermal nerve fiber density in patients with painful diabetic neuropathy.<sup>14</sup> Experimental studies have demonstrated an association between increased LC density and corneal nerve fiber loss in murine models of type 1 diabetes mellitus (T1DM) and type 2 (T2DM).<sup>15,16</sup> We have shown an increase in corneal LC



density in adults with mild diabetic peripheral neuropathy<sup>17</sup> and an increase in LC density and corneal nerve loss in children with T1DM.<sup>4</sup> We have previously shown more severe DPN and greater corneal nerve loss in patients with latent autoimmune diabetes in adults (LADA) compared with patients with T2DM.<sup>18</sup> The purpose of this study was to assess if there are differences in the density of LCs and their association with corneal nerve loss in patients with T1DM, T2DM, or LADA.

## METHODS

### Study Subjects

Subjects with T1DM ( $n = 25$ ), T2DM ( $n = 36$ ), or LADA ( $n = 23$ ) and healthy age-matched controls ( $n = 23$ ) were studied. Patients with a history of connective tissue or infectious disease, malignancy, deficiency of B<sub>12</sub> or folate, chronic renal or liver failure, current or active diabetic foot ulceration, contact lens wear, or ocular or systemic disease (other than diabetes) affecting the cornea were excluded. The research adhered to the tenets of the Declaration of Helsinki and was approved by the Greater Manchester Research Ethics Committee. Each participant provided informed consent prior to participation in the study.

### Clinical and Peripheral Neuropathy Assessment

Lipid profile (total cholesterol, low-density lipoprotein and high-density lipoprotein cholesterol, triglycerides), glycated hemoglobin (HbA1c), and body mass index were measured in each participant. The simplified neuropathy disability score (NDS) was used to examine neurological deficits for vibration, pinprick, temperature perception, and presence or absence of ankle reflexes.<sup>19</sup> The neuropathy symptom profile (NSP) was used to evaluate neurological symptoms<sup>20</sup>; it consists of 38 questions categorized into separate groups of sensory dysfunction, autonomic neuropathy, and weakness of the head and neck, chest, upper limbs, and lower limbs. The vibration perception threshold (VPT) was evaluated using a Neurothesiometer (Scientific Laboratory Supplies, Wilford, Nottingham, UK) on the tip of a big toe. A consultant neurophysiologist undertook electrodiagnostic studies using a Dantec Keypoint system (Dantec Dynamics, Skovlunde, Denmark) equipped with a thermistor (Dantec DISA temperature regulator) to maintain the limb temperature between 32°C and 35°C. Peroneal motor nerve conduction velocity (PMNCV) was tested.

### Corneal Confocal Microscopy

Prior to CCM examination, the ocular surface was assessed using slit-lamp biomicroscopy. CCM examination was performed for both eyes using laser scanning corneal confocal microscopy (Retinal Tomograph III Rostock Cornea Module, Heidelberg Engineering, Heidelberg, Germany) following our published protocol.<sup>21</sup> Six CCM images (three per eye) of the corneal sub-basal nerve plexus from the central cornea were selected for corneal nerve and LC evaluation. The main criteria for the image selection were contrast and quality of the image, position, depth of the sub-basal nerve plexus, and absence of artifacts. Images were analyzed using CCMetrics (The University of Manchester, Manchester, UK) by a single expert in a masked manner.<sup>22</sup> We quantified corneal nerve fiber density (CNFD; total number of

main nerves per square millimeter [no./mm<sup>2</sup>]), corneal nerve branch density (CNBD; total number of branches per square millimeter [no./mm<sup>2</sup>]), corneal nerve fiber length (CNFL; total length of main nerves and nerve branches per square millimeter [mm/mm<sup>2</sup>]).<sup>23</sup>

The same six CCM images were also used to quantify LC density. LCs were identified as bright, white structures.<sup>10</sup> LCs less than 50 μm in length with no dendritic structures were defined as immature cells, and LCs with a length greater than 50 μm and dendritic structures were defined as mature cells. The total LC density (no./mm<sup>2</sup>) was quantified using the NBD feature, and the length of the cell was quantified using the NFL feature in CCMetrics.<sup>4</sup>

### Statistical Analysis

The analysis was carried out using SPSS Statistics 22.0 for Windows (IBM Corporation, Armonk, NY, USA). The Shapiro–Wilk test was employed to assess whether the data were normally distributed. Based on their distribution, data are expressed as mean ± standard deviation (SD) or as median and interquartile range (IQR). Fisher's exact test was used to test the association between two categorical variables. Based on normality, Spearman and Pearson correlations were used to test the association between CCM parameters and LC density. Analysis of variance with Bonferroni correction was used to compare means among groups.  $P < 0.05$  was considered significant. Graphs were created using Prism 7.0 for Windows (GraphPad Software, La Jolla, CA, USA).

## RESULTS

### Demographic and Laboratory Results

Demographic data are presented in Table 1. Age ( $P = 0.6$ ) and sex ( $P = 0.4$ ) were comparable among the groups (Table 1). The duration of diabetes was significantly higher in the T1DM group ( $19.4 \pm 7.6$  years) compared with the LADA group ( $11.9 \pm 9.6$  years;  $P = 0.001$ ) but was comparable to that for the T2DM group ( $15.1 \pm 4.9$  years;  $P = 0.06$ ). HbA1c was significantly higher in patients with T1DM ( $63.0 \pm 15.0$  mmol/mol;  $P < 0.0001$ ), LADA ( $83.0 \pm 25.8$  mmol/mol;  $P < 0.0001$ ), or T2DM ( $66.0 \pm 15.4$  mmol/mol;  $P < 0.0001$ ) compared with controls ( $35.4 \pm 2.9$  mmol/mol), and patients with LADA had a significantly higher HbA1c compared with T1DM ( $P = 0.001$ ) and T2DM ( $P = 0.002$ ). Patients with T2DM had a significantly higher body mass index ( $31.7 \pm 5.2$ ) compared with patients with T1DM ( $26.8 \pm 4.4$ ;  $P = 0.001$ ) or LADA ( $27.1 \pm 4.4$ ;  $P = 0.003$ ) and controls ( $27.4 \pm 4.6$ ;  $P = 0.006$ ). Total cholesterol was significantly lower in the T1DM ( $4.0$  mmol/L; IQR, 3.5–4.7;  $P < 0.0001$ ), LADA ( $4.5$  mmol/L; IQR, 3.8–5.2;  $P = 0.006$ ), and T2DM ( $3.8$  mmol/L; IQR, 3.4–4.7;  $P < 0.0001$ ) groups compared with controls ( $5.3$  mmol/L; IQR, 4.8–5.9) and in the T2DM group compared with the LADA group ( $P = 0.02$ ). Low-density lipoprotein was significantly lower in patients with T1DM ( $2.0$  mmol/L; IQR, 1.8–2.3;  $P < 0.0001$ ), LADA ( $2.4$  mmol/L; IQR, 1.8–2.9;  $P = 0.01$ ), or T2DM ( $1.8$  mmol/L; IQR, 1.3–2.3;  $P < 0.0001$ ) compared with healthy controls ( $3.0$  mmol/L; IQR, 2.7–3.3). High-density lipoprotein was significantly lower in patients with T2DM ( $1.0$  mmol/L; IQR, 0.9–1.2) compared with healthy controls ( $1.6$  mmol/L; IQR, 1.1–1.9;  $P < 0.0001$ ) and patients with T1DM ( $1.6$  mmol/L; IQR, 1.2–2.0;  $P < 0.0001$ ) or LADA ( $1.4$  mmol/L; IQR,

**TABLE 1.** Clinical and Demographic Data in Healthy Controls and Patients With T1DM, LADA, or T2DM

	Control (n = 23)	T1DM (n = 25)	LADA (n = 23)	T2DM (n = 36)	P
Male, n (%)	11 (47.8)	17 (68.0)	18 (50.0)	14 (52.2)	0.4
Age (y), mean ± SD	54.1 ± 11.1	53.3 ± 11.7	50.5 ± 11.5	57.7 ± 7.5	0.6
Diabetes duration (y), mean ± SD	N/A	19.4 ± 7.6*	11.6 ± 9.6	15.1 ± 4.9	<0.0001
BMI (kg/m <sup>2</sup> ), mean ± SD	27.4 ± 4.6	26.8 ± 4.4	27.1 ± 4.4	31.7 ± 5.2*,†,‡	<0.001
HbA1c (%), mean ± SD	5.4 ± 0.2	7.9 ± 1.4*,†	9.7 ± 2.4†	8.2 ± 1.4*,†	<0.001
HbA1c (mmol/mol), mean ± SD	35.4 ± 2.9	63.0 ± 15.0*,†	83.0 ± 25.8†	66.0 ± 15.4*,†	<0.001
Total cholesterol (mmol/L), median (IQR)	5.3 (4.8–5.9)	4.0 (3.5–4.7)†	4.5 (3.8–5.2)†	3.8 (3.4–4.7)*,†	<0.001
HDL (mmol/L), median (IQR)	1.6 (1.1–1.9)	1.6 (1.2–2.0)	1.4 (1.1–1.7)	1.0 (0.9–1.2)*,†,‡	<0.001
Triglycerides (mmol/L), median (IQR)	1.5 (1.0–1.8)	1.0 (0.8–1.4)	1.2 (0.7–2.0)	1.8 (1.1–3.2)*,†,‡	0.003
LDL (mmol/L), median (IQR)	3.0 (2.7–3.3)	2.0 (1.8–2.3)†	2.4 (1.8–2.9)†	1.8 (1.3–2.3)†	<0.001
NDS, median (IQR)	0.0 (0.0–0.0)	2.0 (0.5–4.5)†	2.0 (0.5–6.0)†	2.0 (1.0–5.0)†	<0.001
NSP, median (IQR)	0	2.0 (1.0–7.0)†	4.0 (3.5–8.0)†	2.5 (1.3–7.2)†	0.001
VPT, median (IQR)	5.5 (4.9–10.9)	13.6 (10.3–20.8)†	8.7 (6.1–17.7)	11.1 (10.4–17.3)†	0.01
PMNCV (m/s), median (IQR)	47.7 (45.9–49.4)	42.0 (39.15–43.1)†	42.8 (37.9–44.5)†	44.6 (42.2–46.6)	<0.0001

P represents statistical difference among all groups. All symbols represent statistically significant differences. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Statistically significant differences are in bold.

\* Significant difference compared with LADA.

† Significant difference compared with control.

‡ Significant difference compared with T1DM.

**TABLE 2.** Langerhans Cell Density and Corneal Nerve Parameters in Healthy Controls and Patients With T1DM, LADA, or T2DM

	Control (n = 23)	T1DM (n = 25)	LADA (n = 23)	T2DM (n = 36)	P
Langerhans cell density (no./mm <sup>2</sup> ), median (IQR)					
Mature	3.1 (0.0–9.4)	6.2 (2.3–12.0)	8.3 (3.1–22.5)*	7.5 (2.3–24.5)*	0.059
Immature	17.5 (8.3–42.9)	28.1 (18.7–77.6)	42.7 (31.2–103.1)*	39.3 (11.6–98.1)	0.03
Total	22.5 (9.4–46.9)	34.4 (19.8–95.3)*	47.9 (34.4–138.5)*	51.6 (16.1–107.6)*	0.03
CNFD (no./mm <sup>2</sup> ), median (IQR)	30.2 (28.1–37.5)	23.4 (20.2–28.1)*	22.9 (20.8–27.1)*	26.1 (21.9–30.9)*	<0.001
CNBD (no./mm <sup>2</sup> ), median (IQR)	88.8 (71.9–96.9)	44.8 (28.5–61.6)*	54.2 (41.7–69.8)*	48.4 (32.3–75.0)*	<0.001
CNFL (mm/mm <sup>2</sup> ), mean ± SD	27.8 ± 4.1	19.1 ± 4.2*	20.3 ± 7.4*	21.3 ± 6.3*	<0.001

P represents statistical difference among all groups. Statistically significant differences are in bold.

\* Significant difference compared to control.

1.1–1.7; *P* = 0.001). Triglycerides were significantly higher in patients with T2DM (1.8 mmol/L; IQR, 1.1–3.2) compared with patients with T1DM (1.0 mmol/L; IQR, 0.8–1.4; *P* < 0.0001) or LADA (1.2 mmol/L; IQR, 0.7–2.0; *P* = 0.03) but not controls (1.5 mmol/L; IQR, 1.0–1.8; *P* = 0.07).

### Neuropathy Assessment

NDS was significantly higher in T1DM (2.0; IQR, 0.5–4.5; *P* = 0.01), LADA (2.0; IQR, 0.5–6.0; *P* = 0.006), and T2DM (2.0; IQR, 1.0–5.0; *P* = 0.003) compared with controls and was comparable among diabetes groups. NSP was significantly higher in the T1DM (2.0; IQR, 1.0–7.0; *P* = 0.001), LADA (4.0; IQR, 3.5–8.0; *P* = 0.001), and T2DM (2.5; IQR, 1.3–7.2; *P* = 0.001) groups compared with healthy controls and was comparable among the diabetes groups. VPT was significantly higher in T1DM (13.6; IQR, 10.3–20.8; *P* < 0.0001) and T2DM (11.1; IQR, 10.4–17.3; *P* = 0.002) but not in LADA (8.7; IQR, 6.1–17.7; *P* = 0.1) compared with controls (5.5; IQR, 4.9–10.9). PMNCV was significantly lower in T1DM (42.0 m/s; IQR, 39.1–43.1; *P* = 0.002) and LADA (42.8 m/s; IQR, 37.9–44.5; *P* = 0.001), but not in T2DM (44.6 m/s; IQR, 42.19–46.6; *P* = 0.1) compared with healthy controls (47.7 m/s; IQR, 45.9–49.4) (Table 1). The severity of abnormality in both VPT and PMNCV was indicative of a mild neuropathy.

### Corneal Confocal Microscopy

CNFD was significantly lower in T1DM (23.4/mm<sup>2</sup>; IQR, 20.2–28.1; *P* < 0.0001), LADA (22.9/mm<sup>2</sup>; IQR, 20.8–27.1; *P* < 0.0001), and T2DM (26.1/mm<sup>2</sup>; IQR, 21.9–30.9; *P* = 0.002) compared with controls (30.2/mm<sup>2</sup>; IQR, 28.1–37.5), with no significant difference between T1DM and LADA (*P* = 0.7), T1DM and T2DM (*P* = 0.2), or LADA and T2DM (*P* = 0.09). CNBD and CNFL were also significantly lower in T1DM (CNBD: 44.8/mm<sup>2</sup>, IQR, 28.5–61.6, *P* < 0.0001; CNFL: 19.1 mm/mm<sup>2</sup> ± 4.2, *P* < 0.0001), LADA (CNBD: 54.2/mm<sup>2</sup>, IQR, 41.7–69.8, *P* < 0.0001; CNFL: 20.3 mm/mm<sup>2</sup> ± 7.4, *P* < 0.0001), and T2DM (CNBD: 48.4/mm<sup>2</sup>, IQR, 32.3–75.0, *P* < 0.0001; CNFL: 21.3 mm/mm<sup>2</sup> ± 6.3, *P* < 0.0001) compared with controls (CNBD: 88.8/mm<sup>2</sup>, IQR, 71.9–96.9; CNFL: 27.8 mm/mm<sup>2</sup> ± 4.1). There was no significant difference in CNBD and CNFL between T1DM and LADA (*P* = 0.1, *P* = 0.9), T2DM and LADA (*P* = 0.4, *P* = 0.9), or T1DM and T2DM (*P* = 0.3, *P* = 0.7) (Table 2).

### Langerhans Cells

Total LC density was significantly higher in patients with T1DM (34.4/mm<sup>2</sup>; IQR, 19.8–95.3; *P* = 0.05), LADA (47.9/mm<sup>2</sup>; IQR, 34.4–138.5; *P* = 0.002), or T2DM (51.6/mm<sup>2</sup>; IQR, 16.1–107.6; *P* = 0.05) compared with controls (22.5/mm<sup>2</sup>; IQR, 9.4–46.9) (Table 2, Figs. 1 and 2).

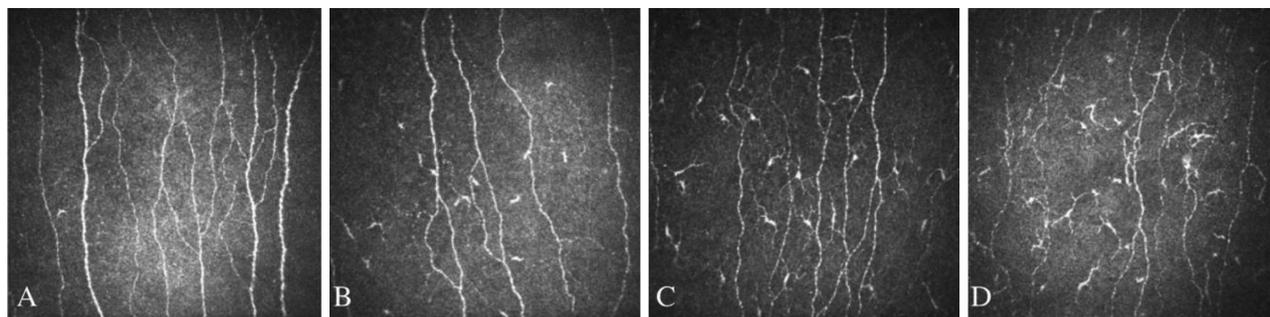


FIGURE 1. CCM images of a healthy control (A) and age-matched patient with T1DM (B), LADA (C), and T2DM (D).

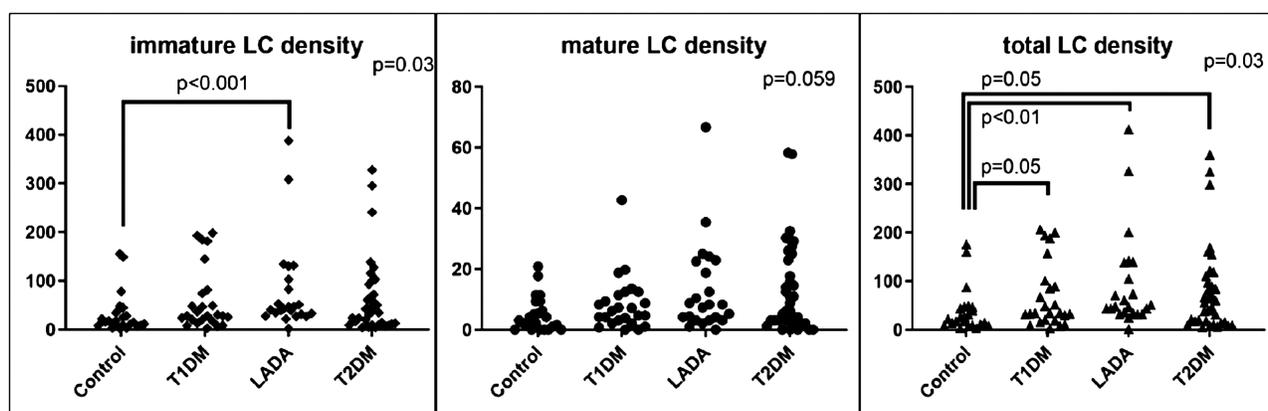


FIGURE 2. Immature LC density (A), mature LC density (B), and total LC density (C) in controls and patients with T1DM, LADA, or T2DM.

There was no significant difference between T1DM and LADA ( $P = 0.1$ ), T2DM and LADA ( $P = 0.8$ ), or T1DM and T2DM ( $P = 0.3$ ). Mature LC density was higher in patients with LADA ( $8.3/\text{mm}^2$ ; IQR, 3.1–22.5;  $P = 0.01$ ) or T2DM ( $7.5/\text{mm}^2$ ; IQR, 2.3–24.5;  $P = 0.02$ ) but not in patients with T1DM ( $6.2/\text{mm}^2$ ; IQR, 2.3–12.0;  $P = 0.1$ ) compared with controls ( $3.1/\text{mm}^2$ ; IQR, 0.0–9.4). There was no significant difference between T1DM and LADA ( $P = 0.2$ ), T2DM and LADA ( $P = 0.8$ ), or T1DM and T2DM ( $P = 0.4$ ). Immature LCs were significantly higher in patients with LADA ( $42.7/\text{mm}^2$ ; IQR, 31.2–103.1;  $P = 0.002$ ), but their numbers did not differ in patients with T1DM ( $28.1/\text{mm}^2$ ; IQR, 18.7–77.6;  $P = 0.06$ ) or T2DM ( $39.3/\text{mm}^2$ ; IQR, 11.6–98.1;  $P = 0.06$ ) compared with controls ( $17.5/\text{mm}^2$ ; IQR, 8.3–42.9). Sixty-five percent of controls and 95% of patients with diabetes had mature LCs in their central cornea. Both patients and controls had immature LCs in their central corneas.

There was a significant negative correlation between CNBD and mature ( $r = -0.5$ ,  $P = 0.008$ ), immature ( $r = -0.4$ ,  $P = 0.02$ ), and total ( $r = -0.5$ ,  $P = 0.01$ ) LC density and between CNFL and immature LC density ( $r = -0.4$ ,  $P = 0.03$ ) in patients with T1DM. There was no significant association between corneal nerve parameters and LC density in patients with T2DM and LADA (Table 3).

### Neuropathy According to Toronto Consensus Among Patients With Diabetes

Patients were divided into two groups: those with DPN ( $n = 25$ ) and those without DPN ( $n = 59$ ) according to the Toronto consensus,<sup>24</sup> which requires the presence of symptoms (abnormal NSP) or signs of neuropathy (NDS > 2 or VPT > 15) and abnormal peroneal nerve conduction velocity (PMNCV < 40 m/s). In our cohort, 36% of subjects with T1DM, 22% of subjects with T2DM, and 34% of subjects with LADA had DPN, without a significant difference among the groups ( $P = 0.4$ ). Considering all patients with diabetes, CNFD was significantly lower in patients with DPN ( $20.8/\text{mm}^2$ ; IQR, 18.74–26.82) compared with those without DPN ( $25.0/\text{mm}^2$ ; IQR, 21.87–29.16;  $P = 0.03$ ), whereas CNBD (with DPN:  $52.1/\text{mm}^2$ , IQR, 29.27–65.15; without DPN:  $50.0/\text{mm}^2$ , IQR, 40.17–67.70;  $P = 0.7$ ) and CNFL (with DPN:  $18.9 \text{ mm}/\text{mm}^2$ , IQR, 16.32–22.57; without DPN:  $20.9 \text{ mm}/\text{mm}^2$ , IQR, 17.18–24.31;  $P = 0.2$ ) did not differ significantly. Mature LCs (with DPN:  $9.4/\text{mm}^2$ , IQR, 3.51–22.70; without DPN:  $6.2/\text{mm}^2$ , IQR, 2.08–14.06;  $P = 0.2$ ), immature LCs (with DPN:  $46.42/\text{mm}^2$ , IQR, 25.52–128.85; without DPN:  $39.58/\text{mm}^2$ , IQR, 14.06–71.87;  $P = 0.2$ ), and total LCs (with DPN:  $61.2/\text{mm}^2$ , IQR, 31.63–148.85; without DPN:  $43.74/\text{mm}^2$ , IQR, 17.49–89.58;  $P = 0.2$ ) did not

TABLE 3. Correlation Between Langerhans Cell Density and Corneal Nerve Parameters in Patients With T1DM, LADA, or T2DM

	T1DM			LADA			T2DM		
	CNFD	CNBD	CNFL	CNFD	CNBD	CNFL	CNFD	CNBD	CNFL
Mature LC density (no./mm <sup>2</sup> )	-0.2 ( <i>P</i> = 0.3)	-0.5 ( <i>P</i> = 0.008)	-0.4 ( <i>P</i> = 0.5)	-0.1 ( <i>P</i> = 0.6)	-0.3 ( <i>P</i> = 0.1)	-0.2 ( <i>P</i> = 0.4)	0.0 ( <i>P</i> = 0.8)	-0.1 ( <i>P</i> = 0.7)	-0.1 ( <i>P</i> = 0.7)
Immature LC density (no./mm <sup>2</sup> )	-0.2 ( <i>P</i> = 0.3)	-0.4 ( <i>P</i> = 0.029)	-0.4 ( <i>P</i> = 0.038)	-0.1 ( <i>P</i> = 0.8)	-0.3 ( <i>P</i> = 0.1)	-0.1 ( <i>P</i> = 0.5)	0.0 ( <i>P</i> = 0.9)	-0.2 ( <i>P</i> = 0.2)	-0.3 ( <i>P</i> = 0.1)
Total LC density (no./mm <sup>2</sup> )	-0.2 ( <i>P</i> = 0.3)	-0.5 ( <i>P</i> = 0.015)	-0.4 ( <i>P</i> = 0.07)	-0.1 ( <i>P</i> = 0.8)	-0.4 ( <i>P</i> = 0.07)	-0.2 ( <i>P</i> = 0.4)	0.0 ( <i>P</i> = 0.8)	-0.2 ( <i>P</i> = 0.2)	-0.42 ( <i>P</i> = 0.2)

differ significantly between patients with and without DPN. In patients with DPN, CNBD was inversely correlated with mature ( $r = -0.4$ ,  $P = 0.03$ ), immature ( $r = -0.5$ ,  $P = 0.01$ ), and total LC ( $r = -0.5$ ,  $P = 0.007$ ) density.

## DISCUSSION

CCM has shown corneal nerve loss in patients with T1DM,<sup>25</sup> T2DM,<sup>26–28</sup> or LADA.<sup>29</sup> Recent studies have shown that corneal nerve loss may be more severe in patients with LADA compared with T2DM<sup>18</sup> and in patients with T2DM compared with T1DM.<sup>30,31</sup> However, in the current study, we show comparable corneal nerve loss in patients with T1DM, T2DM, and LADA, with a significantly lower CNFD in patients with DPN. The differences in relation to the severity of corneal nerve loss in different types of diabetes found in previous studies and in relation to DPN may reflect the severity of DPN and the method used to quantify corneal nerve parameters.<sup>28,32,33</sup> In this respect, Andersen et al.<sup>28</sup> showed a reduction in CNFD but no difference in CNBD or CNFL between patients with and without DPN. However, their patients with T2DM had excellent glycemic control, and they used automated corneal nerve quantification which is not as sensitive in detecting a reduction in nerve branches. Furthermore, the relatively low specificity reported for CCM simply reflects the fact that small fiber abnormalities detected by CCM occur earlier than large fiber abnormalities, whereas the criteria used to define DPN are biased toward the assessment of large fibers.<sup>31</sup>

Inflammation may play a major role in the development of diabetic peripheral neuropathy.<sup>34</sup> In the present study we show an increase in LCs in patients with T1DM, T2DM, or LADA and an association between increased LC density and reduced CNBD. Studies have suggested a pathophysiological interaction between nerves and LCs,<sup>35</sup> and they are located close to peripheral nerves in the skin and cornea.<sup>36</sup> Furthermore, small nerve fibers can influence immune cell activity by releasing cytokines and neuropeptides.<sup>37</sup> LCs also express neurotrophic factors such as ciliary neurotrophic factor which can promote nerve regeneration.<sup>38</sup> Previous studies have reported increased LC density and corneal nerve loss in immune-mediated conditions such as Behçet's disease,<sup>39</sup> multiple sclerosis,<sup>40</sup> and chronic inflammatory demyelinating polyneuropathy.<sup>41</sup> In patients with diabetes, increased TNF- $\alpha$ <sup>42</sup> and corneal<sup>4,17</sup> and epidermal<sup>13,43</sup> LCs have been reported. Using first-generation CCM, we reported an increase in LC density in patients with mild diabetic neuropathy but did not assess for differences between T1DM and T2DM.<sup>17</sup> In a more recent study, with third-generation CCM and higher resolution, we have reported an increase in both mature and immature LCs in children with T1DM.<sup>4</sup> In both studies, there was no association between LC density and the severity of corneal nerve damage. To our knowledge, our study is the first to report an association between corneal nerve loss and an increase in LC density in patients with T1DM but not LADA or T2DM. The differences between these studies may be attributed to significant differences in age, duration of diabetes, and sample size. Leppin et al.<sup>15</sup> reported a significant association between increased corneal dendritic cells and nerve fiber loss in a mouse model of T1DM, although, Yu et al.<sup>44</sup> reported a positive association between LCs and corneal nerves. The heterogeneity of autoimmune diabetes<sup>45–47</sup> may also explain differences in the degree of inflammation among T1DM, LADA, and T2DM. Hence, the inverse association between LC and corneal nerve

parameters in patients with T1DM could be explained by an impaired function of antigen-presenting cells. However, we cannot exclude the effect of disease duration, glycemic control, and other metabolic abnormalities on this relationship, explaining why we did not find an association between LC density and corneal nerve parameters in patients with LADA and T2DM.

Learning from the field of cardiovascular disease, we know that biomarkers that predict the development of cardiovascular disease are paramount in the management of patients at risk.<sup>48,49</sup> Studies that assess the utility of CCM in quantifying LCs in relation to nerve damage may help establish surrogate imaging markers for diabetic neuropathy.

The limitations of this study include the relatively small sample size in each group and the cross-sectional nature of the study, which prevents conclusions regarding cause and effect between increased LCs and corneal nerve loss in patients with DPN. The patients had mild DPN; therefore, we cannot comment on the role of LCs in subclinical or more advanced DPN. Although large and small fiber measurements were comparable, we acknowledge that differences in glycemic control, lipid metabolism, and hypoglycemic treatment could have had an impact on our findings.

In conclusion, CCM has identified comparable corneal nerve loss and an increase in LC density in patients with T1DM, T2DM, or LADA. There was an association between increased LCs and corneal nerve loss in T1DM. Larger longitudinal studies are required to assess the relationship between LCs and corneal nerves in DPN.

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