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RESEARCH ARTICLE

Corneal confocal microscopy in chronic inflammatory demyelinating polyneuropathy

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Abstract

Objective: There is an unmet need for better diagnostic tools to further delineate clinical subsets of heterogeneous chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and multifocal motor neuropathy (MMN) to facilitate treatment decisions. Corneal confocal microscopy (CCM) is a noninvasive and reproducible nerve imaging technique. This study evaluates the potential of CCM as a diagnostic surrogate in CIDP and MMN. **Methods**: In a cross-sectional prospective approach, 182 patients and healthy controls were studied using CCM to quantify corneal nerve damage and immune cell infiltration. **Results**: Patients with CIDP and MMN had a reduction in corneal nerve fiber (CNF) measures and an increase in corneal immune cell infiltrates. In CIDP, CNF parameters decreased with increasing duration of disease. The number of dendritic cells in proximity to CNFs was increased in patients with early disease and correlated with the degree of motor affection. A further reduction in CNF parameters and an increase in nondendritic cells were observed in patients with painful neuropathy. In CIDP patients with antineuronal antibodies the number of nondendritic cells was increased. **Interpretation**: Our findings suggest that CNF loss may reflect severity of neuropathy and quantification of distinct cells around the CNF plexus may help in stratifying CIDP subtypes, clinical course, and disease activity. However, further longitudinal studies are required before CCM can be considered as a valid surrogate endpoint for patients with CIDP and MMN.

Introduction

Immune-mediated disorders of the peripheral nervous system (PNS) exhibit a wide variety of clinical presentations and can be challenging in their diagnosis and treatment.[1,2] Despite established criteria to diagnose chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), there is significant clinical heterogeneity in relation to clinical course and response to treatment.[3] In atypical cases, CIDP can be difficult to diagnose and a significant number of patients with CIDP remain unrecognized.[4]

In addition, the lack of objective and feasible measures to differentiate such subtypes makes it impossible to predict the responsiveness of available therapies.[5] Thus, there is an unmet need for subclassifying chronic inflammatory disorders of the PNS with noninvasive techniques to better define the underlying pathology and improve systematic categorization.

Corneal confocal microscopy (CCM), a rapid noninvasive ophthalmic imaging technique, has been demonstrated to quantify axonal loss in a variety of peripheral neuropathies including hereditary sensory and autonomic neuropathy,[6] Charcot–Marie–Tooth disease type 1A,[7] Fabry disease,[8] and idiopathic small fiber neuropathy.[9] It has also been widely used to evaluate diabetic neuropathy in multiple studies[10] demonstrating that this technique is

a viable surrogate endpoint for early diagnosis,[11] stratification of neuropathy severity,[12] and assessing the response to treatment.[13] This technique is highly reproducible[14,15] and well-tolerated.[16] An automated and standardized image analysis method for quantification of corneal nerve morphology has also been developed.[17,18]

An emerging body of evidence indicates that small fiber involvement and early axonal involvement is present in CIDP.[19,20] As such, CCM may be a useful measure of nerve damage in patients with CIDP. However, studies exploring corneal involvement in CIDP are limited and conflicting to date. While corneal sensitivity was normal,[21] a recent study using CCM in 16 patients with CIDP demonstrated corneal nerve fiber (CNF) loss.[22] CCM can also quantify the presence and density of Langerhans cells in Bowman's layer of the cornea in patients with diabetes.[23] Using the latest third generation HRT III (Heidelberg retinal tomograph III), it can also be used to classify and quantify Langerhans cells into a mature phenotype (dendritic cells) or an immature phenotype (nondendritic cell) and provide insight into immune alterations in vivo.[24] It has been suggested that direct contact between dendritic cells and the sub-basal nerve plexus, seen in CCM, may trigger nerve fiber damage.[25]

In this study we investigated the potential of CCM as a meaningful diagnostic tool in a large cohort of well-characterized patients with CIDP and multifocal motor neuropathy (MMN) compared to control subjects. Detailed quantification of corneal nerve and immune cell morphology was related to electrophysiological parameters, severity of neuropathy, clinical course, response to therapy, and laboratory findings.

Material and Methods

Patient assessment and diagnostic classification

The study was approved by the local Ethics Committee (Ethics Committee University of Dusseldorf, #4870). All patients gave their written informed consent prior to the inclusion into the study. The study was in accordance with the Declaration of Helsinki. A total of 182 patients and healthy controls were studied of which 88 patients were diagnosed as having CIDP, including 12 neuropathy patients with monoclonal gammopathy of undetermined significance (MGUSN), whereas six patients were classified as suffering from MMN. Patients were recruited between 2014 and 2015 at the Department of Neurology, Dusseldorf, Germany.

The diagnoses of CIDP or MMN were based on the respective criteria of the Peripheral Nerve Society/European Federation of Neurological Societies.[26–28] Patients were diagnosed with MGUSN when immunoglobulin (Ig) M or IgG was detectable in the serum, with and without antineural antibodies.[29,30] Due to the small sample size, MGUSN patients were subsumed into the CIDP group for further analysis as per previous suggestions.[29,31,32]

Eighty-five age- and sex-matched healthy controls were recruited at the Centre for Endocrinology and Diabetes at the University of Manchester, United Kingdom (North Manchester Ethics Committee). These controls had a full blood workup and extensive neurological assessment in the form of clinical examination and neurophysiology to exclude neuropathy. The results of these controls were equivalent to those of a control group of clinically healthy subjects recruited at the Department of Neurology, Dusseldorf, Germany. Since no further assessment was performed with this internal control group, these data were not included in the current study.

Corneal confocal microscopy

All study participants were scanned using a laser CCM (Heidelberg Retinal Tomograph III Rostock Cornea Module [HRT III RCM]; Heidelberg Engineering GmbH, Heidelberg, Germany) as published previously.[18]

Highly experienced examiners performed the CCM scans in a blinded fashion. Approximately 200 images were captured from each eye. Using a section mode, several scans of the entire depth of the cornea were recorded by turning the fine focus of the objective lens backward and forward to focus on the sub-basal nerve plexus at the center of the cornea. The bundles run parallel to the Bowman layer before dividing and turning upward toward the surface to terminate as individual axons underneath the surface epithelium. On average, six high-quality images, showing the fiber layer configured vertically, were analyzed and the mean of these results was calculated. Images that included components of both the nerve fiber layer and epithelium or endothelium were not used for analysis. Images with artifacts derived from compression of the eye by the microscope were also excluded from this analysis.[16] A fully automated image analysis algorithm was used to quantify corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), and corneal nerve fiber length (CNFL) using purpose-written, proprietary software (ACCMetrics; M.A. Dabbah, Imaging Science and Biomedical Engineering, Manchester, UK). Cell infiltrates were analyzed manually in a blinded fashion in the same images that were also used to quantify corneal nerves. Based on their morphology only, cells were classified into dendritic cells (DC) and nondendritic cells (NC) and further subclassified into those with nerve fiber contact (F) or no nerve fiber con-

tact (P-peripheral) (Fig. 1). In addition, total cell number (TC) was calculated. For dendritic cells, nerve fiber contact was regarded as positive if one or more of the dendrites or the silhouette of the cell body contacted a nerve fiber. For nondendritic cells, nerve fiber contact was regarded as positive if the silhouette of the cell body contacted a nerve fiber. These assessments were standardized for the area analyzed.

Laboratory examinations

Standard procedures to establish the diagnosis and to exclude other causes of neuropathy were performed as part of the routine clinical workup, including laboratory diagnostics, cerebrospinal fluid examination, and electrophysiology.

The parameters studied included HbA1c, oral glucose tolerance test, vitamin and folic acid levels, renal parameters and liver function tests, differential cell counts, C-reactive protein, serum electrolytes, immunofixation, serum electrophoresis, thyroid function tests, antinuclear antibodies, antibodies to extractable nuclear antigen, antineutrophil cytoplasmic autoantibody, rheumatoid factor, and serology for infections such as borreliosis. If in the serum a monoclonal immunoglobulin was detected, the presence of Bence Jones proteins in the urine was assessed.

The cerebrospinal fluid was examined for cell count, protein levels, and serology. Albuminocytologic dissociation was regarded positive with elevated cerebrospinal fluid protein (>50 mg/dL) and a leukocyte count of <10/mm.[3]

In addition, the following serum antibodies were determined: anti-CASPR2, anti-MBP, anti-MAG, antimyelin (global test), antimyelin of the peripheral nerve (global test), antiunmyelinated fibers (global test), each for IgM, IgG, and IgA. Furthermore, antiganglioside antibodies were analyzed against GM1, GM2, GM3, GD1a, GD1b, GT1b, and GQ1b (each for IgG and IgM). These analyses were performed by a commercial laboratory, for clinical purposes.

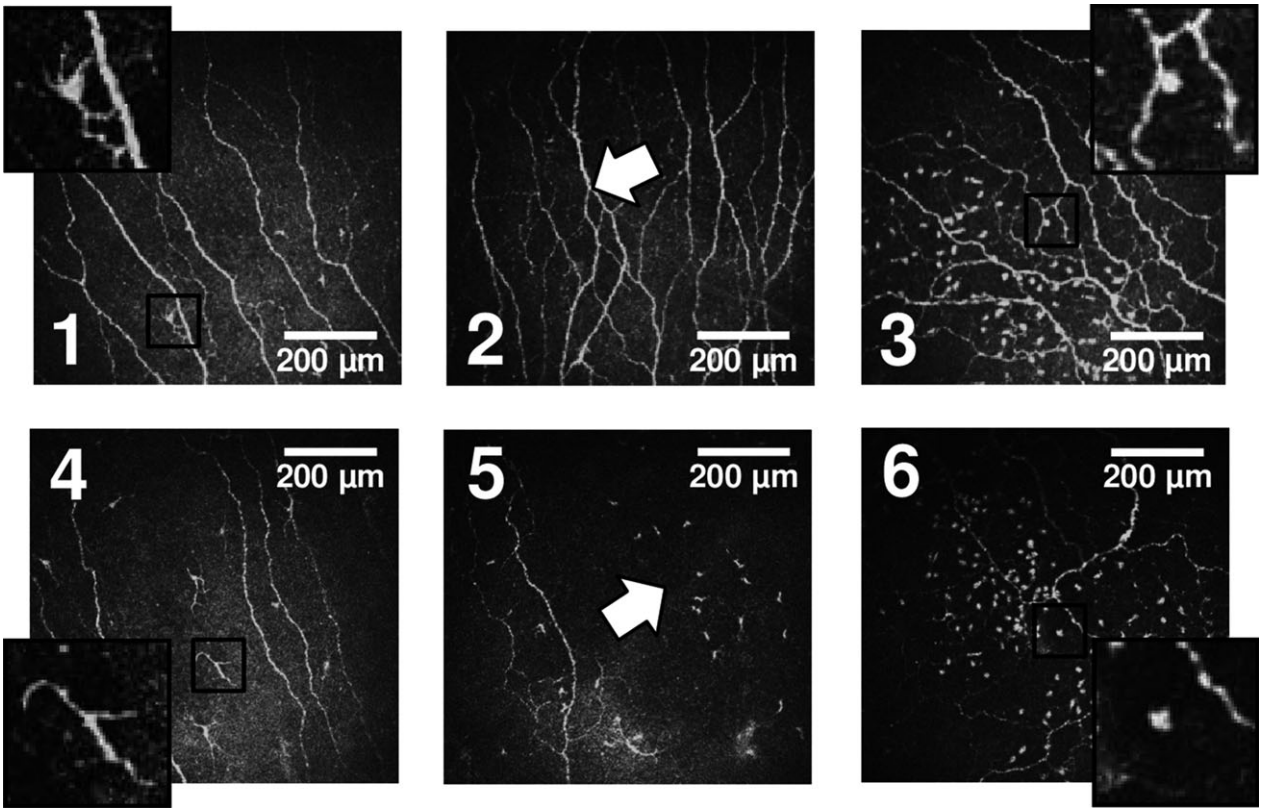

Figure 1. Corneal confocal microscopy (CCM) images. Confocal microscopy images from the sub-basal layer of the cornea. Sub-basal corneal nerves (arrow) in a healthy subject (2) without cell infiltrates are depicted. Decreased density of corneal nerve fibers in a chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) patient (5) with cell infiltrates. CIDP patient (3) with corneal nerve fiber reduction and nondendritic cell infiltrates in proximity to corneal nerve fibers (box) and in the periphery (box) (6). Dendritic cells were classified as in the periphery (box) (4) and in proximity to nerve fibers (box) (1). Magnification as indicated.

Electrophysiological assessment

All electrophysiological assessments[33] included standard nerve conduction studies of sensory and motor nerves of the lower and upper limbs, needle electromyography in affected muscles, quantitative sensory testing, heart rate variability, and sympathetic skin response.

Clinical assessment

Motor dysfunction was quantified using the Inflammatory Neuropathy Cause and Treatment (INCAT) disability score as described previously.[34,35] Cranial nerve affection was regarded positive if the patient presented with at least one cranial nerve affected for more than 5 months, and after excluding other underlying causes of cranial nerve involvement. Treatment decisions were at the discretion of the treating physician, and according to international guidelines.[36] Patients were considered clinically stable when the INCAT score remained unchanged over a period of 6 months. End-of-dose effects were classified positive when patients reported fluctuations or relapses in conjunction with tapering of treatment, at least five times in 6 months, within 14–40 days after therapy (intravenous immunoglobulins [IVIg] or corticosteroids), which was declining after the next therapeutic intervention. Patients were classified as responders if they improved by at least one point on the INCAT score after 4 months of treatment.[37]

Details of the clinical course and the individual symptoms were assessed on the basis of information from the clinical records and a questionnaire designed for this study that included questions from the Neuropathic Pain Symptom Inventory.[38]

Statistical analysis

All data are presented as mean, standard deviation, and *P*-values, and the analysis was performed using GraphPad Prism software version 4.0 (GraphPad Software, Inc., La Jolla, CA). Differences were assessed using unpaired *t*-tests with 95% confidence interval, one-way analysis of variance (ANOVA) with Bonferroni and Dunnett's test, with $P < 0.05$ considered as statistically significant (*P*s: *<0.05, **<0.01, and ***<0.001).

Results

CCM in immune-mediated neuropathies

CNFD, CNBD, and CNFL were significantly reduced in patients with CIDP, MMN, and MGUSN compared to healthy controls (Fig. 2A1–3). All patients had dendritic cells in the cornea. The CIDP group had more dendritic cells in proximity to CNFs compared to the other groups. The majority of dendritic cells in MGUSN patients had fiber contacts (data not shown), while most of the cells in the periphery were nondendritic (Fig. 2A4–5).

CCM and the duration of neuropathy

Patients with inflammatory neuropathy were categorized into five groups based on the time from the appearance of first symptoms: <1 year, 1–2 years, 2–5 years, 5–10 years, and >10 years. CNBD and CNFL were significantly lower compared to healthy controls at all time points (Fig. 2B1–2). Dendritic cells with CNF contacts were significantly increased in patients with <1 year of disease duration, were lower in those with >1 year of disease, but rose progressively with increasing duration of the disease (Fig. 2B3). This was applicable only to dendritic cells since neither nondendritic cells with corneal nerve fiber contact (NCF) nor the total cell number (TC) increased in a duration-dependent manner (data not shown).

None of the patients were free of infiltrating cells in the first 2 years after diagnosis, whereas 18% of patients were without infiltrates 2–5 years after diagnosis, 11% were without cell infiltrates in the 5–10 years group, and 16% were without cell infiltrates in the >10 years group (Fig. 2B4).

CCM and clinical presentation of patients

Patients with motor impairment had significantly higher numbers of dendritic and nondendritic cells, with or without nerve fiber contact (Fig. 3A1–4), and total numbers of cells in proximity to nerve fibers (Fig. 3A5) when compared to the control group. The numbers of dendritic cells with or without nerve fiber contacts were significantly higher in patients with motor compared to sensory impairment (Fig. 3A3–5).

Cell infiltrates (cutoff: >10 cells per mm^2) of dendritic cells with nerve fiber contact (DCF) were found in 16% of patients with motor impairment, 50% of patients with sensory impairment, and 57% of patients with both sensory and motor impairment. Cell infiltrates (cutoff: >10 cells per mm^2) of dendritic cells without CNF contacts (DCP) were found in 100% of patients with motor impairment, 50% with sensory impairment, and 50% of patients with both sensory and motor impairment (Fig. 3A6–7).

Cell infiltrates (cutoff: >10 cells per mm^2) of nondendritic cells without CNF contacts (NCP) were found in 16% of patients with motor impairment, 27% of patients with sensory impairment, and 26% of patients with both

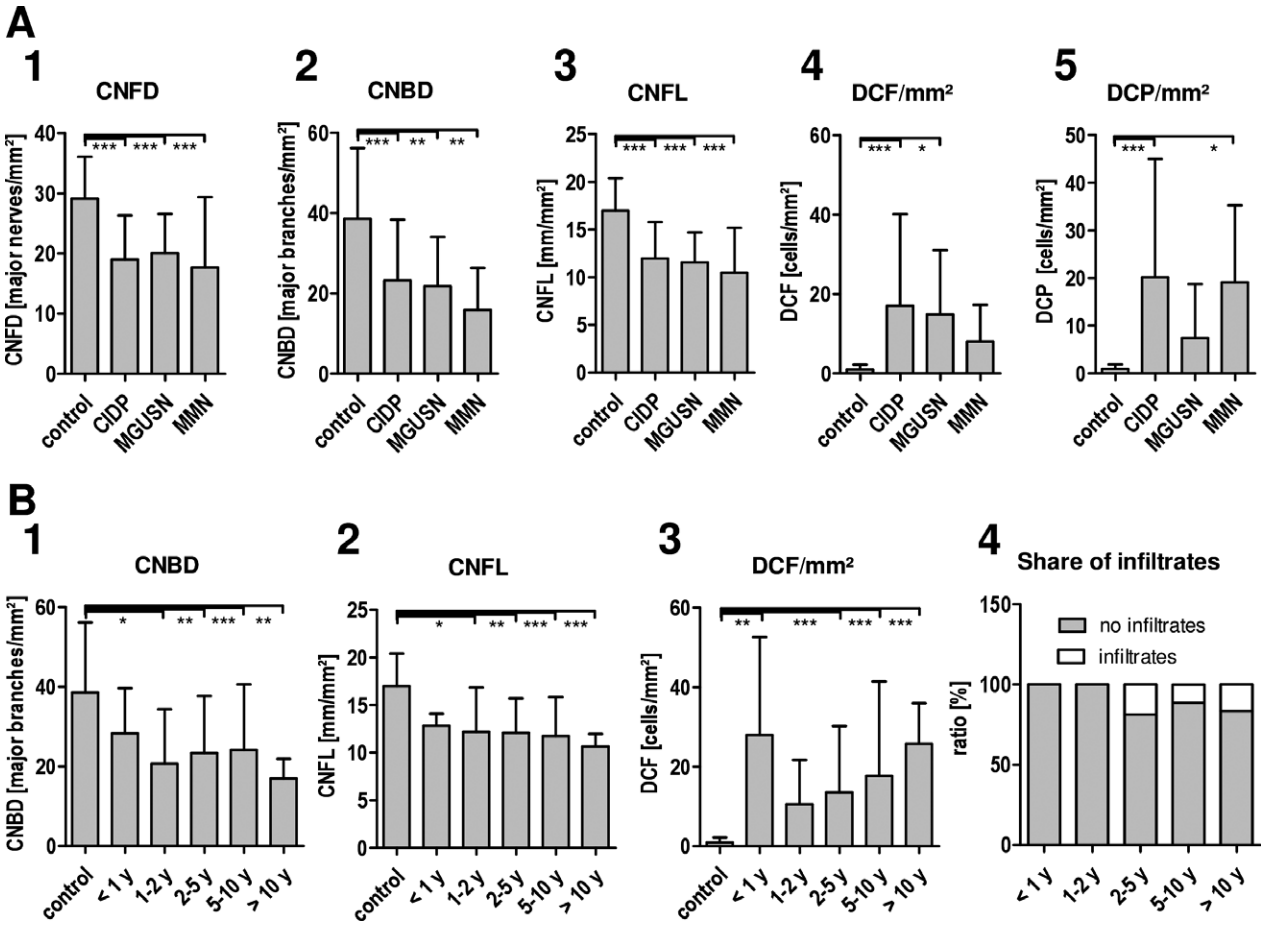

Figure 2. Immune-mediated neuropathies and the duration of neuropathy. (A) 1–5: Corneal nerve fiber density (CNFD) (1), branch density (CNBD) (2) and length (CNFL) (3) in patients with chronic inflammatory demyelinating polyneuropathy (CIDP, $n = 76$), neuropathy with monoclonal gammopathy of undetermined significance (MGUSN, $n = 12$), multifocal motor neuropathy (MMN, n = 6), and healthy controls ($n = 85$). Dendritic cell with fiber contact (DCF) (4), dendritic cell without fiber contact (periphery) (DCP) (5), are graphed. (B) 1–4: Patients grouped according to time since symptom manifestation. Less than 1 year since onset ($n = 3$), 1–2 years ($n = 8$), 2–5 years ($n = 32$), 5–10 years ($n = 35$), and more than 10 years (n = 6) since onset, compared with healthy controls ($n = 86$). Corneal nerve branch density (CNBD) (1) and length (CNFL) (2) are graphed, as well as dendritic cells in proximity to nerve fibers (DCF) (3), and the share of patients with and without infiltrates in total (4). Asterisks define the *P*-values as follows: *<0.05, **<0.01, and ***<0.001.

sensory and motor impairment. Cell infiltrates (cutoff: >10 cells per mm^2) of nondendritic cells with nerve fiber contacts (NCP) were detectable in 16% of patients with motor impairment, 11% of patients with sensory impairment, and 23% of patients with both sensory and motor impairment (data sets not shown).

CCM and INCAT score

CNFL and CNFD revealed significantly lower values in patients with no motor disability than in patients with slight motor disability. Beside this, we did not find a significant correlation between the severity of motor impairment in CIDP and CCM measures (Fig. 3B1–3). Dendritic cells in proximity to CNFs (DCF) were significantly increased in all INCAT groups compared to controls (Fig. 3B4). The significantly lower CNF parameters in patients without motor disability (INCAT = 0) could only be shown for the lower extremities (Fig. S1A1–4). Further measures of clinical neurological impairment and nerve conduction parameters did not correlate with CCM parameters.

CCM and painful neuropathy

CNFD, CNBD, and CNFL were significantly reduced in patients with both painful and painless neuropathy, and CNFD and CNFL were further significantly reduced in patients with painful compared to painless neuropathy (Fig. 41–3). Nondendritic cells without (NCP) and with

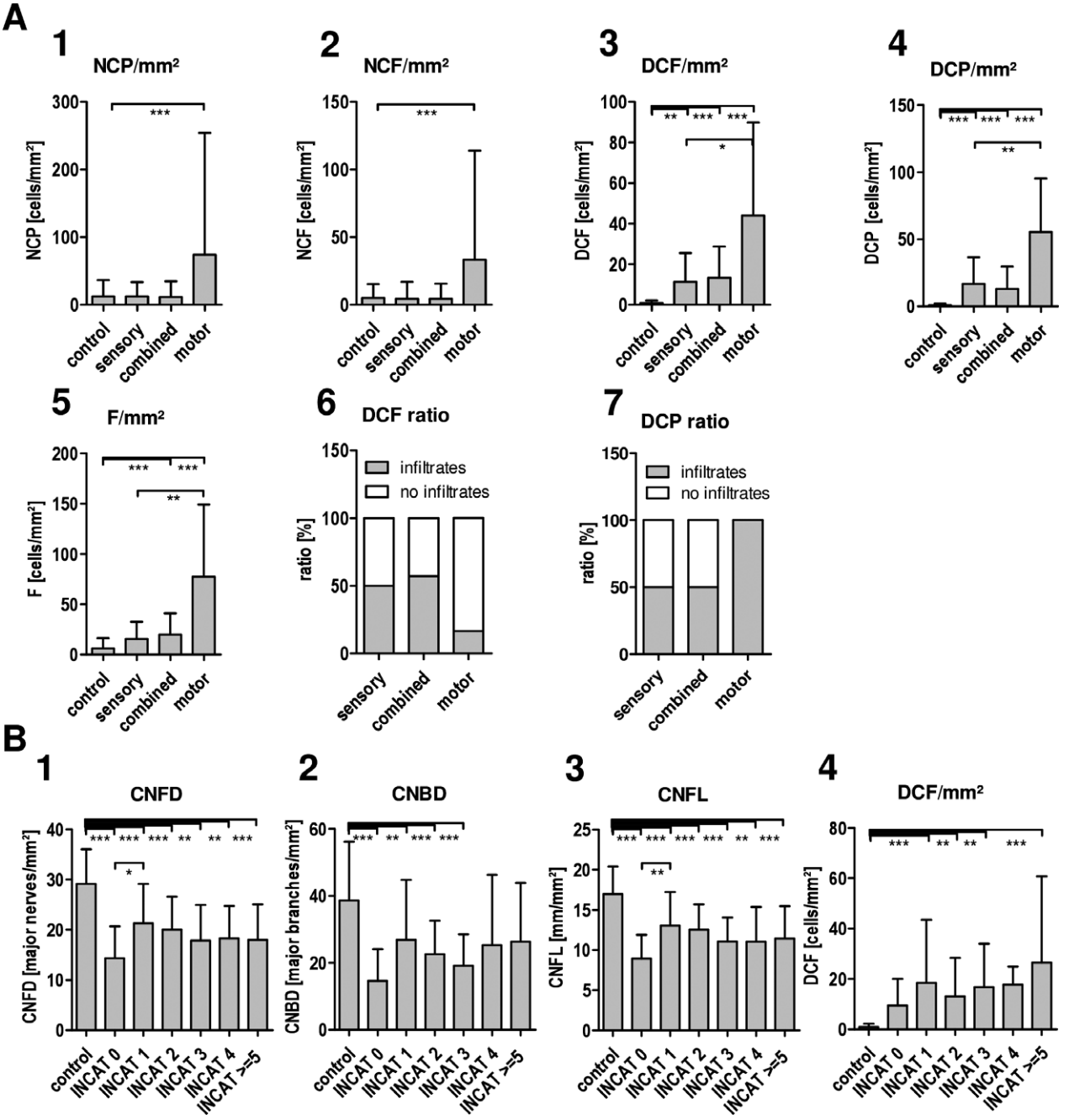

Figure 3. Motor impairment. (A) 1–7: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) patients according to clinical presentation (motor, $n = 6$; sensory, $n = 18$; or combined sensory/motor, $n = 56$, compared with the control group, $n = 86$). Density of nondendritic cell in the periphery (NCP) (1), nondendritic cell in proximity to corneal nerve fibers (NCF) (2), dendritic cell in proximity to corneal nerve fibers (DCF) (3), dendritic cell in the periphery (DCP) (4), and the total number of cells with nerve fiber contacts (F) (5). Ratios of the number of patients with more than 10 cells per mm^2 to the number of patients with less than 10 cells per mm^2 for motor, sensory, or combined impairment are graphed for dendritic cells in proximity to corneal nerve fibers (DCF) (6) and for dendritic cells in the periphery (DCP) (7). (B) 1–4: Motor disability of CIDP patients was quantified using the Inflammatory Neuropathy Cause and Treatment (INCAT) score (control, $n = 85$; INCAT 0, $n = 9$; INCAT 1, $n = 32$; INCAT 2, $n = 21$; INCAT 3, $n = 14$; INCAT 4, $n = 5$; INCAT >5, $n = 8$) and plotted in relation to corneal nerve fiber density (CNFD) (1), branch density (CNBD) (2), and length (CNFL) (3) and density of dendritic cells in proximity to corneal nerve fibers (DCF) (4). Asterisks define the *P*-values as follows: *<0.05, **<0.01, and ***<0.001.

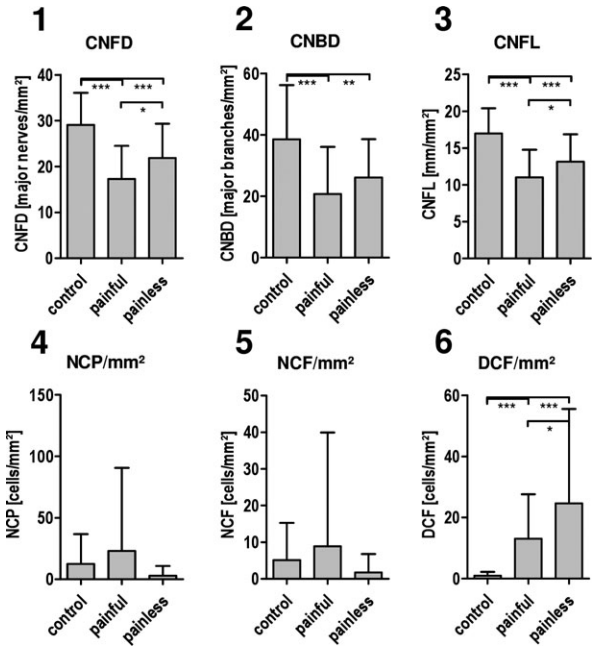

Figure 4. Clinical distribution and painful neuropathy. 1–6: Patients with inflammatory neuropathies classified for the presence of painful (n = 47) compared with painless (n = 20) neuropathy. Corneal nerve fiber density (CNFD) (1), branch density (CNBD) (2), and length (CNFL) (3) and densities of dendritic cells in the periphery (DCP) (4), nondendritic cells in proximity to nerve fibers (NCF) (5), and dendritic cells in proximity to nerve fibers (DCF) (6) are depicted. Asterisks define the P-values as follows: *<0.05, **<0.01, and ***<0.001.

nerve fiber contact (NCF) did not differ between patients with and without painful neuropathy (Fig. 4A4–5). However, the number of dendritic cells with CNF contacts (DCF) was significantly reduced in patients with painful neuropathy and we found a shift toward nondendritic cells with nerve fiber contacts, in patients with painful neuropathy (Fig. 4A6).

CCM and paraclinical laboratory findings

There was no significant difference in CNFD, CNFL, or CNBD in antibody-positive compared to antibody-negative patients (Fig. 5A1–2). The total number of nondendritic cells and the total number of cells were significantly higher in the antibody-positive group (Fig. 5A3–4), but cell numbers in proximity to CNFs did not differ in antibody-positive compared to antibody-negative patients (Fig. 5A5).

There was no difference in CNFD, CNBD, or CNFL between MAG antibody-positive (MAG+) and MAG antibody-negative (MAG−) patients. Total cell and nondendritic cell density was significantly greater in the MAG+ compared to MAG− group (Fig. 5B1–2).

There was no difference in CNFD, CNBD, or CNFL in patients with antibodies against the GM1 ganglioside (GM1+) compared to patients without antibodies to GM1 (GM1−) (Fig. 5C1). However, GM1+ patients had significantly higher total cell and nondendritic cell densities compared to GM1− patients (Fig. 5C2–3).

There was no difference for CNFD, CNBD, and CNFL between patients with MGUS versus MGUS-negative patients (data not shown). In patients with MGUS, there was no difference in the numbers of nondendritic cells with (NCF) and without (NCP) CNF contact and the total cell number (Fig. S1B1–3).

CCM and confounders

In CIDP patients, gender, hypertension, diabetes, and spinal stenosis had no confounding impact on CCM parameters. Regression analysis did not show a confounding effect for the presence of diabetes. There was a slight age-dependent increase for the total cell count in controls, but regression analysis did not show a confounding effect for age.

Discussion

In this study, we have assessed the relationship of CCM with clinical and paraclinical aspects in patients with immune-mediated peripheral neuropathies.

We show that patients with CIDP, MGUSN, and MMN exhibit a significant reduction in CNF parameters. Our findings confirm recently published data demonstrating a reduction in CNF parameters in a cohort of 16 patients with CIDP.[39] Our data are further supported by a previous study showing intraepidermal nerve fiber loss[40] and unmyelinated nerve fiber degeneration in sural nerve biopsies[41] of patients with CIDP. Patients with MMN showed the most marked reduction in CNF parameters, indicative of axonal loss. Although the electrophysiological finding of conduction block in motor but not sensory nerves is a hallmark of MMN,[42] slow progressive axonal degeneration has been shown in the majority of MMN patients.[43] Sensory impairment during the course of MMN with conduction block has been described previously. However, patients showing a reduction in Sural nerve action potential (SNAP) amplitude presented with a more severe disease and more prominent axonal loss.[44] Some authors suggest that a subgroup of MMN patients may subsequently develop electrophysiological sensory abnormalities and this entity may represent an overlap between classical MMN and multifocal acquired demyelinating sensory and motor neuropathy.[45] Further studies with larger patient numbers should evaluate the extent to which MMN patients are affected in CCM and whether

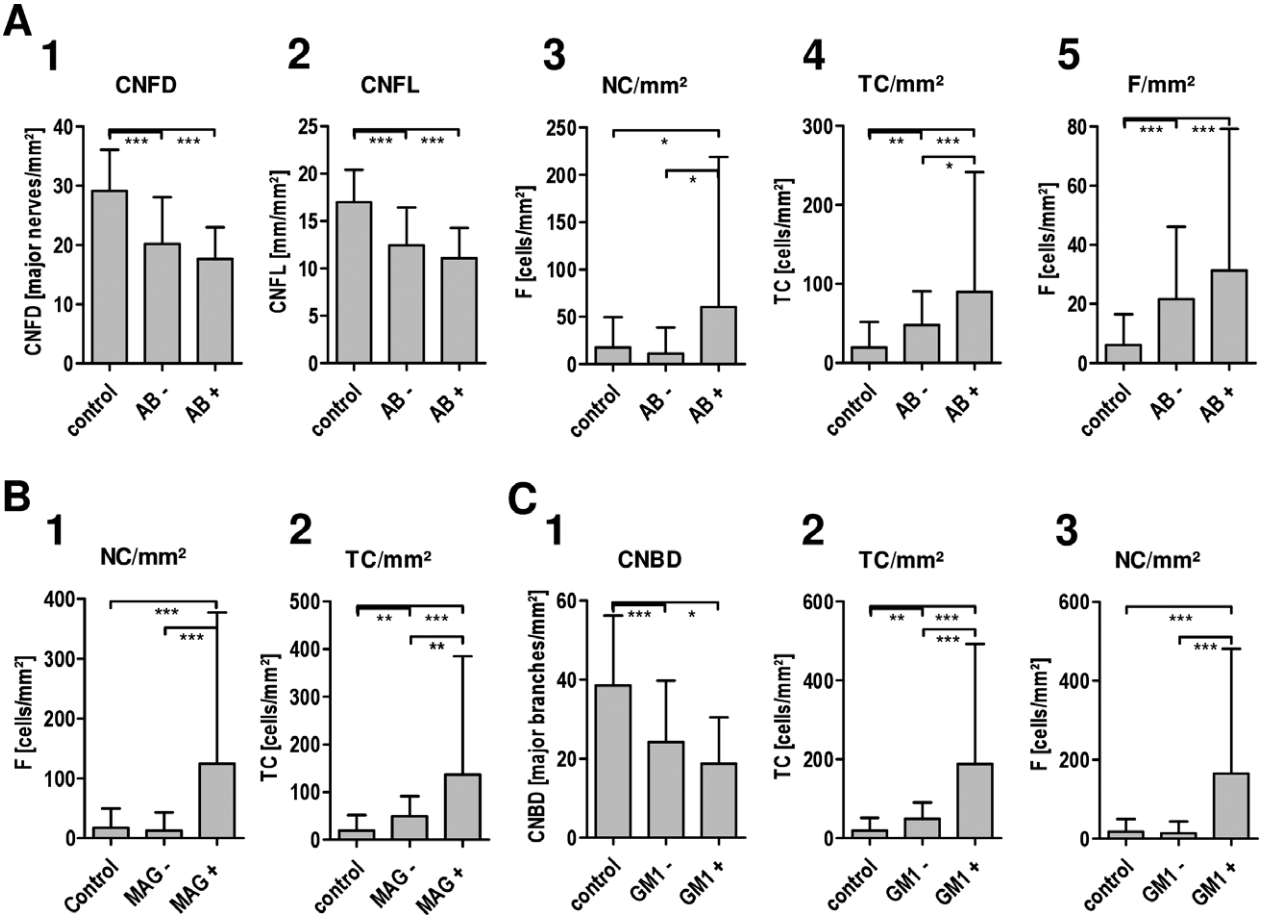

Figure 5. Antineuronal antibodies. (A) 1–5: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) patients with antineuronal antibodies (AB+, $n = 16$) were compared with CIDP patients without antineuronal antibodies (AB−, $n = 61$) and the control group ($n = 86$). Corneal nerve fiber density (CNFD) (1) and length (CNFL) (2), and densities of nondendritic cells (NC) (3), total number of cells (TC) (4), and total number of cells in proximity to nerve fibers (F) (5). (B) 1–2: Comparison between CIDP patients positive for anti-MAG antibodies (MAG+, $n = 6$) and those negative for anti-MAG antibodies (MAG−, $n = 71$) and control group ($n = 85$) for the total number of nondendritic cells (NC) (1) and total number of cells (TC) (2). (C) 1–3: Comparison between CIDP patients positive for anti-GM1 antibodies (GM1+, $n = 4$) and those negative for anti-GM1 antibodies (GM1−, $n = 85$) and control group ($n = 85$) for CNBD (1) and densities of the total number of cells (TC) (2) and nondendritic cells (NC) (3). Asterisks define the *P*-values as follows: *<0.05, **<0.01, and ***<0.001.

this impairment is associated with particular clinical features.

We have previously shown that first generation CCM demonstrates increased density of Langerhans cells in the sub-basal layer of the cornea in patients with diabetes.[23] On the basis of morphology, the latest third generation HRT III can be used to classify and quantify Langerhans cells into dendritic and nondendritic cells and their relationship with CNFs. A recent experimental study in diabetic mice has shown that direct contact between dendritic cells and CNFs in the sub-basal plexus may trigger nerve fiber damage.[25] Here we show a significant increase of cellular infiltrates in the cornea in CIDP, MGUSN, and MMN, which may reflect the immune basis of these conditions.

Duration of symptoms and CCM results

There was a time-dependent decrease in CCM fiber parameters in relation to the duration of the first appearance of symptoms. Within 1 year of symptoms starting, patients had high numbers of cells in proximity to CNFs. Our data suggest that there is an initial severe immune response in CIDP, although this may not always lead to severe and rapid onset of symptoms. This initial severe immune response is clearly recognized in acute inflammatory demyelinating polyneuropathy (AIDP, Guillain–Barré syndrome), which evokes symptoms in less than 4 weeks.[46,47] This suggests not only the need for further longitudinal studies using CCM in CIDP, but also the need to explore the utility of CCM in AIDP.

Although the majority of patients had an increase in the numbers of dendritic cells with nerve fiber contacts, we also identified a subgroup of patients with inflammatory neuropathy who showed no corneal cell infiltrates. This group may represent a distinct clinical entity with a monophasic time course but with a nadir of symptoms after 2 months; and are therefore classified as having CIDP. Further studies may elucidate whether CCM can reliably differentiate patients with ongoing inflammatory activity from patients with a chronic noninflammatory stage of neuropathy.

Increase of dendritic cells in patients with motor symptoms

Motor impairment is a classical feature in CIDP patients, and even patients whose symptoms are primarily sensory frequently develop motor impairment at later stages of the disease.[31,48] The European Federation of Neurological Societies/Peripheral Nerve Society criteria for diagnosing CIDP are based primarily on motor dysfunction.[49] The good response of this entity to IVIg[50] suggests that these patients may have pronounced inflammatory activity in this subgroup. Therefore of relevance we have shown that patients with motor affection had increased cell counts, particularly dendritic cells without fiber contacts. There are previous reports showing that impairment of the blood–nerve barrier (BNB), as a measure of inflammatory activity is more severe in typical CIDP than in other CIDP subtypes.[51]

There is evidence that immunohistochemical detection of cells in the cornea correlates with morphological findings in CCM.[52] The presumption that the dendritic cells observed with CCM are Langerhans cells and not another subset of dendritic cells is supported by previous findings that Langerhans cell-specific surface markers are expressed by dendritic cells in the corneal and limbal epithelium.[53,54] Due to the fact that there are no animal studies for inflammatory neuropathies and that cell differentiation in the current study was based on morphological aspects, it is speculative to specify which immunological subtype was counted.

CCM correlates with INCAT score

No significant INCAT-dependent decrease in CNF parameters was observed, but patients without motor impairment (INCAT = 0) had a significant reduction in CNF parameters compared to patients with INCAT = 1. Whether this is a result of the multiple comparisons being made or that patients without motor impairment have a shorter duration of the disease and associated treatment is purely speculative.

This assumption is further corroborated by the finding that low CNF parameters were also related to a lack of motor symptoms of the lower extremity and of course in classical CIDP, symptoms usually start in the legs.[29] Furthermore, fibers in the cornea are sensory fibers and patients with INCAT = 0 have a primarily sensory neuropathy. Consistent with the relationship between immune cells and lower fiber parameters, cell infiltrates revealed an INCAT-dependent shift to dendritic cells close to CNFs.

CCM in patients with painful neuropathy

The loss of thinly myelinated Aδ-fibers and unmyelinated C-fibers is associated with autonomic dysfunction and neuropathic pain.[55] We show a greater loss of CNFs in CIDP patients with painful neuropathy, which is in keeping with previous studies showing that diabetic patients with painful neuropathy show a greater reduction in corneal nerve parameters compared to diabetic patients with painless neuropathy.[56] Furthermore, in patients with painful neuropathy we detected a shift toward the infiltration of nondendritic cells, which suggests that these cells may play a pathophysiological role in painful neuropathy. Experimental evidence suggests that peripheral mediators such as prostanoids and nerve growth factor contribute to peripheral pain and C-fiber affection.[57] The infiltrates of cells without dendrites detected in this study may be involved in this scenario. There are recent data that show increased epidermal Langerhans cells in patients with painful small fiber neuropathy[58] and Langerhans cell activation in diabetic mice with mechanical allodynia.[59] In this study, differentiation of infiltrating cells was based on morphology, therefore we can only speculate regarding the involvement of specific immunological subtypes of cells.

Antineuronal antibodies in CIDP lead to increased number of nondendritic cells

The underlying etiology of CIDP is not clear, although in a distinct subgroup, neurogenic antibodies are considered to be the driving force of neuroinflammation.

Patients with at least one abnormal antibody had an increase in total cell number and nondendritic cells, in proximity to CNFs. MAG-positive patients showed an increase in nondendritic cells. A previous skin biopsy study of patients with anti-MAG neuropathy has shown a decrease in epidermal nerve fiber density and IgM deposits along myelinated nerve fibers, especially at the paranodal loops.[60]

Ganglioside antibodies such as GM1 or GD1a are established as the cause of acute motor axonal neuropa-

thy.[61,62] Little is known about the cellular pathophysiology in GM1 or GD1a-positive patients, but in some cases, antibodies against specific epitopes are associated with specific clinical features.[63] Patients with autoimmune demyelinating neuropathy with antiglycosphingolipid antibodies including GM1 and GD1b exhibit more severe BNB disruption than those without such antibodies. This barrier disruption may contribute to the damage in autoimmune demyelinating neuropathy.[64] Although there is no well-defined BNB in the cornea, we showed that patients with antibodies against ganglioside GM1 exhibited an increase in nondendritic cells, and patients with MGUS exhibited an increase in nondendritic cells and total cell count. MGUS patients have been shown to have different clinical and electrophysiological patterns, suggesting likely different pathophysiological mechanisms.[65]

Thus, the increase in nondendritic cells appears to be common in antibody-positive patients. The pathophysiological relevance of this observation requires further study as presumably these cells may play a role in B-cell activation.

Conclusion

In conclusion, we show that CCM can identify axonal loss in all major subtypes of immune-mediated neuropathies and alterations of distinct cells around the corneal nerve plexus and correlates with clinical aspects of neuropathy. While the characterization of cells into dendritic and nondendritic cells based on the morphological criteria should be interpreted with caution, it may provide insights into the underlying pathophysiology. CCM may be a useful technique in the diagnosis and management of patients with inflammatory neuropathy and this warrants further studies.

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Author Contributions

M. S. and B. C. K. contributed to the study concept and design; acquisition of data by M. S., L. H., S. B., and I. N. P.; M. S., L. H., R. G., and R. A. M. did the analysis and interpretation of the data; drafting of the manuscript was carried out by M. S. and B. C. K.; M. S., B. C. K., R. A. M., C. W., R. G., and H.-P. H. did the critical revision of the manuscript for important intellectual content; M. S., L. H., and I. N. P. performed the statistical analysis; administrative, technical, and material supports were provided by M. S., B. C. K., and H. P. H; and study supervision was done by M. S. and B. C. K.

Conflict of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Motor disability, therapeutic response, and monoclonal gammopathy. (A) 1–4: Motor disability of patients was quantified using the INCAT score (INCAT 0, $n = 43$; INCAT 1, $n = 34$; INCAT 2, $n = 6$; INCAT >3, $n = 6$; control, $n = 85$). INCAT score for upper and lower extremities was analyzed separately (top row for upper and bottom row for lower extremities). Corneal nerve fiber density (CNFD) (1), nerve fiber length (CNFL) (2), and branch density (CNBD) (3) and density of dendritic cells in proximity to nerve fibers (DCF) (4). (B) 1–3: Comparison between CIDP patients with (MGUS+, $n = 16$), patients without (MGUS−, $n = 71$), and control group ($n = 85$) for the densities of nondendritic cells in the periphery (NCP) (1), in proximity to nerve fibers (NCF) (2) and total number of cells (TC) (3). Asterisks define the *P*-values as follows: *<0.05, **<0.01, and ***<0.001.

