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RESEARCH ARTICLE

Corneal confocal microscopy to detect early immune-mediated small nerve fibre loss in AL amyloidosis

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Abstract

Objective: Light chain (AL) amyloidosis is a life-threatening disorder characterised by extracellular deposition of amyloid leading to dysfunction of multiple organs. Peripheral nerve involvement, particularly small fibre neuropathy, may be associated with poorer survival. Corneal confocal microscopy (CCM) is a rapid and non-invasive imaging technique to quantify corneal small nerve fibres and immune cells in vivo. We aimed to evaluate CCM as a tool for early diagnosis of peripheral nerve involvement in AL amyloidosis. **Methods**: CCM and nerve conduction studies (NCS) were undertaken in 21 newly diagnosed, treatment-naïve AL amyloidosis patients and 21 age- and sex-matched healthy controls. Corneal nerve fibre density (CNFD), corneal nerve branch density and fibre length, and cell infiltrates were quantified in the sub-basal layer of the cornea. **Results**: There was a significant reduction in CNFD and nerve fibre length, even without large fibre affection and an increase in cell density, particularly around corneal nerve fibres in patients with AL amyloidosis compared to controls. Additionally, cell infiltration correlated with reduced nerve fibre density in patients with AL amyloidosis, but reduced CNFD did not correlate with laboratory parameters of organ dysfunction. **Interpretation**: Our study is the first to show that CCM allows rapid non-invasive identification of early small nerve fibre damage associated with immune cell infiltration in patients with AL amyloidosis. CCM detects peripheral nerve involvement more sensitively than NCS.

Introduction

Immunoglobulin light chain (AL) amyloidosis is a life-threatening systemic disease characterised by extracellular deposition of insoluble amyloid fibrils composed of misfolded immunoglobulin light chains produced in the setting of plasma cell dyscrasias. It is typically found in patients with monoclonal gammopathy, smouldering myeloma, or multiple myeloma and is with an incidence rate of approximately 10 per million per year[1,2] alongside wild-type transthyretin amyloidosis the most common form of systemic amyloidosis.[3] Although cardiac and renal involvements are the most frequent organ manifestations of AL amyloidosis, light chain amyloid fibrils may also be deposited in the liver, gastrointestinal tract, soft tissue, and peripheral nervous system (PNS).[4] Peripheral nerve involvement manifests as a symmetric sensorimotor polyneuropathy, carpal tunnel syndrome or small fibre neuropathy.[3,5,6] Small fibre involvement can lead to severe neuropathic pain or result in autonomic symptoms such as postural hypotension, erectile dysfunction, and gastrointestinal dysmotility,[7,8] impacting significantly on the quality of life of AL amyloidosis patients.[9] PNS involvement may also influence treatment decisions as

chemotherapy for the treatment of AL amyloidosis may cause further neurotoxicity. The mean 5-year survival of patients with AL amyloidosis is ~40% and the severity of small fibre damage may have a prognostic value as a lower intraepidermal nerve fibre density (IENFD) has been associated with poorer survival.[10] However, data on small fibre involvement in AL amyloidosis are scarce.[10,11]

Corneal confocal microscopy (CCM) is a rapid, non-invasive imaging technique to quantify small nerve fibres.[12] A reduction in corneal nerve fibre length (CNFL) and nerve fibre density is a hallmark of small fibre neuropathies and corresponds to reduced IENFD in skin biopsies.[13,14] CCM has proven diagnostic value in diabetic neuropathy,[15–17] hereditary neuropathy,[18,19] idiopathic small fibre neuropathy,[20] and immune-mediated neuropathies.[21,22] CCM has also been used to differentiate inflammatory from non-inflammatory neuropathies based on quantification of cellular infiltrates around the subbasal nerve plexus[22–24] and to identify corneal nerve loss in patients with hereditary transthyretin amyloidosis polyneuropathy (ATTRv),[25] particularly avoiding the floor effect of unobtainable sural nerve amplitudes and IENFD.[26] More recently in a study from China, early subclinical loss of corneal nerves had a high diagnostic utility in ATTRv amyloidosis and it also demonstrated immature Langerhans cell clusters at the inferior whorl.[27]

The utility of CCM for identifying small nerve fibre pathology and associated immune-mediated nerve affection in AL amyloidosis has not been investigated to date. We have undertaken quantification of corneal small nerve fibres and immune cell infiltrates and detailed disease phenotyping to assess the diagnostic utility of CCM and to further understand the pathophysiology of small fibre neuropathy in AL amyloidosis.

Patients and Methods

Patients

All examinations were carried out at the Department of Neurology and Department of Haematology and Stem Cell Transplantation of the University Hospital Essen from October 2019 until May 2021. Data from 21 patients with bioptically confirmed AL amyloidosis were collected prior to treatment initiation. All patients underwent a detailed medical history and evaluation with laboratory testing to rule out other causes of neuropathy, for example, exposure to neurotoxic agents such as alcohol or chemotherapy drugs, or confounding neuropathic conditions such as diabetes, thyroid disorders, and vitamin deficiencies. Patients were explicitly surveyed for small fibre-mediated symptoms such as neuropathic pain, signs of gastrointestinal dysmotility, disturbances of bladder function, orthostatic dysregulation, sudomotor and pupillomotor dysfunction. Proteinuria, glomerular filtration rate (GFR), serum N-terminal pro brain natriuretic peptide (NT-proBNP), and the difference between serum levels of involved and uninvolved free light chains (dFLC) as markers of organ dysfunction and disease activity were assessed (for a detailed list of implemented assessments see Table 1).

A control group of 21 age- and sex-matched healthy individuals was recruited from the University of Manchester, United Kingdom (North Manchester Ethics committee). The participants in the control group underwent blood panel testing and extensive neurological and neurophysiological assessments to exclude neuropathy.

This cross-sectional study was approved by the ethics committee of the University Duisburg-Essen (approval number 20-9583-BO) and the North Manchester Ethics committee (control group). Written informed consent to participate was obtained from all participants in the study.

Nerve conduction studies

All patients underwent detailed nerve conduction studies (NCS) measurements of motor and sensory nerves in the upper and lower extremities on both sides. Distal motor latency, nerve conduction velocity, motor and sensory amplitude (CMAP and SNAP) were assessed. Polyneuropathy was defined as affection of at least three of the examined nerves.

Table 1. List of implemented assessments.

History and clinical examination	Large fibre symptoms and signs
	Small fibre symptoms and signs
	Neuropathic conditions other than amyloidosis
Laboratory tests	HbA1c, vitamin B1, B6, B12, folic acid, TSH, ANA, ANCA, rheumatoid factor, immunofixation, serum electrophoresis, glomerular filtration rate, NT-proBNP, dFLC, proteinuria
Nerve conduction studies	Tibial and sural nerves bilaterally
	Right ulnar nerve (see Additional File S1)
Corneal confocal microscopy	Corneal nerve fibre parameters (CNFD, CNFL, CNBD)
	Corneal cell counts (TC, DCF, DCP, NCF, NCP)

HbA1c, glycated haemoglobin; TSH, Thyroid-stimulating hormone; ANA, antinuclear antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; NT-proBNP, N-terminal pro brain natriuretic peptide; dFLC, difference between serum levels of involved and uninvolved free light chains; CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length; CNBD, corneal nerve branch density; TC, total cell count; DCF, dendritic cells with fibre contact; DCP, dendritic cells in the periphery; NCF, non-dendritic cells with fibre contact; NCP, non-dendritic cells in the periphery.

Corneal confocal microscopy

CCM was performed using a Heidelberg Retina Tomograph (HRT III, Rostock Cornea Module, Heidelberg Engineering, Heidelberg, Germany). A local anaesthetic (0.4% benoxinate hydrochloride) was administered immediately before the examination. Viscotears liquid gel was applied to the eye for lubrication and to establish a thin gel bridge between the corneal surface and a sterile, single-use lens cap. Several scan cycles of the entire depth of the cornea were carried out focusing on the sub-basal nerve plexus at the centre of the cornea. At least six images per patient were analysed, meeting established quality criteria.[28] A well-established software (ACCMetrics Image Analysis tool v1.1, University of Manchester, Manchester, UK) was used for automated quantification of corneal nerve fibre density (CNFD, major nerves/mm^2), CNFL (mm/mm^2), and corneal nerve branch density (CNBD, major branches/mm^2). Corneal cells were counted manually by an independent investigator in a blinded fashion using ImageJ software (version 1.41, National Institutes of Health, Bethesda, Maryland, USA) and were classified according to their morphology, i.e. presence of dendritic cell extensions, and their localization relative to the corneal nerve fibres, as previously described.[22] On that basis, four subtypes, dendritic cells with fibre contact (DCF), dendritic cells in the periphery without fibre contact (DCP), non-dendritic cells with fibre contact (NCF), and non-dendritic cells in the periphery without fibre contact (NCP) were identified. As in our clinical routine, based on an internal reference group, a CNFD of 24 fibres/mm^2 (two standard deviations below the mean of the reference group) was regarded as a threshold for supporting the diagnosis of small fibre neuropathy.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 9.1.0 for Windows, GraphPad Software, San Diego, CA, USA). All data are presented as the mean, standard error of the mean, and p values. Differences for cell and nerve fibre parameters between AL amyloidosis patients and healthy controls were assessed using the Mann–Whitney U test. Differences between patients with or without large fibre neuropathy, respectively, and healthy controls were assessed using a Kruskal–Wallis test and Dunn's test as post hoc test. $p < 0.05$ were considered to be statistically significant. Correlations were calculated using Spearman's rank correlation coefficient.

Results

Twenty-one newly diagnosed AL amyloidosis patients (14 male, 7 female, mean age 62.9 ± 12.7 years) were compared to 21 age- and sex-matched healthy controls (14 male, 7 female, mean age 61.1 ± 8.7 years). Small fibre symptoms were reported by 10 patients (47.6%) with neuropathic pain in 7 (33.3%) patients, orthostatic hypotension in 3 (14.3%), and otherwise unexplained diarrhoea/constipation in 2 (9.3%) patients. For detailed clinical and demographic characteristics of the patients with AL amyloidosis, see Table 2.

Nerve conduction studies

NCS revealed sensorimotor polyneuropathy indicating large fibre involvement in 10 AL amyloidosis patients (47.6%). The neuropathy pattern was predominantly axonal in each of these cases (see Additional File S1).

Corneal confocal microscopy

CCM revealed a loss of corneal nerves in AL amyloidosis patients compared to healthy controls (Fig. 1). CNFD (22.29 ± 1.54 vs. 30.61 ± 1.31 fibres/mm^2, $p = 0.0002$) and CNFL (13.95 ± 0.75 vs. 17.46 ± 0.78 mm/mm^2, $p = 0.0077$) were significantly lower, but CNBD (30.14 ± 3.43 vs. 37.24 ± 4.12 branches/mm^2, $p = 0.315$) did not differ significantly in patients with AL amyloidosis and controls. A CNFD below 24 fibres/mm^2 regarded as indicative of small fibre neuropathy was evident in 66.6% (14 out of 21 patients). Of these, 6 patients had normal NCS (42.9%), whilst all but one patient with

Table 2. Characteristics of patients with AL amyloidosis.

Age (mean ± SEM, years)	62.9 ± 12.7
Sex	14 male, 7 female
NT-proBNP (median/range, pg/mL)	3456 (339–114027)
GFR (mean ± SEM, mL/min)	70.5 ± 6.0
Proteinuria (mean/range, g/24 h)	2.53 (0.15–13.66)
dFLC (median/range, mg/L)	391.2 (43–9209.69)
Distal motor weakness (count, percentage)	
Upper limbs	1 (4.8%)
Lower limbs	2 (9.5%)
Sensory symptoms (count, percentage)	
Distal hypaesthesia	10 (47.6%)
Distal paraesthesia	6 (28.6%)
Hyporeflexia (count, percentage)	
Upper limbs	1 (4.8%)
Lower limbs	8 (38.1%)
Gait ataxia	2 (9.5%)
Neuropathic pain (count, percentage)	7 (33.3%)
Autonomic dysfunction (count, percentage)	
Orthostatic hypotension	3 (14.3%)
Diarrhoea/constipation	2 (9.5%)

NT-proBNP, N-terminal pro brain natriuretic peptide; dFLC, difference between serum levels of involved and uninvolved free light chains; GFR, glomerular filtration rate.

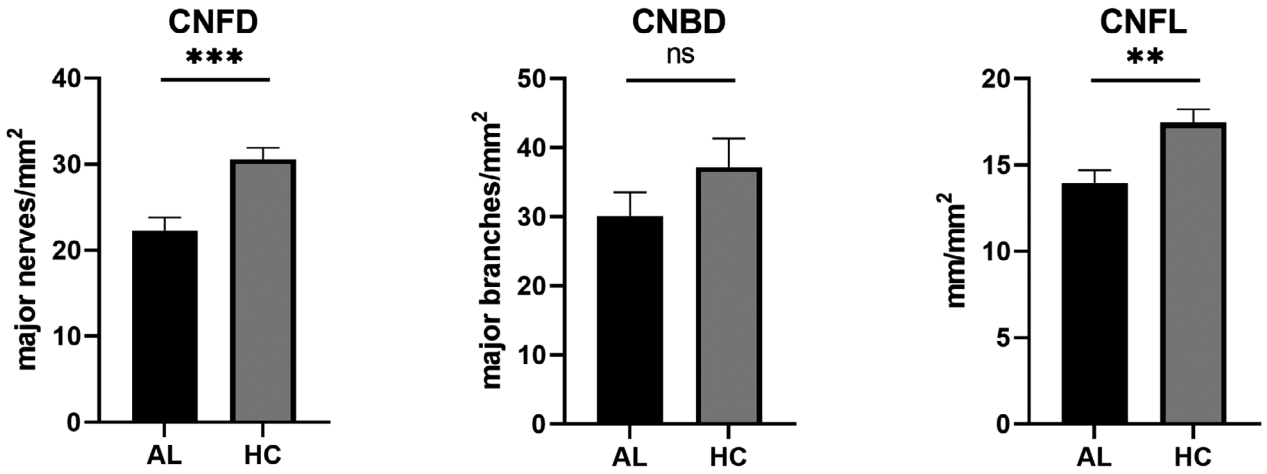


Figure 1. Corneal nerve fibre parameters in patients with AL amyloidosis (black, left) and healthy controls (grey, right). Mean corneal nerve fibre density (CNFD), mean corneal nerve branch density (CNBD), and mean corneal nerve fibre length (CNFL) are displayed. Mean ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$. NS, not significant.

neuropathy on NCS had a loss of small nerve fibres (<24/mm^2) on CCM.

There was an increase in corneal cellular infiltration in patients with AL amyloidosis (Fig. 2). The total corneal cell count (TC) was significantly increased in amyloidosis patients compared to controls (55.48 ± 14.60 vs. 28.08 ± 5.76 cells/mm^2, $p = 0.048$) and the cell-type distribution showed a slight shift towards cells with fibre contact (28.93% vs. 26.01%, Fig. 3). The NCF proportion (20.0% vs. 10.6%) and NCF density (11.10 ± 2.53 vs. 2.99 ± 0.93 cells/mm^2, $p = 0.0038$) were significantly higher with no difference in DCF (4.95 ± 1.30 vs. 4.26 ± 1.12 cells/mm^2, $p = 0.739$), DCP (7.24 ± 1.92 vs. 5.95 ± 1.27 cells/mm^2, $p = 0.631$), and NCP (32.19 ± 12.44 vs. 14.82 ± 3.35 cells/mm^2, $p = 0.218$) between patients with AL amyloidosis and controls. The total number of cells with fibre contact (DCF + NCF) was significantly higher in the AL amyloidosis group (16.85 ± 3.21 vs. 6.99 ± 1.66 cells/mm^2, $p = 0.019$), whereas the numbers of dendritic cells regardless of their localization (DCF + DCP) did not differ (20.48 ± 5.86 vs. 10.27 ± 2.13 cells/mm^2, $p = 0.077$).

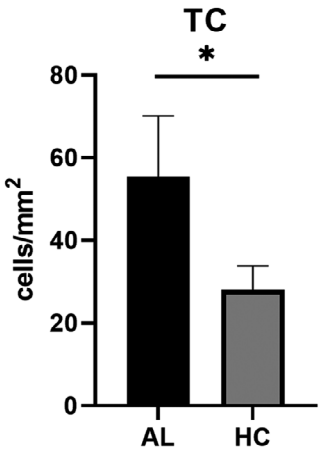
In subgroup analysis, patients without large fibre neuropathy according to NCS (PN−, $n = 11$) had a significantly lower CNFD (23.73 ± 2.18 vs. 30.61 ± 1.31 fibres/mm^2, $p = 0.037$) and higher NCF (12.18 ± 3.74 vs. 2.99 ± 0.93 cells/mm^2, $p = 0.017$) compared to healthy controls (Fig. 4). There was no significant difference in corneal nerve fibre parameters and cell infiltration between patients with (PN+) and without (PN−) large fibre involvement. Patients with (SFS+, $n = 10$) and without (SFS−, $n = 11$) small fibre-mediated symptoms did not differ significantly in CCM parameters (for details see Table 3). Differences between patients with (NP+, $n = 7$) and without (NP−, $n = 14$) neuropathic pain did not reach statistical significance either, but data suggested lower CNFD and CNFL in patients with painful neuropathy and in patients with other small fibre symptoms (see Tables 3 and 4).

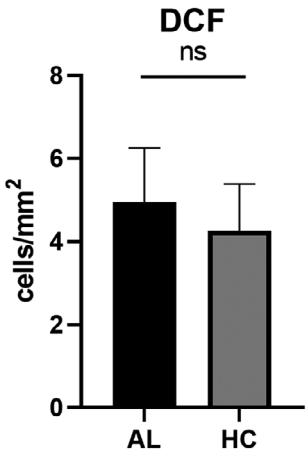
There was a moderate negative correlation ($r = -0.496$, $p = 0.022$) between cells with fibre contact (NCF + DCF) and CNFD (Fig. 5). However, CNFD, TC, NCF, and DCF + NCF did not correlate with proteinuria, GFR, serum NT-proBNP, or dFLC in the AL amyloidosis group (Table 5).

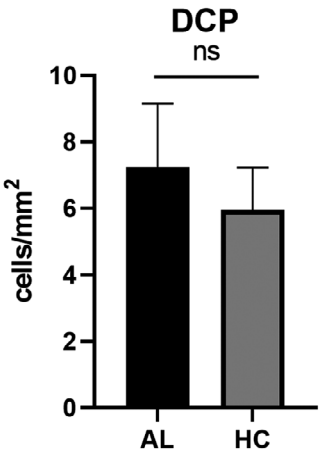
Discussion

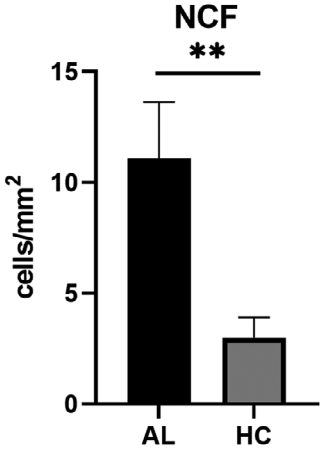
Our study is the first to show that CCM identifies small nerve fibre loss in patients with AL amyloidosis at an early stage when large fibre NCS are still normal. Furthermore, our data demonstrate a negative correlation between corneal nerve fibre loss and immune cell infiltration in AL amyloidosis, but no correlation between

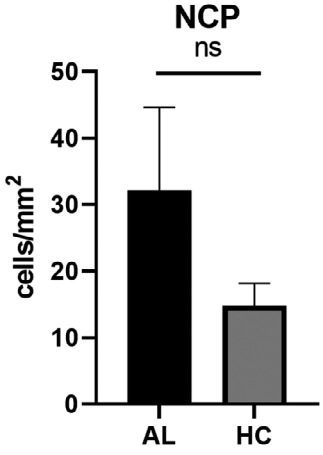
Figure 2. Corneal Langerhans cell counts in patients with AL amyloidosis (black, left) and healthy controls (grey, right). The total cell count (TC), cell counts of dendritic cells with fibre contact (DCF), dendritic cells in the periphery (DCP), non-dendritic cells with fibre contact (NCF), non-dendritic cells in the periphery (NCP), all dendritic cells regardless of their localization (DCP + DCF), and all cells with fibre contact regardless of their morphology (DCF + NCF) are displayed. Mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$. NS, not significant.

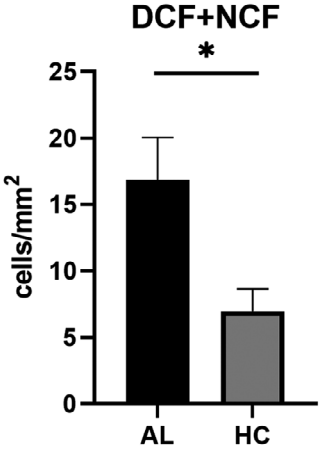


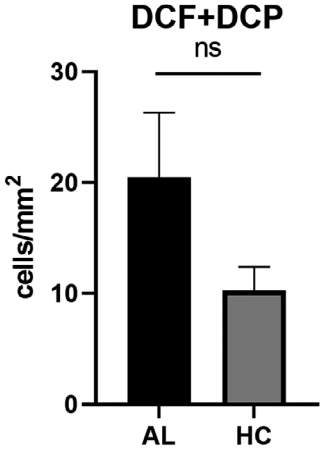












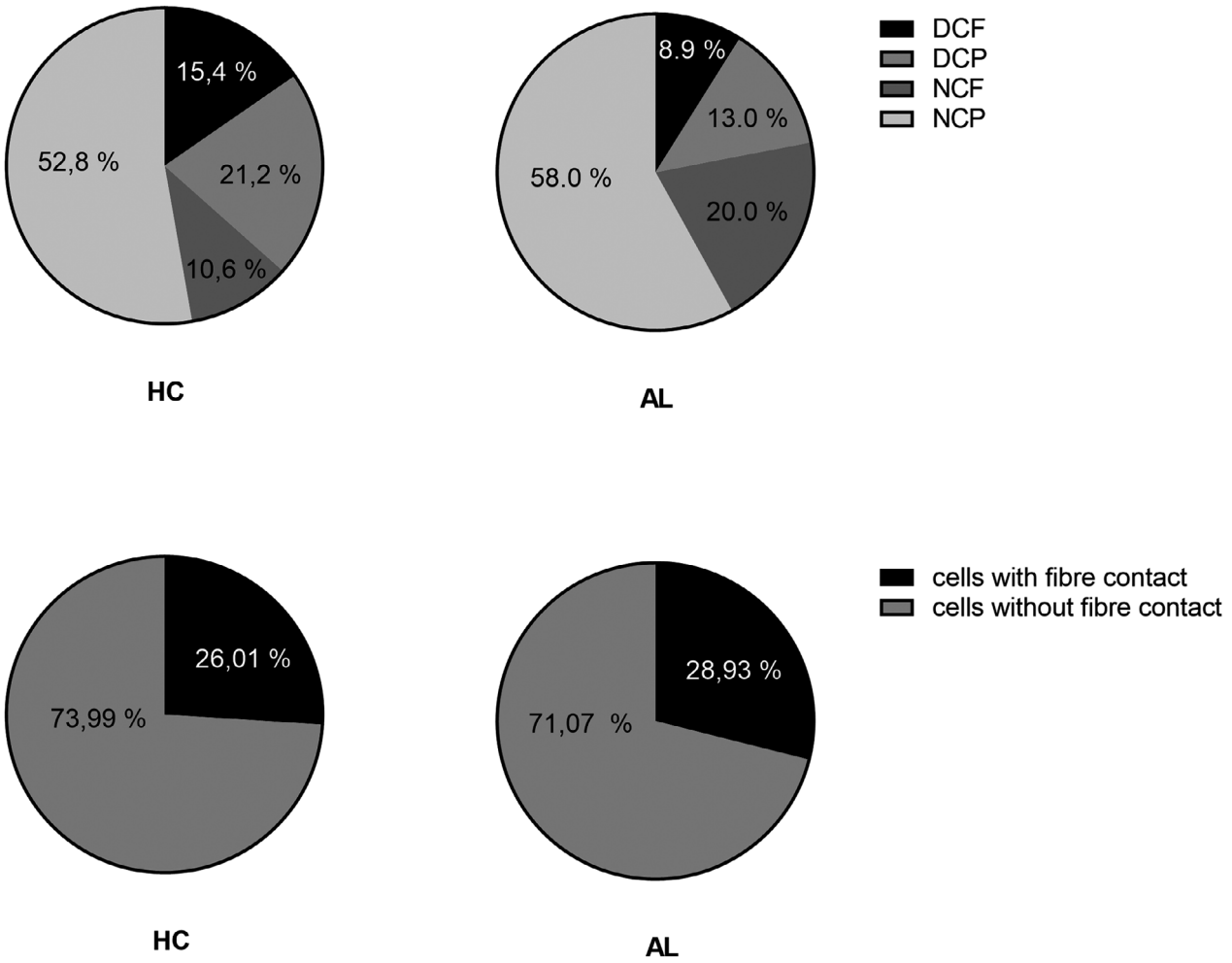


Figure 3. Cell-type distribution in patients with AL amyloidosis (AL) and healthy controls (HC).

corneal nerve fibre loss and laboratory parameters of organ dysfunction. Reduced CNFD in patients without NCS abnormalities indicated early small nerve fibre damage independent of large fibre neuropathy. Only 47.6% showed a predominantly axonal large fibre neuropathy, consistent with the literature,[29,30] whilst CNFD reduction below reference values used in clinical practice was evident in 66.6%. Furthermore, the proportion of patients with CCM abnormalities but normal NCS (42.9%) was higher than the proportion of patients with large fibre neuropathy on NCS and normal CCM results (10%). Our data build on recent studies showing the utility of CCM in patients with ATTRv polyneuropathy,[25] particularly in identifying the full spectrum of disease severity[26] and early small nerve fibre damage.[27] In our study, reduction of CNFD and CNFL did not significantly differentiate between patients with and without small fibre symptoms and patients with and without neuropathic pain, respectively. However, based on our data, one can reasonably assume, that the observed differences would be statistically significant in a larger cohort.

The mechanisms underlying amyloid neuropathy are poorly understood. Several hypotheses mostly referring to hereditary transthyretin amyloid neuropathy have been

Figure 4. Corneal nerve and cell parameters in patients with AL amyloidosis with (PN+, black) and without (PN−, light grey) large fibre neuropathy and healthy controls (HC, dark grey). Mean corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD), corneal nerve fibre length (CNFL), total cell count (TC), dendritic cells with fibre contact (DCF), dendritic cells in the periphery (DCP), non-dendritic cells with fibre contact (NCF), and non-dendritic cells in the periphery (NCP) are displayed. Mean ± SEM. Differences between PN− and PN+ were not significant, bars were omitted for clarity. *$p < 0.05$, **$p < 0.01$. NS, not significant.

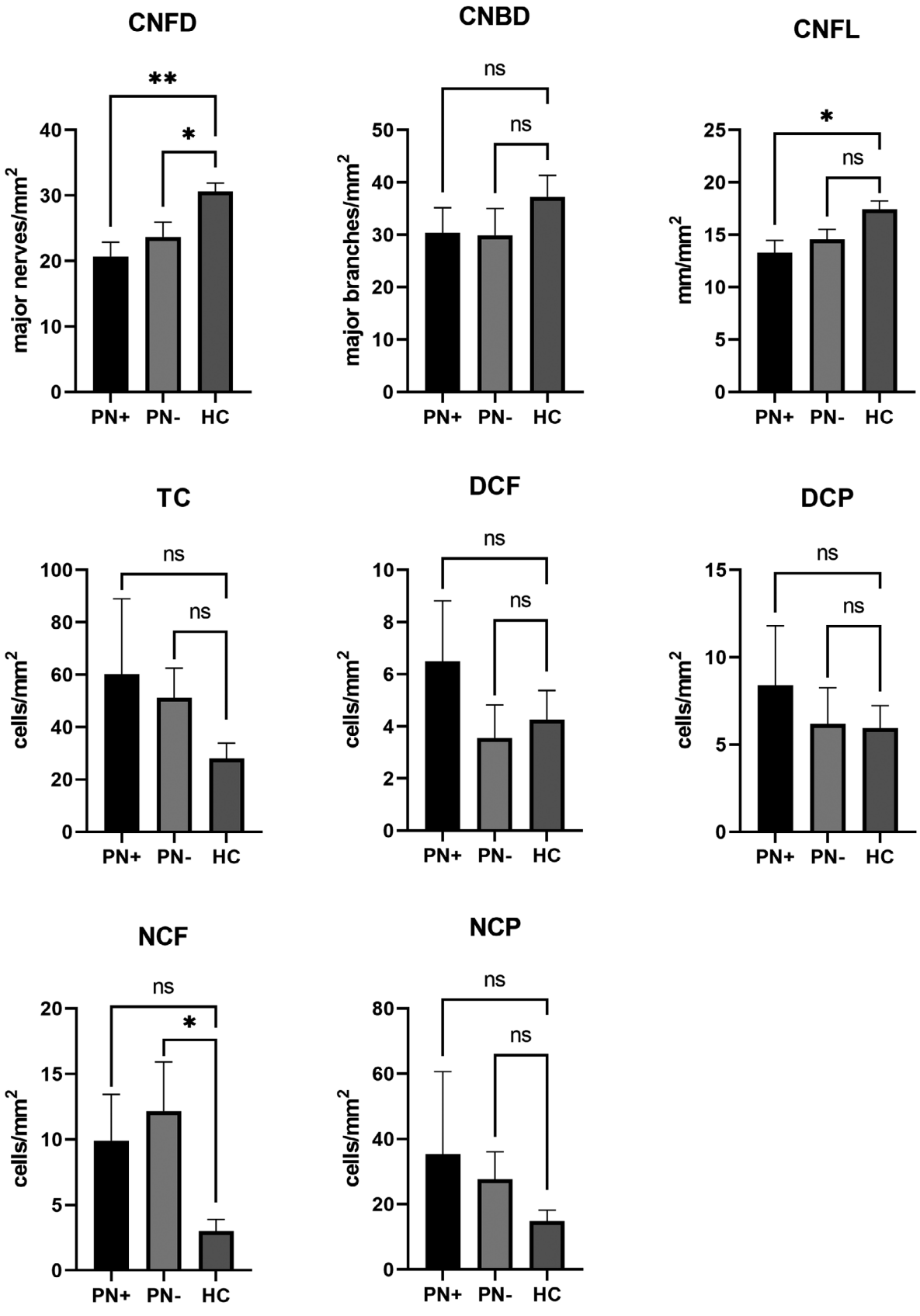


Table 3. Corneal nerve and cell parameters in patients with AL amyloidosis with (SFS+, $n = 10$) and without (SFS−, $n = 11$) small fibre-mediated symptoms.

	SFS+	SFS−	*p* values
CNFD (mean ± SEM, fibres/mm^2)	20.30 ± 1.72	24.09 ± 2.45	0.338
CNFL (mean ± SEM, mm/mm^2)	13.00 ± 0.99	14.82 ± 1.10	0.255
CNBD (mean ± SEM, branches/mm^2)	28.70 ± 3.95	31.45 ± 5.64	0.796
TC (mean ± SEM, cells/mm^2)	59.20 ± 28.96	52.09 ± 11.18	0.456
DCF (mean ± SEM, cells/mm^2)	4.90 ± 2.30	5.00 ± 1.47	0.450
DCP (mean ± SEM, cells/mm^2)	8.10 ± 3.41	6.46 ± 2.09	0.690
NCF (mean ± SEM, cells/mm^2)	8.90 ± 3.53	13.09 ± 3.67	0.288
NCP (mean ± SEM, cells/mm^2)	37.30 ± 25.09	27.55 ± 8.42	0.478
DCF + NCF (mean ± SEM, cells/mm^2)	13.80 ± 5.05	18.09 ± 4.03	0.304
DCP + DCF (mean ± SEM, cells/mm^2)	13.00 ± 5.38	11.45 ± 2.99	0.876

CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length; CNBD, corneal nerve branch density; TC, total cell count; DCF, dendritic cells with fibre contact; DCP, dendritic cells in the periphery; NCF, non-dendritic cells with fibre contact; NCP, non-dendritic cells in the periphery.

Table 4. Corneal nerve and cell parameters in patients with AL amyloidosis with (NP+, $n = 7$) and without (NP−, $n = 14$) neuropathic pain.

	NP+	NP−	*p* values
CNFD (mean ± SEM, fibres/mm^2)	19.00 ± 1.94	23.93 ± 2.00	0.215
CNFL (mean ± SEM, mm/mm^2)	12.71 ± 1.21	14.57 ± 0.94	0.259
CNBD (mean ± SEM, branches/mm^2)	29.43 ± 4.95	30.50 ± 4.63	0.956
TC (mean ± SEM, cells/mm^2)	70.86 ± 40.26	47.79 ± 10.20	0.784
DCF (mean ± SEM, cells/mm^2)	4.43 ± 2.26	5.21 ± 1.65	0.728
DCP (mean ± SEM, cells/mm^2)	6.14 ± 1.52	7.79 ± 2.81	0.594
NCF (mean ± SEM, cells/mm^2)	10.71 ± 4.76	11.29 ± 3.09	0.622
NCP (mean ± SEM, cells/mm^2)	49.57 ± 35.56	23.50 ± 6.90	0.812
DCF + NCF (mean ± SEM, cells/mm^2)	15.14 ± 6.15	17.77 ± 3.83	0.447
DCP + DCF (mean ± SEM, cells/mm^2)	10.57 ± 2.94	13.00 ± 4.20	0.813

CNFD, corneal nerve fibre density; CNFL, corneal nerve fibre length; CNBD, corneal nerve branch density; TC, total cell count; DCF, dendritic cells with fibre contact; DCP, dendritic cells in the periphery; NCF, non-dendritic cells with fibre contact; NCP, non-dendritic cells in the periphery.

proposed, including mechanical compression of nerve fibres, ischemia due to perivascular amyloid deposition and toxic effects of non-fibrillar aggregates.[31–33] Little is known about the involvement of immune mechanisms, although an upregulation of pro-inflammatory cytokines[32,34] and an induction of inflammatory responses through macrophage activation in the sural nerve[35] have been shown previously in patients with amyloidosis. Recently, a potential immune-mediated mechanism for small fibre loss has been proposed in patients with ATTRv neuropathy based on the observation that there was a clustering of immature Langerhans cells at the inferior whorl of the cornea in association with an early reduction in inferior whorl length.[27] In general, reduced CNFD represents axonal loss, while increased corneal cellular infiltration reflects inflammation[22–24] as the majority of morphologically dendritic corneal cells are indeed antigen presenting Langerhans cells.[36–39] In recent longitudinal studies, higher total corneal cell counts were associated with a more progressive disease course in chronic inflammatory demyelinating polyneuropathy[40] and they were reduced in response to immune therapy in inflammatory neuropathies.[41] In mouse models, increased corneal DCF are associated with reduced nerve fibre

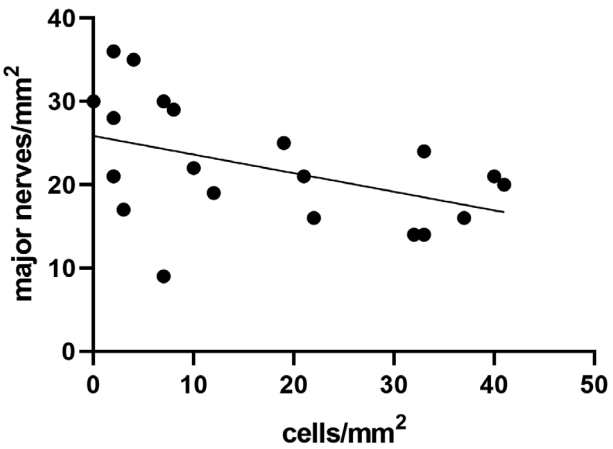


Figure 5. Correlation between cells with fibre contact (DCF + NCF) and corneal nerve fibre density (CNFD) in patients with AL amyloidosis.

density.[42] In this context, our findings of a significant increase in total corneal cellular infiltration, a shift in cell-type distribution towards cells with fibre contact, and a correlation between higher cell counts near nerve fibres and reduced nerve fibre density in patients with AL

Table 5. Correlation of nerve fibre parameters and corneal dendritic cell infiltration with laboratory parameters.

	GFR	NT-proBNP	dFLC
CNFD	$r = 0.146$	$r = -0.391$	$r = -0.035$
	$p = 0.527$	$p = 0.079$	$p = 0.882$
TC	$r = -0.146$	$r = -0.091$	$r = -0.040$
	$p = 0.527$	$p = 0.695$	$p = 0.862$
NCF	$r = -0.180$	$r = -0.094$	$r = -0.270$
	$p = 0.433$	$p = 0.686$	$p = 0.237$
DCF + NCF	$r = -0.311$	$r = 0.087$	$r = -0.158$
	$p = 0.170$	$p = 0.707$	$p = 0.495$

NT-proBNP, N-terminal pro brain natriuretic peptide; dFLC, difference between serum levels of involved and uninvolved free light chains; GFR, glomerular filtration rate; CNFD, corneal nerve fibre density; TC, total cell count; DCF, dendritic cells with fibre contact; NCF, non-dendritic cells with fibre contact.

amyloidosis suggest an unbalanced immune response in amyloid neuropathy.

Importantly, Kokotis et al recently showed that a IENFD was associated with poorer survival in AL amyloidosis.[10] Whether or not reduced CNFD can predict disease progression and treatment response in AL amyloidosis remains to be proven in longitudinal studies. Our cross-sectional data would not support that notion, as the severity of corneal nerve fibre loss did not correlate with NT-proBNP, a prognostic marker of cardiac amyloidosis nor with dFLC which is an established prognostic marker and measure of disease progression[43–46] in light chain amyloidosis.

This is a relatively small cohort study with limitations regarding conclusions about the diagnostic utility of CCM and pathogenic mechanisms in patients with AL amyloidosis. Nevertheless, the rapid non-invasive and reiterative capability of CCM provides a method to easily assess small fibre involvement in relation to neuropathic pain and autonomic symptoms, amyloid load and to assess both the beneficial and potentially neurotoxic effects of treatments for AL amyloidosis.

In conclusion, we show evidence of early small nerve fibre loss associated with corneal immune cell infiltration preceding large fibre neuropathy in patients with AL amyloidosis. Although cross-sectional, our results question prior data concerning the prognostic value of small fibre neuropathy in AL amyloidosis. CCM may be suited for early diagnosis and longitudinal evaluation of small nerve fibre integrity to help determine its value as a potential surrogate marker for treatment response and prognosis in AL amyloidosis.

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Conflict of Interest

K. H. reports personal fees from Bayer, personal fees and other from Sofie Biosciences, personal fees from SIRTEX, non-financial support from ABX, personal fees from Adacap, personal fees from Curium, personal fees from Endocyte, grants and personal fees from BTG, personal fees from IPSEN, personal fees from Siemens Healthineers, personal fees from GE Healthcare, personal fees from Amgen, personal fees from Novartis, personal fees from ymabs, personal fees from Aktis Oncology, personal fees from Theragnostics, personal fees from Pharma15, outside the submitted work. H. C. R. received consulting and lecture fees from Abbvie, AstraZeneca, Vertex and Merck, research funding from Gilead Pharmaceuticals, and is a co-founder of CDL Therapeutics GmbH. MS served on the scientific advisory boards and/or received speaker honoraria, travel funding or honoraria for medical writing from UCB, Biogen Idec; Grifols, Genzyme, Roche, Merck, Novartis, Octapharma, CSL Behring, Sanofi-Aventis, TEVA, and Bayer. A. T., A. C., S. O., M. P., L. K., C. R., R. A. M., T. R., C. K., and T. H. report no competing interests.

Author Contributions

AT designed the study, collected and analysed the data, and wrote the manuscript. A. C., S. O., M. P., L. K., C. R. contributed substantially to data acquisition and revised the manuscript for intellectual content. R. A. M. collected data of the control group and revised the manuscript for important intellectual content. H. C. R., T. R., K. H., and C. K. revised the manuscript for important intellectual content. M. S. and T. H. contributed equally to data acquisition and interpretation, conceptualization of the study and revised the manuscript for important intellectual content.

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Supporting Information

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

File S1. Additional Table 1. NCS results in patients with AL amyloidosis.

