RESEARCH PAPER

Retinal ganglion cell loss in neuromyelitis optica: a longitudinal study

Frederike C Oertel,[1] Joachim Havla,[2] Adriana Roca-Fernández,[3] Nathaniel Lizak,[1,4] Hanna Zimmermann,[1] Seyedamirhosein Motamedi,[1] Nadja Borisow,[1] Owen B White,[5] Judith Bellmann-Strobl,[1,6] Philipp Albrecht,[7] Klemens Ruprecht,[8] Sven Jarius,[9] Jacqueline Palace,[3] Maria Isabel Leite,[3] Tania Kuempfel,[2] Friedemann Paul,[1,6,8] Alexander U Brandt[1,10]

For numbered affiliations see end of article.

Correspondence to
Professor Friedemann Paul, NeuroCure Clinical Research Center, Charité, Charitéplatz 1, Berlin, 10117, Germany; friedemann.paul@charite.de

FCO and JH are joint first authors.
FP and AUB are joint senior authors.

Received 9 March 2018
Revised 4 May 2018
Accepted 16 May 2018
Published Online First 19 June 2018

ABSTRACT

Objectives Neuromyelitis optica spectrum disorders (NMOSD) are inflammatory conditions of the central nervous system and an important differential diagnosis of multiple sclerosis (MS). Unlike MS, the course is usually relapsing, and it is unclear, if progressive neurodegeneration contributes to disability. Therefore, we aimed to investigate if progressive retinal neuroaxonal damage occurs in aquaporin4-antibody-seropositive NMOSD.

Methods Out of 157 patients with NMOSD screened, 94 eyes of 51 patients without optic neuritis (ON) during follow-up (F/U) and 56 eyes of 28 age-matched and sex-matched healthy controls (HC) were included (median F/U 2.3 years). The NMOSD cohort included 60 eyes without (Eye^{ON-}) and 34 eyes with a history of ON prior to enrolment (Eye^{ON+}). Peripapillary retinal nerve fibre layer thickness (pRNFL), fovea thickness (FT), volumes of the combined ganglion cell and inner plexiform layer (GCIP) and the inner nuclear layer (INL) and total macular volume (TMV) were acquired by optical coherence tomography (OCT).

Results At baseline, GCIP, FT and TMV were reduced in Eye^{ON+} (GCIP $p<2e^{-16}$; FT $p=3.7e^{-4}$; TMV $p=3.7e^{-12}$) and in Eye^{ON-} (GCIP p=0.002; FT p=0.040; TMV $p=6.1e^{-6}$) compared with HC. Longitudinally, we observed GCIP thinning in Eye^{ON-} (p=0.044) but not in Eye^{ON+}. Seven patients had attacks during F/U; they presented pRNFL thickening compared with patients without attacks (p=0.003).

Conclusion This study clearly shows GCIP loss independent of ON attacks in aquaporin4-antibody-seropositive NMOSD. Potential explanations for progressive GCIP thinning include primary retinopathy, drug-induced neurodegeneration and retrograde neuroaxonal degeneration from lesions or optic neuropathy. pRNFL thickening in the patients presenting with attacks during F/U might be indicative of pRNFL susceptibility to inflammation.

INTRODUCTION

Neuromyelitis optica spectrum disorders (NMOSD) are relapsing autoimmune inflammatory diseases of the central nervous system (CNS)[1] and an important differential diagnosis of multiple sclerosis (MS), the most common autoimmune inflammatory disorder of the CNS.[2] Unlike MS, which is thought to be a B-cell-mediated and T-cell-mediated demyelinating disease with early axonal pathology,[3] NMOSD is considered a complement-dependent and antibody-mediated astrocytopathy, with 60%–80% of patients having detectable autoantibodies against the astrocytic water channel aquaporin-4 (AQP4-ab).[1 4 5]

Chronic neurodegeneration and disability progression during the progressive stage are observed in more than half of all patients with MS[6] where there is no efficient treatment available.[7] In contrast, patients with NMOSD virtually always present with monophasic or relapsing disease courses[8] and it remains unclear, if progressive disease independent of clinical attacks also occurs in NMOSD.[9 10]

In 55% of patients with NMOSD, optic neuritis (ON) is the first clinical manifestation and frequently leads to severe structural damage of the afferent visual pathway with resulting functional impairment.[11–13] The axon of the retinal ganglion cell and the ganglion cell itself are highly affected by ON and therefore are suitable targets to investigate neuroaxonal damage in NMOSD.[14] Optical coherence tomography (OCT) is a non-invasive interferometric technique, which has been shown to provide high-resolution images of these retinal structures allowing an accurate quantification of their alterations.[14]

We and others recently found evidence for ON-independent retinal changes in NMOSD,[15–17] which supports experimental data from animal models, suggesting an underlying astrocytopathy.[10 18] Therefore, we aimed to investigate if progressive retinal neuroaxonal damage occurs in NMOSD. For this, we longitudinally followed AQP4-ab seropositive patients with NMOSD and healthy controls (HC) with OCT. We excluded all eyes with an acute ON during follow-up (F/U) and investigated eyes without previous ON and those with a history of ON separately to isolate ON independent effects.

METHODS

Patients

One hundred and fifty-seven AQP4-ab seropositive patients with NMOSD from longitudinal observational cohort studies at the NeuroCure Clinical Research Center (NCRC) at

To cite: Oertel FC, Havla J, Roca-Fernández A, *et al*. *J Neurol Neurosurg Psychiatry* 2018;**89**:1259–1265.

Charité — Universitätsmedizin Berlin, Germany (EA1/131/09), the Ludwig Maximilians Universität Munich, Germany (Z427-14) and the Nuffield Center for Clinical Neurosciences at Oxford University, UK (REC 16/SC/0224) were screened for this study. Inclusion criteria were a definite diagnosis of AQP4-ab seropositive NMOSD according to 2015 international consensus criteria,[19] complete longitudinal clinical and OCT imaging data with minimum F/U of 1 year, and age between 18 and 75 years at baseline. Eyes with ophthalmological comorbidities, insufficient OCT image quality, ON <5 months before F/U as well as patients with ON in both eyes during F/U were excluded. For comparison, we included longitudinal data of 28 matched HCs from the NCRC's research database with equal median duration of F/U. High-contrast visual acuity (VA) was acquired under best correction in a subset of 22 patients with NMOSD from Berlin and Oxford (28 eyes without a history of ON, 11 eyes with a history of ON; F/U (median (min–max)) (1.1 (0.7–3.3)) with Early Treatment in Diabetes Retinopathy Study charts at 20 ft distance with retroilluminated charts. All participants gave written informed consent. The study was conducted in accordance with the current version of the Declaration of Helsinki and the applicable German and British laws.

Optical coherence tomography

All centres used Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) with automatic real-time (ART) function for image averaging. The combined ganglion cell and inner plexiform layer volume (GCIP), inner nuclear layer volume (INL) and total macular volume (TMV) was calculated as a 3 mm diameter cylinder around the fovea from a macular volume scan (*Berlin*: 25°×30°, 61 vertical B-scans, 11≤ART≤18; *Munich*: 20°×20°, 25 vertical B-scans, 21≤ART≤49; *Oxford*: 69% 30°×25° 61 vertical B-scans, 27% 30°×15° 37 horizontal B-scans, 2% 30°×25° 61 horizontal B-scans, 2% 30°×15° 19 horizontal B-scans, 7≤ART≤22).[20] The fovea thickness (FT) was defined as mean thickness of the 1 mm diameter cylinder around the fovea from the same scan.[16] The peripapillary RNFL (pRNFL) was measured with activated eye tracker using 3.4 mm ring scans around the optic nerve (12°, 1536 A-scans, 1≤ART≤99) and the most inner 3.5 mm ring of a star-and-ring scan around the optic nerve (12°, 768 A-scans, 22≤ART≤32). Segmentation of all layers was performed semi-automatically using software provided by the OCT manufacturer (Eye Explorer 1.9.10.0 with viewing module 6.3.4.0, Heidelberg Engineering). One experienced rater (FCO) carefully checked all scans for sufficient quality and segmentation errors and corrected if necessary.[21] OCT data in this study are reported according to the APOSTEL recommendations.[22]

Statistical methods

Group differences between NMOSD and HC were tested by X^2 test for sex and Wilcoxon rank sum test for age. The primary outcome was the change of GCIP over F/U; secondary outcomes were changes in pRNFL, INL, FT and TMV. Cross-sectional differences of OCT values and VA between all groups were analysed pairwise by generalised estimating equation models to account for intereye within-patient correlations of monocular measurements. Longitudinal analysis of OCT and VA values was performed by the linear mixed-effects model: OCTvalue~time from baseline×group+(1+time from baseline|patient/eye)+(1|age)+(1|sex) and results are reported for (time from baseline×group). Marginal and conditional coefficients of determination for the models were estimated by pseudo-R^2 for mixed-effect models. To exclude influences of contralateral ON, we included the contralateral ON status as random effect in a subanalysis. Annual loss was estimated for each individual as change to baseline at last visit divided by F/U time. For an exploratory subgroup analysis in patients with NMOSD with other, non-ON attacks during F/U, we defined the visit before first attack as new baseline. For VA analysis, we defined the first visit with best-corrected VA assessments as new baseline. All tests and graphical representations were performed with R V.3.3.1,[23] with packages beeswarm, psych, geepack, ggplot, lme4, lmer, MuMIn, Rmisc and multcomp. Statistical significance was established at $p<0.05$.

RESULTS

Cohort description and follow-up

From the 157 patients with NMOSD screened, data from 51 patients with NMOSD (94 eyes) with a median F/U time of 2.3 years (range 1.0–3.5 years) from Berlin (n=23), Munich (n=11) and Oxford (n=17) fulfilled all inclusion criteria (figure 1 and table 1). The study cohort included 76% Caucasians, 10% African-Caribbeans, 8% Asians, 2% Middle Eastern, and 2% people of mixed origin; for 2% ethnicity was not available. Expanded Disability Status Scale assessment at baseline was available for 31 patients with NMOSD (table 1). Furthermore, we included OCT data from 28 age-matched and sex-matched HCs.

Group differences at baseline

First, we analysed group differences at baseline between eyes from patients with NMOSD with history of ON ($Eyes^{ON+}$), eyes from patients with NMOSD without previous ON ($Eyes^{ON-}$) and eyes from HC. As expected, GCIP in $Eyes^{ON+}$ was lower than in HC ($p<2e^{-16}$), representing well-established ganglion cell damage after ON (table 2). GCIP in $Eyes^{ON-}$ was also lower than in HC eyes ($p=0.002$), but higher than in $Eyes^{ON+}$ ($p=7.4e^{-12}$) (figure 2A). Two eyes (3.3%) of Eye^{ON-} had GCIP values <2 SD of the mean GCIP of Eye^{ON-}. A subset of Eye^{ON-} having experienced ON neither in the ipsilateral nor in the contralateral eye also showed reduced GCIP (0.60±0.05 mm^3) compared with HC (0.63±0.04 mm^3, $p=0.016$). We found no pRNFL reduction in $Eyes^{ON-}$ in comparison with HC eyes, but as expected $Eyes^{ON+}$ had reduced pRNFL as a marker of retinal axonal loss compared with $Eyes^{ON-}$ ($p=7.9e^{-10}$) and HC ($p=5.0e^{-11}$) (figure 2B). INL was comparable between the three groups. Also, TMV was reduced in $Eyes^{ON+}$ ($p=3.7e^{-12}$) and $Eyes^{ON-}$ ($p=6.1e^{-6}$) compared with HC (figure 2C). $Eyes^{ON-}$ showed still higher TMV than $Eyes^{ON+}$ ($p=1.0e^{-5}$). FT was reduced in $Eyes^{ON+}$ ($p=3.7e^{-4}$) and again in $Eyes^{ON-}$ ($p=0.040$) in comparison with HC (figure 2D).

OCT changes during follow-up

Longitudinally, we observed a thinning of GCIP in $Eyes^{ON-}$ (B=−0.004 SE=0.001 p=0.022; annual loss −0.00415±0.01200 mm^3). This effect remained significant against HC (annual loss HC −0.00005±0.00466 mm^3; p=0.044; figure 3). The longitudinal effect in Eye^{ON-} did not differ between eyes without a history of any ON (annual loss −0.00468±0.01310 mm^3) compared with eyes with history of a contralateral ON before baseline (−0.00413±0.00779 mm^3; p=0.805). $Eyes^{ON+}$ revealed no longitudinal GCIP changes compared with HC (annual loss −0.00094±0.0119 mm^3; p=0.960), which we interpret as a potential flooring effect resulting from previous ON episodes. pRNFL, TMV, FT and INL did not change in $Eyes^{ON-}$ or $Eyes^{ON+}$ longitudinally compared

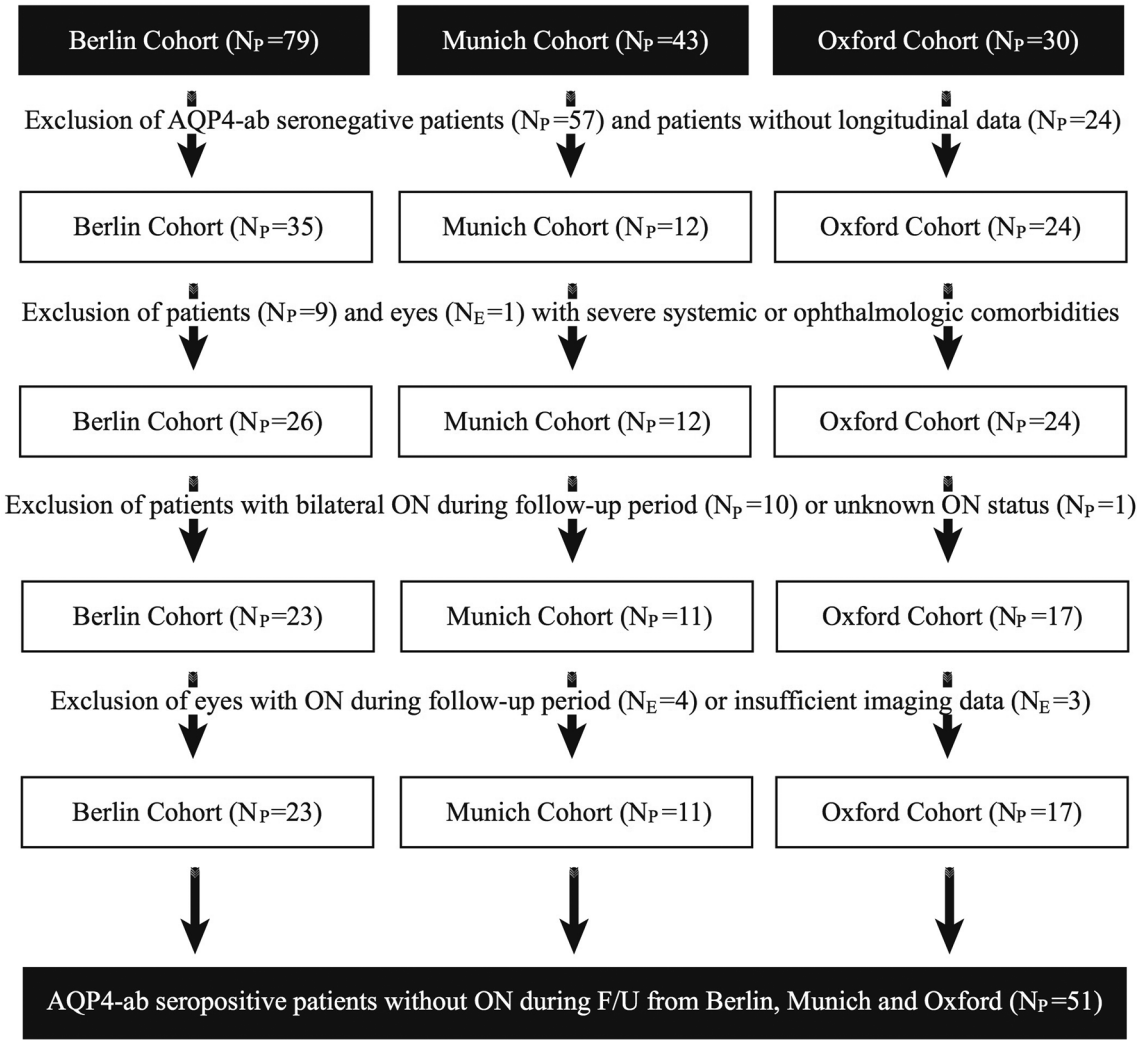

Figure 1 NMOSD cohort selection. AQP4-ab, aquaporin-4 autoantibodies; F/U, follow-up; NMOSD, neuromyelitis optica spectrum disorders; $N_P$number of patients; N_E, number of eyes; ON, optic neuritis.

with HCs (table 3). We excluded potential influences of ethnicity and treatment by incorporating respective factors in additional models (data not shown). In a subanalysis, GCIP change was not different between $Eyes^{ON-}$ treated with rituximab or azathioprine (p=0.710). Also, we excluded potential influences of contralateral ON in $Eyes^{ON-}$ (n=2) on longitudinal GCIP changes by including contralateral ON during F/U as additional factor, which led to the same p -value as without (p=0.044).

Seven patients with NMOSD (10 eyes) experienced attacks during F/U (two patients with one LETM, one patient with four LETMs, four patients (eyes) with contralateral ON). These patients showed pRNFL thickening (1.56±4.39 µm) compared with patients with NMOSD without any attacks during F/U (−1.04±3.21 µm; B=1.912 SE=0.597, p=0.003; figure 3), but no differences in GCIP (p=0.513), TMV (p=0.670), INL (p=0.970) or FT (p=0.330).

Table 1 Demographic overview

	NMOSD	HC
Subjects (N)	51	28
Number of eyes (N)	94	56
Sex (female in %)	84.3	78.6
Age at baseline (years, mean±SD)	47.3±14.4	43.1±9.8
F/U time (years, median (range))	2.3 (1.0–3.5)	2.3 (1.0–3.3)
Disease duration at baseline (years, mean±SD)	5.7±5.4	–
EDSS at baseline (median (range))	3 (0–6)	–
Eyes with a history of ON (N (%))	34 (36.2%)	–
Time from last ON to baseline (months, median (range))	46 (5–263)	–
Patients with attacks during F/U (N (%))	7 (14%)	–

Age (W=867, p=0.118) and sex (χ^2=0.110, p=0.740) did not differ between patients with NMOSD and HCs.
EDSS, Expanded Disability Status Scale; F/U, follow-up; HC, healthy controls; N, number of patients; NMOSD, neuromyelitis optica spectrum disorders; ON, optic neuritis.

High-contrast visual acuity

At baseline, VA was not significantly lower in Eye^{ON+} ((logMAR) 0.41±0.69) compared with Eye^{ON-} ((logMAR) 0.01±0.25, p=0.052). VA improved slightly in both groups longitudinally (Eye^{ON-} (logMAR) −0.02±0.07; Eye^{ON+} (logMAR) −0.21±0.40) without discrepancy (p=0.054). VA of Eye^{ON-} was within normal range compared with published data.[24]

DISCUSSION

We observed longitudinal GCIP loss in AQP4-ab seropositive patients with NMOSD without a history of ON. Using

Table 2 Baseline OCT results of patients with NMOSD and HCs.

	Baseline HC	Baseline NMOSD		Baseline Eyes^{ON-} vs HC			Baseline Eyes^{ON+} vs HC			Baseline Eyes^{ON-} vs Eyes^{ON+}		
	Mean±SD	Eyes^{ON-} mean±SD	Eyes^{ON+} mean±SD	B	SE	P values	B	SE	P values	B	SE	P value
GCIP (mm^3)	0.63±0.04	0.59±0.06	0.43±0.12	−0.04	0.01	0.002	−0.19	0.02	$<2.0e^{-16}$	−0.15	0.02	$7.4e^{-12}$
pRNFL (µm)	98.5±9.2	96.7±11.9	67.0±24.2	−2.51	2.59	0.330	−30.22	4.60	$5.0e^{-11}$	−27.91	4.54	$7.9e^{-10}$
INL (mm^3)	0.27±0.03	0.27±0.03	0.28±0.04	−0.01	0.01	0.340	−0.010	0.01	0.250	0.013	0.01	0.054
FT (µm)	278±19	267±21	262±20	−9.99	4.85	0.040	−17.79	4.99	$3.7e^{-4}$	−6.25	1.64	$1.3e^{-4}$
TMV (mm^3)	2.37±0.10	2.25±0.15	2.11±0.19	−0.14	0.03	$6.1e^{-6}$	−0.27	0.04	$3.7e^{-12}$	−0.11	0.02	$1.0e^{-5}$

B, estimate; Eyes ON$^-$, patients with NMOSD without a history of ON; Eyes ON$^+$, patients with NMOSD with a history of ON; FT, fovea thickness; GCIP, combined ganglion cell and inner plexiform layer; HC, healthy control; INL, inner nuclear layer; NMOSD, neuromyelitis optica spectrum disorders; OCT, optical coherence tomography; ON, optic neuritis; pRNFL, peripapillary retinal nerve fibre layer; TMV, total macular volume.

intraretinal layer segmentation and three-dimensional macular OCT, our results may be indicative of neuroaxonal damage both at baseline and progressively during F/U. In contrast, in eyes from patients with a new NMOSD-related attack, we did not observe pRNFL loss, but an increase in pRNFL thickness during F/U.

Cross-sectional retinal imaging studies in NMOSD have shown conflicting results as to whether inner retinal layer thinning can

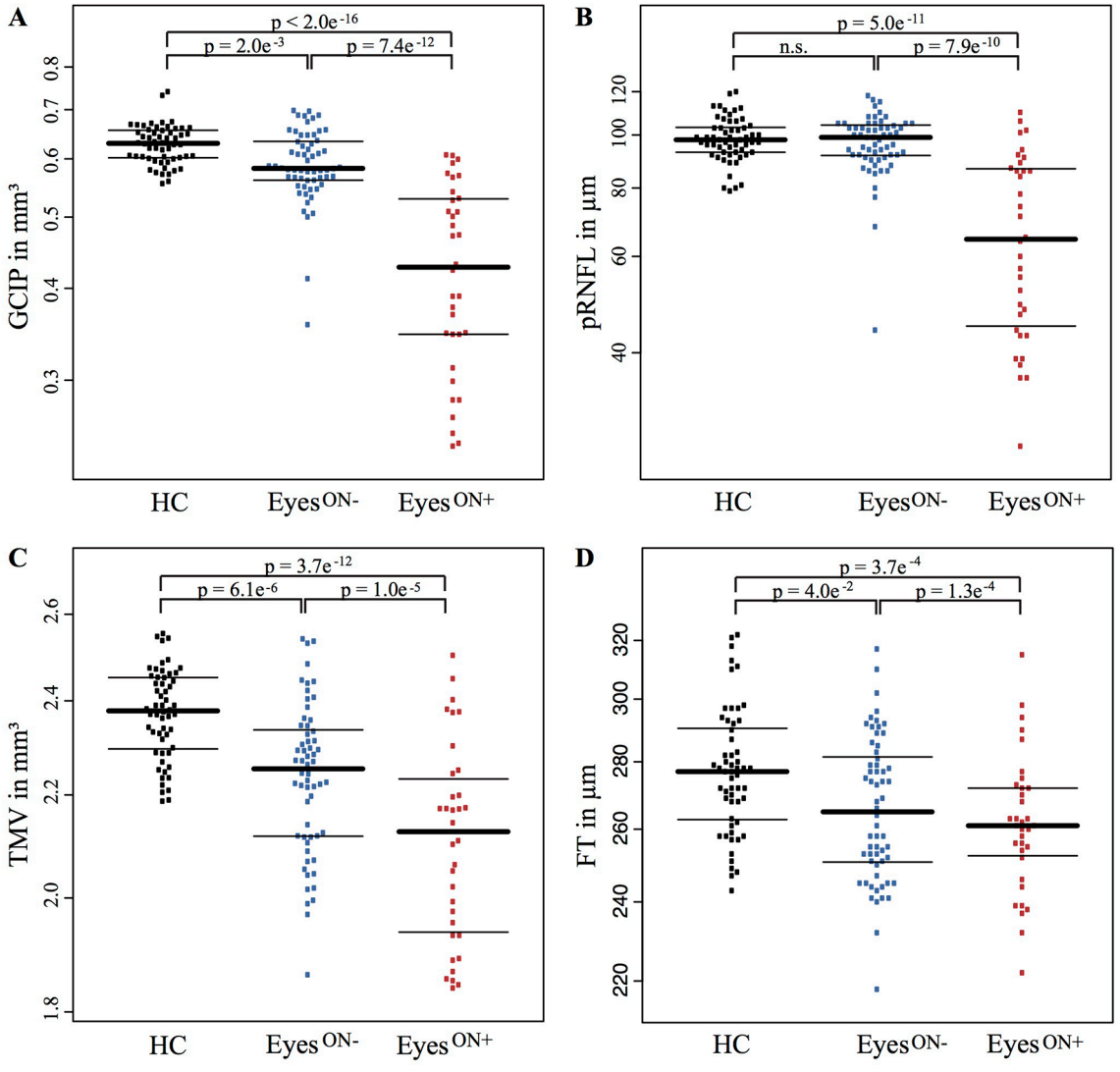

Figure 2 Beeswarm plots of cross-sectional OCT data for HC (black, left), NMOSD Eyes^{ON-} (blue, middle) and NMOSD Eyes^{ON+} (red, right) (median±IQR, single eyes as dots) for (A) GCIP, (B) pRNFL, (C) TMV and (D) FT. Eyes^{ON-}, patients with NMOSD without a history of ON; Eyes^{ON+}, patients with NMOSD with a history of ON; FT, fovea thickness; GCIP, combined ganglion cell and inner plexiform layer; HC, healthy control; NMOSD, neuromyelitis optica spectrum disorders; n.s., not significant; OCT, optical coherence tomography; pRNFL, peripapillary retinal nerve fibre layer; TMV, total macular volume.

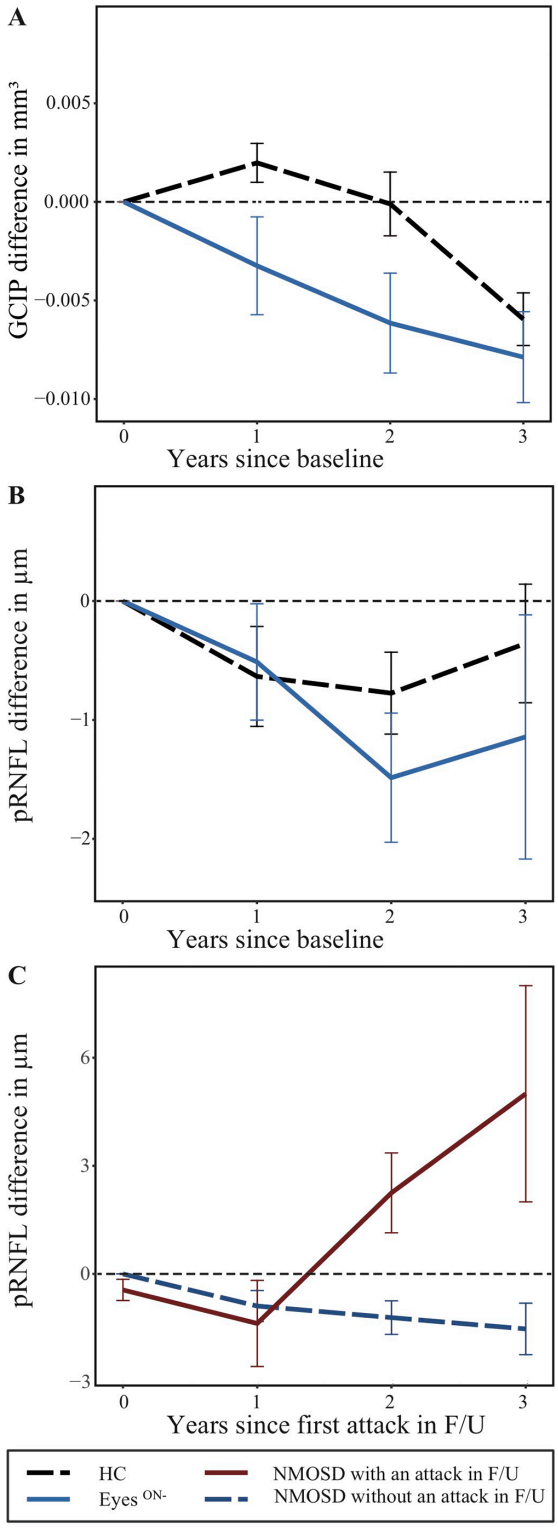

Figure 3 Plots of longitudinal OCT data. Plotted change for rounded time since baseline for (A) GCIP and (B) pRNFL for Eyes^{ON-} (blue, continuous) and HC (black, open-worked). Eyes^{ON+} are not shown due to high noise suggestive of a flooring effect. (C) pRNFL changes in patients with NMOSD with (dark red, continuous) and without (dark blue, open-worked) any attack during F/U independent from ON status. Line as connected means±SE. Eyes^{ON-}, NMOSD eyes without a history of ON; F/U, follow-up; GCIP, combined ganglion cell and inner plexiform layer; HC, healthy control; NMOSD, neuromyelitis optica spectrum disorders; ON, optic neuritis; OCT, optical coherence tomography; pRNFL, peripapillary retinal nerve fibre layer.

occur independently from ON in patients with NMOSD: While some studies reported reduced pRNFL or GCIP thickness in eyes without history of ON as potential evidence for ON-unrelated damage,[15 25 26] others did not find differences compared with HC.[11 27 28]

We suggest that at least some of the reported neuroaxonal damage in NMOSD might be caused by a primary retinopathy. In the human retina, three types of astrocytic cells can be distinguished: (1) Müller cells, damage to which was previously suspected to cause foveal changes in NMOSD in cross-sectional and animal studies,[15 16 29 30] (2) elongated retinal astrocytes are located in the RNFL, (3) whereas star-shaped astrocytes reside in the ganglion cell layer.[31] Astrocytic dysfunction could lead to neuroaxonal damage in the retina and other affected brain regions, as has been reported in brainstem and chiasm, which was recently also shown in animal models of NMOSD.[18 32] Microstructural changes have been reported in biopsies from spinal cord lesions,[33] in spinal cord atrophy in AQP4-ab seropositive patients with NMOSD without previous myelitis[34] and fovea thickness as well as optic radiation changes in AQP4-ab seropositive patients without previous ON[16]– all suggesting a primary astrocytopathy.

Besides a disease-related retinopathy, drug-induced neuroaxonal damage has to be considered. For example, it is well established that aggressive immunosuppressive treatment by cytostatic or cytotoxic drugs as well as methylprednisolone might induce neuronal damage.[35] Patients with NMOSD are regularly on these treatments, and methylprednisolone still presents the primary option for treating acute attacks. In an exploratory analysis comparing patients on rituximab treatment versus those on azathioprine, we did not detect any differences. However, our study was most likely underpowered to investigate drug-related changes.

Alternatively, retrograde neuroaxonal degeneration has to be considered as an explanation for GCIP thinning.[36] In NMOSD, ON often spans several segments of the optic nerve, and chiasmal crossover is reported in some cases.[37] In our study, 3.3% of eyes without history of ON showed severely reduced GCIP at baseline, indicative of chiasmal affection and crossing-over during acute ON, which was clinically apparent only unilaterally. On the other hand, also patients who never experienced ON showed decreased GCIP compared with HC at baseline pointing towards a pathology independent from bygone optic nerve damage. Although, subclinical ONs, which might have occurred before the study, could be considered. Both are, however, unable to explain the observed GCIP loss during F/U: first, we accounted for contralateral ONs in F/U. And second, we did not observe pRNFL or VA loss that go along with GCIP thinning. It should be kept in mind that this study did not account for potential retrograde degeneration originating from posterior visual pathway damage, which is mandatory for future confirmatory studies.

Interesting is our exploratory finding of a mild pRNFL thickening in the few patients presenting with a NMOSD-related attack during F/U. Although this should not be overstated in light of the small sample size, it could indicate that astrocytes in regions not directly affected by an acute lesion or attack could be affected during an attack elsewhere in the CNS. But a mildly swollen pRNFL might also be an effect of prednisolone treatment in acute state or covert subclinical axonal damage in the pRNFL in line with an underlying optic neuropathy, which limits certain conclusions in this context. This finding might also explain conflicting results of two previous longitudinal studies: Manogaran *et al* did not find significant pRNFL or macular thinning in a case series of nine patients with NMOSD from

Table 3 Longitudinal OCT results of patients with NMOSD and HCs.

	Absolute change in HC	Absolute change in Eyes^{ON-} Eyes^{ON+}		Longitudinal change in Eyes^{ON-} vs HC					Longitudinal change in Eyes^{ON+} vs HC				
	Mean±SD	Mean±SD	Mean±SD	B	SE	R^2_{marg}	R^2_{cond}	P values	B	SE	R^2_{marg}	R^2_{cond}	P values
GCIP (mm^3)	0.00±0.01	−0.01±0.02	0.00±0.02	−0.003	0.002	0.123	0.995	0.044	8.8e^{-5}	0.002	0.580	0.997	0.960
pRNFL (µm)	−0.61±2.00	−0.68±3.52	−0.94±3.09	−0.104	0.312	0.016	0.980	0.740	−0.113	0.257	0.454	0.995	0.663
INL (mm^3)	0.00±0.01	0.00±0.01	0.00±0.01	−6.7e^{-5}	0.001	0.019	0.957	0.947	−5.0e^{-5}	0.001	0.019	0.960	0.960
FT (µm)	0.52±4.16	−0.32±4.94	2.74±11.4	−0.047	0.510	0.077	0.991	0.927	0.936	1.093	0.142	0.994	0.396
TMV (mm^3)	0.00±0.02	−0.01±0.03	−0.01±0.03	−0.001	0.003	0.234	0.997	0.740	−0.003	0.003	0.442	0.999	0.380

B, estimate; Eyes ON$^-$, patients with NMOSD without a history of ON; Eyes ON$^+$, patients with NMOSD with a history of ON; FT, fovea thickness; GCIP, combined ganglion cell and inner plexiform layer; HC, healthy control; INL, inner nuclear layer; NMOSD, neuromyelitis optica spectrum disorders; OCT, optical coherence tomography; ON, optic neuritis; pRNFL, peripapillary retinal nerve fibre layer;TMV, total macular volume.

Canada and two, 4 years apart visits.[38] Bouyon *et al* reported pRNFL thinning over 18 months in 30 patients with NMOSD from France.[39]

Given the rarity of NMOSD in Europe, an important strength of our study is the large sample size.[40] Furthermore, we were able to distinguish disease-related changes from physiological changes by including a matched HC cohort. We also thoroughly excluded potential confounders like AQP4-ab seronegative patients, or patients within 5 months after an acute ON, in which ongoing neuroaxonal degeneration from ON might still be present. Important limitations of our study include the lack of consistent longitudinal functional measurements in HCs and sensitive functional tests, which prohibit investigating the functional relevance of these changes, and the lack of HCs from all study-sites. Furthermore, our study cannot conclusively investigate the influence of disease-modifying therapy, ethnicity and contralateral ON because of limited sample size for these subgroup analyses.

Our findings, if confirmed, challenge the notion that disease-related damage occurs only attack dependent in NMOSD, which is the hallmark of today's immunosuppressive therapy of NMOSD. If confirmed, our data suggest that targeting a progressive retinopathy might be important for monitoring and treatment of the disease. The clinical relevance of these changes still needs to be investigated. It also remains to be shown, if similar observations can be made also in other areas of the CNS.

Author affiliations
[1]NeuroCure Clinical Research Center, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[2]Institute of Clinical Neuroimmunology, Ludwig Maximilians University, Munich, Germany
[3]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK
[4]Monash School of Medicine, Monash University & The Alfred Hospital, Melbourne, Victoria, Australia
[5]Central Clinical School, Department of Neurosciences, Monash University, Melbourne, Victoria, Australia
[6]Experimental and Clinical Research Center, Max Delbrueck Center for Molecular Medicine and Charité—Universitätsmedizin Berlin, Berlin, Germany
[7]Department of Neurology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
[8]Department of Neurology, Charité—Universitätsmedizin Berlin, Berlin, Germany
[9]Molecular Neuroimmunology Group, Department of Neurology, University of Heidelberg, Heidelberg, Germany
[10]Department of Neurology, University of California, Irvine, Irvine, CA, United States

Acknowledgements The authors would like to thank Dr Janine Mikolajczak, Charlotte Bereuter, Jan Lödige and Ivonne Hinz for their excellent technical support. SJ would like to thank Mrs Anna Eschlbeck and the Nikon Imaging Center at the University of Heidelberg for excellent technical assistance.

Contributors FCO: data collection, OCT data processing, data analysis, writing of the manuscript. JH: data acquisition and collection, study coordination. ARF: data collection. NL: data collection, data analysis. HZ: data acquisition, study coordination, supervision of OCT data processing. SM: data analysis. NB/JBS/JP/MIL/TK: data acquisition, study coordination. OBW/PA/KR: study coordination. SJ: data acquisition. FP: data acquisition, study coordination, study concept. AUB: study concept, design, study coordination, data analysis, writing of the manuscript. All authors revised the manuscript for intellectual content and read and approved the final manuscript.

Funding The project was supported with grants from the German Ministry for Education and Research (BMBF/KKNMS; Competence Network Multiple Sclerosis (to FP, KR), from the Deutsche Forschungsgemeinschaft (DFG, grant Exc. 257 to FP, AUB), from the German Federal Ministry of Economic Affairs and Energy (EXIST 03EFEBE079 to AUB), from the German Ministry of Education and Research (N2-ADVISIMS 16GW0079 to FP and AUB), from the National Multiple Sclerosis Society (to FP), from the Guthy-Jackson Charitable Foundation (to FP, AUB) and from Novartis (to HZ). MIL and JP were funded for highly specialised service for neuromyelitis optica—NHS, UK, grants. JP also received research support from the MS society and Guthy-Jackson Charitable Foundation. FCO received financial support by the MD student research stipend from the Berlin Institute of Health (BIH).

Competing interests FCO has nothing to disclose. JH reports personal fees and non-financial support from Merck, grants, personal fees and non-financial support from Novartis, personal fees from Roche, non-financial support from Bayer Healthcare, personal fees from Santhera, personal fees and non-financial support from Biogen, personal fees and non-financial support from Sanofi Genzyme, all outside the submitted work. ARF is sponsored by Abide Therapeutic outside of the submitted work and reports no potential conflicts of interest. NL has nothing to disclose. HZ reports grants from Novartis, during the conduct of the study. SM has nothing to disclose; he is named as inventor on a patent application describing OCT image analysis. NB has nothing to disclose. JBS received speaking fees and travel grants from Bayer Healthcare, Sanofi-Aventis/Genzyme, Biogen and Teva Pharmaceuticals, unrelated to the present scientific work. PA reports grants, personal fees and non-financial support from Allergan, personal fees and non-financial support from Bayer Healthcare, grants, personal fees and non-financial support from Biogen, grants, personal fees and non-financial support from Ipsen, grants, personal fees and non-financial support from Merz Pharmaceuticals, personal fees and non-financial support from Merck, grants, personal fees and non-financial support from Novartis, non-financial support from Sanofi-Aventis/Genzyme, personal fees and non-financial support from Teva Pharmaceuticals, grants, personal fees and non-financial support from Roche, outside the submitted work. KR reports research support from Novartis and Merck Serono as well as speaking fees or travel grants from Bayer Healthcare, Biogen Idec, Merck Serono, Sanofi-Aventis/Genzyme, Teva Pharmaceuticals, Roche, Novartis and the Guthy-Jackson Charitable Foundation, all unrelated to this work. TK received travel expenses and personal compensations from Bayer Healthcare, Teva Pharmaceuticals, Merck, Novartis Pharma, Sanofi-Aventis/Genzyme, Roche and Biogen as well as grant support from Bayer-Schering AG, Novartis and Chugai Pharma, unrelated to this work. OW has nothing to disclose. SJ has nothing to disclose. MIL reports personal fees from Biogen Idec and grants from Novartis, outside the submitted work. JP reports grants Journal of Neurology, Neurosurgery and Psychiatry and personal fees from Merck Serono, grants and personal fees from Biogen Idec, personal fees from Teva Pharmaceuticals, grants from Chugai, grants and personal fees from MedImmun, grants and personal fees from Alexion, grants and personal fees from Novartis, personal fees from Roche, grants from Genzyme, grants and personal fees from ABIDE, personal fees from TG Therapeutics, outside the submitted work. In addition, JP has a patent Isis: Diagnosing Multiple Sclerosis. FP reports research grants and speaker honoraria from Bayer, Teva, Genzyme, Merck, Novartis, MedImmune and is member of the steering committee of the OCTIMS study (Novartis), all unrelated to this work. AUB is cofounder and holds shares of commercial entities Motognosis and Nocturne. He is named as inventor on several patent applications describing serum biomarkers

for MS, perceptive visual computing for tracking of motor dysfunction and OCT image analysis.

Patient consent Not required.

Ethics approval NeuroCure Clinical Research Center (NCRC) at Charité — Universitätsmedizin Berlin, Germany (EA1/131/09), the Ludwig Maximilians Universität Munich, Germany (Z427- 14) and the Nuffield Center for Clinical Neurosciences at Oxford University, UK (REC 16/SC/0224).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Jarius S, Paul F, Fechner K, *et al*. Aquaporin-4 antibody testing: direct comparison of M1-AQP4-DNA-transfected cells with leaky scanning versus M23-AQP4-DNA-transfected cells as antigenic substrate. *J Neuroinflammation* 2014;11:129.
2 Thompson AJ, Banwell BL, Barkhof F, *et al*. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* 2018;17:162–73.
3 Trapp BD, Peterson J, Ransohoff RM, *et al*. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998;338:278–85.
4 Zekeridou A, Lennon VA. Aquaporin-4 autoimmunity. *Neurol Neuroimmunol Neuroinflamm* 2015;2:e110.
5 Uzawa A, Mori M, Kuwabara S. Neuromyelitis optica: concept, immunology and treatment. *J Clin Neurosci* 2014;21:12–21.
6 Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol* 2015;14:183–93.
7 Dörr J, Paul F. The transition from first-line to second-line therapy in multiple sclerosis. *Curr Treat Options Neurol* 2015;17:354.
8 Wingerchuk DM, Pittock SJ, Lucchinetti CF, *et al*. A secondary progressive clinical course is uncommon in neuromyelitis optica. *Neurology* 2007;68:603–5.
9 Jarius S, Ruprecht K, Wildemann B, *et al*. Contrasting disease patterns in seropositive and seronegative neuromyelitis optica: a multicentre study of 175 patients. *J Neuroinflammation* 2012;9:14.
10 Lucchinetti CF, Guo Y, Popescu BF, *et al*. The pathology of an autoimmune astrocytopathy: lessons learned from neuromyelitis optica. *Brain Pathol* 2014;24:83–97.
11 Schneider E, Zimmermann H, Oberwahrenbrock T, *et al*. Optical Coherence Tomography Reveals Distinct Patterns of Retinal Damage in Neuromyelitis Optica and Multiple Sclerosis. *PLoS One* 2013;8:e66151.
12 Ramanathan S, Prelog K, Barnes EH, *et al*. Radiological differentiation of optic neuritis with myelin oligodendrocyte glycoprotein antibodies, aquaporin-4 antibodies, and multiple sclerosis. *Mult Scler* 2016;22:470–82.
13 Schmidt F, Zimmermann H, Mikolajczak J, *et al*. Severe structural and functional visual system damage leads to profound loss of vision-related quality of life in patients with neuromyelitis optica spectrum disorders. *Mult Scler Relat Disord* 2017;11:45–50.
14 Oertel FC, Zimmermann H, Paul F, *et al*. Optical coherence tomography in neuromyelitis optica spectrum disorders: potential advantages for individualized monitoring of progression and therapy. *Epma J* 2018;9:21–33.
15 Jeong IH, Kim HJ, Kim NH, *et al*. Subclinical primary retinal pathology in neuromyelitis optica spectrum disorder. *J Neurol* 2016;263:1343–8.
16 Oertel FC, Kuchling J, Zimmermann H, *et al*. Microstructural visual system changes in AQP4-antibody-seropositive NMOSD. *Neurol Neuroimmunol Neuroinflamm* 2017;4:e334.
17 Tian DC, Su L, Fan M, *et al*. Bidirectional degeneration in the visual pathway in neuromyelitis optica spectrum disorder (NMOSD). *Mult Scler* 2017:135245851772760.
18 Felix CM, Levin MH, Verkman AS. Complement-independent retinal pathology produced by intravitreal injection of neuromyelitis optica immunoglobulin G. *J Neuroinflammation* 2016;13:275.
19 Wingerchuk DM, Banwell B, Bennett JL, *et al*. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015;85:177–89.
20 Oberwahrenbrock T, Weinhold M, Mikolajczak J, *et al*. Reliability of Intra-Retinal Layer Thickness Estimates. *PLoS One* 2015;10:e0137316.
21 Tewarie P, Balk L, Costello F, *et al*. The OSCAR-IB consensus criteria for retinal OCT quality assessment. *PLoS One* 2012;7:e34823.
22 Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, *et al*. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology* 2016;86:2303–9.
23 R Development Core Team. R: a language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria, 2008. http://www.R-project.org.
24 Yates JT, Harrison JM, O'Connor PS, *et al*. Contrast sensitivity: characteristics of a large, young, adult population. *Am J Optom Physiol Opt* 1987;64:519–27.
25 Syc SB, Saidha S, Newsome SD, *et al*. Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis. *Brain* 2012;135(Pt 2):521–33.
26 Akaishi T, Kaneko K, Himori N, *et al*. Subclinical retinal atrophy in the unaffected fellow eyes of multiple sclerosis and neuromyelitis optica. *J Neuroimmunol* 2017;313:10–15.
27 Merle H, Olindo S, Donnio A, *et al*. Retinal peripapillary nerve fiber layer thickness in neuromyelitis optica. *Invest Ophthalmol Vis Sci* 2008;49:4412–7.
28 Fernandes DB, Raza AS, Nogueira RG, *et al*. Evaluation of inner retinal layers in patients with multiple sclerosis or neuromyelitis optica using optical coherence tomography. *Ophthalmology* 2013;120:387–94.
29 Sabater L, Giralt A, Boronat A, *et al*. Cytotoxic effect of neuromyelitis optica antibody (NMO-IgG) to astrocytes: an in vitro study. *J Neuroimmunol* 2009;215:31–5.
30 Nicchia GP, Pisani F, Simone L, *et al*. Glio-vascular modifications caused by Aquaporin-4 deletion in the mouse retina. *Exp Eye Res* 2016;146:259–68.
31 de Hoz R, Rojas B, Ramírez AI, *et al*. Retinal macroglial responses in health and disease. *Biomed Res Int* 2016;2016:1–13.
32 Kurosawa K, Misu T, Takai Y, *et al*. Severely exacerbated neuromyelitis optica rat model with extensive astrocytopathy by high affinity anti-aquaporin-4 monoclonal antibody. *Acta Neuropathol Commun* 2015;3:82.
33 Hayashida S, Masaki K, Yonekawa T, *et al*. Early and extensive spinal white matter involvement in neuromyelitis optica. *Brain Pathol* 2017;27:249–65.
34 Ventura RE, Kister I, Chung S, *et al*. Cervical spinal cord atrophy in NMOSD without a history of myelitis or MRI-visible lesions: Table 1. *Neurology - Neuroimmunology Neuroinflammation* 2016;3:e224.
35 Diem R, Hobom M, Maier K, *et al*. Methylprednisolone increases neuronal apoptosis during autoimmune CNS inflammation by inhibition of an endogenous neuroprotective pathway. *J Neurosci* 2003;23:6993–7000.
36 Gabilondo I, Martínez-Lapiscina EH, Martínez-Heras E, *et al*. Trans-synaptic axonal degeneration in the visual pathway in multiple sclerosis. *Ann Neurol* 2014;75:98–107.
37 Raz N, Vaknin A, Chokron S, *et al*. Functional MRI as a tool for assessing chiasmal visual defect in a patient with neuromyelitis optica. *J Neurol Neurosurg Psychiatry* 2010;81:1174–5.
38 Manogaran P, Traboulsee AL, Lange AP. Longitudinal study of retinal nerve fiber layer thickness and macular volume in patients with Neuromyelitis Optica Spectrum disorder. *J Neuroophthalmol* 2016;36:363–8.
39 Bouyon M, Collongues N, Zéphir H, *et al*. Longitudinal follow-up of vision in a neuromyelitis optica cohort. *Multiple Sclerosis Journal* 2013;19:1320–2.
40 Mori M, Kuwabara S, Paul F. Worldwide prevalence of neuromyelitis optica spectrum disorders. *J Neurol Neurosurg Psychiatry* 2018;89:555–6.