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Original research

Astrocytic outer retinal layer thinning is not a feature in AQP4-IgG seropositive neuromyelitis optica spectrum disorders

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ABSTRACT

Background Patients with anti-aquaporin-4 antibody seropositive (AQP4-IgG+) neuromyelitis optica spectrum disorders (NMOSDs) frequently suffer from optic neuritis (ON) leading to severe retinal neuroaxonal damage. Further, the relationship of this retinal damage to a primary astrocytopathy in NMOSD is uncertain. Primary astrocytopathy has been suggested to cause ON-independent retinal damage and contribute to changes particularly in the outer plexiform layer (OPL) and outer nuclear layer (ONL), as reported in some earlier studies. However, these were limited in their sample size and contradictory as to the localisation. This study assesses outer retinal layer changes using optical coherence tomography (OCT) in a multicentre cross-sectional cohort.

Method 197 patients who were AQP4-IgG+ and 32 myelin-oligodendrocyte-glycoprotein antibody seropositive (MOG-IgG+) patients were enrolled in this study along with 75 healthy controls. Participants underwent neurological examination and OCT with central postprocessing conducted at a single site.

Results No significant thinning of OPL (25.02±2.03 µm) or ONL (61.63±7.04 µm) were observed in patients who were AQP4-IgG+ compared with patients who were MOG-IgG + with comparable neuroaxonal damage (OPL: 25.10±2.00 µm; ONL: 64.71±7.87 µm) or healthy controls (OPL: 24.58±1.64 µm; ONL: 63.59±5.78 µm). Eyes of patients who were AQP4-IgG+ (19.84±5.09 µm, p=0.027) and MOG-IgG + (19.82±4.78 µm, p=0.004) with a history of ON showed parafoveal OPL thinning compared with healthy controls (20.99±5.14 µm); this was not observed elsewhere.

Conclusion The results suggest that outer retinal layer loss is not a consistent component of retinal astrocytic damage in AQP4-IgG+ NMOSD. Longitudinal studies are necessary to determine if OPL and ONL are damaged in late disease due to retrograde trans-synaptic axonal degeneration and whether outer retinal dysfunction occurs despite any measurable structural correlates.

INTRODUCTION

Neuromyelitis optica spectrum disorders (NMOSDs) are relapsing autoimmune disorders affecting the central nervous system (CNS).[1] Common clinical attacks in NMOSD include optic neuritis (ON), acute myelitis and area postrema syndrome.[2] Serum autoantibodies to aquaporin-4 (AQP4-IgG) are detectable in 60%–80% of patients with NMOSD .[3 4]

AQP4 is an astrocytic water channel in the CNS.[5] In the retina, astrocytes are mainly located in the inner neuroaxonal layers of the retina, but AQP4 is additionally highly expressed in retinal Müller cells.[6] These glial cells have diverse functions, such as regulation of water homeostasis and neurotransmitter recycling, and are located around the fovea

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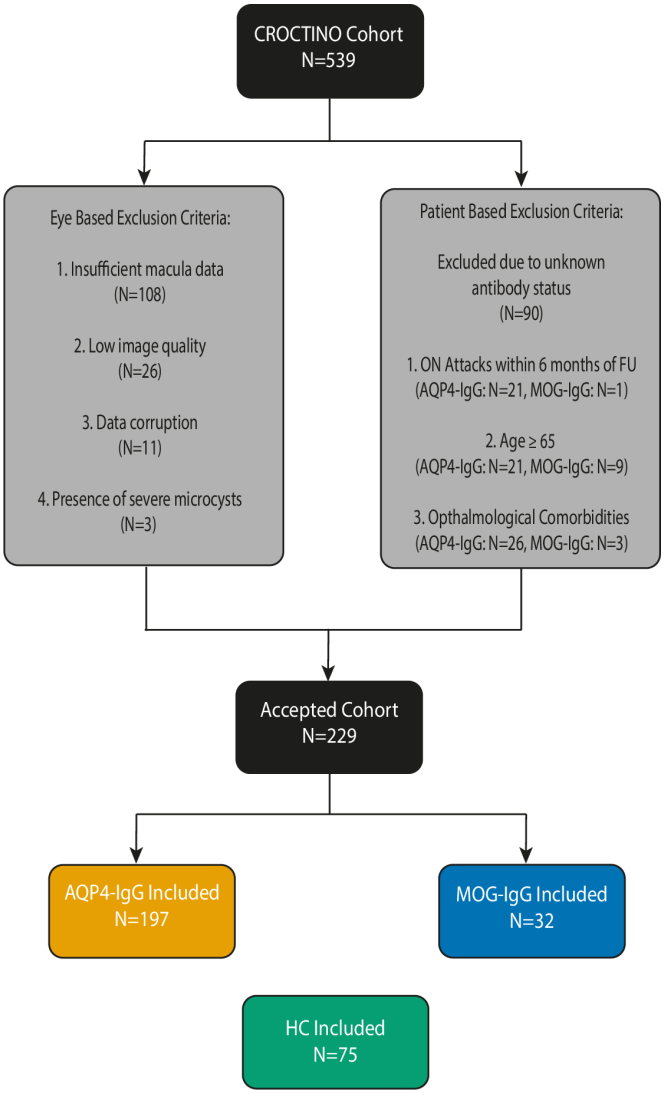

Figure 1 Cohort design and exclusion criteria: from the original 539 patients recruited in the CROCTINO cohort, 108 patients were excluded due to missing macular data. Of the remaining 431 patients in the segmentation cohort, a further 40 patients were excluded due to anomalies in their OCT scans (OSCAR-IB criteria; primarily due to low image quality (26 patients) or the presence of microcysts (3 patients) or other pathologies) or due to data corruption (11 patients). We also excluded patients with unknown antibody status (90 patients). Of the remaining 301 patients, the cohort was split based on AQP4-IgG or MOG-IgG seropositivity and a further set of exclusion criteria were applied based on age (being ≥65 years), ophthalmological comorbidities (eg, glaucoma) and in instances where follow-ups occurred within 6 months of an ON attack. AQP4-IgG, anti-aquaporin-4 antibody; HC, healthy control; MOG-IgG, anti-myelin-oligodendrocyte-glycoprotein antibody; OCT, optical coherence tomography; ON, optic neuritis.

spanning the entire thickness of the retina.[7] Of particular interest is also the Henle Fibre outer nuclear layer (ONL) boundary of the parafovea where AQP4 channels are highly expressed.[8]

A primary and attack-independent astrocytopathy in NMOSD has been suggested to contribute to retinal neurodegeneration and to Müller cell-associated parafoveal changes.[9–13] Recent studies suggested potential astrocytopathy-related outer retinal layer (ORL) thinning in AQP4-IgG seropositive NMOSD but were limited in their sample size and in parts contradictory on the exact layers in which these changes occur.[8 11] It thereby remains unclear if ORLs, especially the ONL are also potentially affected by primary retinal astrocytopathy in AQP4-IgG seropositive NMOSD.

Representing the largest international NMOSD dataset collected so far, the CROCTINO study (*Collaborative Retrospective Study on retinal optical coherence tomography (OCT) in Neuromyelitis Optica*) overcomes one of the common weaknesses of NMOSD studies—being limited to small and homogenous sample populations.[14 15] Using OCT data from over 20 centres worldwide, reliable quantitative and qualitative retinal assessment becomes possible, and controversial questions such as ORL changes in AQP4-IgG seropositive NMOSD can be clarified. Apart from patients who were AQP4-IgG seropositive, the CROCTINO cohort also includes patients with antibodies to myelin-oligodendrocyte-glycoprotein (MOG-IgG); a group that is now believed to be a distinct disease entity.[14 16–18] While clinically similar and undergoing comparable retinal neurodegeneration after ON, MOG-IgG-associated disease (MOGAD) lacks an identifiable astrocytopathy component and is thereby an appropriate diseased control group for patients who were AQP4-IgG seropositive when investigating astrocytic changes.[10 19]

In this study, we investigated if ORL thinning, specifically in the foveal and macular ONL, occurs in patients who were AQP4-IgG seropositive compared with healthy controls (HCs) and with patients with MOGAD as a diseased control group.

METHODS

Cohort design

A total of 539 patients with NMOSD were recruited between 2000 and 2018 as part of CROCTINO (stratified data of centres by device type and number of patients are summarised in the online supplemental file 1).[14] Patients with (1) diseases potentially confounding OCT analyses (including glaucoma, diabetic retinopathy, retinal surgery and ametropia greater than ±6 diopters), (2) a history of ON within the last 6 months before baseline, (3) no evidence of seropositivity for AQP4-IgG or MOG-IgG[20 21] and (4) no macular OCT data were excluded. Cell-based assays were used for the detection of AQP4-IgG and MOG-IgG antibodies in serum samples from all patients. Clinical data (antibody serology, disease duration, frequency of ON, location of ON, date of ON, Expanded Disability Standard Scale and treatment received) were collected from all patients. We also included 75 HCs (recruited from Barcelona, Isfahan, Mangalore and Berlin), who were neither age nor sex matched to either cohort.

Optical coherence Tomography

Retinal examinations were conducted at each centre using the following OCT devices: Spectralis SD-OCT, Heidelberg Engineering, Heidelberg, Germany (Spectralis), Cirrus HD-OCT, Carl Zeiss Meditec Inc, Dublin, California, USA (Cirrus) and Topcon 3D-OCT, Topcon Corp, Tokyo, Japan (Topcon). With respect to each device and each centre, two scans were collected: (1) a 3.4 mm diameter peripapillary ring scan around the optic nerve head for Spectralis SD-OCT (for Cirrus and Topcon devices: extracted from optic disc volume scans), and (2) a macular volume scans, centred on the fovea.[14] Scans were categorised and uploaded onto a central server to be accessed for further processing.

All OCT images fulfilled the OSCAR-IB criteria[22 23] (see figure 1—images from 29 patients not fulfilling these criteria were excluded) and results were presented in line with the

Table 1 Demographic overview

	HC	AQP4-IgG	MOG-IgG
Subjects (N)	75	197	32
Number of eyes (N)	148	317	55
Age (years, mean±SD)	32.3±9.6	41.8±12.1	36.5±13.7
Sex (male, N (%))	25 (33.8)	24 (12.2)	10 (31.2)
EDSS (median (IQR))	–	3.5 (2.0–5.0)	2.0 (1.5–2.5)
Average age at onset (years, median (IQR))	–	32.9 (24.9–42.4)	30.0 (17.6–42.5)
Patients with a history of ON (N (%))	–	142 (72.1)	24 (75.0)
Median number of ON episodes (median, IQR)	–	1.00 (0.00–3.00)	2.00 (1.00–4.00)
Disease duration (years, mean±SD)	–	7.1±6.7	4.8±7.8
Ethnicity (N (%))	White (57 (761)) Asian (16 (21.3)) Hispanic (1 (1.3)) Other (1 (1.3))	White (105 (53.3) Asian (56 (28.4)) African American (11 (5.6)) Other (25 (12.7))	White (19 (59.4)) Asian (13 (40.6))
Current treatment (N (%))		Rituximab (51 (25.9)) Azathioprine (42 (21.3)) Mycophenolate Mofetil (31 (15.7)) Methotrexate (4 (2.0)) Other or missing (69 (35.0))	Rituximab (6 (18.8)) Azathioprine (6 (18.8)) Prednisone (6 (18.8)) Mycophenolate mofetil (5 (15.6)) Other or missing (9 (28.1))
OCT device (N (%))	Spectralis (75 (100))	Spectralis (139 (70.6)) Cirrus (38 (19.3)) Topcon (20 (10.2))	Spectralis (25 (78.1)) Cirrus (3 (9.4)) Topcon (4 (12.5))

Cirrus: Cirrus HD-OCT, Carl Zeiss Meditec Inc, Dublin, California, USA; Spectralis: SD-OCT, Heidelberg Engineering, Heidelberg, Germany;Topcon: Topcon 3D-OCT, Topcon Corp, Tokyo Japan.
AQP4-IgG, anti-aquaporin-4 antibody; EDSS, Expanded Disability Standard Scale; HCs, healthy controls; MOG-IgG, anti-myelin-oligodendrocyte-glycoprotein antibody; N, number of subjects; ON, optic neuritis.

APOSTEL V.2.0 recommendations.[24] Peripapillary retinal nerve fibre layer (pRNFL) thickness was derived using a device-specific protocol and centred around the optic nerve head. Segmentation of all layers in macular volume scans were performed semiautomatically and processed with an in-house proprietary software (SAMIRIX).[25] For the purposes of this study, the macular retinal layers were segmented in the following layers: macular retinal nerve fibre layer (mRNFL), ganglion cell and inner plexiform layer (GCIP), inner nuclear layer (INL), outer plexiform layer (OPL), ONL, the outer plexiform and nuclear layer (OPNL), photoreceptor layer (PR, inner photoreceptor segments to Bruch's membrane) and the total retinal thickness (RT, calculated as the thickness consisting of the RNFL (defined as layer no. 3 per Staurenghi *et al*[26]) to the Bruch's membrane (layer no. 14). All scans were checked and, where necessary, manual correction of the automatic segmentation was conducted using SAMIRIX by experienced raters (FCO, CB and SS for ring scans, HZ, FCO and AL for macular scans) at a single site at the Charité—Universitätsmedizin Berlin. To assure comparability with previously published data on ORL changes in NMOSD, the macular volume data were further segregated into one of three export protocols: (1) a 5 mm diameter cylinder omitting a 1 mm diameter around the fovea (5 mm study), (2) a 3 mm diameter cylinder omitting a 1 mm diameter around the fovea (3 mm study) and (3) a 1 mm mean thickness around the fovea (1 mm study). Results are reported for the 5 mm study on Spectralis devices; confirmatory results based on the 3 mm and 1 mm study as well as for Cirrus and Topcon devices are set out in the online supplemental file 1.

Statistical methods

Data were stratified in cohorts by (1) antibody status and (2) ON history (contralateral eyes of patients with a history of unilateral ON are classified not fulfilling the ON history criteria). The data were further bifurcated by OCT device (Spectralis, Cirrus or Topcon) to mitigate any device-specific aberrations. For continuous cohort data (age, average age at onset and disease duration) on each of the AQP4-IgG, MOG-IgG and HC cohorts, the Student's t-test was employed. Cross-sectional group comparisons of the OCT values were conducted using linear mixed-effect models with age and sex as fixed and centre and patient-ID as random effects; where necessary, models were corrected for age and sex. Marginal and conditional coefficients of determination for the models were estimated by pseudo-R^2 for mixed-effect models. Significance was established at $p<0.05$. Statistical

Table 2 Group comparison between HC and patients who were AQP4-IgG and MOG-IgG seropositive at baseline (Spectralis devices only)

				AQP4-IgG vs HC			AQP4-IgG vs MOG-IgG			MOG-IgG vs HC		
	HC	AQP4-IgG	MOG-IgG	B	SE	P	B	SE	P	B	SE	P
Number of eyes	148	317	55									
pRNFL in µm (mean±SD)	99.17±9.76	78.46±24.13	74.33±23.44	−20.22	2.86	<0.001	0.34	4.33	0.937	−29.40	2.75	<0.001
mRNFL in µm (mean±SD)	35.25±3.13	28.09±6.60	27.62±5.43	−6.12	0.69	<0.001	−0.15	1.38	0.913	−6.98	0.66	<0.001
GCIP in µm (mean±SD)	80.62±6.14	65.81±13.03	66.16±11.85	−14.74	1.45	<0.001	−2.18	2.95	0.461	−15.16	1.33	<0.001
INL in µm (mean±SD)	39.64±2.51	39.85±3.57	41.55±4.14	0.34	0.39	0.384	−1.93	0.87	0.028	1.79	0.53	0.001
OPL in µm (mean±SD)	24.58±1.64	25.02±2.03	25.10±2.00	0.28	0.24	0.241	−0.21	0.44	0.634	−0.01	0.29	0.986
ONL in µm (mean±SD)	63.59±5.78	61.63±7.04	64.71±7.87	−0.01	0.83	0.993	−1.77	1.80	0.327	0.69	0.93	0.457
OPNL in µm (mean±SD)	89.23±6.95	86.65±7.21	89.81±8.61	−0.41	0.85	0.634	−1.54	1.85	0.406	−0.14	0.93	0.878
PR in µm (mean±SD)	80.80±2.38	80.35±2.94	81.49±3.59	−0.30	0.33	0.363	−0.07	0.68	0.923	0.20	0.39	0.610
RT in µm (mean±SD)	324.47±13.24	300.76±20.11	306.6±17.99	−20.16	2.37	<0.001	−6.61	4.77	0.169	−18.91	2.49	<0.001

AQP4-IgG, anti-aquaporin-4 antibody; B, estimate; GCIP, ganglion cell and inner plexiform layer; HC, healthy control; INL, inner nuclear layer; MOG-IgG, anti-myelin-oligodendrocyte-glycoprotein antibody; mRNFL, macular retinal nerve fibre layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor layer; pRNFL, peripapillary retinal nerve fibre layer; RT, total retinal thickness.

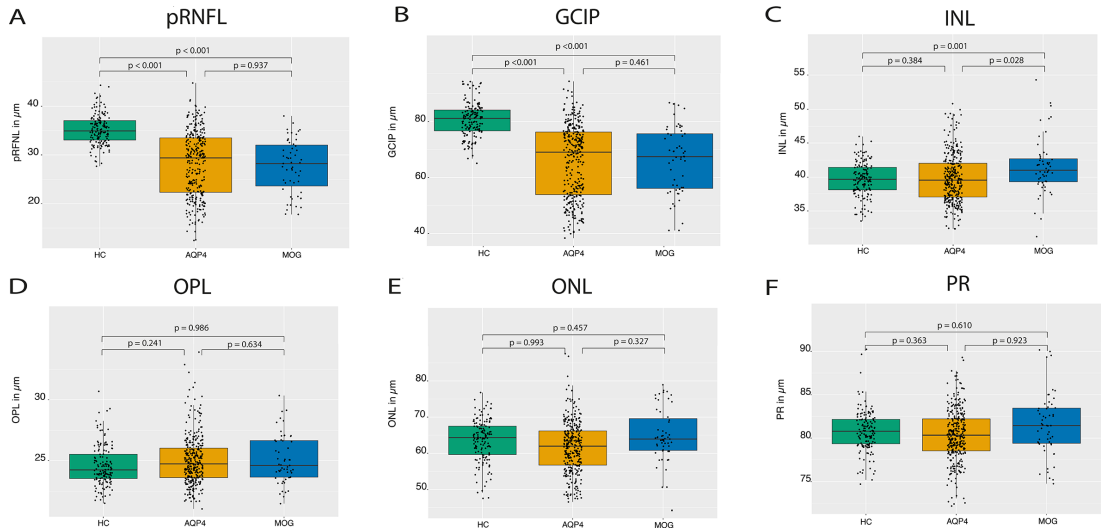

Figure 2 Group comparison of HC and patients who were AQP4-IgG and MOG-IgG seropositive at baseline: boxplots of mean OCT values with individual eyes (jitter) in HC (left, green), patients with AQP4-IgG (middle, yellow) and patients with MOG-IgG (right, blue). (A) pRNFL; (B) GCIP; (C) INL; (D) OPL; (E) ONL; and (F) PR. AQP4, aquaporin-4; HC, healthy control; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; MOG, myelin-oligodendrocyte-glycoprotein; OCT, optical coherence tomography; ONL, outer nuclear layer; OPL, outer plexiform layer; PR, photoreceptive layer; pRNFL, peripapillary retinal nerve fibre layer

analyses were conducted using R (V.4.0.0) (RStudio Inc, Boston, Massachusetts, USA).[27]

RESULTS

Cohort description

In total, 197 patients who were AQP4-IgG seropositive fulfilled the inclusion criteria (figure 1, table 1). We also included 75 unmatched HCs and 32 patients who were MOG-IgG seropositiveas control groups.

Neuroaxonal damage measured by pRNFL, mRFNL and GCIP was comparable in patients who were AQP4-IgG seropositive (pRNFL: 78.46±24.13 µm, mRNFL: 28.09±6.60 µm, GCIP: 65.81±13.03 µm) and MOG-IgG seropositive (pRNFL: 74.33±23.44 µm, mRNFL: 27.62±5.43 µm, GCIP: 66.16±11.85 µm) making MOGAD a highly relevant comparative disease control group for our investigation of ORLs (table 2).

Limited outer retinal changes in AQP4-IgG seropositive NMOSD

No significant thinning of macular OPL and ONL in patients who were AQP4-IgG seropositive (irrespective of ON status) were observed compared with HC or patients who were MOG-IgG seropositive using the 5 mm diameter macular data (table 2, figure 2). No significant changes were observed when the OPL and ONL values were analysed as the combined OPNL. Previous studies described ORL thinning only in the foveal and parafoveal area as a sign of AQP4-IgG-induced Müller cell damage.[8 11] We therefore repeated our analyses in both 3 mm and the 1 mm diameter volumes around the fovea, but these narrower volumes showed again no relevant OPL or ONL thinning in patients who were AQP4-IgG seropositive compared with HC or patients who were MOG-IgG seropositive (see online supplemental data). Additionally, while these previous studies reported changes in the inner segment layer of the photoreceptors, this was not seen in our study.[8 11]

After a previous description[11] of ORL changes in patients who were AQP4-IgG seropositive with a history of ON, we also examined ORL differences separately in eyes with a history of ON. AQP4-IgG seropositive eyes with a history of ON (AQP4-ON) did not display any thinning of ONL and OPL compared with patients without a history of ON (AQP4-NON) or HC, despite severe neuroaxonal loss measured by pRNFL and GCIP layer (table 3, figure 3). Comparing patients who were AQP4-IgG and MOG-IgG seropositive, both groups had a comparable neuroaxonal loss (pRNFL, GCIP)—in the whole group as well as in respect of ON and non-ON eyes (table 2, figure 2). AQP4-ON (B=−1.54, SE=0.69 µm, p=0.027) as well as MOG-ON (B=−2.51, SE=0.87 µm, p=0.004) showed an OPL thinning in the fovea (1 mm diameter) compared with HC, but no difference was observed between AQP4-ON and MOG-ON (p=0.100). Also, no significant correlation between ethnicity and current therapies on outer retinal thickness was found (data not shown).

DISCUSSION

Our study suggests that neither macular OPL nor ONL loss occurs in AQP4-IgG seropositive NMOSD, regardless of ON phenotype, as compared with HC and patients who were MOG-IgG seropositive. The MOG-IgG cohort presented a unique opportunity to contrast our AQP4-IgG seropositive cohort with a highly relevant comparator group, which most likely has no astrocytopathy-component.[28]

Our results differ from those published by You *et al* in 2019[8] and Filippatou et al in 2020.[11] In both studies, thinning was observed in the ONL and the inner segment of the photoreceptor layers. In the case of You *et al*, who utilised Spectralis SD-OCT devices for the image acquisition, foveal thinning was observed along with a reduction in b-wave amplitudes in full-field electroretinography (ERG) suggestive of Müller cell dysfunction.[8] Filippatou *et al*, who employed Cirrus-SD-OCT for the image acquisition, also described thinning of the fovea in the 5 mm diameter macular area around the fovea.[11] Both studies suggested the ORL changes to be caused by a primary retinal astrocytopathy with AQP4-IgG associated glial dysfunction in Müller cells.[29] These pathological responses could account for the associated thinning observed in the ONL in these studies. However, other exogenous factors

Table 3 OCT results in patients who were AQP4-IgG seropositive stratified by history of on (Spectralis devices only)

	AQP4-ON	AQP4-NON	MOG-ON	MOG-NON	AQP4-ON vs AQP4-NON			AQP4-ON vs HC			AQP-NON vs HC			AQP4-ON vs MOG-ON			AQP4-NON vs MOG-NON		
Number of eyes	232	85	43	12	B	SE	P	B	SE	P	B	SE	P	B	SE	P	B	SE	P
pRNFL in µm (mean±SD)	72.84±24.47	96.09±12.99	68.03±22.95	95.33±7.32	−25.18	3.93	<0.001	−29.56	3.57	<0.001	−9.29	5.15	0.07	2.63	6.12	0.667	6.03	5.44	0.275
GCIP in µm (mean±SD)	62.94±12.73	77.11±7.56	63.45±11.96	75.88±6.01	−14.74	2.06	<0.001	−19.60	1.49	<0.001	−0.50	1.48	0.735	−4.00	3.20	0.215	3.22	3.36	0.344
OPL in µm (mean±SD)	25.06±2.01	24.71±1.79	25.28±2.08	24.45±1.55	0.26	0.38	0.498	0.34	0.26	0.184	0.09	0.38	0.804	0.34	0.51	0.509	−0.12	0.96	0.899
ONL in µm (mean±SD)	62.53±7.45	63.14±6.62	66.09±8.08	59.76±4.58	−0.19	1.49	0.901	−0.18	0.92	0.847	−0.82	1.40	0.560	−2.84	2.12	0.183	2.76	3.58	0.446
OPNL in µm (mean±SD)	87.58±7.67	87.85±6.78	84.21±5.68	91.37±8.69	0.00	1.53	0.879	0.27	0.95	0.775	0.37	1.42	0.794	−2.51	2.21	0.259	2.67	3.51	0.450
PR in µm (mean±SD)	80.89±2.93	79.80±2.94	82.01±3.45	79.62±3.58	0.75	0.57	0.187	−0.19	0.36	0.595	−1.08	0.59	0.071	−0.58	0.77	0.454	1.36	1.44	0.348

AQP4, aquaporin-4 ; B, estimate; GCIP, ganglion cell and inner plexiform layer; HC, healthy control; MOG, myelin-oligodendrocyte-glycoprotein; NON, non-optic neuritis; ON, optic neuritis; ONL, outer nuclear layer; OPL, outer plexiform layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor layer; pRNFL, peripapillary retinal nerve fibre layer.

cannot be ruled out as contributory, such as cohort composition and study methodologies.

On a cohort level, our population is larger (197 patients who were AQP4-IgG seropositive vs 22 and 51 by You *et al* and Filippatou *et al*, respectively)[8 11] and more diverse than prior studies, which minimises potential type I errors. While You *et al* did not specify the ethnic composition of their cohort, the cohort in Filippatou *et al* had a relatively even distribution between Caucasian Americans (43%) and African Americans (53%) with a minor subset of Asian Americans (4%)—describing a pronounced ONL thinning in African Americans. African American patients with multiple sclerosis (MS) are also known to suffer from faster and often more aggressive disease course in general, which could also be true for other neuroinflammatory diseases like NMOSD.[30 31] Our AQP4-IgG seropositive cohort included an ethnically diverse dataset acquired worldwide with a lower African American patient composition (5.6%), which might have contributed to the less profound foveal ONL changes.[11 32]

Recently, it has been hypothesised that the neuroplastic characteristics of the INL may act as a barrier to retrograde (but not anterograde) trans-synaptic axonal degeneration—rectified to the ORLs—in patients with MS following ON.[33] This limited neuroplastic ability is hypothesised to rest with the bipolar, amacrine and horizontal cells, which feed into the synaptic tree at the level of the INL, and raises questions as to whether such protective mechanisms may also play a limited part in NMOSD and whether it remains so as we age.[33] The average age of participants in the two other studies were relatively older (mean age for both being 47 years), whereas for our AQP4-IgG cohort it was 42 years. Previously reported studies concerning cohorts of similar demographic distribution to ours reported no significant correlation between age and retinal thickness.[34 35] However, age-related changes in the retina cannot be ruled out and ORLs may be more susceptible to change with increasing age and/or disease duration. It is well-known that the plasticity of the CNS markedly reduces over time, and as a corollary, the regenerative properties of the INL may also be affected thereby diminishing its protective effects in reducing retrograde (trans-synaptic) axonal degeneration.[36] The retina is also a vascularised organ, particularly at the interface between inner and outer retina, where the deep vascular plexus intercepts the boundary between the INL and OPL.[37] Should the blood–retina barrier be compromised in the boundary between the INL and OPL, it is conceivable that the protective abilities of the INL may be circumvented and thereby mediating glial dysfunction in the Müller cells. This may have been what was observed in the OPL from the 1 mm AQP4-ON and MOG-ON cohort given the relative location of the OPL to the INL. To that end, while disease duration did not reveal to any correlates with OPL ($p=0.805$) or ONL ($p=0.835$) values, we cannot exclude time-dependent effects in a cross-sectional analysis. We believe that this area warrants more research to quantify if (1) age is a factor, (2) ON damages the barrier function and (3) the INL does indeed play a role as a dam to retrograde axonal degeneration in NMOSD.

A strength of our study rests on its cohort size and composition, which mirrors that of a global population. This result derives from a consortium of expert NMOSD researchers enabling the enrolment of participants through a multicentre strategy. This approach was designed to overcome many of the earlier NMOSD study limitations, for example small and homogeneous sample populations. Additionally, the use of differing OCT devices compounds complexities in OCT comparisons and a high degree of caution is needed in order to rely on differing platforms interchangeably.[38] Thus, our study focuses on use of

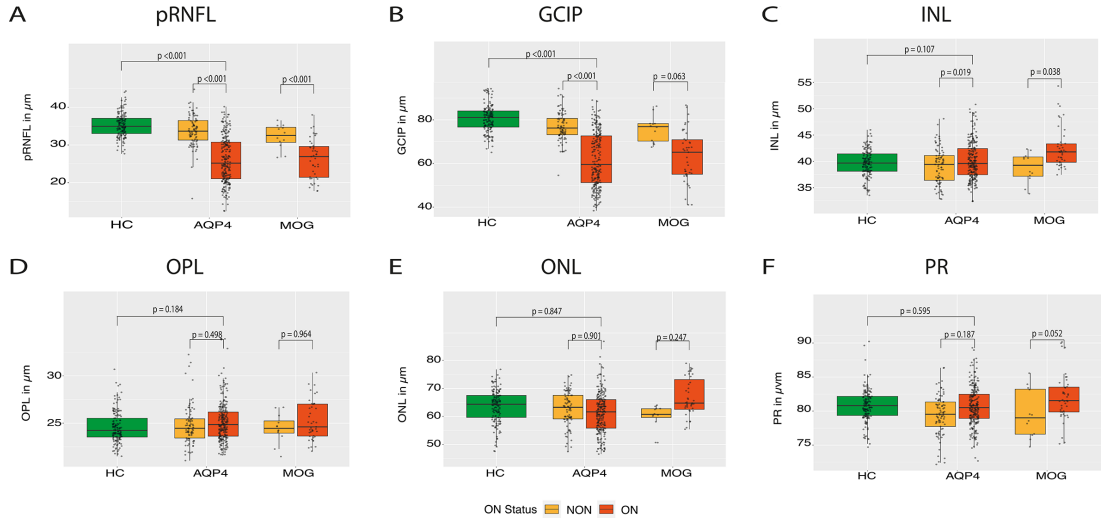

Figure 3 OCT results stratified by ON status (tested with Spectralis devices): boxplots of mean OCT values with individual eyes (jitter) in HC (left, green), AQP4-IgG cohort (middle) and MOG-IgG cohort (right). Seropositive patients with a history of ON are highlighted with light yellow and seropositive patients without a history of ON are highlighted in orange. (A) pRNFL; (B) GCIP; (C) INL; (D) OPL; (E) ONL; and (F) PR. AQP4, aquaporin-4; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; MOG, myelin-oligodendrocyte-glycoprotein; OCT, optical coherence tomography; ONL, outer nuclear layer; OPL, outer plexiform layer; PR, photoreceptive layer; pRNFL, peripapillary retinal nerve fibre layer.

three widely available OCT devices, and obtained confirmatory results with each of them; of these, two were also employed respectively in the studies by You *et al*[8] and Filippatou *et al*.[11]

Limitations of the current study should also be considered. First, the HCs and patients with MOGAD were not matched, which makes it difficult to rule out age-related and gender-related affects. Notably, retinal thickness decreases with age and males generally exhibit higher GCIP and RT.[25] Also, no ERG or functional visual pathway assessments were conducted, which could have potentially shown more subtle functional impairment of ORLs without associated tissue loss. Outer retinal studies are additionally complicated by Henle Fibre morphologies as OCT beam placement plays a major role in how this layer is depicted; the high level of irregularity and variability in these morphologies add a level of subjectiveness in the quantification and correction of outer layer segmentation and analyses.[39] Finally, Cirrus and Topcon measurements could not be utilised as confirmatory cohorts as there lacked sufficient HCs examined with these devices. Nonetheless, the current findings provide insights into relationships between retinal layer changes and axonal damage that have not previously been recognised; as no ORL changes can be observed on account of a primary astrocytopathy in NMOSD, it potentially alleviates the burden of monitoring the ORLs when tracking disease progression and reinforces the need to focus primarily on the inner layers, particularly the RNFL and the GCIP layer.

CONCLUSION

Our results show no evidence of macular ORL changes as a major component of retinal damage in patients who were seropositive AQP4-IgG NMOSD and patients with MOGAD. Further studies will be necessary to clarify (1) if OPL and ONL are damaged in late disease stages due to retrograde trans-synaptic axonal degeneration across the damaged INL barrier and (2) if outer retinal dysfunction without a measurable structural correlate occurs. Longitudinal studies could help quantify changes in the ORLs alongside disease progression.

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Patient consent for publication Consent obtained directly from patient(s).

Ethics approval Written informed consent was obtained from all patients prior to the commencement of the study and institutional review board approvals for retrospective data use were obtained or waived from each centre in accordance with

the Declaration of Helsinki (1964) in its currently applicable version at their own discretion and in accordance with relevant local laws. The study also conformed to all relevant best practice guidelines and ethical standards of each centre.

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Supplement

Center	Number of Patients	Device type
Bangkok, Thailand	25	Cirrus
Barcelona, Spain	13	Cirrus and Spectralis
Belo Horizonte, Brazil	57	Spectralis
Berlin, Germany	76	Spectralis
Düsseldorf, Germany	11	Spectralis
Goyang; Korea	50	Topcon
Isfahan, Iran	40	Spectralis
Istanbul, Turkey	8	Cirrus
Lyon, France	8	Spectralis
Mangalore, India	40	Spectralis
Maracaibo, Venuzuela	3	Spectralis
Michigan, USA	5	Spectralis
Milan, Italy	30	Spectralis
Munich, Germany	11	Spectralis
New York City, USA	6	Cirrus and Spectralis
Odense, Denmark	9	Spectralis
Oxford, United Kingdom	48	Spectralis
Petah Tikva, Israel	25	Cirrus
Sao Paolo, Brazil	9	Spectralis
Seattle, USA	30	Cirrus
Strasbourg, France	25	Spectralis
Total	**539**	

Table 1: Participating centers with number of patients included in this study and the devices used for the OCT imaging

	HC	AQP4 -IgG	MOG-IgG	AQP4-IgG vs HC			AQP4 -IgG vs MOG-IgG			MOG-IgG vs HC		
				B	SE	p	B	SE	p	B	SE	p
Number of eyes	148	317	55									
mRNFL μm (mean±SD)	17.42± 2.33	17.59± 2.22	17.56±2. 60	-1.39	0.27	<0.001	-0.45	0.41	0.262	-1.58	0.42	<0.001
GCIP μm (mean±SD)	66.30± 19.47	64.81± 21.53	64.76±17 .87	-20.62	2.24	<0.001	-4.52	4.01	0.261	-22.67	2.43	<0.001
INL μm (mean±SD)	40.98± 6.55	39.54± 6.04	41.02±7. 18	-0.75	0.71	0.295	-3.19	1.19	0.007	0.36	1.11	0.746
OPL μm (mean±SD)	27.24± 6.86	27.08± 7.72	27.12±7. 26	-1.18	0.98	0.226	-1.39	1.32	0.293	-0.74	1.41	0.601
ONL μm (mean±SD)	78.61± 11.37	77.85± 11.83	79.10±13 .98	2.91	1.43	0.043	-0.66	2.43	0.787	4.18	2.36	0.079
OPNL μm (mean±SD)	105.86 ±9.15	104.93 ±9.35	106.22±1 1.86	1.81	1.07	0.091	-1.34	2.05	0.514	3.00	1.61	0.064
PR μm (mean±SD)	87.89± 3.51	87.82± 3.71	88.41±3. 05	-0.54	0.42	0.203	-0.24	0.75	0.750	0.03	0.60	0.953
RT μm (mean±SD)	318.50 ±23.80	314.70 ±25.27	318.12±2 5.86	-20.80	2.73	<0.001	-10.37	4.72	0.030	-24.46	3.52	<0.001

Table 2: 3mm Study - Cross-sectional OCT results (Spectralis devices only). Abbreviations: mRNFL, macular retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor

layer; RT, total retinal thickness; HC, healthy control, AQP4-IgG, aquaporin-4 immunoglobulin G; MOG-IgG, myelin oligodendrocyte glycoprotein immunoglobulin-G.

	HC	AQP4-IgG	MOG-IgG	AQP4-IgG vs HC			AQP4 -IgGvs MOG-IgG			MOG-IgG vs HC		
				B	SE	p	B	SE	p	B	SE	p
Number of eyes	148	317	55									
mRNFLμm (mean±SD)	13.38± 1.57	13.09± 1.48	13.03± 1.55	-0.05	0.18	0.758	0.12	0.27	0.648	-0.53	0.21	0.012
GCIP μm (mean±SD)	32.43± 10.68	25.13± 10.45	24.93± 9.28	-6.97	1.22	<0.001	-1.59	1.97	0.420	-7.23	1.50	<0.001
INL μm (mean±SD)	18.95± 5.36	18.93± 4.77	19.66± 4.76	-0.53	0.57	0.353	-1.51	0.95	0.114	0.43	0.75	0.567
OPL μm (mean±SD)	20.99± 5.14	20.49± 5.49	19.90± 4.60	-1.30	0.64	0.044	-0.92	0.99	0.334	-1.64	0.73	0.002
ONL μm (mean±SD)	88.66± 8.67	89.45± 10.25	90.73± 14.31	0.64	1.23	0.600	-1.71	2.33	0.463	1.65	1.66	0.321
OPNL μm (mean±SD)	109.74 ±9.23	109.94 ±10.26	110.63 ±14.57	-0.72	1.15	0.530	-2.69	2.19	0.221	-0.19	1.58	0.904
PR μm (mean±SD)	94.31± 2.95	93.53± 3.37	93.66± 3.20	-0.63	0.39	0.106	-0.06	0.69	0.934	-0.43	0.46	0.348
RT μm (mean±SD)	268.70 ±23.45	260.55 ±20.91	261.76 ±23.82	-9.21	2.40	<0.001	-6.09	4.06	0.135	-8.99	3.11	<0.001

Table 3: 1mm Study - Cross-sectional OCT results (Spectralis devices only). Abbreviations: mRNFL, macular retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor layer; RT, total retinal thickness; HC, healthy control, AQP4-IgG, aquaporin-4 immunoglobulin G; MOG-IgG, myelin oligodendrocyte glycoprotein immunoglobulin-G.

	AQP4-ON	AQP4-NON	AQP4-ON vs. AQP4-NON			AQP4-ON vs HC			AQP4-NON vs HC		
			B	SE	p	B	SE	p	B	SE	p
Number of eyes	232	85									
mRNFL μm (mean±SD)	17.20±2.44	18.32±2.83	-1.09	0.31	<0.001	-1.64	0.30	<0.001	-0.71	0.45	0.116
GCIP μm (mean±SD)	59.33±16.21	76.21±15.98	-21.67	2.69	<0.001	-27.35	2.34	<0.001	-1.00	2.24	0.655

INL μm (mean±SD)	42.12±6.98	38.68±7.20	0.75	0.95	0.433	0.07	0.76	0.923	-1.26	1.33	0.348
OPL μm (mean±SD)	27.08±7.41	27.21±7.12	0.47	1.09	0.662	-0.61	1.04	0.562	-1.59	1.56	0.313
ONL μm (mean±SD)	83.28±12.43	70.32±13.22	3.52	1.89	0.066	3.70	1.62	0.024	-0.01	2.09	0.995
OPNL μm (mean±SD)	110.35±10.22	97.53±10.4	3.88	1.57	0.141	3.02	1.22	0.014	-1.17	1.60	0.469
PR μm (mean±SD)	88.67±2.90	87.84±3.34	0.91	0.63	0.148	-0.40	0.47	0.391	-1.49	0.61	0.170
RT μm (mean±SD)	317.90±22.87	318.58±31.96	-16.88	3.51	<0.001	-25.27	2.98	<0.001	-6.15	4.07	0.134

Table 4: 3mm Study - Cross-sectional AQP4-ON vs AQP4-NON OCT results (Spectralis devices only).
Abbreviations: mRNFL, macular retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor layer; RT, total retinal thickness; HC, healthy control, AQP4-IgG, aquaporin-4 immunoglobulin G; MOG-IgG, myelin oligodendrocyte glycoprotein immunoglobulin-G.

	AQP4-ON	**AQP4-NON**	**AQP4-ON vs. AQP4-NON**			**AQP4-ON vs. HC**			**AQP4-NON vs HC**		
			B	**SE**	**p**	**B**	**SE**	**p**	**B**	**SE**	**p**
Number of eyes	232	85									
mRNFL μm (mean±SD)	13.10±1.54	13.05±1.36	-0.05	0.22	<0.001	0.06	0.20	0.764	-0.33	0.20	0.135
GCIP μm (mean±SD)	22.45±9.56	30.79±10.11	-6.91	1.49	<0.001	-7.86	1.30	<0.001	-2.58	1.64	0.116
INL μm (mean±SD)	18.99±5.06	18.99±5.06	0.24	0.77	0.756	0.30	0.62	0.625	-1.62	0.81	0.047
OPL μm (mean±SD)	21.30±5.75	21.30±5.75	-1.38	0.83	0.100	-1.54	0.69	0.027	-0.60	0.87	0.494
ONL μm (mean±SD)	87.96±8.81	87.96±8.81	3.41	1.77	0.056	1.76	1.38	0.204	-0.98	1.49	0.513
OPNL μm (mean±SD)	109.26±9.12	109.26±9.12	-3.94	3.25	0.227	0.21	1.30	0.872	-1.76	1.45	0.225
PR μm (mean±SD)	93.93±3.07	93.39±3.07	0.45	0.56	0.427	-0.67	0.43	0.118	-0.75	0.50	0.132
RT μm (mean±SD)	265.42±21.79	265.42±21.79	-3.95	3.25	0.227	-8.21	2.65	0.002	-7.58	3.37	0.025

Table 5. 1mm Study - Cross-sectional AQP4-ON vs AQP4-NON OCT results (Spectralis devices only).
Abbreviations: mRNFL, macular retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor layer; RT, total retinal thickness; HC, healthy control, AQP4-IgG, aquaporin-4 immunoglobulin G; MOG-IgG, myelin oligodendrocyte glycoprotein immunoglobulin-G.

	MOG-ON	**MOG-NON**	**MOG-ON vs. MOG-NON**			**MOG-ON vs. HC**			**MOG-NON vs HC**		
			B	**SE**	**p**	**B**	**SE**	**p**	**B**	**SE**	**p**
Number of eyes	43	12									
mRNFL μm (mean±SD)	13.15±1.51	12.79±1.65	-0.15	0.44	0.729	0.62	0.25	0.013	-0.36	0.30	0.237
GCIP μm (mean±SD)	22.40±7.43	30.26±10.65	-12.20	2.49	<0.001	-11.84	1.69	<0.001	-0.64	2.22	0.773
INL μm (mean±SD)	20.05±4.50	18.84±5.30	0.05	1.14	0.968	0.35	0.90	0.384	0.42	1.14	0.711
OPL μm (mean±SD)	19.82±4.78	20.05±4.30	-2.00	1.28	0.127	-2.51	0.87	0.004	-0.15	1.09	0.887
ONL μm (mean±SD)	95.38±10.69	80.95±16.23	17.53	3.44	<0.001	5.73	1.69	0.001	-6.46	2.35	0.007

OPNL μm (mean±SD)	115.20±11.64	101.00±15.67	15.86	2.06	<0.001	3.03	1.69	0.075	-6.21	2.28	0.007
PR μm (mean±SD)	93.85±2.70	93.26±4.12	1.72	0.90	0.063	-0.14	0.52	0.793	-0.96	0.73	0.191
RT μm (mean±SD)	264.52±19.08	255.95±31.41	5.35	5.66	0.352	-10.04	3.56	0.005	-7.76	4.89	0.114

Table 6. 1mm Study - Cross-sectional MOG ON vs MOG-NON OCT results (Spectralis devices) Abbreviations: mRNFL, macular retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OPNL, outer plexiform and nuclear layer; PR, photoreceptor layer; RT, total retinal thickness; HC, healthy control, AQP4-IgG, aquaporin-4 immunoglobulin G; MOG-IgG, myelin oligodendrocyte glycoprotein immunoglobulin-G; ON, optic neuritis; NON, non-optic neuritis.

	Cirrus		Topcon	
	AQP4-IgG	**MOG-IgG**	**AQP4-IgG**	**MOG-IgG**
Number of eyes	47	5	36	8
mRNFL μm (mean±SD)	25.40±6.82	23.16±3.94	25.37±5.30	28.05±5.85
GCIP μm (mean±SD)	65.98±11.14	68.00±6.39	65.61±10.94	67.28±10.46
INL μm (mean±SD)	42.01±3.54	44.72±3.92	38.45±2.69	38.73±0.97
OPL μm (mean±SD)	24.13±1.86	26.10±2.55	26.22±1.46	27.07±1.10
ONL μm (mean±SD)	64.30±6.01	65.82±9.67	56.51±5.35	65.79±9.02
PR μm (mean±SD)	78.32±2.36	79.25±2.11	83.34±2.13	86.68±3.40
RT μm (mean±SD)	300.14±18.63	307.06±18.29	295.51±13.78	313.60±10.57

Table 7. 5mm Study - Cross-sectional OCT results (Topcon and Cirrus devices) Abbreviations: mRNFL, macular retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PR, photoreceptor layer; RT, total retinal thickness; HC, healthy control, AQP4-IgG, aquaporin-4 immunoglobulin G; MOG-IgG, myelin oligodendrocyte glycoprotein immunoglobulin-G.