

Macular changes of neuromyelitis optica through spectral-domain optical coherence tomography

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Abstract

• **AIM:** To evaluate the thickness of the retinal layers in the macula using spectral-domain optical coherence tomography (SD-OCT) in patients with neuromyelitis optica (NMO).

• **METHODS:** Spectralis SD-OCT, utilizing automated macular layer segmentation, was performed in 26 NMO patients and 26 healthy controls. Visual function including visual field tests and pattern visual evoked potential were recorded in study subjects.

• **RESULTS:** Forty-one eyes from 26 NMO patients and 52 eyes from 26 age- and sex-matched healthy controls were included. Besides total macular volume, peri-papillary retinal nerve fiber layer (RNFL) thickness, the thickness of macular RNFL, ganglion cell layer (GCL) and inner plexiform layer (IPL) were also significantly reduced in NMO patients compared to those in healthy controls ($P < 0.000$). No differences were found in the thickness of macular inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL) between the two groups. Reversely, the outer retinal layer (ORL) was shown to be thicker in NMO than controls ($P < 0.05$). Compared with the peri-papillary RNFL thickness, the GCL thickness was demonstrated to correlate with visual function better.

• **CONCLUSION:** The study provides *in vivo* evidence of retinal neural loss in NMO patients and demonstrates a

better structure-function correlation between retinal ganglion cell and visual function than peri-papillary RNFL does. In addition, no evidence of primary neural damage is found. Besides, the photoreceptor cells and retinal pigments epithelial (RPE) cells presumably proliferated in compensation in NMO after retinal neural loss.

• **KEYWORDS:** neuromyelitis optica; optical coherence tomography; macular layer thickness; ganglion cell loss

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INTRODUCTION

Neuromyelitis optica (NMO) is an inflammatory, autoimmune, demyelinating disease that often attack the optic nerve initially, causing optic neuritis (ON) [1-2]. Approximately 50% of NMO patients initially present with ON. NMO is highly prevalent in Asia [1], accounting for a large percentage (20%-48%) of demyelinating disease in Asians [2]. Historically, NMO was thought to be a subtype of multiple sclerosis (MS), a more common demyelinating disorder, whereas many recent advances, especially the discovery of NMO-IgG directed against aquaporin-4 (AQP4), the major water channel in astrocytes in the central nervous system (CNS), clearly identified NMO as a separate disease from MS [3]. ON is a common feature in NMO, involving primary inflammation, demyelination, and axonal injury of the optic nerve. Approximately one-half of NMO patients present with isolated ON, of which about 20% is bilateral [4]. Profound and persistent visual loss is a hallmark of ON in NMO, while most MS patients recover significant visual acuity following ON [5].

Optical coherence tomography (OCT), a noninvasive imaging technique, has been commonly used worldwide for clinical diagnosis of many ocular diseases including those resulting from axonal loss in diseases affecting the anterior optic pathway including NMO and MS. Research using OCT measurements in NMO and MS has primarily focused on the evaluation of the peri-papillary retinal nerve fiber layer (pRNFL), which was used to detect axonal loss, monitor treatment efficacy and help differentiate NMO from MS. Reductions in macular thickness measured using OCT then

have been interpreted as a consequence of pRNFL loss. In the past few years with the advance of OCT technique and segmentation protocol of macular retinal layers, different retinal layers, especially inner retinal layers including RNFL, ganglion cell layer (GCL), and inner plexiform layer (IPL) have been investigated in MS and NMO cases. A thinning of the inner retinal layers has been measured through OCT^[6-8] and pathologically demonstrated through post-mortem assessment^[9]. It has also been demonstrated that this GCL thinning correlated better with visual loss than did changes in pRNFL thickness in MS^[10]. Several studies have also reported the involvement of retinal deeper layers, such as the ON associated microcystic macular edema (MME) of the inner nuclear layer (INL) and the outer nuclear layer (ONL)^[6,11-15]. However, several questions about the effects of NMO on macular layer thickness remain open. For example, does NMO really affect other retinal layers and does macular inner layers thinning correlate better with visual loss than pRNFL in NMO? Moreover, little is known about the thickness of the outer retinal layer (ORL) including photoreceptor cells and retinal pigments epithelial (RPE) in NMO. Furthermore, the effect of positivity for NMO-IgG on the retinal supporting cells, Müller cells, which also contain the AQP4 and are located in the INL, remains ambiguous.

In this cross-sectional study we used high resolution spectral-domain optical coherence tomography (SD-OCT) with retinal segmentation protocol to assess if NMO patients exhibit quantitative *in vivo* retinal neuronal loss, in particular ganglion cell loss, and to evaluate correlations between retinal neuronal loss with visual function in NMO. We also sought to determine if NMO patients may exhibit OCT features of supporting cells associated pathology as recently described INL/ONL changes^[6,11] and to investigate whether the outer retinal structure including photoreceptor cells and RPE cells are involved in NMO cases.

SUBJECTS AND METHODS

This cross-sectional observational study was performed at the Shanghai First People's Hospital, School of Medicine, Shanghai Jiao Tong University. The research protocols were approved by the institutional review board and Ethics Committee of Shanghai First People's Hospital, and carried out in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from each subject after an explanation of the nature of the study. The results of the current study were reported according to the APOSTEL recommendations^[16].

Study Population Subjects with NMO were recruited from the Department of Neurology and the Department of Ophthalmology of Shanghai First People's Hospital. NMO was defined according to the 2006 Wingerchuk criteria^[17]. All patients had their diagnosis confirmed by their neurologist (Ling ZF). In the current study, indirect immunofluorescent

(IIF, Neurology Mosaics IIFT Assay, Euroimmun Medizinische Labordiagnostika AG, Germany) was used to detect serum NMO-IgG and anti-myelin oligodendrocyte glycoprotein (MOG) antibodies. Among the 26 included NMO patients, all had a history of clinical episodes of ON, and among the 7 patients with unilateral ON, only the affected eyes were included. Fourteen patients were NMO-IgG positive, for the NMO-IgG negative patients, longitudinally extensive transverse myelitis (LETM) exists. The normal subjects consisted of normal healthy volunteers recruited from Shanghai First People's Hospital and surrounding areas. The exclusion criteria for all subjects were the following: 1) younger than 18 or older than 65 years of age; 2) extra-axial compressive etiology by neuroimaging; 3) CNS manifestation of infectious diseases; 4) brain MRI abnormalities other than those suggestive of NMO^[18]; 5) serum anti-MOG antibodies positive; 6) refractive error beyond -6.0 to +3.0 diopters; 7) intraocular pressure (IOP) >22 mm Hg; or 8) any ocular disease that may affect optical coherence tomography angiography (OCTA) measurements, such as glaucoma and diabetic retinopathy. Eyes with a best-corrected visual acuity (BCVA) less than 0.01 were excluded from analysis to minimize the effect of fixation problem on OCTA measurements.

Visual Function Testing All subjects underwent retinal functional examinations including BCVA assessment and Humphrey visual field test after recruitment. Pattern visual evoked potential (PVEP) results of NMO subjects were extracted retrospectively from the medical record. BCVA was assessed by a Snellen 20-foot wall chart and was converted to a logarithm of the minimum angle of resolution (logMAR) for statistical analysis. Visual field tests were performed with the Humphrey Field Analyzer II (Carl Zeiss Meditec, Inc.). The system was set for the 30-2 threshold test, size III white stimulus, and standard SITA algorithm. A reliable visual field test was defined as one with <20% fixation losses, <20% of false positive, and <20% of false negative responses^[19]. Any result not matching these criteria was repeated and excluded after two failed repetitions. We evaluated visual field abnormalities quantitatively using visual field index (VFI), mean deviation (MD) and pattern standard deviation (PSD). MD is a measure of overall visual field loss and represents severity of global damage, while PSD is a measure of focal loss, more related to localized functional damage. PVEP results were evaluated and analyzed through the latency and amplitude of P100.

Optical Coherence Tomography Data Acquisition and Processing On the same day of visual function tests, all subjects underwent pRNFL and segmented macular layers examinations with SD-OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) after pupil dilation with 1% tropicamide eye drops. The OCT test was

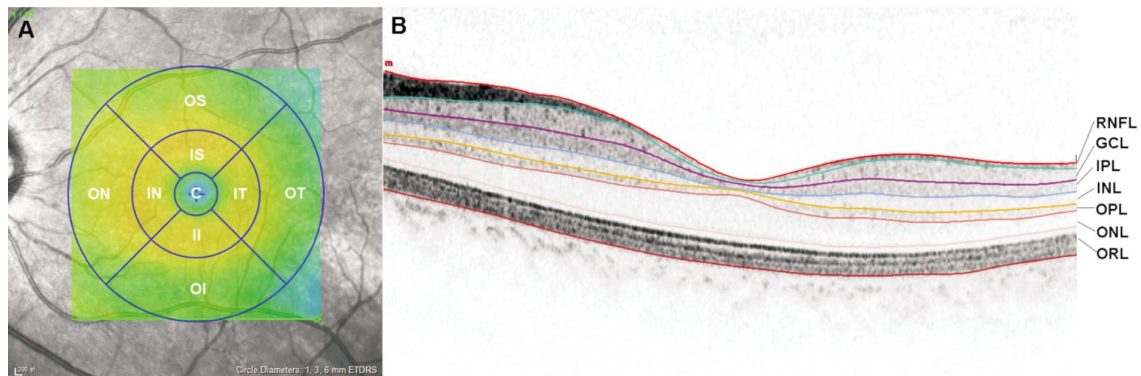


Figure 1 ETDRS map and boundaries of segmented macular layers A: The green square indicates the 6×6 mm² cube scan area during the OCT examination. The ETDRS map and its nine quadrants are shown. The diameters of the three circles on the ETDRS map are 1 mm, 3 mm and 6 mm. C: Central; IS: Inner superior; II: Inner inferior; IN: Inner nasal; IT: Inner temporal; OS: Outer superior; OI: Outer inferior; ON: Outer nasal; OT: Outer temporal. B: A schematic representation of a B scan where the different color lines correspond to the retinal layer boundaries as identified during our segmentation process. RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer; INL: Inner nuclear layer; OPL: Outer plexiform layer; ONL: Outer nuclear layer; ORL: Outer retinal layer.

conducted by the same operator and images were analyzed with the automatic analysis protocol the OCT comes with (HSF Region Finder, version 2.5.8, Heidelberg Engineering, Heidelberg, Germany). Afterwards the hand-correction was done by another experienced operator. Both the two operators were blind to the patients' diagnosis during the process. The thickness of pRNFL was measured with a 3.4 mm circular scan protocol, which offered 7 quadrants of pRNFL thickness (μm): nasal quadrant (N), nasal superior quadrant (NS), nasal inferior quadrant (NI), temporal quadrant (T), temporal superior quadrant (TS), temporal inferior quadrant (TI) and average thickness of pRNFL. Only the data of average pRNFL thickness was extracted in the present study. For each cube scan within a 6×6 mm² region centered on the macular area, we segmented 97 B-scans and obtained the thickness for each evaluated layer using the included automated segmentation algorithm, which was previously validated and provided an average macular volume (mm³) and all sublayers thickness of 9 quadrants according to the Early Treatment Diabetic Retinopathy Study (ETDRS) map. The ETDRS quadrant map and macular layers segmentation are shown in Figure 1. All the images were reviewed with respect to their quality according to the OSCAR-IB criteria^[20-21]. Eyes of which the images quality was poor were discarded.

Statistical Analysis All analyses were performed using SPSS 22.0 (IBM) or SAS (9.4). Because both eyes of each control and some NMO patients were included in this study, and eyes of the same patient would be expected to have some degree of intercorrelation with respect to visual function and macular layers thickness, generalized estimating equation (GEE) models allowed us to adjust for these within-patient intereye correlations. In this investigation, GEE models were used to compare patient and healthy control groups with respect to macular layers thickness values (average overall and each quadrant). GEE models including age, sex, IOP,

and refractive error in the model were also used for primary analyses that examined the relation of visual function to macular layers thickness, and models including pRNFL as well were used to examine the relation of visual function to GCC/GCL thickness. Due to the exploratory nature of the study no adjustment for multiple comparisons was made.

RESULTS

Out of the available cohort of 31 NMO subjects, 5 subjects (one was under age 18, one was over age 65, one had glaucoma, and two were positive for anti-MOG antibodies) and 11 eyes (7 did not have episodes of ON, 2 had refractive error of more than -6.0 diopters, and 2 had poor image quality) were excluded. Therefore, a total of 41 eyes from 26 NMO patients, and 52 eyes from 26 age- and sex-matched healthy controls were included in the study. All the NMO eyes included had at least one episode of ON. Subject characteristics are described in Table 1. No significant differences were observed in gender, age, and IOP between the 2 groups. Three normal subjects (11.5%) and 3 NMO subjects (11.5%) had hypertension and all were taking systemic antihypertensive medications. No subjects had diabetes. As expected, the NMO group had significantly worse visual function examination (BCVA and visual field test) results as well as optic nerve damage (pRNFL thickness). Table 2 and Figure 2 show the macular layer thickness measurements according to the ETDRS map between NMO and normal subjects. Total macular volume and macular RNFL thickness were significantly reduced in NMO patients compared to that in healthy controls ($P < 0.000$), in each of the 9 quadrants as well as the whole area of ETDRS map. The GCL and IPL thicknesses were likewise significantly reduced in NMO patients compared to those in healthy controls ($P < 0.000$) in total area or subregions. Similarly, the ganglion cell complex (GCC), consisting of the three layers above, was also thinner than that of the normal group.

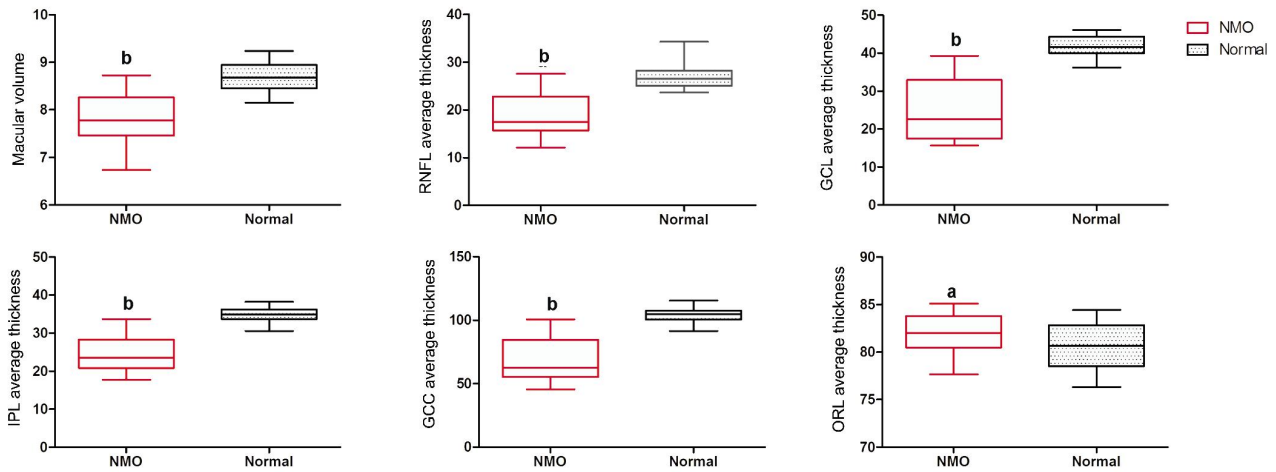


Figure 2 Box plots illustrating the inter-quartile range for macular volume and average whole area thickness of different macular layers in NMO patients and normal controls RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer; GCC: Ganglion cell complex; ORL: Outer retinal layer; NMO: Neuromyelitis optica. ^a*P*<0.05, ^b*P*<0.01.

Table 1 Characteristics of NMO eyes and normal eyes

Characteristics	NMO group	Normal group	<i>P</i>
Eyes, <i>n</i>	41	52	
Gender (M/F)	6/20	6/20	1.000
Age (a)	42.41±9.98	42.06±10.54	0.921
Hypertension	3 (11.5%)	3 (11.5%)	1.000
Anti-hypertensive	3 (11.5%)	3 (11.5%)	1.000
Diabetes mellitus	0	0	
BCVA Snellen	0.72 (0.01, 1.00)	1 (0.80, 1.00)	0.000
BCVA (logMAR)	0.15 (0, 2.0)	0 (0, 0.10)	0.000
Spherical equivalent (D)	-2.43 (-10.88, 1.38)	-0.19 (-4.38, 1.50)	0.008
IOP (mm Hg)	19.45±2.81	14.84±2.78	0.874
Visual field test			
VFI	0.65 (0.12, 1.00)	0.99 (0.96, 1.00)	0.000
MD (dB)	-12.16 (-32.52, -0.51)	-1.11 (-5.22, 0.95)	0.000
PSD (dB)	9.91 (1.58, 26.46)	1.66 (0.96, 3.94)	0.000
pRNFL thickness (μm)	74.92±31.34	118.32±8.65	0.000

NMO: Neuromyelitis optica; BCVA: Best-corrected visual acuity; VFI: Visual field index; MD: Mean deviation; PSD: Pattern standard deviation; pRNFL: Peri-papillary retinal nerve fiber layer.

Conversely, the ORL, consisting of retinal photoreceptor cells and RPE cells, was thicker for NMO than the normal controls (Table 2). Among the 7 macular layers evaluated, the thickness of INL, outer plexiform layer (OPL), and ONL for NMO did not differ from controls, whether in single quadrant or the whole area (data not shown), indicating that no changes were found in the deep layer of the macular. A pseudo-color thickness map of these layers is shown in Figure 3 for the average of the controls and NMO patients. For further correlation analysis, only 24 eyes of the NMO group were finally included according to the criteria for the reliable visual function test mentioned above. In the NMO group, the thicknesses of inner layers of the whole area, as a reflection of retinal neural loss, along with the peri-papillary RNFL thickness as the reflection of optic nerve damage, were selected to be analyzed with the visual function

parameters including VFI, MD, and PSD as well as the latency and amplitude of P100 of PVEP. A correlation analysis showed that the thicknesses of both GCL and GCC, were significantly correlated with the visual function testing results, as well as the pRNFL thickness did (Table 3). The age and IOP were not significantly correlated with visual function (data not shown). GEE including age, sex, IOP, and refractor errors in the model was also used to adjust for RNFL and GCL/GCC layer thicknesses when assessing OCT correlations with visual function. When GCL or GCC layer thickness was adjusted for pRNFL thickness it retained its significant correlations with visual field VFI (GCL: coefficient=0.029, *P*=0.007; GCC: coefficient=0.017, *P*=0.006), MD (GCL: coefficient=0.248, *P*=0.001; GCC: coefficient=0.481, *P*=0.010), PSD (GCL: *r*=-0.736, *P*=0.001; GCC: coefficient=-0.309, *P*=0.012).

Table 2 OCT macular volume and thickness measurements (µm)

Parameters	NMO eyes (n=41)	Normal eyes (n=56)	P
Macular volume (mm ³)	7.83±0.52	8.67±0.30	0.000
Macular RNFL thickness			
C	7.63±3.94	11.18±1.86	0.000
IS	18.25±4.25	23.87±3.50	0.000
II	19.00±5.15	24.58±2.60	0.000
IN	17.25±3.33	20.13±1.85	0.000
IT	17.00 (11.00, 22.00)	17.00 (15.00, 19.00)	0.125
OS	23.79±7.89	39.39±4.49	0.000
OI	25.21±8.32	40.89±4.98	0.000
ON	26.42±10.60	47.11±6.66	0.000
OT	17.00 (13.00, 20.00)	18.00 (16.00, 23.00)	0.000
Average	19.01±4.44	27.00±2.36	0.000
Macular GCL thickness			
C	8.00 (6.00, 49.00)	14.00 (9.00, 24.00)	0.000
IS	24.00 (13.00, 50.00)	54.00 (46.00, 60.00)	0.000
II	28.92±11.92	53.03±4.23	0.000
IN	21.50 (14.00, 55.00)	53.00 (38.00, 61.00)	0.000
IT	24.54±9.61	48.11±4.21	0.000
OS	26.13±6.53	38.68±2.89	0.000
OI	25.83±5.97	36.11±3.42	0.000
ON	27.71±7.75	42.68±3.25	0.000
OT	24.71±7.03	38.55±3.87	0.000
Average	24.90±7.98	41.89±2.64	0.000
Macular IPL thickness			
C	15.00 (12.00, 38.00)	19.00 (16.00, 27.00)	0.000
IS	27.42±7.06	41.76±2.35	0.000
II	27.08±7.24	41.42±2.56	0.000
IN	27.08±7.98	42.53±2.90	0.000
IT	26.92±6.88	41.95±3.11	0.000
OS	24.00±3.66	30.95±1.99	0.000
OI	23.25±3.66	28.84±2.34	0.000
ON	24.17±4.23	32.76±2.27	0.000
OT	25.83±3.99	33.76±2.52	0.000
Average	24.68±4.74	34.86±1.80	0.000
Macular GCC thickness			
C	29.50 (21.00, 109.00)	44.00 (35.00, 65.00)	0.000
IS	66.00 (44.00, 112.00)	119.00 (104.00, 142.00)	0.000
II	75.00±22.58	119.03±8.40	0.000
IN	63.50 (43.00, 120.00)	115.00 (92.00, 136.00)	0.000
IT	67.75±17.75	107.16±7.13	0.000
OS	73.92±16.62	109.03±6.67	0.000
OI	74.29±16.16	105.84±9.15	0.000
ON	78.29±21.60	122.55±8.88	0.000
OT	67.83±11.35	91.08±6.93	0.000
Average	68.59±16.85	103.85±5.07	0.000
Macular ORL thickness			
C	88.13±3.71	88.53±2.69	0.590
IS	82.79±3.02	81.32±3.09	0.170
II	81.67±2.35	79.74±2.74	0.006
IN	83.79±2.62	82.61±2.69	0.093
IT	83.08±2.32	81.26±2.85	0.041
OS	79.83±2.66	78.76±2.98	0.157
OI	78.04±2.48	76.29±2.44	0.000
ON	80.00 (75.00, 85.00)	78.00 (75.00, 83.00)	0.010
OT	79.50 (76.00, 83.00)	78.00 (74.00, 83.00)	0.051
Average	81.92±2.08	80.56±2.34	0.039

NMO: Neuromyelitis optica; C: Central, IS: Inner superior; II: Inner inferior, IN: Inner nasal; IT: Inner temporal; OS: Outer superior; OI: Outer inferior; ON: Outer nasal; OT: Outer temporal; RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer; GCC: Ganglion cell complex; ORL: Outer retinal layer.

However, when RNFL thickness was adjusted for GCL layer thickness, it lost its significant correlations with VFI (coefficient=0.001, *P*=0.845) and PSD (coefficient=0.065, *P*=0.218).

DISCUSSION

SD-OCT has several compelling methodological characteristics including high resolution that can detect tiny changes of neuroretina and significant structure-function

Table 3 Regression coefficient matrix between structural variables and visual function in subjects with NMO using GEE model

Average retinal layer thickness	Visual field			PVEP	
	VFI (%)	MD (dB)	PSD (dB)	P100 Latency (ms)	P100 amplitude (uV)
RNFL	0.056 ^b	1.712 ^b	-1.354 ^a	1.561	0.476
GCL	0.038 ^b	0.311 ^b	-0.880 ^b	-0.339 ^b	0.440 ^b
IPL	0.045	1.575	-1.181 ^b	1.134	1.002
GCC	0.016 ^b	0.433 ^b	-0.372 ^b	0.253	0.301 ^b
pRNFL	0.007 ^b	0.193 ^b	-0.142 ^b	-0.136	0.146 ^b

VFI: Visual field index; MD: Mean deviation; PSD: Pattern standard deviation; PVEP: Patten visual evoked potential; RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer; GCC: Ganglion cell complex; pRNFL: Peri-papillary RNFL. ^a*P*<0.05, ^b*P*<0.01.

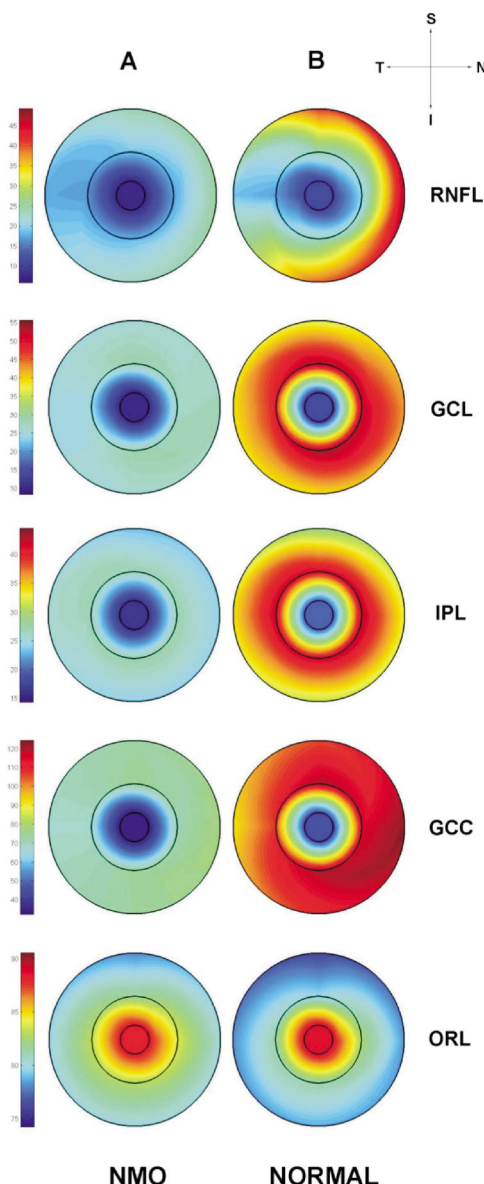


Figure 3 Pseudo-color thickness map generated from cube scan based on the thickness of the layers evaluated The three circles correspond to the central area, inner circle and outer circle of ETDRS map. The cross arrow in the right top corner indicates the quadrants distribution of the map: S: Superior; I: Inferior; N: Nasal; T: Temporal. A line: NMO; B line: Normal Controls. Each row: RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer; GCC: Ganglion cell complex; ORL: Outer retinal layer.

correlations, which make it equipped to detect and monitor the course of disease-related neurodegeneration and to document the neuroprotective and potentially neurorestorative effects of therapeutic agents in many neuro-ophthalmology diseases^[22-23]. Retinal axons are unmyelinated, making the retina a unique structure within the CNS. Given this and the predilection of NMO to affect the optic nerves, the eye has been proposed as a model within which to study the neurodegenerative processes associated with NMO. A large number of studies using OCT has demonstrated the degeneration of optic nerve in NMO through the measurements of peri-papillary RNFL thickness^[7,24-26]. The advent of OCT segmentation protocol not only allows objective evaluation of optic nerve damage upon proximal RNFL axons, but also enables the observation of retinal neuronal architecture. In the current study, we found that both macular GCC layer and its composing RNFL, GCL, and IPL single layers were statistically thinner in NMO when compared to normal controls. The findings are also in agreement with a number of previous studies^[6,8,27], which evaluated macular full thickness or GCIPL (GCL+IPL) thickness, but not specifically the GCL, in patients with NMO. In the past, the GCL and IPL could not be distinguished by traditional OCT techniques, such as Fourier-domain OCT or Cirrus HD-OCT. With the advance of OCT techniques, the spectralis SD-OCT used in the current study can solve this problem. Compared with GCIPL, the thinning of GCL, where the ganglion cell bodies are located, demonstrated the retinal neuronal loss more significantly. These findings are consistent with the hypothesis that optic nerve demyelination results in retrograde axonal degeneration, culminating in ganglion cell death^[28]. Similar to that observed in MS eyes, the current study also shows that GCL or GCC thinning correlated better with visual function loss than did changes in peri-papillary RNFL thickness in NMO patients. OCT characteristics of peri-papillary RNFL thickness have been proposed as a primary outcome measure in clinical trials of neuroprotectants in ON. However, predominant structure-function correlation between GCL thickness and validated visual functional measures found in this systematic retinal layer segmentation analysis

provide support that retinal neuronal morphology, particularly of the GCL, may serve as a better instrument than peri-papillary RNFL to estimate the severity of NMO and examine NMO associated neurodegeneration within the eye. Following complete degeneration, it might be reasonable to anticipate equal numbers of axonal and ganglion cell death, in which case differences in structure-function correlations between RNFL thickness and GCL thickness may not be expected. A possible explanation of the differences is that astrogliosis (which principally occurs in the RNFL) may contribute to falsely increased measures of RNFL thickness^[9]. In other words, the presence of astrogliosis may confound the ability of OCT to measure RNFL thickness as accurately as GCL thickness. This may be a contributing factor underlying the better structure-function correlations observed with GCL thickness than RNFL thickness.

Several studies evaluated the retinal deeper layer changes of MS patients and demonstrated a loss of INL neurons (neurons from bipolar, horizontal, and amacrine cells) in post mortem studies^[9], and reduction of INL thickness has also been found through OCT macular segmentation analysis^[11]. Saidha *et al*^[11] defined a subset of MS patients with predominantly macular thinning (normal pRNFL thickness with average macular thickness <5th percentile) and found those patients had significant thinning of both the INL and ONL, when compared with other patients with MS. On the basis of these findings, it was hypothesized that a primary process, independent of optic neuropathy, is targeting retinal neurons in some MS patients. However, this point of primary retinal pathology remained controversial. In the study conducted by Brandt *et al*^[29], the results did not support the conclusion of a distinct macular thinning predominant OCT phenotype in MS. On the other hand, there were also studies reporting MME of INL in NMO patients, and demonstrating MME may possibly contribute to poor visual outcomes independently of optic nerve damage^[6,12-13]. Except for neurons, the INL is also where the Müller cells bodies are located. The AQP4 is not only found in astrocytes in the CNS, but is also found in other supporting cells, including the retina Müller cells. Some researchers speculated that the MME was caused by the dysfunction/deletion of AQP4 in Müller cells in NMO patients^[12-13]. Bearing this in mind, it is worth considering the possibility of the primary retinal pathology in NMO patients. In the current study, we did not find evidence for this process. No statistical difference was shown between INL thickness of NMO patients and the normal group in the present study. However, this could not eliminate the possibility of primary retinal damage in NMO. It might be due to the coexistence of MME and INL neurons loss.

The current study is also the first to evaluate separately the ORL of patients with NMO. Interestingly, while macular

inner layers are shown to be thinner in NMO, analysis of ORL showed strikingly different tendency. The ORL, containing the inner and outer segments of photoreceptor cells as well as RPE cells, seems to be thicker in NMO compared with controls. A possible explanation is the compensatory proliferation of photoreceptor cells and RPE cells, protecting against axonal degeneration and retinal neuronal loss, thereby resulting in visual function preservation. Histological studies are needed to investigate the mechanisms of ORL thickening.

This study has a number of limitations warranting discussion. The majority of NMO patients in this study had episodes of ON. To more precisely and accurately characterize the role of OCT segmentation measures in NMO pathophysiology, the enrollment of greater numbers of not only NMO but also LETM patients in future studies is warranted. Moreover, anti-MOG antibody positive NMO-IgG seronegative opticospinal inflammatory disease, as an emerging nosologic entity with an overlap in phenotype with NMO^[30-33], also needs to be investigated to explore its retinal morphologic characteristics in the future studies. To investigate if macular segmentation could be used to differentiate NMO from MS in the early stage, MS patients with or without ON should also be included in future studies. Limited participant enrollment to this study and under powering may have contributed to the lack of significance of some of the analyses. Further, to research whether axonal degeneration after the optic nerve insult, or primary retinal neural damage contributes to the involvement of retinal ganglion cells, studies on macular segmentation of NMO patients without ON episodes and abnormal pRNFL characteristics are needed.

In summary, with the high-resolution SD-OCT and validated macular segmentation protocol, the present study provided *in vivo* evidence of retinal neural loss in NMO patients and a better structure-function correlation between retinal ganglion cell and visual function than pRNFL does. In contrast to previous studies, no involvement of INL was found in the NMO patients according to the current study. We also investigated the photoreceptor cells and RPE cells in NMO patients innovatively and demonstrated an increase in the thickness of ORL.

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