Retinal Changes in Double-Antibody Seronegative Neuromyelitis Optica Spectrum Disorders

Frederike C. Oertel, MD/PhD, Hanna G. Zimmermann, PhD, Seyedamirhosein Motamedi, PhD,
Charlotte Bereuter, BSc, Luca Magdalena Manthey, Fereshteh Ashtari, MD, Rahele Kafieh, PhD,
Alireza Dehghani, MD, Mohsen Pourazizi, MD, Lekha Pandit, MD, DM, PhD, Anitha D'Cunha, PhD,
Orhan Aktas, MD, Philipp Albrecht, MD, Marius Ringelstein, MD, Elena H. Martinez-Lapiscina, MD, PhD, MPH,
Bernardo F. Sanchez Dalmau, MD, PhD, Pablo Villoslada, MD, PhD, Nasrin Asgari, MD, Romain Marignier, MD,
Alvaro Cobo-Calvo, MD, PhD, Letizia Leocani, MD, PhD, Marco Pisa, MD, Marta Radaelli, MD,
Jacqueline Palace, BM (Hons), FRCP, DM, Adriana Roca-Fernandez, PhD, Maria Isabel S. Leite, MD, DPhil, FRCP,
Srilakshmi Sharma, MBCHB, PhD, Jerome De Seze, MD, Thomas Senger, MD,
Michael R. Yeaman, PhD, MSc, BS, Terry J. Smith, MD, PhD, Lawrence J. Cook, PhD, Alexander U. Brandt, MD,
and Friedemann Paul, MD, as the Guthy-Jackson Charitable Foundation

Correspondence
Dr. Paul
friedemann.paul@charite.de

Abstract

Background and Objectives

To systematically describe the clinical picture of double-antibody seronegative neuromyelitis optica spectrum disorders (DN-NMOSD) with specific emphasis on retinal involvement.

Methods

Cross-sectional data of 25 people with DN-NMOSD (48 eyes) with and without a history of optic neuritis (ON) were included in this study along with data from 25 people with aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorder (AQP4-NMOSD, 46 eyes) and from 25 healthy controls (HCs, 49 eyes) for comparison. All groups were matched for age and sex and included from the collaborative retrospective study of retinal optical coherence tomography (OCT) in neuromyelitis optica (CROCTINO). Participants underwent OCT with central postprocessing and local neurologic examination and antibody testing. Retinal neurodegeneration was quantified as peripapillary retinal nerve fiber layer thickness (pRNFL) and combined ganglion cell and inner plexiform layer thickness (GCIPL).

Results

This DN-NMOSD cohort had a history of [median (inter–quartile range)] 6 (5; 9) attacks within their 5 ± 4 years since onset. Myelitis and ON were the most common attack types. In DN-NMOSD eyes after ON, pRNFL ($p < 0.001$) and GCIPL ($p = 0.023$) were thinner compared with eyes of HCs. Even after only one ON episode, DN-NMOSD eyes already had considerable neuroaxonal loss compared with HCs. In DN-NMOSD eyes without a history of ON, pRNFL ($p = 0.027$) and GCIPL ($p = 0.022$) were also reduced compared with eyes of

HCs. However, there was no difference in pRNFL and GCIPL between DN-NMOSD and AQP4-NMOSD for the whole group and for subsets with a history of ON and without a history of ON—as well as between variances of retinal layer thicknesses.

Discussion

DN-NMOSD is characterized by severe retinal damage after ON and attack-independent retinal neurodegeneration. Most of the damage occurs during the first ON episode, which highlights the need for better diagnostic markers in DN-NMOSD to facilitate an earlier diagnosis as well as for effective and early treatments. In this study, people with DN-NMOSD presented with homogeneous clinical and imaging findings potentially suggesting a common retinal pathology in these patients.

Introduction

Neuromyelitis optica spectrum disorders (NMOSD) are chronic inflammatory diseases of the CNS.[1-3] Optic neuritis (ON) and myelitis are clinical hallmarks of the disease.[1-3] In most people with NMOSD, an antibody against aquaporin-4 (AQP4-IgG), an astrocytic water channel, can be detected.[4] In a subset of AQP4-IgG seronegative people with NMOSD, an antibody against myelin oligodendrocyte glycoprotein (MOG-IgG) can be found.[5-8] We and others were able to show that the clinical picture and pathology differs significantly between AQP4-IgG and MOG-IgG seropositive people—thereby leading to the definition of MOG-IgG–associated diseases (MOGAD) as a new disease entity.[9-14] However, there is another subset of people who are categorized as part of the NMO spectrum but have neither AQP4-IgG nor MOG-IgG, which is commonly referred to as double-antibody seronegative NMOSD (DN-NMOSD).

In the past, it was assumed that double-antibody negativity in NMOSD is a result of test imprecisions, for example, by low antibody concentrations or improper testing time points (e.g., after steroid treatment or long-last immunosuppression) or by low sensitivity of the assays (e.g., ELISA). Because nowadays mostly cell-based antibody assays with high sensitivity are used and testing is performed repetitively, it becomes increasingly unlikely that all these people have unrecognized AQP4-IgG or MOG-IgG—although a few cases might still be false negative.[1,15,16] Thus, in accurately identified DN-NMOSD, the disease might either be caused by an antibody against a different, not yet identified, target molecule, as supported by a recent study describing MOG-IgA in a subset of people with DN-NMOSD,[17] or might be a not primarily antibody-mediated disease. These options are not exclusive, and both might be true for a subset of people with DN-NMOSD.[17] In the long term, it seems inevitable that DN-NMOSD will be separated from the NMO spectrum as one or multiple disease entities, similar to MOGAD.[18]

DN-NMOSD–specific disease information are lacking. Despite the fast progress of NMOSD research, people with MOGAD and DN-NMOSD are still often combined into one AQP4-IgG seronegative group to increase sample sizes for observational and interventional studies.[19,20] However, from multiple studies comparing people with MOGAD and people with AQP4-IgG seropositive NMOSD (AQP4-NMOSD), it is nowadays clear that these diseases can be distinguished by multiple clinical and imaging features and are characterized by a unique disease course and therapeutic response.[9,21-23] It is likely that DN-NMOSD can also be separated from its differential diagnoses using distinct features. Case series support this hypothesis.[24-26] The only systematic analysis of DN-NMOSD so far described demographic, clinical, and imaging features of people with DN-NMOSD to cluster in a multiple sclerosis (MS)-like group, a NMOSD-like group, and a group with low brain lesion count—potentially suggesting multiple disease entities within the DN-NMOSD group.[18] Retinal involvement in DN-NMOSD has not been investigated in DN-NMOSD so far.

The missing antibody status in DN-NMOSD often leads to delayed diagnosis because the involvement of more than one anatomical region is currently required for the diagnosis. Thus, specific diagnostic markers are necessary to distinguish DN-NMOSD from its differential diagnoses early on. Retinal changes seem to be promising contributors because afferent visual system changes play a prominent role in the disease and can be quantified in high resolution using retinal optical coherence tomography (OCT).[2,27,28] Owing to the rarity of the disease, systematic and conclusive descriptions of DN-NMOSD are only possible in large, and—because of ethnical differences—preferentially internationally acquired, data sets. The CROCTINO (Collaborative Multicenter Study of Retinal OCT in NMOSD) cohort includes >500 people with NMOSD from expert centers worldwide.[11,21,29-31] In this study, we aimed to describe the clinical picture and afferent visual system involvement in DN-NMOSD systematically using the CROCTINO cohort.

Table 1 Cohort Description for DN-NMOSD, AQP4-NMOSD, and HCs

	DN-NMOSD	AQP4-NMOSD	HC
Subjects, N	25	25	25
Eyes, N	48	46	49
Ethnicity, N (%)			
Asian	2 (8)	9 (36)	4 (16)
White	23 (92)	15 (60)	21 (84)
Other	0 (0)	1 (4)	0 (0)
Age, year, mean ± SD	32 ± 8	33 ± 8	31 ± 7
Sex, male, N (%)	7 (28)	3 (12)	9 (36)
EDSS, median (IQR)	2 (1–3)	1.5 (1–3.75)	
Time since onset, year, mean ± SD	5 ± 4	7 ± 6	
Eyes with a history of ON, N (%)	23 (48)	28 (61)	

Abbreviation: AQP4-NMOSD = people with aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorder; DN-NMOSD = people with double-antibody seronegative neuromyelitis optica spectrum disorder; HC = healthy control; IQR = interquartile range; N = number.

Methods

Study Design and Cohort Selection

This study included adult individuals with NMOSD fulfilling 2015 International Panel for NMO diagnosis criteria. We included all people with confirmed DN-NMOSD as well as matched cohorts of people with AQP4-NMOSD and healthy controls (HCs). All data were acquired in context of the international CROCTINO study, and this specific data set was obtained from 10 international centers between 2000 and 2018 (eTable 1). Inclusion criteria were (1) OCT data acquired by Spectralis spectral domain (SD) OCT devices, (2) the absence of diseases potentially confounding OCT analyses (such as glaucoma, retinal surgery, and ametropia >6 diopters), and (3) confirmed serostatus for AQP4-IgG and MOG-IgG in serum samples by cell-based assay on the discretion of each center. We excluded eyes with an ON attack less than 6 months before OCT. Clinical data were also collected at the discretion of each center including number of and time since last ON attack, treatment history, and time since disease onset. Measurements of visual function were heterogeneous across centers and thus not included in this analysis.

Optical Coherence Tomography

OCT examinations were conducted at each center using Spectralis SD-OCT devices (Heidelberg Engineering, Heidelberg, Germany). Five graders performed all OCT data reading at Charité-Universitätsmedizin Berlin as previously described.[31] All included OCT data fulfilled OSCAR-IB quality criteria[32,33] and are reported in accordance with the Apostel V.2.0 recommendations.[34,35] Seven eyes (2 DN-NMOSD, 4 AQP4-NMOSD, 1 HC) were excluded because of insufficient image quality. Macular thicknesses (including macular volume [MV], combined ganglion cell and inner plexiform layer [GCIPL], and inner nuclear layer [INL]) were calculated using a 5-mm diameter annulus around the fovea excluding the central 1-mm diameter cylinder from a volume scan. The peripapillary retinal nerve fiber layer thickness (pRNFL) was measured using a 12° or 3.5-mm diameter ring scan centered on the optic nerve head.

Statistical Analysis

Groups were differentiated by diagnosis, antibody status, and history of ON. If not stated otherwise, continuous data were described as mean ± SD and noncontinuous data were described either as median and interquartile range (IQR) for noncontinuous numeric data or as number and percentages for factor variables. Group comparisons for eye-based continuous variables (e.g., retinal thicknesses) were conducted by a mixed linear model using patient ID as the random effect to correct for intrasubject intereye dependencies (random intercept). Group comparisons for patient-based continuous variables (e.g., age) were conducted by unpaired t-test. Group comparisons for factor variables (e.g., sex) were conducted by χ^2 test. Statistical analyses were performed using R (Version 4.2.1) (RStudio Inc., Boston, MA).[36] Statistical significance for this study was established as $p < 0.05$; borderline significance was defined as $p < 0.06$. All data of this project are available from the corresponding author by reasonable request—as well as within the article.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was conducted according to the current version of the Declaration of Helsinki and the applicable local laws. All participating people gave written informed consent. All data are reported in line with the STROBE guidelines.

Results

Cohort

Table 1 summarizes the characteristics of the study cohort. Twenty-five people (48 eyes) with a diagnosis of DN-NMOSD were compared with 25 people (46 eyes) with a diagnosis of AQP4-NMOSD as the most important differential diagnosis and with 25 HCs (49 eyes). All control groups were age-matched (HC: $p = 0.36$; AQP4-NMOSD: $p = 0.55$) and sex-matched (HC: $p = 0.63$; AQP4-NMOSD: $p = 0.29$) with DN-NMOSD.

Clinical Phenotype in DN-NMOSD

In people with DN-NMOSD, the age at disease onset was 27 ± 9 years and this cross-sectional data set was acquired 5 ± 4 years after onset. This DN-NMOSD cohort had a history of 6 (5; 9) attacks (displayed as median [IQR]) since onset. The minimum and maximum numbers of attacks since onset were 2 and 13, respectively. Myelitis and ON were the most common attack types, followed by brainstem syndromes

Table 2 Clinical Characteristics for DN-NMOSD

Attack type	Number of DN-NMOSD affected, N (%)	Number of attacks per affected person with DN-NMOSD, median (IQR)
Myelitis	21 (84)	4 (4–8)
Optic neuritis	17 (68)	2 (1–3)
Bilateral ON (consecutive or simultaneous)	6 (24)	2 (1–3.75)
Simultaneous bilateral ON	4 (16)	1 (1–1)
Brainstem syndromes	11 (44)	1 (1–1)
Area postrema syndrome	3 (12)	1 (1–1)

Abbreviations: DN-NMOSD = people with double-antibody seronegative neuromyelitis optica spectrum disorder; IQR = interquartile range; N = number.

(Table 2). Four people (16%) with DN-NMOSD had a history of simultaneous bilateral ON. None of the subjects had a history of symptomatic brain lesions or narcolepsy.

Twenty-four of 25 people (96%) with DN-NMOSD were treated: Rituximab ([N (%)]: 12 (48)) and azathioprine ([N (%)]: 6 (24)) were the most common treatment choices, followed by glatiramer acetate ([N (%)]: 2 (8)) and a combination of azathioprine and glatiramer acetate ([N (%)]: 2 (8)). Two subjects were treated with mycophenolate mofetil and IV steroids, respectively.

Neuroaxonal Loss Dependent and Independent of ON

In DN-NMOSD eyes after ON (N = 23), pRNFL (relative loss: 26%, $p < 0.001$), MV (relative loss: 6%, $p < 0.001$), mRNFL (relative loss: 27%, $p < 0.001$), and GCIPL (relative loss: 21%, $p = 0.023$) were thinner compared with eyes of HCs (Table 3, Figure 1). After only one ON episode, DN-NMOSD eyes (N = 13) already had considerable neuroaxonal loss compared with HCs (pRNFL: 79.3 ± 14.8 μm, relative loss: 21%, B = 21.6, SE = 4.4, $p < 0.001$; MV: 308.9 ± 15.2 μm, relative loss: 5%, B = 16.1, SE = 6.0, $p = 0.011$; mRNFL: 28.8 ± 4.6 μm, relative loss: 22%, B = 8.0, SE = 1.2, $p < 0.001$; GCIPL: 65.2 ± 13.0 μm, relative loss: 20%, B = 15.6, SE = 3.4, $p < 0.001$). After 2 ON episodes, DN-NMOSD eyes (N = 6) did not have more neuroaxonal loss compared with DN-NMOSD eyes after one ON episode (pRNFL: 67.5 ± 26 μm, relative loss: 15% not significant (n.s.); MV: 303.3 ± 18.5 μm, relative loss: 2%, n.s.; mRNFL: 26.1 ± 5.5 μm, relative loss 9%, n.s.; GCIPL: 63.8 ± 11.9 μm, relative loss: 2%, n.s.) (Figure 2).

In DN-NMOSD eyes without a history of ON (N = 25), pRNFL (relative loss: 11%, $p = 0.027$) and GCIPL (relative loss: 9%, $p = 0.022$) were reduced compared with eyes of HCs. There was no difference between eyes with (N = 10, pRNFL: 92.2 ± 12.4 μm; GCIPL: 75.8 ± 10.9 μm) and without a history of contralateral ON (N = 15, pRNFL: 89.1 ± 25.2 μm; GCIPL: 73.2 ± 12.6 μm, n.s.). Even eyes without a history of contralateral ON had significantly thinner GCIPL (B = 8.5, SE = 3.3, $p = 0.015$) than HCs—the pRNFL difference between these groups was borderline significant (B = 11.6, SE = 5.9, $p = 0.058$).

## Retinal Changes Do Not Differ Between DN-NMOSD and AQP4-NMOSD

Comparing DN-NMOSD with AQP4-NMOSD, pRNFL and GCIPL did not differ between groups with and without a history of ON. In addition, pRNFL and GCIPL did not differ between DN-NMOSD and AQP4-NMOSD after one ON episode (in AQP4-NMOSD N = 18, pRNFL: 67.7 ± 30.2 μm; GCIPL: 61.7 ± 14.7 μm) and after 2 ON episodes (in AQP4-NMOSD N = 5; pRNFL: 62.4 ± 21.3 μm; GCIPL: 57.3 ± 3.3 μm) (Figure 1). Sector-specific pRNFL thicknesses

Table 3 Cross-Sectional Comparisons for Retinal Layer Thicknesses in DN-NMOSDs vs AQP4-NMOSDs and HCs

	Absolute values					DN-NMOSD vs AQP4-NMOSD						DN-NMOSD vs HC					
	DN-NMOSD		AQP4-NMOSD			ON vs ON			NON vs NON			ON vs HC			NON vs HC		
	ON	NON	ON	NON	HC	B	SE	p Value	B	SE	p Value	B	SE	p Value	B	SE	p Value
pRNFL [μm, mean ± SD]	74.2 ± 21.5	90.3 ± 21.1	65.6 ± 26.9	97.4 ± 19.8	100.9 ± 9.3	11.2	8.0	0.173	−4.5	7.9	0.577	−22.7	4.5	**<0.001**	−10.1	4.4	**0.027**
MV [μm, mean ± SD]	305.3 ± 15.8	316.5 ± 20.7	292.7 ± 15.8	313.0 ± 16.6	326.0 ± 15.2	15.5	5.3	**0.006**	5.7	7.2	0.435	−18.6	4.8	**<0.001**	−9.3	5.5	0.098
mRNFL [μm, mean ± SD]	27.1 ± 5.0	30.4 ± 4.2	25.9 ± 5.3	33.3 ± 4.8	37.1 ± 2.6	1.4	1.3	0.503	−3.4	1.4	0.366	−8.6	0.8	**<0.001**	−8.6	0.7	0.080
GCIPL [μm, mean ± SD]	64.3 ± 11.8	74.2 ± 11.8	60.5 ± 12.9	77.2 ± 11.5	81.2 ± 6.1	3.7	3.4	0.373	−6.1	3.5	0.250	−13.3	1.9	**0.023**	−9.3	1.9	**0.022**
INL [μm, mean ± SD]	41.6 ± 2.6	41.5 ± 4.3	40.6 ± 4.4	38.7 ± 2.6	39.4 ± 3.2	1.9	0.9	**0.034**	2.8	1.0	0.543	1.1	0.7	0.503	2.5	0.8	0.302

Abbreviations: AQP4-NMOSD = people with aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorder; B = estimate; DN-NMOSD = people with double-antibody seronegative neuromyelitis optica spectrum disorder; GCIPL = combined ganglion cell and inner plexiform layer; HC = healthy control; INL = inner nuclear layer; mRNFL = macular retinal nerve fiber layer; MV = total macular volume; NON = no optic neuritis; ON = optic neuritis; pRNFL = peripapillary retinal nerve fiber layer; SE = standard error.

Figure 1 Retinal Layer Thicknesses in DN-NMOSD, AQP4-NMOSD, and HCs
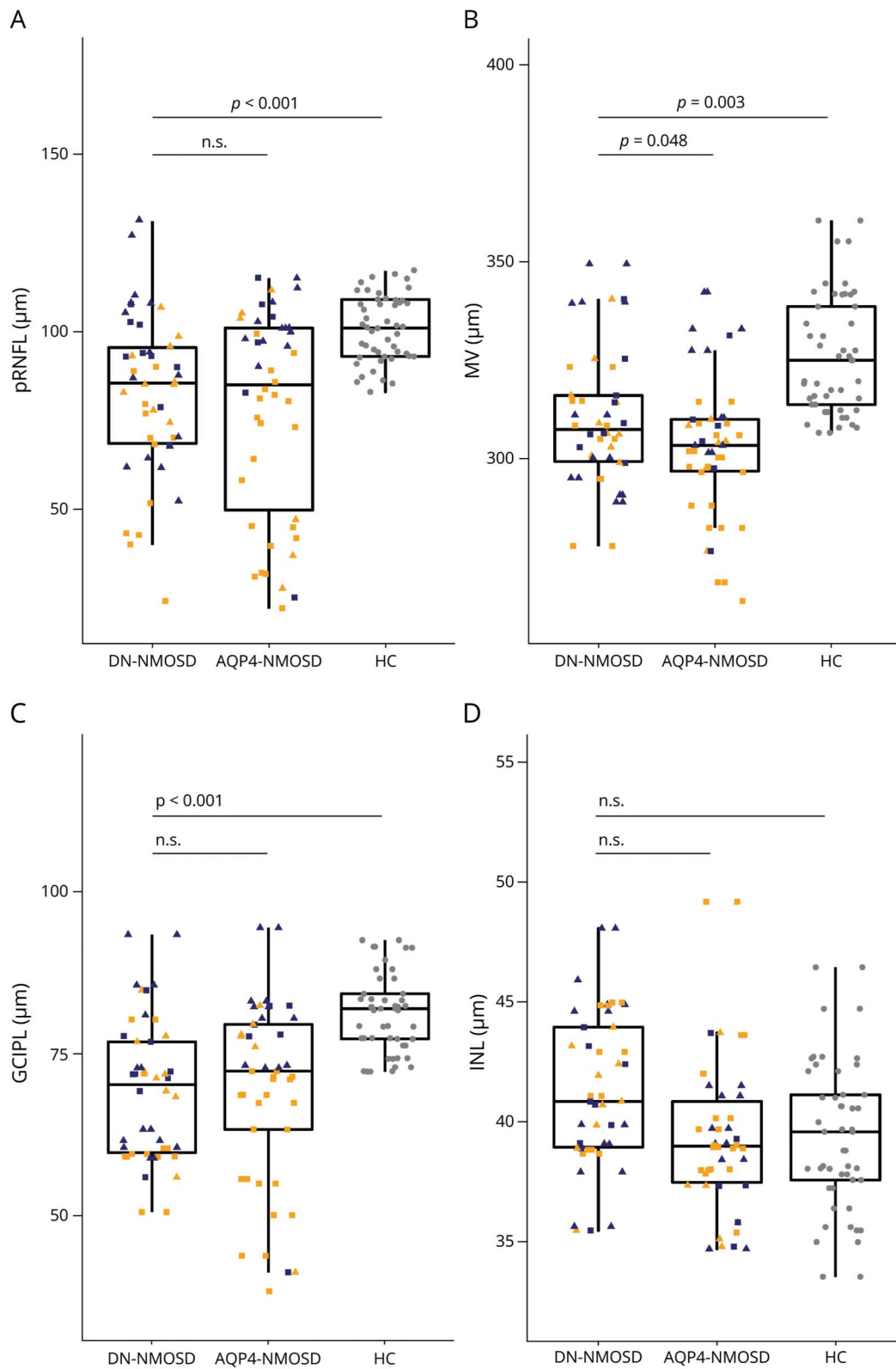
Box plots with dotted overlay for single-eye–based values for eyes with a history of ON (orange) and without a history of ON (blue) as well as for HC eyes (gray). Dots are shaped depending on the history of contralateral ON (history of contralateral ON: square, no history of contralateral ON: triangle, HC: circle). AQP4-NMOSD = people with aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorder; DN-NMOSD = people with double-antibody sero-negative neuromyelitis optica spectrum disorder; GCIPL = combined ganglion cell and inner plexiform layer; HC = healthy control; INL = inner nuclear layer; MV = macular volume; n.s. = not significant; ON = optic neuritis; pRNFL = peripapillary retinal nerve fiber layer.

Figure 2 Retinal Neurodegeneration After ON in DN-NMOSD and AQP4-NMOSD

Mean layer thickness (dot) with standard error of the mean (whiskers) for eyes without ON (NON, blue) as well as for eyes after 1 ON, 2 ON, and ≥3 ON (orange) of people with DN-NMOSD (left) and AQP4-NMOSD (right). Owing to the low sample size, none of the within-gro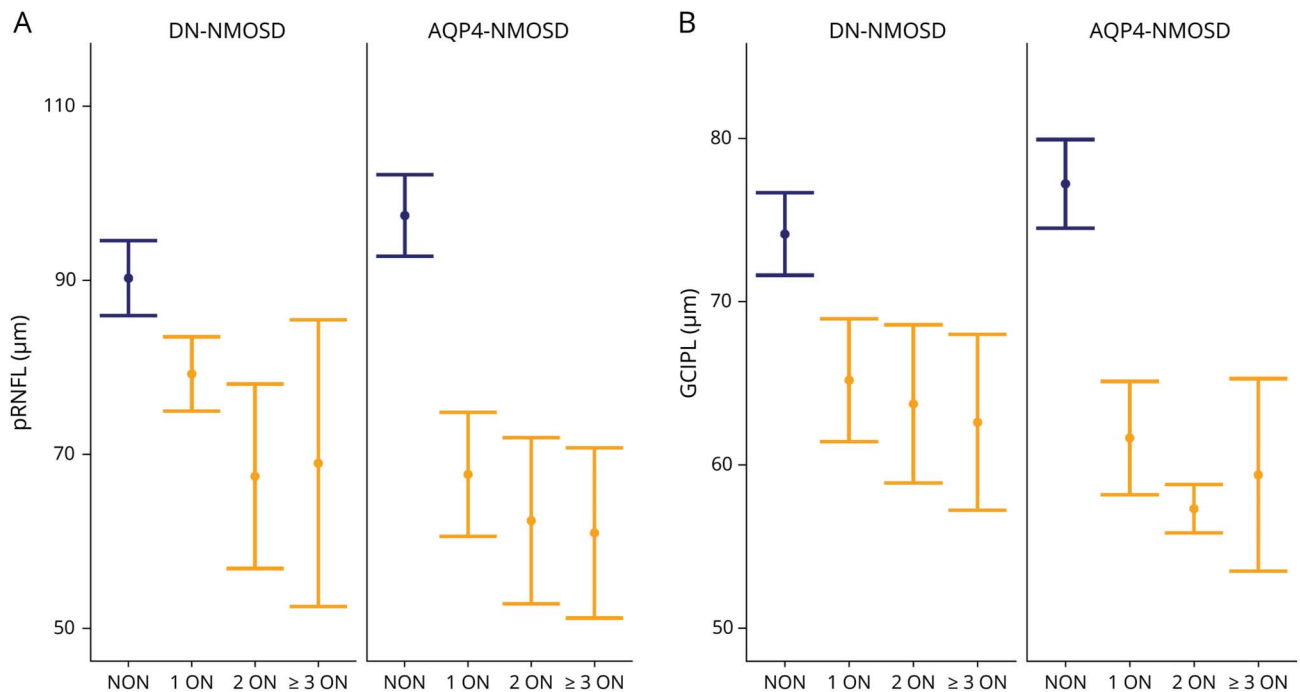up comparisons (NON vs 1 ON, 1 ON vs 2 ON, 2 ON vs ≥3 ON) was statistically significant. AQP4-NMOSD = people with aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorder; DN-NMOSD = people with double-antibody seronegative neuromyelitis optica spectrum disorder; GCIPL = combined ganglion cell and inner plexiform layer; ON = optic neuritis; pRNFL = peripapillary retinal nerve fiber layer.

(temporal, temporal-superior, temporal-inferior, nasal, nasal-superior, nasal-inferior) after ON did not differ between DN-NMOSD and AQP4-NMOSD (data not shown).

Only MV was slightly higher in DN-NMOSD after ON compared with AQP4-NMOSD (relative difference: 4%, $p = 0.006$), which was also the case in the subset of eyes with a history of one ON episode (in AQP4-NMOSD: 294.1 ± 16.9 μm, B = 15.6, SE = 7.1, $p = 0.039$). This might be because of the small, but significant, increase of INL in eyes after ON in DN-NMOSD compared with AQP4-NMOSD (Table 3).

The Levene test did not show a difference in variances for retinal layer thicknesses between DN-NMOSD and AQP4-NMOSD for the whole data set and for subsets with a history of ON and without a history of ON (n.s., data not shown).

Discussion

Our study describes systematically the clinical picture of DN-NMOSD with focus on afferent visual system damage. Twenty-eight percent of the DN-NMOSD cohort were male, which is a slightly more balanced gender distribution than that described in AQP4-NMOSD.[37,38] The disease onset, course, and severity of this DN-NMOSD cohort were comparable with known demographics of AQP4-NMOSD.[37] Our study specifically reports severe ON-associated neuroaxonal loss

with predominant damage after the first ON attack and still considerable, but less, damage after subsequent ON attacks. This study also suggests pRNFL and GCIPL loss in DN-NMOSD independent of ON compared with HCs. This effect was not driven by contralateral ON.

Retinal neurodegeneration in this DN-NMOSD cohort after ON compared with non-ON eyes (16 μm pRNFL) was not significantly different from AQP4-NMOSD—which might also be because of the low sample size—but is still numerically lower and comparable with numbers known from studies in people with MS (15 μm pRNFL loss).[39] Like in AQP4-NMOSD, however, the most dramatic damage was caused by the first ON attack, which might be helpful to facilitate an earlier diagnosis after the first manifestation.[31] The existence of attack-independent damage in AQP4-NMOSD has been a controversy over the past decade, but nowadays studies worldwide have confirmed existing, although subtle, retinal neurodegeneration independent of ON in AQP4-NMOSD.[13,21,31,40-44] In DN-NMOSD, ON-independent retinal neuroaxonal loss seems to be less subtle as measured here by pRNFL and GCIPL loss compared with HCs. This progressive retinal tissue loss has to be confirmed longitudinally, but also seems to be more in the ranges known from MS.[45] Interestingly, we also found differences in INL thickness: After ON, eyes in the DN-NMOSD cohort had thicker INL than eyes in the AQP-NMOSD cohort. While this could

be by pure chance because of the small sample size, this thicker INL could alternatively also point toward less degenerative processes in this layer or more ongoing inflammatory edema outside of attacks in DN-NMOSD.[46,47] Longitudinal studies will be necessary to further investigate this phenomenon.

It remains unclear whether people with DN-NMOSD belong to one homogeneous disease entity with a common pathology and clinical picture or whether they in fact are an artificially assembled group of different disease entities. One potential hint toward the latter might be a larger variety of clinical phenotypes and damage patterns as seen previously on MRI.[18] In this study, we did not find a difference in variances for retinal damage patterns between DN-NMOSD and AQP4-NMOSD, potentially pointing toward a more homogeneous retinal pathology in DN-NMOSD.

The strengths of our study rest on its cohort size and international acquisition, which allowed us to perform a systematic analysis in this rare disease. The comparison with AQP4-NMOSD in the same study further allowed us to describe the disease in perspective to its most important differential diagnosis. However, limitations must be considered: First, no functional assessments, optic nerve involvement on orbital MRI, and description of acute management were included in this study. Especially the lack of imaging of the retina and optic nerve during the acute attack limits our understanding of acute ON-related damage patterns in DN-NMOSD. Second, the ON diagnosis was performed clinically, and we do not have homogeneous paraclinical measures to confirm ON status across the study.[48] Last but not least, the antibody testing, in particular for MOG-IgG, was performed at most centers as part of the clinical routine, not in CSF, and not at predefined or consistent time points. This together with the center-specific use of different cell-based assays might have led to differences in test sensitivity. We also cannot exclude a potential influence of the center-distribution and/or center-specific effects on our results.

To conclude, DN-NMOSD is characterized by severe retinal neurodegeneration after ON and attack-independent retinal damage. Most of the damage occurs during the first ON episode. This highlights the need for diagnostic imaging markers for DN-NMOSD to facilitate an earlier diagnosis as well as for effective attack treatments. Further studies will be necessary to better understand the clinical picture and pathology of DN-NMOSD as well as the utility of OCT for diagnosing and monitoring DN-NMOSD.

Study Funding

Disclosure

Appendix Authors

Name	Location	Contribution
Frederike C. Oertel, MD/PhD	Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité—Universitätsmedizin Berlin; Neuroscience Clinical Research Center; Department of Neurology, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Hanna G. Zimmermann, PhD	Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité—Universitätsmedizin Berlin; Neuroscience Clinical Research Center, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin; Einstein Center Digital Future, Berlin, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design
Seyedamirhosein Motamedi	Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité—Universitätsmedizin Berlin; Neuroscience Clinical Research Center, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Charlotte Bereuter	Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité—Universitätsmedizin Berlin; Neuroscience Clinical Research Center, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Luca Magdalena Manthey	Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité—Universitätsmedizin Berlin; Neuroscience Clinical Research Center, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany	Analysis or interpretation of data

Continued

Name	Location	Contribution
Fereshteh Ashtari	Kashani MS Center. Isfahan University of Medical Sciences, Isfahan, Iran	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Rahele Kafieh	School of advanced technologies in medicine and Medical Image and Signal processing Research Center, Isfahan University of Medical Sciences, Iran	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Alireza Dehghani	Department of Ophthalmology, Isfahan Eye Research Center, Isfahan University of Medical Sciences, Iran	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Mohsen Pourazizi	Department of Ophthalmology, Isfahan Eye Research Center, Isfahan University of Medical Sciences, Iran	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Lekha Pandit, MD, DM, PhD	Department of Neurology, KS Hegde Medical Academy, Nitte University, Mangalore, India	Major role in the acquisition of data
Anitha D'Cunha, PhD	Department of Neurology, KS Hegde Medical Academy, Nitte University, Mangalore, India	Major role in the acquisition of data
Orhan Aktas, MD	Department of Neurology, Medical Faculty, Heinrich Heine University Düsseldorf, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Philipp Albrecht	Department of Neurology, Medical Faculty, Heinrich Heine University Düsseldorf; Department of Neurlogy, Kliniken Maria Hilf Mönchengladbach, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Marius Ringelstein, MD	Department of Neurology; Centre for Neurology and Neuropsychiatry, Landschaftsverband Rheinland-Klinikum Düsseldorf, Medical Faculty, Heinrich Heine University Düsseldorf, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Elena H. Martinez-Lapiscina, MD, PhD, MPH	Hospital Clinic of Barcelona-Institut d'Investigacions, Biomèdiques August Pi Sunyer, (IDIBAPS), Barcelona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data

Name	Location	Contribution
Bernardo F. Sanchez Dalmau, MD, PhD	Hospital Clinic of Barcelona-Institut d'Investigacions, Biomèdiques August Pi Sunyer, (IDIBAPS), Barcelona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Pablo Villoslada, MD	Hospital Clinic of Barcelona-Institut d'Investigacions, Biomèdiques August Pi Sunyer, (IDIBAPS), Barcelona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Nasrin Asgari	Departments of Neurology, Slagelse Hospitals, Denmark, Institute of Regional Health Research; Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Romain Marignier	Service de Neurologie, Sclérose en Plaques, Pathologies de la Myéline et Neuro-inflammation, Centre de Référence des Maladies Inflammatoires Rares du Cerveau et de la Moelle, Hôpital Neurologique Pierre Wertheimer, Hospices Civils de Lyon, France	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Alvaro Cobo-Calvo, MD, PhD	Service de Neurologie, Sclérose en Plaques, Pathologies de la Myéline et Neuro-inflammation, Centre de Référence des Maladies Inflammatoires Rares du Cerveau et de la Moelle, Hôpital Neurologique Pierre Wertheimer, Hospices Civils de Lyon, France; Centre d'Esclerosi Múltiple de Catalunya (Cemcat). Department of Neurology/Neuroimmunology, Hospital Universitari Vall d'Hebron. Universitat Autònoma de Barcelona, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Letizia Leocani	Experimental Neurophysiology Unit, Institute of Experimental Neurology (INSPE) Scientific Institute San Raffaele and University Vita-Salute San Raffaele, Milan, Italy	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Marco Pisa, MD	Experimental Neurophysiology Unit, Institute of Experimental Neurology (INSPE) Scientific Institute San Raffaele and University Vita-Salute San Raffaele, Milan, Italy	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Marta Radaelli	Experimental Neurophysiology Unit, Institute of Experimental Neurology (INSPE) Scientific Institute San Raffaele and University Vita-Salute San Raffaele, Milan, Italy	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data

Name	Location	Contribution
Jacqueline Palace, BM (Hons), FRCP, DM	Department of Neurology, Oxford University Hospitals, NHS Foundation Trust, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Adriana Roca-Fernandez	Department of Neurology, Oxford University Hospitals, NHS Foundation Trust, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Maria Isabel S. Leite, MD, DPhil, FRCP	Department of Neurology, Oxford University Hospitals, NHS Foundation Trust, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Srilakshmi Sharma, MBCHB, PhD	Department of Ophthalmology, Oxford University Hospitals, National Health Service Trust, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Jerome De Seze	Neurology Service, University Hospital of Strasbourg, France	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Thomas Senger, MD	Neurology Service, University Hospital of Strasbourg, France	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Michael R. Yeaman, PhD, MSc, BS	Department of Medicine, Divisions of Molecular Medicine & Infectious Diseases, Harbor-University of California at Los Angeles (UCLA) Medical Center, and Lundquist Institute for Biomedical Innovation, Torrance; Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design
Terry J. Smith, MD, PhD	Departments of Ophthalmology and Visual Sciences, Kellogg Eye Center; Division of Metabolism, Endocrine and Diabetes, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design
Lawrence J. Cook, PhD	Department of Pediatrics, University of Utah	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design

Name	Location	Contribution
Alexander U. Brandt	Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité—Universitätsmedizin Berlin; Neuroscience Clinical Research Center, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design
Friedemann Paul, MD	Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité—Universitätsmedizin Berlin; Neuroscience Clinical Research Center; Department of Neurology, Charité—Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design

References

1. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology*. 2015;85(2):177-189. doi:10.1212/WNL.0000000000001729
2. Oertel FC, Zimmermann H, Paul F, Brandt AU. Optical coherence tomography in neuromyelitis optica spectrum disorders: potential advantages for individualized monitoring of progression and therapy. *EPMA J*. 2018;9(1):21-33. doi:10.1007/s13167-017-0123-5
3. Jarius S, Paul F, Franciotta D, et al. Mechanisms of disease: aquaporin-4 antibodies in neuromyelitis optica. *Nat Clin Pract Neurol*. 2008;4:202-214. doi:10.1038/ncpneuro0764
4. Zekeridou A, Lennon VA. Aquaporin-4 autoimmunity. *Neurol Neuroimmunol Neuroinflamm*. 2015;2(4):e110. doi:10.1212/NXI.0000000000000110
5. Cobo-Calvo A, Ruiz A, Maillart E, et al. Clinical spectrum and prognostic value of CNS MOG autoimmunity in adults: the MOGADOR study. *Neurology*. 2018;90(21):e1858-e1869. doi:10.1212/WNL.0000000000005560
6. Jarius S, Wildemann B, Paul F. Neuromyelitis optica: clinical features, immunopathogenesis and treatment. *Clin Exp Immunol*. 2014;176(2):149-164. doi:10.1111/cei.12271
7. Kitley J, Woodhall M, Waters P, et al. Myelin-oligodendrocyte glycoprotein antibodies in adults with a neuromyelitis optica phenotype. *Neurology*. 2012;79(12):1273-1277. doi:10.1212/WNL.0b013e31826aac4e
8. Hamid SHM, Whittam D, Mutch K, et al. What proportion of AQP4-IgG-negative NMO spectrum disorder patients are MOG-IgG positive? A cross sectional study of 132 patients. *J Neurol*. 2017;264(10):2088-2094. doi:10.1007/s00415-017-8596-7
9. Pache F, Zimmermann H, Mikolajczak J, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 4: afferent visual system damage after optic neuritis in MOG-IgG-seropositive versus AQP4-IgG-seropositive patients. *J Neuroinflammation*. 2016;13(1):282. doi:10.1186/s12974-016-0720-6
10. Oertel FC, Outteryck O, Knier B, et al. Optical coherence tomography in myelin-oligodendrocyte-glycoprotein antibody-seropositive patients: a longitudinal study. *J Neuroinflammation*. 2019;16(1):154. doi:10.1186/s12974-019-1521-5
11. Oertel FC, Sotirchos ES, Zimmermann HG, et al. Longitudinal retinal changes in MOGAD. *Ann Neurol*. 2022;92(3):476-485. doi:10.1002/ana.26440
12. Hacohen Y, Palace J. Time to separate MOG-Ab-associated disease from AQP4-Ab-positive neuromyelitis optica spectrum disorder. *Neurology*. 2018;90(21):947-948. doi:10.1212/WNL.0000000000005619
13. Oertel FC, Havla J, Roca-Fernández A, et al. Retinal ganglion cell loss in neuromyelitis optica: a longitudinal study. *J Neurol Neurosurg Psychiatry*. 2018;89(12):1259-1265. doi:10.1136/jnnp-2018-318382
14. Banwell B, Bennett JL, Marignier R, et al. Diagnosis of myelin oligodendrocyte glycoprotein antibody-associated disease: international MOGAD Panel proposed criteria. *Lancet Neurol*. 2023;22(3):268-282. doi:10.1016/S1474-4422(22)00431-8
15. Waters P, Reindl M, Saiz A, et al. Multicentre comparison of a diagnostic assay: aquaporin-4 antibodies in neuromyelitis optica. *J Neurol Neurosurg Psychiatry*. 2016;87(9):1005-1015. doi:10.1136/jnnp-2015-312601
16. 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. doi:10.1186/1742-2094-9-14

17. Ayroza Galvão Ribeiro Gomes AB, Kulsvehagen L, Lipps P, et al. Immunoglobulin A antibodies against myelin oligodendrocyte glycoprotein in a subgroup of patients with central nervous system demyelination. *JAMA Neurol.* 2023;80(9):989–995. doi: 10.1001/jamaneurol.2023.2523

18. Yeo T, Probert F, Jurynczyk M, et al. Classifying the antibody-negative NMO syndromes: clinical, imaging, and metabolomic modeling. *Neurol Neuroimmunol Neuroinflamm.* 2019;6:e626. doi:10.1212/NXI.0000000000000626

19. Cree BAC, Bennett JL, Kim HJ, et al. Inebilizumab for the treatment of neuromyelitis optica spectrum disorder (N-MOmentum): a double-blind, randomised placebo-controlled phase 2/3 trial. *Lancet.* 2019;394(10206):1352-1363. doi:10.1016/S0140-6736(19)31817-3

20. Sun J, Sun X, Zhang N, et al. Analysis of brain and spinal cord lesions to occult brain damage in seropositive and seronegative neuromyelitis optica. *Eur J Radiol.* 2017;94: 25-30. doi:10.1016/j.ejrad.2017.07.002

21. Lu A, Zimmermann HG, Specovius S, et al. Astrocytic outer retinal layer thinning is not a feature in AQP4-IgG seropositive neuromyelitis optica spectrum disorders. *J Neurol Neurosurg Psychiatry.* 2022;93(2):188-195. doi:10.1136/jnnp-2021-327412

22. 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(4):470-482. doi:10.1177/1352458515593406

23. Kitley J, Waters P, Woodhall M, et al. Neuromyelitis optica spectrum disorders with aquaporin-4 and myelin-oligodendrocyte glycoprotein antibodies: a comparative study. *JAMA Neurol.* 2014;71(3):276-283. doi:10.1001/jamaneurol.2013.5857

24. Khadka B, Bhattarai AM, Dhakal B, Karki A, Acharya A, Poudel R. Seronegative neuromyelitis optica spectrum disorder with longitudinally extending transverse myelitis and optic neuritis: a case report. *Ann Med Surg (Lond).* 2022;78:103757. doi: 10.1016/j.amsu.2022.103757

25. Digala L, Katyal N, Narula N, Govindarajan R. Eculizumab in the Treatment of aquaporin-4 seronegative neuromyelitis optica spectrum disorder: a case report. *Front Neurol.* 2021;12:660741. doi:10.3389/fneur.2021.660741

26. Alshurafa ZH, Alkhateeb MO. Seronegative Neuromyelitis optica spectrum disorder: an unusual presentation of acute brainstem syndrome. *Am J Case Rep.* 2020;21: e922590. doi:10.12659/AJCR.922590

27. Graves JS, Oertel FC, Van der Walt A, et al. Leveraging visual outcome measures to advance therapy development in neuroimmunologic disorders. *Neurol Neuroimmunol Neuroinflamm.* 2022;9(2):e1126. doi:10.1212/NXI.0000000000001126

28. Oertel FC, Zimmermann HG, Brandt AU, Paul F. Novel uses of retinal imaging with optical coherence tomography in multiple sclerosis. *Expert Rev Neurother.* 2019; 19(1):31-43. doi:10.1080/14737175.2019.1559051

29. Specovius S, Zimmermann HG, Oertel FC, et al. Cohort profile: a collaborative multicentre study of retinal optical coherence tomography in 539 patients with neuromyelitis optica spectrum disorders (CROCTINO). *BMJ Open.* 2020;10: e035397. doi:10.1136/bmjopen-2019-035397

30. Oertel FC, Zimmermann HG, Motamedi S, et al. Diagnostic value of intereye difference metrics for optic neuritis in aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorders. *J Neurol Neurosurg Psychiatry.* 2023;94(7):560-566. doi: 10.1136/jnnp-2022-330608

31. Oertel FC, Specovius S, Zimmermann HG, et al. Retinal optical coherence tomography in neuromyelitis optica. *Neurol Neuroimmunol Neuroinflamm.* 2021;8(6). doi: 10.1212/nxi.0000000000001068

32. Tewarie P, Balk L, Costello F, et al. The OSCAR-IB consensus criteria for retinal OCT quality assessment. *PLoS ONE.* 2012;7(4):e34823. doi:10.1371/journal.pone.0034823

33. Schippling S, Balk LJ, Costello F, et al. Quality control for retinal OCT in multiple sclerosis: validation of the OSCAR-IB criteria. *Mult Scler.* 2015;21(2):163-170. doi: 10.1177/1352458514538110

34. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology.* 2016;86(24):2303-2309. doi:10.1212/WNL.0000000000002774

35. Aytulun A, Cruz-Herranz A, Aktas O, et al. APOSTEL 2.0 recommendations for reporting quantitative optical coherence tomography studies. *Neurology.* 2021;97(2): 68-79. doi:10.1212/WNL.0000000000012125

36. R Development Core Team. *R: A Language and Environment for Statistical Computing [online].* R Foundation for Statistical Computing; 2008. R-project.org

37. Pandit L, Asgari N, Apiwattanakul M, et al. Demographic and clinical features of neuromyelitis optica: a review. *Mult Scler.* 2015;21(7):845-853. doi:10.1177/1352458515572406

38. Wu Y, Geraldes R, Juryńczyk M, Palace J. Double-negative neuromyelitis optica spectrum disorder. *Mult Scler.* 2023;29(11-12):1353-1362. doi:10.1177/13524585231199819

39. Petzold A, Balcer LJ, Calabresi PA, et al. Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol.* 2017;16(10):797-812. doi:10.1016/S1474-4422(17)30278-8

40. Oertel FC, Kuchling J, Zimmermann H, et al. Microstructural visual system changes in AQP4-antibody–seropositive NMOSD. *Neurol Neuroimmunol Neuroinflamm.* 2017; 4(3):e334. doi:10.1212/NXI.0000000000000334

41. Filippatou AG, Vasileiou ES, He Y, et al. Optic neuritis-independent retinal atrophy in neuromyelitis optica spectrum disorder. *J Neuroophthalmol.* 2022;42(1):e40-e47. doi: 10.1097/WNO.0000000000001282

42. Filippatou AG, Vasileiou ES, He Y, et al. Evidence of subclinical quantitative retinal layer abnormalities in AQP4-IgG seropositive NMOSD. *Mult Scler.* 2021;27(11): 1738-1748. doi:10.1177/1352458520977771

43. Yamamura T, Nakashima I. Foveal thinning in neuromyelitis optica: a sign of retinal astrocytopathy? *Neurol Neuroimmunol Neuroinflamm.* 2017;4(3):e347. doi:10.1212/NXI.0000000000000347

44. Ringelstein M, Kleiter I, Ayzenberg I, et al. Visual evoked potentials in neuromyelitis optica and its spectrum disorders. *Mult Scler.* 2014;20(5):617-620. doi:10.1177/1352458513503053

45. Cordano C, Nourbakhsh B, Yiu HH, et al. Differences in age-related retinal and cortical atrophy rates in multiple sclerosis. *Neurology.* 2022;99(15):e1685-e1693. doi: 10.1212/WNL.0000000000200977

46. Cordano C, Yiu HH, Oertel FC, et al. Retinal INL thickness in multiple sclerosis: a mere marker of neurodegeneration? *Ann Neurol.* 2021;89(1):192-193. doi:10.1002/ana.25933

47. Balk LJ, Coric D, Knier B, et al. Retinal inner nuclear layer volume reflects inflammatory disease activity in multiple sclerosis; a longitudinal OCT study. *Mult Scler J Exp Transl Clin.* 2019;5(3):2055217319871582. doi:10.1177/2055217319871582

48. Petzold A, Fraser CL, Abegg M, et al. Diagnosis and classification of optic neuritis. *Lancet Neurol.* 2022;21(12):1120-1134. doi:10.1016/S1474-4422(22)00200-9