Original research

Diagnostic value of intereye difference metrics for optic neuritis in aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorders

Frederike Cosima Oertel,1,2,3 Hanna G Zimmermann,1,2,4 Seydamirhosein Motamedi,1,2 Claudia Chien,1,2,4 Orhan Aktas,5 Philipp Albrecht,1,4 Marius Ringelstein,1,2,4,6 Anitha Dcunha,1,7 Lekha Pandit,2,7 Elena H Martinez-Lapiscina,2,8 Bernardo Sanchez-Dalmau,2,8 Pablo Villoslada,2,8,9 Jacqueline Palace,2,10 Adriana Roca-Fernández,2,10 Maria Isabel Leite,10 Srilakshmi M Sharma,1,11 Letizia Leocani,1,12 Marco Aurélio Lana-Peixoto,13 Mariana Andrade Fontenelle,13 Joachim Havla,1,14 Fereshteh Ashtari,1,15 Rahele Kafieh,15 Alireza Dehghani,17 Mohsen Pourazizi,17 Romain Marignier,18 Alvaro Cobo-Calvo,18,19 Nasrin Asgari,20,21 Anu Jacob,22,23 Saif Huda,22,24 Yang Mao-Draayer,24 Ari J Green,25 Rachel Kenney,25 Michael R Yeaman,26,27 Terry J Smith,28,29 Lawrence Cook,30 Alexander U Brandt,1,2,31 Friedemann Paul,1,2,32 Axel Petzold1,33,34

ABSTRACT

Background The novel optic neuritis (ON) diagnostic criteria include intereye differences (IED) of optical coherence tomography (OCT) parameters. IED has proven valuable for ON diagnosis in multiple sclerosis but has not been evaluated in aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorders (AQP4+NMOSD). We evaluated the diagnostic accuracy of intereye absolute (IEAD) and percentage difference (IEPD) in AQP4+NMOSD after unilateral ON >6 months before OCT as compared with healthy controls (HC).

Methods Twenty-eight AQP4+NMOSD after unilateral ON (NMOSD-ON), 62 HC and 45 AQP4+NMOSD without a history of ON (NMOSD-NON) were recruited by 13 centres as part of the international Collaborative Retrospective Study on retinal OCT in Neuromyelitis Optica study. Mean thickness of peripapillary retinal nerve fibre layer (pRNFL) and macular ganglion cell and inner plexiform layer (GCIP) were quantified by Spectralis spectral domain OCT. Threshold values of the ON diagnostic criteria (pRNFL: IEAD: 5 µm, IEPD: 5%; GCIP: IEAD: 4 µm, IEPD: 4%) were evaluated using receiver operating characteristics and area under the curve (AUC) metrics.

Results The discriminative power was high for NMOSD-ON versus HC for IEAD (pRNFL: AUC 0.92, specificity 85%, sensitivity 75%) as well as versus AQP4+NMOSD (AUC 0.94, specificity 82%, sensitivity 89%; GCIP: AUC 0.93, specificity 98%, sensitivity 86%). The discriminative power was high/moderate for NMOSD-NON for IEAD (pRNFL: AUC 0.92, specificity 82%, sensitivity 86%; GCIP: AUC 0.87, specificity 85%, sensitivity 75%) and for IEPD (pRNFL: AUC 0.90, specificity 82%, sensitivity 89%; GCIP: AUC 0.88, specificity 82%, sensitivity 82%).

Conclusions Results support the validation of the IED metrics as OCT parameters of the novel diagnostic ON criteria in AQP4+NMOSD.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The novel optic neuritis (ON) diagnostic criteria include intereye differences (IED) of neuroaxonal optical coherence tomography (OCT) parameters.

⇒ IED has proven valuable for ON diagnosis in multiple sclerosis but has not been evaluated in aquaporin-4 antibody seropositive neuromyelitis optica spectrum disorders (AQP4+NMOSD).

WHAT THIS STUDY ADDS

⇒ In this cohort study that included 73 AQP4+NMOSD patients and 62 healthy controls from 13 centres, the discriminative power of intereye differences of neuroaxonal OCT parameters was high for AQP4+NMOSD with a history of one unilateral ON event versus healthy controls (specificity ≥82%, sensitivity ≥75%) as well as versus AQP4+NMOSD without a history of ON (specificity ≥77%, sensitivity ≥75%).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ OCT parameters of novel diagnostic ON criteria are applicable in AQP4+NMOSD.

© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Oertel FC, Zimmermann HG, Motamedi S, et al. J Neurol Neurosurg Psychiatry Epub ahead of print: [please include Day Month Year]. doi:10.1136/jnnp-2022-330608
**BACKGROUND**

Long-awaited diagnostic criteria and a new classification schema for optic neuritis (ON) have recently been defined by an expert consortium. As part of these criteria, the increasing value of optical coherence tomography (OCT) as an accurate method for quantitative assessment of retinal neurodegeneration after ON in clinical diagnosis and monitoring has been recognised.

In addition to monocular atrophy of the peripapillary retinal nerve fibre layer (pRNFL) and of the combined ganglion cell and inner plexiform layer (GCIPL), retinal asymmetry of pRNFL and GCIPL can be used as a diagnostic tool for retinal neurodegeneration after ON. 

In multiple sclerosis (MS), an intereye absolute difference (IEAD) of >3–5 μm and intereye percentage difference (IEPD) of >3%–5% are diagnostic for a history of unilateral ON and can reach a specificity up to 97% and sensitivity of up to 100%. Yet, outside of MS cohorts, ON associations with intereye differences have not been validated in other disorders.

Patients with aquaporin-4 antibody (AQP4-IgG) seropositive neuromyelitis optica spectrum disorder (NMOSD) frequently suffer from severe and recurrent ON episodes. Due to the relative rarity of these patients as compared with MS, diagnosis and appropriate treatment are often delayed. Furthermore, ON in AQP4-IgG seropositive NMOSD can present with an atypical clinical picture more commonly than in MS. In turn, inaccurate and/or delayed diagnoses can significantly negatively affect long-term clinical outcomes. Thus, IEAD and IEPD could be valuable additions to the diagnostic workup of ON in AQP4-IgG seropositive NMOSD. Yet, it remains unclear if the threshold values defined by the novel ON diagnostic criteria and established in MS are also applicable in AQP4-IgG seropositive NMOSD. Therefore, this study aims to evaluate the diagnostic accuracy of reported IEAD and IEPD values for AQP4-IgG seropositive NMOSD patients with one unilateral ON event (NMOSD-ON) as compared with healthy controls (HC).

**METHODS**

**Cohort design**

We included AQP4-IgG seropositive NMOSD patients with a history of one unilateral ON (NMOSD-ON) >6 months before OCT measurements and two groups for comparison: (1) HC and (2) AQP4-IgG seropositive NMOSD patients without a history of ON (NMOSD-NON). All NMOSD and HC subjects were recruited as part of the international Collaborative Retrospective Study on retinal OCT in Neuromyelitis Optica (CROCTINO) study and their data were acquired by 13 international centres between 2000 and 2018 (online supplemental table 1). Inclusion criteria were: (1) OCT data acquired by Spectralis SD-OCT devices, (2) the absence of diseases potentially confounding OCT analyses (such as glaucoma, retinal surgery and ametropia >6 D) and (3) the absence of (further) ON attacks within 6 months before the examination date. Clinical data including time since disease onset, time since ON and treatment history were collected at the discretion of each centre. AQP4-IgG antibodies were tested at the discretion of each centre.

**Table 1** Demographic overview

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>NMOSD-NON</th>
<th>NMOSD-ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>62</td>
<td>45</td>
<td>28</td>
</tr>
<tr>
<td>Eyes (n)</td>
<td>124</td>
<td>90</td>
<td>56</td>
</tr>
<tr>
<td>Patients with a disease duration &lt;10 years (n)</td>
<td>.</td>
<td>43</td>
<td>21</td>
</tr>
<tr>
<td>Patients with ON as first manifestation (n)</td>
<td>.</td>
<td>.</td>
<td>17</td>
</tr>
<tr>
<td>Age (year, mean±SD)</td>
<td>37.7±10.2</td>
<td>39.0±10.4</td>
<td>38.8±12.1</td>
</tr>
<tr>
<td>Sex (male, n (%))</td>
<td>20 (32)</td>
<td>2 (4)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Time since ON (year, median (min−max))</td>
<td>.</td>
<td>2.8 (0.7–19.5)</td>
<td></td>
</tr>
<tr>
<td>Time since onset (year, mean±SD)</td>
<td>.</td>
<td>3.8±4.0</td>
<td>6.5±5.6</td>
</tr>
</tbody>
</table>

**Table 2** Comparison of IEAD and IEPD value for GCIPL and pRNFL

<table>
<thead>
<tr>
<th></th>
<th>NMOSD-ON versus HC</th>
<th>NMOSD-ON versus NMOSD-NON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T value</td>
<td>P value</td>
</tr>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL IEAD (%)</td>
<td>−6.885</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pRNFL IEAD (µm)</td>
<td>−6.483</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCIPL IEAD (%)</td>
<td>−6.566</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCIPL IEAD (µm)</td>
<td>−6.338</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First attack only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL IEAD (%)</td>
<td>−6.162</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pRNFL IEAD (µm)</td>
<td>−5.969</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCIPL IEAD (%)</td>
<td>−6.570</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCIPL IEAD (µm)</td>
<td>−6.357</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Recent onset (&lt;10years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL IEAD (%)</td>
<td>−6.527</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pRNFL IEAD (µm)</td>
<td>−6.054</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCIPL IEAD (%)</td>
<td>−5.768</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GCIPL IEAD (µm)</td>
<td>−5.530</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**D) and (3) the absence of (further) ON attacks within 6 months after the examination date. Clinical data including time since disease onset, time since ON and treatment history were collected at the discretion of each centre. AQP4-IgG antibodies**
were detected in serum samples of all NMO patients by cell-based assays.\textsuperscript{17} Data are reported according to the Enhancing the Quality and Transparency Of Health Research reporting guidelines.\textsuperscript{18}

**Optical coherence tomography**

All OCT examinations were conducted at each centre using Spectralis SD-OCT devices (Heidelberg Engineering, Heidelberg, Germany). pRNFL thickness was measured using a 12° or 3.5 mm diameter peripapillary ring scan. Macular GCIPL thickness was calculated using a 5 mm diameter annulus around the fovea excluding the central 1 mm diameter cylinder from a volume scan. All OCT data reading was performed at Charité Universitätsmedizin Berlin by five graders as previously described.\textsuperscript{14} All included OCT images fulfilled OSCAR-IB criteria\textsuperscript{19,20} and are presented adhering to the Apostel V.2.0 recommendations.\textsuperscript{21,22}

**Statistical analyses**

Statistical analyses were conducted using R (V.4.2.1) (RStudio, Boston, Massachusetts, USA).\textsuperscript{23} Data were stratified in cohorts by diagnosis and history of ON. Continuous data were described as mean±SD, if not stated otherwise. IEAD was calculated as absolute difference between eyes in pRNFL or GCIPL, respectively. IEPD was calculated as IEAD divided by the higher pRNFL or GCIPL value, respectively (comparing both eyes of the patient). Age, IEAD and IEPD were compared between groups employing unpaired t-test. Significance was established at p<0.05. The level of diagnostic accuracy was evaluated using receiver operating characteristics (ROC) curves. Area under the curve (AUC) measurements were rated as *low/no discriminative power* (for AUC <0.7), *moderate discriminative power* (for AUC 0.7–0.9) and *high discriminative power* (for AUC >0.9).

**Data availability**

All data used in this project are available within the article and by reasonable request from the corresponding author.

**RESULTS**

**Cohort**

Characteristics of the study cohort are summarised in table 1. Twenty-eight patients (56 eyes) with a diagnosis of AQP4-IgG seropositive NMO and a history of unilateral ON (NMO-ON) were compared with 62 healthy controls (HC, 124 eyes) and 45 patients with AQP4-IgG seropositive NMO without a history of ON (NMO-NON, 90 eyes). All control groups were age-matched with NMO-ON (HC: p=0.881, NMO-NON: p=0.942).

Twenty-one NMO-ON (42 eyes, age: 36.2±11.5, sex (male, n (%)): 2 (10)) and 43 NMO-NON (86 eyes, age: 38.4±10.3, sex (male, n (%)): 2 (5)) had a disease duration <10 years. For 17 NMO-ON (34 eyes, age: 39.2±12.2, sex (male, n (%)): 2 (12)), the unilateral ON episode was their first disease manifestation.

**Intereye percentage and absolute differences**

The IEAD and IEPD of pRNFL and GCIPL was higher in NMO-ON compared with HC and NMO-NON (table 2)
Neuro-inflammation

and figures 1 and 2). This result was highly significant for both parameters (p<0.001) when comparing the whole cohort as well as subsets with (a) the unilateral ON episode as the first attack and (b) disease duration <10 years.

Diagnostic sensitivity and specificity of intereye differences in NMOSD

The discriminative power for IEAD and IEAD was high (AUC >0.9) for HC versus NMOSD-ON. This outcome was true for patients overall, and for patients with a disease duration <10 years (figures 3 and 4). The discriminative power for IEAD and IEAD was also high or moderate (AUC 0.8–0.9) for separating NMOSD-NON versus NMOSD-ON.

Evaluating previously reported threshold values (IEAD: 5 µm, IEAD: 5% for pRNFL and IEAD: 4 µm, IEAD: 4% GCIP), NMOSD-ON could be separated with high discriminative power from HC using IEAD for pRNFL with a specificity of 82% and a sensitivity of 86% (AUC 0.95, positive predictive value (PPV) 0.68, negative predictive value (NPV) 0.93). Likewise, discriminative power was high using IEAD for pRNFL with a specificity of 87% and a sensitivity of 89% (AUC 0.96, PPV 0.76, NPV 0.95). NMOSD-ON can be equally well distinguished from HC using IEAD for GCIP with a specificity of 98% and a sensitivity of 75% (AUC 0.93, PPV 0.94, NPV 0.90) and using IEAD for GCIP with a specificity of 96% and a sensitivity of 82% (AUC 0.94, PPV 0.90, NPV 0.92).

NMOSD-ON can be separated with high to moderate discriminative power from NMOSD-NON using IEAD for pRNFL with a specificity of 77% and a sensitivity of 86% (AUC 0.92, PPV 0.70, NPV 0.90) and using IEAD for pRNFL with a specificity of 82% and a sensitivity of 89% (AUC 0.94, PPV 0.75, NPV 0.92). NMOSD-ON can equally well be separated from NMOSD-NON using IEAD for GCIP with a specificity of 85% and sensitivity of 75% (AUC 0.87, PPV 0.76, NPV 0.85) and using IEAD for GCIP with a specificity of 82% and a sensitivity of 82% (AUC 0.88, PPV 0.74, NPV 0.88). ROC results of IEAD and IEAD did not differ significantly for any discrimination (data not shown).

DISCUSSION

The identification of prior ON episodes is a valuable step in the diagnostic workup and differential diagnosis of multiple autoimmune neuroinflammatory diseases such as AQP4-IgG seropositive NMOSD and MS.2 24 Whereas IEAD and IEAD previously proved their diagnostic value in the ON workup of patients with MS,2 3 our study demonstrates for the first time a high diagnostic accuracy of intereye difference metrics for the identification of AQP4-IgG seropositive NMOSD patients with a prior unilateral ON episode (>6 months before OCT), as compared with NMOSD patients without prior ON episodes and HC. The data also provide support for the macular GCIP IEAD (4 µm), and IEAD (4%) thresholds as defined in the novel ON diagnostic criteria as having excellent diagnostic sensitivity and specificity for ON in AQP4-IgG seropositive NMOSD. Intereye difference metrics thereby prove to not be specific or limited to a particular demyelinating disease, but rather a reflection of the thinning of the RNFL and GCIP that occurs after ON associated with many autoimmune neuroinflammatory diseases. The fact that...
these findings were consistent across an array of international centres is also encouraging with respect to uniform application of these methods and outcome interpretations with minimised centre bias globally.

The IEPD and IEAD performed equally well in our study suggesting that intereye difference metrics may not require the establishment of separate reference values for different OCT devices. Rather, it is conceivable that the IEPD threshold could be used across heterogeneous devices and cohorts. Interestingly, both pRNFL and GCIPL results provided comparable assessments in this cohort, extending the application of intereye differences. Whereas pRNFL may be the faster and more convenient metric in a standard clinical setting, GCIPL undergoes less edematous change and may therefore have special utility earlier after acute ON episodes or in clinical ON trials. The effects of recurrent ON attacks and of chiasmic involvement on the IEPD and IEAD, as commonly seen in NMOSD, are not known. Therefore, future studies should interrogate the validity of these measurements longitudinally in NMOSD-ON.

A strength of our study rests on its size and multicentre setting since the cohort was derived from a consortium of expert NMOSD researchers in context of the international CROCTINO cohort. Hence, the significance of the results suggests that potential differences in use of OCT across broad
geographic distances are overcome by the methods of acquisition and analysis used here. Yet, limitations of the current study should also be considered. First, the cohorts were not matched for gender or degree of loss of neurological function, preventing statistical evaluation. Furthermore, the prevalence of unilateral ON in our cohort is higher than expected in a real-world setting, thereby influencing PPV and NPV calculations. Also, no functional metrics such as visually evoked potentials, or MRI were included in this study, thus subclinical contralateral optic nerve involvement, which would affect IED measurements, cannot be excluded. Therefore, it remains uncertain if there is added diagnostic value of MRI and visually evoked potentials. This determination will be important for future, especially prospective, studies to further improve on diagnostic sensitivity. Nonetheless, the current findings show the astonishing value of quantitative intereye differences for ON diagnosis in AQP4-IgG seropositive NMOSD. This might be of value in making a clinical diagnosis and defining inclusion criteria for clinical trials in NMOSD.

Author affiliations
1 NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
2 Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
3 Department of Neurology, University of California San Francisco, San Francisco, California, USA
4 Einstein Center Digital Future, Berlin, Germany
5 Department of Neurology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
6 Centre for Neurology and Neuropsychiatry, Landschaftsverband Rheinland-Klinikum Düsseldorf, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany
7 Department of Neurology, KS Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India
8 Hospital Clinic of Barcelona-Institut d’Investigacions, Biomèdiques August Pi Sunyer, (IDIBAPS), Barcelona, Spain
9 Wu Tsai Neurosciences Institute, Stanford University, Palo Alto, California, USA
10 Department of Neurology, Oxford University Hospitals, National Health Service Trust, Oxford, UK
11 Department of Ophthalmology, Oxford University Hospitals, National Health Service Trust, Oxford, UK
12 Experimental Neurophysiology Unit, Institute of Experimental Neurology (INSEPE) Scientific Institute San Raffaele and University Vita-Salute San Raffaele, Milan, Italy
13 CIEM MS Research Center, University of Minas Gerais, Medical School, Belo Horizonte, Brazil
14 Institute of Clinical Neuroimmunology, LMU Hospital, Ludwig-Maximilians Universität München, Munich, Germany
15 Kashani MS Center, Isfahan University of Medical Sciences, Isfahan, Iran
16 School of advanced technologies in medicine and Medical Image and Signal Processing Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
17 Department of Ophthalmology, Isfahan Eye Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
18 Neurology, Multiple Sclerosis, Myelin Disorders and Neuroinflammation, Pierre Wertheimer Neurological Hospital, Hospices Civils de Lyon, Lyon, France
19 Centre d’Esclerosi Multiple de Catalunya (Cemcat), Department of Neurology/Neuroimmunology, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain
20 Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark
21 Departments of Neurology, Slagelse Hospitals Denmark, Institute of Regional Neurology, University of Southern Denmark, Odense, Denmark
22 Department of Neurology, The Walton Centre NHS Foundation Trust, Liverpool, UK
23 Department of Neurology, Cleveland Clinic Abu Dhabi, Abu Dhabi, UAE
24 Department of Neurology, University of Michigan Medical School, Ann Arbor, Michigan, USA
25 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, California, USA
26 Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California, USA
27 Departments of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan Medical School, Ann Arbor, Michigan, USA
28 Division of Metabolism, Endocrine and Diabetes, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA
29 Department of Pediatrics, University of Utah, Salt Lake City, Utah, USA
30 Department of Neurology, University of California, Irvine, California, USA
31 Department of Neurology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany
32 Moorfield’s Eye Hospital, The National Hospital for Neurology and Neurosurgery, Queen Square Institute of Neurology, University College London, London, UK
33 Neuro-ophthalmology Expert Center, Amsterdam UMC, Amsterdam, Netherlands
34 Neuro-ophthalmology Expert, Amsterdam UMC, Amsterdam, Netherlands

Twitter Elena H Martinez-Lapiscina @elenahlapiscina@ and Joachim Havla @NeuroVisionLab

Contributors FCO: conceptualisation (support), data curation (support), formal analysis (lead), investigation (equal), project administration (equal), resources (equal), visualisation (lead), writing – original draft, writing – review and editing (equal); HZ: data curation (equal), formal analysis (support), project administration (equal), resources (equal), validation (lead), writing – review and editing (equal); SM: data curation (equal), project administration (equal), resources (equal), writing – review and editing (equal); CB: data curation (support), project administration (equal), resources (equal), writing – review and editing (equal), CC: data curation (equal), project administration (equal), resources (equal), writing – review and editing (equal); OA: resources (equal), writing – review and editing (equal); PA: resources (equal), writing – review and editing (equal); MR: resources (equal), writing – review and editing; AD: resources (equal), writing – review and editing (equal); LL: resources (equal), writing – review and editing (equal); MJ: resources (equal), writing – review and editing (equal); LL: resources (equal), writing – review and editing (equal); AB: resources (equal), writing – review and editing equal; AI: resources (equal), writing – review and editing (equal); AJG: resources (equal), writing – review and editing (equal); KS: resources (equal), writing – review and editing (equal); AL: resources (equal), writing – review and editing (equal); LM: resources (equal), writing – review and editing (equal); YM: resources (equal), writing – review and editing (equal); AC-C: resources (equal), writing – review and editing (equal); PS: resources (equal), writing – review and editing (equal); YA: resources (equal), writing – review and editing (equal); JA: resources (equal), writing – review and editing (equal); JA: resources (equal), writing – review and editing (equal); MPo: resources (equal), writing – review and editing (equal); MA: resources (equal), writing – review and editing (equal); JH: resources (equal), writing – review and editing (equal); RP: resources (equal), writing – review and editing (equal); JH: resources (equal), writing – review and editing (equal); MG: resources (equal), writing – review and editing (equal); AM: resources (equal), writing – review and editing (equal); AC-C: resources (equal), writing – review and editing (equal); NA: resources (equal), writing – review and editing (equal); KKS: resources (equal), writing – review and editing (equal); RL: resources (equal), writing – review and editing (equal); RM: resources (equal), writing – review and editing (equal); Rk: resources (equal), writing – review and editing (equal); RF: resources (equal), writing – review and editing (equal); PA: resources (equal), writing – review and editing (equal); AG: resources (equal), writing – review and editing (equal); A: resources (equal), writing – review and editing (equal); AF: resources (equal), writing – review and editing (equal); YPD: resources (equal), writing – review and editing (equal); FN: resources (equal), writing – review and editing (equal); FP: funding acquisition (equal), project administration (equal), resources (equal), supervision (support), writing – review and editing (equal); FF: funding acquisition (equal), project administration (equal), resources (equal), supervision (support), writing – review and editing (equal); AP: conceptualisation (lead), formal analysis (support), methodology (lead), supervision (lead), writing – review and editing (equal).

Funding regarding the CROCTINO project (to FP), the German Neurology Association (DGN) (to FCO) and the German Ministry for Economic Affairs and Climate Action (to HGZ). FCO received fellowship funding from the American Academy of Neurology (AAN) and the German Ministry from Economic Affairs and Climate Action (to FP), the German Neurology Association (DGN) (to FCO) and the German Ministry for Economic Affairs and Climate Action (to HGZ). FCO received fellowship funding from the American Academy of Neurology (AAN) and the National Multiple Sclerosis Society (NMSS).

Competing interests The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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

Ethics approval The study was approved by local ethics committees (Charité Universitätsmedizin Berlin: EA1/097/18) and conducted in accordance with the applicable laws as well as the current version of the Declaration of Helsinki. All participants gave written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have
been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use and license their derivative works are on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use and license their derivative works are on different terms, provided the original work is

---

**REFERENCES**