Aus dem Exzellenzcluster NeuroCure der Medizinischen Fakultät

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DISSERTATION

Strukturelle und funktionelle Veränderungen des afferenten visuellen Systems bei Patienten mit Neuromyelitis optica-Spektrum-Erkrankungen

zur Erlangung des akademischen Grades

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von

Hanna Gwendolyn Zimmermann

aus Dachau

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# Zusammenfassung

## Abstrakt

Neuromyelitis optica-Spektrum-Erkrankungen (NMOSD) bezeichnen eine inflammatorische Autoimmunerkrankung des zentralen Nervensystems (ZNS), die vorwiegend durch Optikusneuritis (ON) und/oder Myelitis gekennzeichnet ist. In der Mehrzahl der Betroffenen sind pathogene Serumantikörper gegen Aquaporin-4 (AQP4-IgG) nachweisbar. In einem Teil der AQP4-IgG-seronegativen NMOSD-Patienten sowie in Patienten mit rezidivierender ON oder Myelitis konnten Antikörper gegen Myelin-Oligodendrozyten-Glykoprotein (MOG-IgG) nachgewiesen werden. Patienten mit MOG-IgG-assoziierter Enzephalomyelitis (MOG-EM) wurden häufig klinisch als milderer Phänotyp mit besserer Remission und Langzeitprognose im Vergleich zu AQP4-IgG-positiven NMOSD-Patienten beschrieben.

Ziel dieser Arbeit war die Charakterisierung der strukturellen und funktionellen Schädigung des afferenten visuellen Systems bei NMOSD und MOG-EM.

In einer multimodalen Magnetresonanztomographie-Untersuchung zeigten NMOSD-Patienten im Vergleich zu gesunden Kontrollen keine signifikanten Verminderungen der grauen und weißen Substanz sowie kortikaler Dicken. Mit Hilfe von Diffusions-Tensor-Bildgebung und optischer Kohärenztomographie (OCT) konnte allerdings gezeigt werden, dass es bei NMOSD zu einer Abnahme der fraktionellen Anisotropie in der Sehstrahlung kommt, welche mit einer Verdünnung der retinalen Nervenfaserschicht (RNFL) assoziiert ist. Bei AQP4-IgG-positiven NMOSD-Patienten kam es auch ohne vorherige ON zu einer solchen Abnahme. Zudem zeigten diese Patienten auch eine Reduktion der fovealen Dicke. Bei AQP4-IgG-positiven NMOSD-Patienten war zudem die funktionelle Konnektivität der visuellen Netzwerke erhöht, was auf einen maladaptiven plastischen Prozess hindeutet.

Es wurde gezeigt, dass Blutgefäße in der inneren Retina OCT-Dickenmessungen der RNFL bei NMOSD beeinflussen, wobei bei dünnerer RNFL der Einfluss ausgeprägter ist. Eine multizentrische Untersuchung zeigte, dass es bei MOG-EM und AQP4-IgG-positiven NMOSD-Patienten bei vergleichbarer Erkrankungsdauer in ähnlicher Dimension zu ON-assoziierter neuro-axonaler Schädigung der Retina kommt. Allerdings hatten MOG-EM-Patienten eine deutlich höhere ON-Schubrate als die NMOSD Patienten. Daraus schlussfolgerten wir, dass es bei MOG-EM durch die akkumulierte

Schädigung - trotz besserer Remission beim einzelnen Schub - zu ähnlicher Schädigung kommt wie bei AQP4-IgG-positiven NMOSD-Patienten mit weniger, aber dafür schwereren Schüben.

Mit Hilfe eines Fragebogens (NEI-VFQ) wurde die sehbezogene Lebensqualität von Patienten mit NMOSD untersucht. Dabei wurde festgestellt, dass diese eine signifikant niedrigere sehbezogene Lebensqualität haben als Patienten mit multipler Sklerose (MS). Dies erklärte sich dadurch, dass NMOSD-Patienten schwerwiegendere ON-Episoden durchliefen, die zudem häufiger beide Augen betrafen, als MS-Patienten.

Zusammengefasst führt ON bei NMOSD-Patienten neben retinalem neuro-axonalen Schaden zu anterograder Neurodegeneration der Sehstrahlung, möglicherweise maladaptiver Reorganisation des visuellen Netzwerks und eingeschränkter sehbezogener Lebensqualität. Inwieweit es schubunabhängig zu einer direkten, möglicherweise gegen AQP4 gerichteten ZNS-Schädigung kommt, muss noch näher untersucht werden.

# Abstract

Neuromyelitis optica spectrum disorders (NMOSD) describe an autoimmune disease of the central nervous system (CNS), predominantly characterized by optic neuritis (ON) and/or myelitis. In the majority of patients pathogenic serum antibodies against aquaporin-4 (AQP4-IgG) can be detected. In a subset of AQP4-IgG seronegative NMOSD patients, as well as in patients with recurrent ON or myelitis, antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG) could be detected. Patients with MOG-IgG associated encephalomyelitis (MOG-EM) have been described as a milder phenotype with better remission and long-term outcomes as compared to AQP4-IgG positive NMOSD patients.

The objective of this work was the characterization of structural and functional afferent visual system damage in NMOSD and MOG-EM.

A multimodal magnetic resonance imaging study did not show a reduction of either grey or white matter, nor cortical thicknesses in NMOSD patients in comparison to healthy controls. However, diffusion tensor imaging and optical coherence tomography showed that fractional anisotropy of the optic radiation was reduced in NMOSD patients, which was associated with thinner retinal nerve fiber layer (RNFL). Even AQP4-IgG positive NMOSD patients without previous ON showed this reduction, and, additionally, reduced

foveal thickness. Furthermore, the functional connectivity of the visual networks was shown to be increased in AQP4-IgG positive NMOSD patients, indicating a maladaptive process.

We showed that blood vessels of the inner retina influenced OCT-derived thickness measurements in NMOSD, with a more pronounced effect in thinner RNFL.

A multicenter study showed similar retinal neuro-axonal damage in MOG-EM and AQP4-IgG positive NMOSD patients with comparable disease duration. However, MOG-EM patients had a significantly higher ON relapse rate than NMOSD patients. We concluded that accumulative damage in MOG-EM patients, despite better remission in a single attack, leads to similar damage as in AQP4-IgG positive NMOSD patients with less, but more severe attacks.

Applying a questionnaire (NEI-VFQ), we investigated vision-related quality of life in patients with NMOSD. We found that NMOSD patients have a significantly reduced vision-related quality of life compared to patients with multiple sclerosis (MS). This could be explained by more severe ON episodes in NMOSD, that present more frequently in both eyes, compared to MS.

To conclude, besides retinal neuro-axonal damage, ON in NMOSD leads to anterograde neurodegeneration of the optic radiation, potentially maladaptive reorganization of the visual networks and impairment in vision-related quality of life. To what extent an attack-independent and direct, potentially AQP4-targeted CNS damage occurs in these patients has to be further investigated.

# Einführung

## Neuromyelitis optica-Spektrum-Erkrankungen

Der Begriff Neuromyelitis optica (NMO) wurde erstmals 1849 durch den Franzosen Eugène Devic geprägt, um die simultane oder zeitlich kurz aufeinanderfolgende Symptomatik von Sehnerventzündungen (Optikusneuritis, ON) und Rückenmarksentzündungen (Myelitis) zu beschreiben [1]. Lange Zeit wurde die Erkrankung unter dem Namen Devic-Syndrom als eine Variante der Multiplen Sklerose (MS) betrachtet oder als MS fehlklassifiziert. Erst im Jahr 2004 führte eine Entdeckung von Lennon et al. dazu, dass NMO als eine eigenständige Erkrankung unabhängig von MS betrachtet wurde: Ein Serum-Antikörper konnte in ca. 60 – 70% von Patienten mit NMO, aber nicht in MS-Patienten nachgewiesen werden [2]. Wenig später gelang der gleichen Gruppe der Nachweis, dass die entdeckten Antikörper an Aquaporin-4 (AQP4), einen astrozytischen Wasserkanal, binden [3]. Seitdem gilt die NMO als eigenständige Erkrankung, die als Astrozytopathie klar von MS zu unterscheiden ist [4]. Ein internationales Gremium hat 2006 Diagnosekriterien für NMO veröffentlicht, in denen neben einem klinischem Bild bestehend aus typischen Schüben wie ON und Myelitis und seltener auch Schüben im Hirnstamm oder auch zerebral, erstmalig auch die Detektion von AQP4-IgG als wichtigstes Kriterium berücksichtigt wurde [5]. Für Patienten mit NMO-typischen Symptomen, die nicht die Diagnosekriterien erfüllten, wurde ursprünglich der Begriff Neuomyelitis optica-Spektrum-Erkrankungen (*Neuromyelitis optica spectrum disorders,* NMOSD) geprägt [6]. 2015 wurden diese Kriterien nochmals aktualisiert: Der Begriff NMO wurde zunehmend ersetzt durch NMOSD, was nun übergreifend für alle Patienten aus dem NMO-Spektrum verwendet wird. Unterschieden wird lediglich nach NMOSD mit positivem oder negativem bzw. unbekanntem AQP4-IgG-Status. In letzterem Fall gelten strengere Regeln an das klinische Krankheitsbild und die paraklinischen Befunde, insbesondere aus der Magnetresonanztomographie (MRT), um die Diagnose einer NMOSD stellen zu können [7].

## MOG-Antikörper in neuroinflammatorischen Erkrankungen

Antikörper gegen Myelin-Oligodendrozyten-Glykoprotein (MOG-IgG) waren vor allem bei Kindern und seltener in Erwachsenen mit akuter disseminierter Enzephalomyelitis bekannt [8]. MOG-IgG wurden aber auch in einem Teil von AQP4-IgG negativen

Patienten nachgewiesen, sowie in Patienten mit isolierter, häufig rezidivierender ON oder Myelitis, welche nicht die NMOSD-Diagnosekriterien erfüllen [9,10]. Jedoch sind die Angriffsziele der beiden Antikörper verschiedene: AQP4-IgG greifen AQP4-Wasserkanäle auf Astrozyten-Endfüßen an, MOG-IgG dagegen Oligodendrozyten, die Myelin exprimieren. Vor dieser Arbeit veröffentlichte Studien deuten darauf hin, dass MOG-IgG-positive NMOSD-Patienten tendenziell einen milderen Krankheitsverlauf haben als Patienten mit AQP4-IgG [11,12].

Es ist momentan noch nicht abschließend geklärt, ob es sich bei MOG-IgG um pathogene Antikörper handelt, die die Definition einer eigenen Krankheitsentität rechtfertigen, oder ob es sich um überlappende Krankheitssyndrome handelt, die bei noch unklarer Primärerkrankung auch bei MS [13] und NMOSD vereinzelt auftreten können. So erfüllen vielen Patienten mit MOG-IgG die offiziellen Diagnosekriterien der NMOSD [7], bei anderen mit isolierten, oft rekurrierende Myelitiden oder ON ist dies jedoch nicht der Fall [14]. Wegen der unterschiedlichen Immunpathogenese wird daher von vielen Seiten dafür plädiert, die sogenannte MOG-IgG assoziierte Enzephalomyelitis (MOG-EM) als eigenständige Erkrankung unabhängig von der „klassischen" AQP4-IgG-assoziierten NMOSD zu bewerten [15]. Dieser Einordnung bin ich auch im Rahmen dieses Promotionsvorhaben gefolgt.

## Sehnerventzündung und retinaler neuro-axonaler Schaden

Im Rahmen von MS, NMOSD und MOG-EM kommt es häufig zu einer Entzündung des Nervus Opticus (ON). Diese geht meist mit Verschwommensehen, Rotentsättigung und teilweise Augenbewegungsschmerzen einher [16]. Meist remittieren die Symptome spontan, bei NMOSD sind ON-Episoden allerdings häufig therapierefraktär [1]. Bei ON kommt es durch retrograde Neurodegeneration zu Atrophie der retinalen Nervenfaserschicht (*retinal nerve fiber layer*, RNFL) und Ganglienzellschicht [17].

## Fragestellungen

Ziel der Arbeit ist eine bessere Charakterisierung der Schädigung im visuellen System bei Patienten mit NMOSD und MOG-EM-assoziierter ON.

Zwar ist bekannt, dass ON in NMOSD zu schwerer wiegenden Veränderungen führt als beispielsweise in MS [18]. Dies macht sich durch stärkere Verdünnung der RNFL und Ganglienzellschicht sowie durch häufigeres Vorkommen makulärer Mikrozysten in der inneren Körnerschicht (*inner nuclear layer,* INL) bemerkbar [19]. Noch weitgehend

ungeklärt oder widersprüchlich sind jedoch die Unterschiede zwischen Patienten mit AQP4-IgG und MOG-IgG. Zudem ist wenig über die konkreten Auswirkungen auf die visuelle Lebensqualität der Patienten bekannt.

Die vorliegende Arbeit untersuchte sechs zusammenhängende Fragestellungen, die jeweils einzeln publiziert wurden und wie folgt zusammengefasst werden können:

1. Gibt es Schädigung der grauen und weißen Substanz im Gehirn von NMOSD Patienten, und wie ist gegebenenfalls der Zusammenhang mit einer vorangegangenen ON [20]?

2. Welche mikrostrukturellen Veränderungen gibt es im afferenten visuellen System bei Patienten mit NMOSD [21]?

3. Welche Veränderungen der funktionellen Konnektivität im visuellen Kortex sind mit einer Schädigung nach ON bei Patienten mit NMOSD assoziiert [22]?

4. Wie wirken sich die Blutgefäße der inneren Retina auf RNFL-Messungen bei ON-Patienten mit massiver RNFL Verdünnung aus [23]?

5. Was zeichnet die Patienten mit MOG-EM in Bezug auf Krankheitsverlauf und vor allem ON-assoziierte strukturelle und funktionelle Veränderungen aus [24–27]?

6. Wie wirken sich neuro-axonale Schädigung der Retina in Patienten mit NMOSD und MOG-EM sowie deren Einschränkungen der visuellen Funktion auf die visuelle Lebensqualität aus [28]?

# Methodik

## Patienten

Alle Fragestellungen wurden im Rahmen der longitudinalen Beobachtungsstudie „NMO" untersucht, die seit 2013 in der AG Klinische Neuroimmunologie am NeuroCure Clinical Research Center, Charité - Universitätsmedizin Berlin durchgeführt wird. Einschlusskriterien beinhalten ein Alter von mindestens 18 Jahren, Einwilligungsfähigkeit und schriftliche Einwilligung, Vorliegen einer NMOSD oder verwandten Erkrankung, insbesondere rezidivierende ON und longitudinale extensive transverse Myelitis (LETM). Ausschlusskriterien sind diagnostizierte MS nach revidierten McDonald-Kriterien 2010, übliche Kontraindikationen für eine MRT-Untersuchung sowie relevante Augenerkrankungen mit Einschränkung für die Auswertung der OCT-Untersuchung (insbesondere Glaukom, diabetische Retinopathie).

Das Studienprotokoll sieht jährliche Visiten vor, im Rahmen derer neben einer ausführlichen neurologischen Untersuchung u.a. jeweils im folgenden beschriebenen bildgebenden und funktionellen Untersuchungen durchgeführt werden.

Die Studienprotokolle wurden von den lokalen Ethikkommissionen der Charité und ggf. der weiteren teilnehmenden Kliniken genehmigt und in Einklang mit der Deklaration von Helsinki (1964), den Richtlinien zur guten klinischen Praxis (ICH-GCP) und in Deutschland geltendem Recht durchgeführt. Alle Studienteilnehmer gaben ihr schriftliches Einverständnis zur Teilnahme.

## Untersuchungsmethoden

*Optische Kohärenztomographie (OCT)*

OCT Aufnahmen wurden mit einem Spectralis SD-OCT von Heidelberg Engineering (Heidelberg, Deutschland) erhoben. Von jedem Patienten wurde ein peripapillärer Ringscan und ein Volumenscan der Makula zur Analyse herangezogen. Beim peripapillären Ringscan handelt es sich um ein Standard-Protokoll des Geräteherstellers (Scanwinkel 12°, 768 oder 1536 A-scans, 16-100 Mittelungen) und dient der Erhebung der Dicke der peripapillären Nervenfaserschichtdicke (pRNFL). Der makuläre Volumenscan (25°x30°, 61 vertikale oder horizontale B-scans, 768 A-scans pro B-scan, 12 – 15 Mittelungen) wurde zur Analyse weiterer intraretinaler Schichten herangezogen. Diese wurden jeweils innerhalb eines 6mm-Durchmesser umfassenden Kreises um die Fovea centralis als Volumen extrahiert. Neben der kombinierten Ganglienzell- und inneren plexiformen Schicht (GCIP) und der INL wurden die äußeren Makulaschichten, zusammengefasst ab der äußeren plexiformen Schicht bis zur Bruch'schen Membran, bestimmt. Die Foveadicke wurde als durchschnittliche Dicke aller Schichten (Innere Grenzmembran bis Bruch'sche Membran) in einem 1mm-Durchmesser umfassenden Kreis um die Fovea centralis gemessen. Die Position der retinalen Blutgefäße wurde mit Hilfe des Programms OCTSEG automatisch bestimmt und, wenn notwendig, manuell korrigiert [29].

*Visuelle Funktion*

Die visuelle Funktion wurden unter standardisierten Bedingungen bezüglich Beleuchtung und Abstand mit dem Functional Vision Analyzer Optec 6500 P (Stereo Optical Co., Chicago, Illinois) erhoben. Hochkontrastvisusmessungen erfolgten mit ETDRS-Tafeln. Zur besseren Vergleichbarkeit wurden alle Werte in logMAR-Einheiten (*Logarithm of the*

*Minimum Angle of Resolution*) umgerechnet. Die Kontrastsensitivität wurde mit dem *Functional Acuity Contrast Test* (FACT) erhoben und als *Area Under the Log Contrast Sensitivity Function* (AULCSF) in einem Wert zusammengefasst [30].

*Magnetresonanztomographie*

Alle MRT-Untersuchungen wurden am *Berlin Center for Advanced Neuroimaging* (BCAN) im selben 3-Tesla Scanner durchgeführt (MAGNETOM Trio, Siemens, Erlangen). Lediglich die beiden für diese Arbeit meistrelevanten MRT-Methoden werden hier kurz detaillierter beschrieben.

Die Diffusionsbildgebung, genauer das Diffusion Tensor Imaging (DTI), ist eine sehr sensitive MRT-basierte Methode zur Erfassung mikrostruktureller Schäden von Fasertrakten, die auf Messung der Brown'schen Molekularbewegung von Wassermolekülen beruht [31]. Aus einem ermittelten Tensorprofil können verschiedene Parameter abgeleitet werden, welche Rückschlüsse über die Schädigung des Trakts erlauben. Die Fraktionelle Anisotropie (FA), das Längenverhältnis der Tensorhauptachsen, gilt als Marker für neurodegenerative Schädigung [32].

Funktionelle MRT (fMRT) des Gehirns im Ruhezustand (*resting state*) beruht auf der Annahme, dass es auch bei ruhendem Gehirn eine Grundaktivität gibt, die mit der lokalen neuronalen Aktivität assoziiert ist. Diese wird aus dem *blood oxygen level dependen*t (BOLD)-Signal, also durch Messung des Sauerstoffanteils im Blut, bestimmt.

Im Gegensatz zu langwierigen aufgaben-basierten fMRT Untersuchungen dauert die resting state fMRT, bei der der Patient ruhig im MRT liegt, lediglich 5–10 min, und ist damit potentiell auch für den Einsatz in der klinischen Routine geeignet. Aus spontanen Fluktuationen des BOLD-Signals können Rückschlüsse auf die funktionelle Konnektivität einzelner Systeme gezogen werden [33,34].

*NEI-VFQ*

Der *National Eye Institute Visual Function Questionnaire* (NEI-VFQ) ist ein Fragebogen zur Erhebung der sehbezogenen Lebensqualität. In dieser Arbeit wurde die deutsche Version der 39-Item Auflage herangezogen [35]. Aus den 39 Items werden 12 Skalen berechnet: Allgemeiner Gesundheitszustand, allgemeine Sehkraft, Augenschmerzen, Nahsicht, Fernsicht, soziale Funktionsfähigkeit, psychisches Befinden, Ausübung sozialer Rollen, Abhängigkeit von anderen, Probleme mit dem Autofahren, Farbensehen und peripheres Sehen. Frühere Studien haben gezeigt, dass die sehbezogene

Lebensqualität von MS-Patienten mit dem Ausmaß des neuro-axonalen Schaden der Retina zusammenhängt [36]. Der NEI-VFQ-Summenscore berechnet sich als Durchschnitt aller Subskalen, ausgenommen „Allgemeiner Gesundheitszustand". Der Fragebogen wurde von den meisten Studienteilnehmern selbst ausgefüllt, bei einigen erfolgte er aufgrund starker Sehstörungen in Interviewform.

## Statistik

Messwerte wurden entweder als Mittelwert und Standardabweichung oder Median und Spannweite angegeben. Die Statistik und Abbildungen wurden mit Hilfe von des Programms R (Versionen 3.1.0, 3.1.2, 3.2.2, 3.3.0) mit u.a. den Paketen „psych", „MASS", „ggplot2", und „geepack" durchgeführt [37]. Für alle Tests wurde das Signifikanzlevel bei $p<0,05$ festgelegt. Zum Vergleich von Häufigkeiten wurde der Chi-Quadrat-Test oder Fisher's Exact Test, für den Vergleich von Messwerten der Mann-Whitney-U-Test verwendet. Bei Gruppenvergleichen und Korrelationen von augenbezogenen Parametern, wie OCT-Parameter und Sehschärfe, wurden verallgemeinerte Schätzgleichungen (*Generalized estimating equations,* GEEs) angewandt. GEE-Modelle können Abhängigkeiten beider Augen eines Probanden berücksichtigen und eignen sich daher besonders gut für augenbezogene Parameter. Weitere verwendete statistische Verfahren werden im entsprechenden Ergebnisteil kurz beschrieben.

# Ergebnisse

## Schädigung grauer und weißer Substanz

*MRT-OCT I* [20]

Es handelt sich hier um eine multimodale MRT-Querschnittsuntersuchung zur Untersuchung der Schädigung grauer und weißer Substanz in verschiedenen Hirnregionen bei NMOSD. Die demographischen Daten der 21 Patienten und 21 alters- und geschlechts-gematchten Kontrollen sind in Tabelle 1 in der Publikation [20] zusammengefasst. MRT-Analysen beinhalteten manuelle computergestützte Läsionsquantifizierung, Voxel-basierte Morphometrie (VBM), Bestimmung kortikaler Dicken und subkortikaler Volumen (Freesurfer) und DTI mit *Tract based spatial statistics* (TBSS).

NMOSD-Patienten hatten ein größeres Läsionsvolumen der weißen Substanz und im Trend auch eine höhere Anzahl von Läsionen im Vergleich zu gesunden Kontrollen.

Eine explorative VBM Analyse zeigte verringertes Volumen in mehreren Regionen der grauen Substanz bei NMOSD-Patienten im Vergleich zu gesunden Kontrollen. Nach Korrektur für multiple Vergleiche wurden keine Unterschiede zwischen Patienten und Kontrollgruppe mehr beobachtet. Auch die Freesurfer-Analyse kortikaler Dicken und subkortikaler Volumen zeigte keine signifikanten Veränderungen in NMOSD-Patienten.

Bei einem Grenzwert von p<0,05 zeigte die regionale Analyse der DTI Sequenzen eine Minderung der FA in der Sehstrahlung, auch nach Korrektur für multiple Vergleiche. Die TBSS-Analyse zeigte reduzierte FA in mehreren Teilbereichen des Okzipitallappens. Die pRNFL war signifikant dünner in NMOSD-Patienten im Vergleich zu gesunden Kontrollen und korrelierte positiv mit der FA der Sehstrahlung.

## Mikrostrukturelle Veränderungen im afferenten visuellen System

*MRT-OCT II* [21]

Hierfür wurde eine kombinierte OCT-MRT Studie mit Schwerpunkt auf Traktographie der Sehstrahlung und detaillierter Netzhautanalyse durchgeführt. NMOSD-Patienten (n=25) wurden in zwei Gruppen aufgeteilt: Sechs AQP4-IgG-seropositive NMOSD-Patienten mit LETM ohne ON (NMOSD-LETM) und 19 AQP4-IgG-seropositive NMOSD-Patienten mit mindestens einer ON-Episode (NMOSD-ON).

Die demographischen Daten der Patienten und 26 gesunden Kontrollen sind in Tabelle 1 in der Publikation [21] zusammengefasst. Bei allen Teilnehmern erfolgte eine OCT-Untersuchung mit Bestimmung von pRNFL, makulärer GCIP und Foveadicke sowie eine MRT-Untersuchung mit probabilistischer Traktographie auf Basis von DTI.

Im Vergleich zu gesunden Kontrollen war die Foveadicke in der Gruppe der NMOSD-LETM-Patienten in ähnlichem Ausmaß reduziert wie in der NMOSD-ON-Gruppe. Gleichzeitig waren pRNFL und GCIP als Marker von axonalem und neuronalem Verlust in der NMOSD-ON Gruppe im Vergleich zu gesunden Kontrollen reduziert, jedoch nicht in der NMOSD-LETM Gruppe. Diese Ergebnisse konnten in einer unabhängigen Kohorte bestätigt werden. Die probabilistische Traktographie zeigte eine Reduktion der FA in der Sehstrahlung in der NMOSD-ON Gruppe und, zu einem etwas geringeren Ausmaß, in der NMOSD-LETM Gruppe. Die FA der Sehstrahlung in NMOSD-LETM zeigte keine Korrelation zu fovealer Dicke, pRNFL oder GCIP. Bei NMOSD-ON-Patienten gab es eine positive Korrelation zwischen FA der Sehstrahlung und GCIP. Die Sehschärfe der

Patienten in der NMOSD-LETM-Gruppe war nicht reduziert. In keiner der beiden Patientengruppen gab es eine Korrelation zwischen Sehschärfe und FA der Sehstrahlung oder fovealer Dicke.

## Funktionelle Konnektivität im visuellen Netzwerk

*Resting State & OCT* [22]

Für diese Untersuchung wurden 31 AQP4-IgG-positive NMOSD-Patienten und 31 gematchte gesunde Kontrollen mit Resting State fMRT untersucht. Dabei wurde die funktionelle Konnektivität großer Netzwerke bestimmt. Die demographischen Daten sind in Tabelle 1 in der Publikation [22] zusammengefasst. Die funktionelle Konnektivität des primären und sekundären visuellen Netzwerks wurde auf Assoziationen zu retinalem neuro-axonalen Schaden (OCT) und visueller Funktion (Visus und Kontrastempfindlichkeit) untersucht.

NMOSD-Patienten zeigten im Vergleich zu gesunden Kontrollen eine deutlich höhere funktionelle Konnektivität auf beiden Seiten des primären visuellen Netzwerks. Zu einem geringeren Ausmaß war das auch im sekundären visuellen Netzwerk, genauer gesagt im Okzipitalpol, beidseits der Fall. Die Veränderungen waren in Patienten mit vorangegangener ON stärker als in Patienten ohne ON. Erhöhte funktionelle Konnektivität des primären visuellen Netzwerks korrelierte mit reduziertem Visus und Kontrastempfindlichkeit sowie mit dünnerer GCIP, allerdings nicht mit pRNFL. Für das sekundäre visuelle Netzwerk konnte keine Korrelation zu Visus, Kontrastempfindlichkeit, GCIP oder pRNFL festgestellt werden. In keinem der anderen Netzwerke konnten Veränderungen der funktionellen Konnektivität in der NMOSD Gruppe festgestellt werden. Weder für das primäre noch für das sekundäre visuelle Netzwerk hatte die Zeit seit der letzten ON-Episode einen Einfluss auf die funktionelle Konnektivität.

## Einfluss retinaler Blutgefäße auf die Nervenfaserschichtdicke

*Blutgefäß-Analyse* [23]

Hierfür wurde in pRNFL-Messungen von 40 Patienten (Alter 44,7 ± 15,4 Jahre, 39 weiblich) mit NMOSD oder MOG-EM die Blutgefäße segmentiert und die Messungen sowie deren Korrelation zur visuellen Funktion mit und ohne Blutgefäßbereiche untersucht.

Messungen der pRNFL exklusive der Blutgefäßbereiche waren dünner als inklusive der Blutgefäßbereiche. Der relative Einfluss der Blutgefäße auf die pRNFL Dicke nahm mit

dünnerer pRNFL zu. In Augen mit einer pRNFL <60µm war der relative Beitrag der Blutgefäße signifikant größer als in Augen mit einer pRNFL Dicke >60µm.

# Schädigung im afferenten visuellen System bei Patienten mit MOG-EM

*MOG-EM I - IV* [24–27]

Diese vierteilige Artikel-Serie über MOG-IgG in NMOSD und verwandten Erkrankungen ist aus einer retrospektiven multizentrischen Studie hervorgegangen. Bei der Studie handelt es sich um ein internationales Kooperationsprojekt. Hierfür wurden 614 Serumproben von 522 Teilnehmern gesammelt und zentral in Heidelberg auf MOG-IgG untersucht. So konnten 50 Patienten mit MOG-IgG aus elf universitären Zentren identifiziert werden [24]. Lediglich auf Teil 4 der Serie *(MOG-EM IV)* soll in dieser Arbeit im Detail eingegangen werden. Dieser beschreibt detailliert Untersuchungsergebnisse aus OCT-Untersuchungen von MOG-IgG-positiven Patienten nach ON und vergleicht diese zu Daten von AQP4-IgG-positiven NMOSD-Patienten nach ON bei ähnlicher Erkrankungsdauer sowie zu gesunden Kontrollen [27].

Für diese Veröffentlichung wurden neben Patienten mit MOG-EM aus der Kohortenstudie am NCRC noch Patienten aus den anderen Zentren eingeschlossen, für die retrospektiv OCT-Aufnahmen und weitere visuelle Untersuchungsergebnisse verfügbar waren [27]. Zusätzlich zu den 16 Patienten mit MOG-EM wurde eine Kontrollkohorte von 16 altersgematchten AQP4-IgG-positiven NMOSD-Patienten mit ON sowie eine gesunde Kontrollkohorte analysiert. Die demographischen Daten sind in Tabelle 1 in der Publikation [27] zusammengefasst.

Alle 16 MOG-EM-Patienten hatten mindestens eine Episode von ON (Median 4,5 Episoden, Spannweite 1 – 13). Weder das Alter bei Erkrankungsbeginn noch die Krankheitsdauer zum Untersuchungszeitpunkt unterschied sich zwischen der MOG-EM- und der AQP4-IgG-positiven NMOSD-Gruppe. Dagegen hatten MOG-EM-Patienten eine signifikant höhere Schubrate, besonders wenn nur die ON Schübe verglichen wurden. OCT-Aufnahmen und funktionelle Untersuchungen von zwei MOG-EM-Patientinnen (beide mit beidseitiger ON) mussten wegen Augenerkrankungen ausgeschlossen werden. Zwei Augen von zwei weiteren MOG-EM Patientinnen wurden aufgrund akuter ON zum Untersuchungszeitpunkt von der Analyse ausgeschlossen. Im Vergleich zu gesunden Kontrollen waren pRNFL und GCIP in den ON Augen der MOG-EM-Patienten ähnlich verdünnt wie die ON-Augen der AQP4-IgG-positiven NMOSD-Patienten.

Dagegen war die INL-Dicke der ON-Augen in der MOG-EM-Gruppe im Vergleich zu gesunden Kontrollen signifikant erhöht, nicht aber bei der AQP4-IgG-positiven NMOSD-Gruppe. Ein direkter Vergleich von ON-Augen der MOG-EM-Patienten mit der AQP4-IgG-positiven NMOSD-Patienten zeigte keine signifikanten Unterschiede in den retinalen Schichtdicken. Makuläre Mikrozysten traten in beiden Gruppen in vergleichbarer Häufigkeit auf. Der Visus war zwar bei den ON Augen der AQP4-IgG-positiven Gruppe stärker betroffen als in der MOG-EM-Gruppe, dies war statistisch aber nicht signifikant. In MOG-EM-Patienten korrelierte die pRNFL- und GCIP-Verdünnung mit einer höheren Anzahl an vorangegangenen ON-Episoden, was in AQP4-IgG-positiven Patienten nicht der Fall war. pRNFL und GCIP in Augen mit nur einer ON-Episode waren im Vergleich zu gesunden Kontrollen bei MOG-EM deutlich weniger stark verdünnt als bei AQP4-IgG-positiver NMOSD. Der weitere Schaden durch eine zweite ON-Episode war dagegen bei MOG-EM stärker als bei AQP4-IgG-positiver NMOSD.

## Sehbezogene Lebensqualität

*NEI-VFQ & OCT* [28]

In diese Querschnittsstudie wurden 31 Patienten mit NMOSD oder MOG-EM eingeschlossen [28]. Da es keine signifikanten Unterschiede bei NEI-VFQ, OCT und Sehfunktion zwischen diesen Patientenkollektiven gab, wurden der Einfachheit halber alle diese Patienten als „NMOSD-Kohorte" zusammengefasst. Weiterhin wurden 31 in Alter, Geschlecht und ON Anamnese ähnliche MS-Patienten eingeschlossen. Die demographischen Daten sind in Tabelle 1 im Artikel [28] zusammengefasst. Alle Patienten füllten den NEI-VFQ-Fragebogen aus und es erfolgte eine neurovisuelle Untersuchung mit Hochkontrastvisus, Kontrastsensitivität und OCT-Aufnahmen mit Analyse der pRNFL. Bei der NMOSD-Kohorte wurden auch die Schichtdicken der GCIP und der INL analysiert.

NMOSD-Patienten schnitten signifikant schlechter im NEI-VFQ-Summenscore ab und zeigten damit eine deutlich schlechtere sehbezogene Lebensqualität als MS-Patienten. Alle Sub-Skalen waren in der NMOSD-Kohorte ebenfalls niedriger als in MS. Eine multivariate lineare Regressionsanalyse zeigte, dass dies durch die Wechselwirkung schwererer und zugleich häufiger beide Augen betreffende ON-Episoden bei NMOSD-Patienten begründet ist. ON in einem Auge bei NMOSD, ON in einem oder beiden Auge unabhängig von der Diagnose, oder Diagnose NMOSD alleine zeigten jeweils keinen signifikanten Einfluss auf den NEI-VFQ-Summenscore. Dies wurde ähnlich in allen

Subskalen beobachtet. NEI-VFQ-Ergebnisse der NMOSD-Kohorte wurden auch mit den Werten einer publizierten Kontrollkohorte verglichen [35]. Der Summenscore und die meisten Subskalen (außer „Abhängigkeit von Anderen" und „Farbensehen") waren signifikant niedriger in NMOSD. Fünfzehn der NMOSD-Patienten (48,3%) wiesen reduzierten NEI-VFQ Werte außerhalb der Standardabweichung der Referenzkohorte auf. Bei den 16 Patienten, deren Werte innerhalb der Standardabweichung der Referenzkohorte lagen, handelte es sich um sieben NMOSD-LETM Patienten ohne ON und acht Patienten mit nur einem von ON betroffenen Auge (n=8), und somit um nur einen Patienten mit ON in beiden Augen. In der MS Kohorte lagen Ergebnisse von nur drei Patienten (9,7%) außerhalb normaler Grenzen.

In Übereinstimmung mit der Literatur war die NMOSD-Kohorte stärker von pRNFL-Verdünnung und Sehbeeinträchtigung (Visus und Kontrastsensibilität) betroffen als die MS Kohorte. In der NMOSD Kohorte korrelierten schlechtere NEI-VFQ-Summenscores mit dünnerer pRNFL und GCIP sowie mit dickerer INL.


# Diskussion

In dieser Arbeit wurden Veränderungen im visuellen System aufgrund von NMOSD oder MOG-EM besser charakterisiert. Die Arbeit zeigt, dass a) bei NMOSD-Patienten zerebrale Veränderungen vor allem im visuellen System zu finden sind und mit dem retinalen axonalen Schaden assoziiert sind [20], b) mikrostrukturelle Veränderungen in Retina und Sehstrahlung bei AQP4-IgG-positiven NMOSD-Patienten auch ohne vorherige ON auftreten [21], c) es bei NMOSD zu erhöhter funktioneller Konnektivität der visuellen Netzwerke kommt [22], d) Blutgefäße der inneren Retina bei NMOSD-Patienten mit sehr dünner RNFL einen größeren relativen Fehler bei pRNFL Messungen verursachen [23], e) MOG-EM-assoziierte ON aufgrund der hohen Schubfrequenz dieser Patienten mittel- bis langfristig zu ähnlichem retinalen neuro-axonalen Schaden führt wie bei AQP4-IgG-positiven NMOSD-Patienten [27] und f) NMOSD und MOG-EM zu stärkerem und häufiger beidseitigem neuro-axonalen Schaden der Netzhaut führen als MS, was die visuelle Funktion und sehbezogene Lebensqualität massiv stärker beeinträchtigt [28].

Vorkommen und Pathogenese zerebraler Schädigung bei NMOSD sind umstritten. In den NMOSD Kohorten der beiden hier vorgestellten MRT-Studien hatten die meisten Patienten, in Übereinstimmung mit existierenden Studien, kleinere unspezifische

Läsionen in der weißen Substanz [38]. Diese waren zwar in unserer gesunden Kontrollkohorte fast ebenso prävalent wie bei NMOSD-Patienten, das T2-Läsionsvolumen war aber bei NMOSD-Patienten deutlich erhöht. In unserer multimodalen MRT Untersuchung konnten wir zeigen, dass lediglich die DTI mikrostrukturelle Veränderungen bei NMOSD zeigt. Alle betroffenen Regionen waren im oder in direkter Nachbarschaft des visuellen Systems oder direkt funktional mit diesem verbunden. Zudem korrelierte die FA der Sehstrahlung mit der RNFL, was auf anterograde Degeneration aufgrund vorangegangener ON hindeutet. Aufgrund dessen haben wir diese Zusammenhänge in einer NMOSD Kohorte mit ausschließlich AQP4-IgG-positiven Patienten noch näher untersucht. Dabei wurde eine noch aussagekräftigere DTI-Analyse gewählt, die probabilistische Traktographie, und mit GCIP und Foveadicke zusätzliche retinale OCT-Parameter herangezogen. Zum einen konnte hier ein Zusammenhang von neuronalem retinalen Schaden (Verdünnung der GCIP) und Neurodegeneration im posterioren visuellen System (FA der Sehstrahlung) in NMOSD-ON-Patienten bestätigt werden. Darüber hinaus zeigten sich aber auch mikrostrukturelle Veränderungen in Form von dünnerer Fovea und reduzierter FA auch in NMOSD-LETM-Patienten ohne ON. Diese Befunde weisen auf eine schubunabhängige, direkt gegen AQP4 gerichtete Schädigung hin. Diese wirkt sich klinisch aber offenbar nicht aus, zumindest bei unserer Untersuchung mit sehr kleiner Fallzahl (NMOSD-LETM n=6) wurde kein Zusammenhang zu visueller Funktion festgestellt.

Eine Untersuchung von AQP4-IgG-positiven NMOSD-Patienten mit resting state fMRT zeigte eine Erhöhung der funktionellen Konnektivität im Vergleich zu gesunden Kontrollen im Sinne einer Reorganisation der visuellen Netzwerke. Zudem war die Erhöhung bei Patienten mit vorangegangener ON deutlich höher als bei Patienten ohne ON. Die funktionelle Konnektivität korrelierte mit der visuellen Funktion. Diese Ergebnisse legen nahe, dass es sich hier um einen maladaptiven plastischen Prozess handelt.

Für die Quantifizierung der RNFL mit OCT wird üblicherweise ein peripapillärer Ringscan herangezogen. Dieser hat den Vorteil, dass so alle Nervenbündel aus dem gesamten Gesichtsfeld miteinbezogen werden; ein Nachteil ist jedoch, dass in diesem Bereich auch die Hauptzweige der Blutgefäße der inneren Retina verlaufen und mit in die Dickenmessungen der pRNFL eingehen. Bei einigen NMOSD- oder MOG-EM-Patienten kommt es im Verlauf der Erkrankung bzw. nach sehr starken ON-Episoden zu einer fast vollständigen Atrophie der RNFL. Aufgrund der Blutgefäße bleibt jedoch stets eine gewisse Grunddicke als Messfehler erhalten. Unsere Studie hat gezeigt, dass bei pRNFL

Dicke unter 60 μm, was bei NMOSD assoziierter ON keineswegs eine Seltenheit ist, der relative Fehler durch die Blutgefäße signifikant höher ist als bei Patienten mit pRNFL über 60 μm. Dies sollte bei Verlaufsstudien berücksichtigt werden.

Patienten mit NMOSD-typischen Symptomen und MOG-IgG waren zuvor als tendenziell milderer Phänotyp mit schwächeren Schüben und besserer Remission beschrieben worden [11,12]. Die einzige vorherige OCT-Studie vergleicht Patienten mit MOG-IgG und AQP4-IgG nach initialer ON [39]. Hier sind AQP4-IgG positive Patienten deutlich stärker betroffen. Da aber keine Messungen im Verlauf der Erkrankung gezeigt werden, bleibt unklar, wie es um den Langzeitverlauf der Patienten steht [39]. Damit stehen diese Daten nicht in Widerspruch zu unseren Ergebnissen.

Es wurde vielfach gezeigt, dass ON in NMOSD zu stärkerer struktureller und funktioneller visueller Beeinträchtigung kommt und häufiger beide Augen betroffen sind als in MS [19]. Allerdings ist unsere Studie die erste, die zeigt, wie viel stärker dadurch NMOSD-Patienten im Alltag eingeschränkt sind. Eine multivariate lineare Regressionsanalyse zeigte, dass die Wechselwirkung von besonders starker Schädigung und beidseitiger Beeinträchtigung hauptsächlich für den Verlust sehbezogener Lebensqualität in NMOSD-Patienten verantwortlich ist. Solange nur ein Auge betroffen ist, bleibt die sehbezogene Lebensqualität also weitgehend erhalten. Dies betont die Notwendigkeit frühzeitiger effektiver medikamentöser Therapien für NMOSD Patienten, um einer ON-Episode auf dem noch nicht betroffenen Auge nach Möglichkeit vorzubeugen und so die sehbezogene Lebensqualität des Patienten zu erhalten.

Mit der vorliegenden Arbeit konnten die pathologischen Veränderungen des visuellen Systems bei NMOSD und MOG-EM-Patienten sowie deren Auswirkung auf die visuelle Funktion und sehbezogene Lebensqualität präziser charakterisiert werden. Um festzustellen, inwieweit es subklinische, möglicherweise durch AQP4-IgG-getriebene ZNS-Schädigung gibt, sind weitere Studien mit größerer Fallzahl notwendig. Für NMOSD sind dringend gezieltere Therapieansätze notwendig, um ON-Episoden vorzubeugen oder den neuro-axonalen Schaden zu begrenzen [40]. OCT sollte als Outcome-Parameter in klinischen Studien eingesetzt werden, um den neuroprotektiven Effekt von Medikamenten zu quantifizieren.

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# Eidesstattliche Versicherung

„Ich, Hanna Zimmermann, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Strukturelle und funktionelle Veränderungen des afferenten visuellen Systems bei Patienten mit Neuromyelitis optica-Spektrum-Erkrankungen" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst."

Datum                                        Unterschrift

# Anteilserklärung an erfolgten Publikationen

Hanna Gwendolyn Zimmermann hatte folgenden Anteil an den folgenden Publikationen:

*OCT-MRT I* [20]

Florence Pache, **Hanna Zimmermann**, Carsten Finke, Anna Lacheta; Sebastian Papazoglou, Jens Wuerfel, Bernd Hamm, Klemens Ruprecht; Friedemann Paul, Alexander U. Brandt, Michael Scheel **Brain Parenchymal Damage in Neuromyelitis Optica Spectrum Disorder - A Multimodal MRI Study** European Radiology, 2016

Beitrag im Einzelnen:

Erhebung und Zusammenstellung von OCT- und visuellen Daten, Qualitätskontrolle und Segmentierung der OCT-Daten, Teile der statistischen Analyse, Überarbeitung des Manuskripts.

*OCT-MRT II* [21]

Frederike C. Oertel, Joseph Kuchling, **Hanna Zimmermann**, Claudia Chien, Felix Schmidt, Benjamin Knier, Judith Bellmann-Strobl, Thomas Korn, Michael Scheel, Alexander Klistorner, Klemens Ruprecht, Friedemann Paul, Alexander U. Brandt **Microstructural visual system changes in AQP4-antibody seropositive NMOSD** Neurology: Neuroimmunology & Neuroinflammation, 2017

Beitrag im Einzelnen:

Erhebung von OCT- und visuellen Daten, Qualitätskontrolle der OCT Daten, Supervision der OCT Segmentierung, Überarbeitung des Manuskripts.

*Resting state & OCT* [22]

Carsten Finke, **Hanna Zimmermann**, Florence Pache, Frederike C. Oertel, Velina Sevdalinova Chavarro, Yelyzaveta Kramarenko, Judith Bellmann-Strobl, Klemens Ruprecht, Alexander U. Brandt, Friedemann Paul**; Association of Visual Impairment in Neuromyelitis Optica Spectrum Disorder With Visual Network Reorganization,** JAMA Neurology, 2017

Beitrag im Einzelnen:

Erhebung von OCT- und visuellen Daten, Qualitätskontrolle der OCT-Daten, Supervision der OCT-Segmentierung, Teile der statistische Analyse, Interpretation der Ergebnisse, Überarbeitung des Manuskripts.

*Blutgefäß-Analyse* [23]

Frederike Cosima Oertel*, **Hanna Zimmermann*,** Janine Mikolacjzak, Maria Weinhold, Ella Maria Kadas, Timm Oberwahrenbrock, Florence Pache, Friedemann Paul, Alexander U. Brandt; **Influence of blood vessels on peri-papillary retinal nerve fiber layer thickness measurements in patients with neuromyelitis optica spectrum disorders.** Neurology: Neuroimmunology & Neuroinflammation, 2016

Beitrag im Einzelnen:

Erhebung von OCT- und visuellen Daten, Qualitätskontrolle der OCT-Daten, Supervision der OCT-Segmentierung, Interpretation der Ergebnisse, Supervision der statistischen Analyse, Schreiben von *Introduction* und *Methods* des Manuskripts.


*MOG-EM I* [24]

Sven Jarius, Klemens Ruprecht, Ingo Kleiter, Nadja Borisow, Nasrin Asgari, Kalliopi Pitarokoili, Florence Pache, Oliver Stich, Lena-Alexandra Beume, Martin W. Hümmert, Corinna Trebst, Marius Ringelstein, Orhan Aktas, Alexander Winkelmann, Mathias Buttmann, Alexander Schwarz, **Hanna Zimmermann,** Alexander U. Brandt, Diego Franciotta, Marco Capobianco, Joseph Kuchling, Jürgen Haas, Mirjam Korporal-Kuhnke, Soeren Thue Lillevang, Kai Fechner, Kathrin Schanda, Friedemann Paul, Brigitte Wildemann, Markus Reindl; in cooperation with the Neuromyelitis Optica Study Group (NEMOS); **MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin** Journal of Neuroinflammation, 2016.

Beitrag im Einzelnen:

Erhebung und Zusammenstellung klinischer und paraklinischer Daten, Überarbeitung des Manuskripts.


MOG-EM II [25]

Sven Jarius, Klemens Ruprecht, Ingo Kleiter, Nadja Borisow, Nasrin Asgari, Kalliopi Pitarokoili, Florence Pache, Oliver Stich, Lena-Alexandra Beume, Martin W. Hümmert,

Marius Ringelstein, Corinna Trebst, Alexander Winkelmann, Alexander Schwarz, Mathias Buttmann, **Hanna Zimmermann,** Joseph Kuchling, Diego Franciotta, Marco Capobianco, Eberhard Siebert, Carsten Lukas, Mirjam Korporal-Kuhnke,

Jürgen Haas, Kai Fechner, Alexander U. Brandt, Kathrin Schanda, Orhan Aktas, Friedemann Paul, Markus Reindl, and Brigitte Wildemann; in cooperation with the Neuromyelitis Optica Study Group (NEMOS) **MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome.** Journal of Neuroinflammation, 2016.;

Beitrag im Einzelnen:

Erhebung und Zusammenstellung klinischer und paraklinischer Daten, Überarbeitung des Manuskripts.

MOG-EM III [26]

Sven Jarius, Ingo Kleiter, Klemens Ruprecht, Nasrin Asgari, Kalliopi Pitarokoili, Nadja Borisow, Martin W. Hümmert, Corinna Trebst, Florence Pache, Alexander Winkelmann, Lena-Alexandra Beume, Marius Ringelstein, Oliver Stich, Orhan Aktas, Mirjam Korporal-Kuhnke, Alexander Schwarz, Carsten Lukas, Jürgen Haas, Kai Fechner, Mathias Buttmann, Judith Bellmann-Strobl, **Hanna Zimmermann,** Alexander U. Brandt, Diego Franciotta, Kathrin Schanda, Friedemann Paul, Markus Reindl, and Brigitte Wildemann; in cooperation with the Neuromyelitis Optica Study Group (NEMOS), **MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 3: Brainstem involvement - frequency, presentation and outcome,** Journal of Neuroinflammation, 2016.

Beitrag im Einzelnen:

Erhebung und Zusammenstellung klinischer und paraklinischer Daten, Überarbeitung des Manuskripts.

MOG-EM IV [27]

Florence Pache*, **Hanna Zimmermann*,** Janine Mikolajczak, Sophie Schumacher, Anna Lacheta, Frederike C Oertel, Judith Bellmann-Strobl, Sven Jarius, Brigitte Wildemann, Markus Reindl, Amy Waldman, Kerstin Soelberg, Nasrin Asgari, Marius Ringelstein, Orhan Aktas, Nikolai Gross, Mathias Buttmann, Thomas Ach, Klemens

Ruprecht, Friedemann Paul, Alexander U Brandt, **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,** Journal of Neuroinflammation, 2016.

*) Gleichermaßen beitragende Erstautorinnen in alphabetischer Reihenfolge.

Beitrag im Einzelnen:

Erhebung von OCT- und visuellen Daten der Berliner Patienten, Zusammenstellung aller Daten, Qualitätskontrolle und Segmentierung aller OCT-Daten, statistische Analyse, Interpretation der Ergebnisse, Schreiben des Manuskripts

*NEI-VFQ & OCT* [28]

Felix Schmidt*, **Hanna Zimmermann*,** Janine Mikolajczak, Frederike C. Oertel, Florence Pache, Maria Weinhold, Johann Schinzel, Judith Bellmann-Strobl, Klemens Ruprecht, Friedemann Paul, Alexander U. Brandt, **Severe structural and functional visual system damage leads to profound loss of vision-related quality of life in patients with neuromyelitis optica spectrum disorders,** Multiple Sclerosis and Related Disorders, 2017

*) Gleichermaßen beitragende Erstautoren in alphabetischer Reihenfolge.

Beitrag im Einzelnen:

Erhebung und Zusammenstellung von OCT- und visuellen Daten, Qualitätskontrolle der OCT-Daten, Supervision der OCT-Segmentierung, Teile der statistischen Analyse, Schreiben von *Methods* und *Results* des Manuskripts.

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Unterschrift, Datum und Stempel des betreuenden Hochschullehrers

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Unterschrift der Doktorandin

# Druckexemplare der ausgewählten Publikationen

Pache F, Zimmermann H, Finke C, Lacheta, Papazoglou, Kuchling J, Wuerfel J, Hamm B, Ruprecht K, Paul F, Brandt AU, Scheel M; Brain parenchymal damage in neuromyelitis optica spectrum disorder - A multimodal MRI study. Eur Radiol. 2016
Journal Impact Factor: 3.967

Oertel FC, Kuchling J, Zimmermann H, Chien C, Schmidt F, Knier B, Bellmann-Strobl J, Korn T, Scheel M, Klistorner A, Ruprecht K, Paul F, Brandt AU. Microstructural visual system changes in AQP4-antibody-seropositive NMOSD. Neurol Neuroimmunol Neuroinflamm. 2017

Finke C, Zimmermann H, Pache F, Oertel FC, Chavarro VS, Kramarenko Y, Bellmann-Strobl J, Ruprecht K, Brandt AU, Paul F; Association of Visual Impairment in Neuromyelitis Optica Spectrum Disorder With Visual Network Reorganization, JAMA Neurol. 2018
Journal Impact Factor: 10.029

Oertel FC*, Zimmermann H*, Mikolajczak J, Weinhold M, Kadas EM, Oberwahrenbrock T, Pache F, Bellmann-Strobl J, Ruprecht K, Paul F, Brandt AU.
Contribution of blood vessels to retinal nerve fiber layer thickness in NMOSD. Neurol Neuroimmunol Neuroinflamm. 2017

Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, Pache F, Stich O, Beume LA, Hümmert MW, Trebst C, Ringelstein M, Aktas O, Winkelmann A, Buttmann M, Schwarz A, Zimmermann H, Brandt AU, Franciotta D, Capobianco M, Kuchling J, Haas J, Korporal-Kuhnke M, Lillevang ST, Fechner K, Schanda K, Paul F, Wildemann B, Reindl M; in cooperation with the Neuromyelitis Optica Study Group (NEMOS). MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin. J Neuroinflammation. 2016
Journal Impact Factor: 5.102

Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, Pache F, Stich O, Beume LA, Hümmert MW, Ringelstein M, Trebst C, Winkelmann A, Schwarz A, Buttmann M, Zimmermann H, Kuchling J, Franciotta D, Capobianco M, Siebert E, Lukas C, Korporal-Kuhnke M, Haas J, Fechner K, Brandt AU, Schanda K, Aktas O, Paul F, Reindl M, Wildemann B; in cooperation

with the Neuromyelitis Optica Study Group (NEMOS). MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. J Neuroinflammation. 2016
Journal Impact Factor: 5.102


Jarius S, Kleiter I, Ruprecht K, Asgari N, Pitarokoili K, Borisow N, Hümmert MW, Trebst C, Pache F, Winkelmann A, Beume LA, Ringelstein M, Stich O, Aktas O, Korporal-Kuhnke M, Schwarz A, Lukas C, Haas J, Fechner K, Buttmann M, Bellmann-Strobl J, Zimmermann H, Brandt AU, Franciotta D, Schanda K, Paul F, Reindl M, Wildemann B; in cooperation with the Neuromyelitis Optica Study Group (NEMOS). MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 3: Brainstem involvement - frequency, presentation and outcome. J Neuroinflammation. 2016
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Pache F*, Zimmermann H*, Mikolajczak J, Schumacher S, Lacheta A, Oertel FC, Bellmann-Strobl J, Jarius S, Wildemann B, Reindl M, Waldman A, Soelberg K, Asgari N, Ringelstein M, Aktas O, Gross N, Buttmann M, Ach T, Ruprecht K, Paul F, Brandt AU; in cooperation with the Neuromyelitis Optica Study Group (NEMOS). 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
Journal Impact Factor: 5.102


Schmidt F*, Zimmermann H*, Mikolajczak J, Oertel FC, Pache F, Weinhold M, Schinzel J, Bellmann-Strobl J, Ruprecht K, Paul F, Brandt AU. Severe structural and functional visual system damage leads to profound loss of vision-related quality of life in patients with neuromyelitis optica spectrum disorders. Mult Scler Relat Disord. 2017
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# Pache et al. Eur. Radiol. 2016 (OCT-MRT I)

Die Publikation

ist abrufbar unter:

Oertel & Kuchling et al. N2 2017 (OCT-MRT II)

# Microstructural visual system changes in AQP4-antibody–seropositive NMOSD

OPEN

Frederike C. Oertel*
Joseph Kuchling, MD*
Hanna Zimmermann, MEng
Claudia Chien, MSc
Felix Schmidt, MD
Benjamin Knier, MD
Judith Bellmann-Strobl, MD
Thomas Korn, MD
Michael Scheel, MD
Alexander Klistorner, MD
Klemens Ruprecht, MD
Friedemann Paul, MD
Alexander U. Brandt, MD

Correspondence to
Dr. Brandt:
alexander.brandt@charite.de

## ABSTRACT

**Objective:** To trace microstructural changes in patients with aquaporin-4 antibody (AQP4-ab)-seropositive neuromyelitis optica spectrum disorders (NMOSDs) by investigating the afferent visual system in patients without clinically overt visual symptoms or visual pathway lesions.

**Methods:** Of 51 screened patients with NMOSD from a longitudinal observational cohort study, we compared 6 AQP4-ab–seropositive NMOSD patients with longitudinally extensive transverse myelitis (LETM) but no history of optic neuritis (ON) or other bout (NMOSD-LETM) to 19 AQP4-ab–seropositive NMOSD patients with previous ON (NMOSD-ON) and 26 healthy controls (HCs). Foveal thickness (FT), peripapillary retinal nerve fiber layer (pRNFL) thickness, and ganglion cell and inner plexiform layer (GCIPL) thickness were measured with optical coherence tomography (OCT). Microstructural changes in the optic radiation (OR) were investigated using diffusion tensor imaging (DTI). Visual function was determined by high-contrast visual acuity (VA). OCT results were confirmed in a second independent cohort.

**Results:** FT was reduced in both patients with NMOSD-LETM ($p = 3.52e^{-14}$) and NMOSD-ON ($p = 1.24e^{-16}$) in comparison with HC. Probabilistic tractography showed fractional anisotropy reduction in the OR in patients with NMOSD-LETM ($p = 0.046$) and NMOSD-ON ($p = 1.50e^{-5}$) compared with HC. Only patients with NMOSD-ON but not NMOSD-LETM showed neuroaxonal damage in the form of pRNFL and GCIPL thinning. VA was normal in patients with NMOSD-LETM and was not associated with OCT or DTI parameters.

**Conclusions:** Patients with AQP4-ab–seropositive NMOSD without a history of ON have microstructural changes in the afferent visual system. The localization of retinal changes around the Müller-cell rich fovea supports a retinal astrocytopathy. *Neurol Neuroimmunol Neuroinflamm* 2017;4:e334; doi: 10.1212/NXI.0000000000000334

## GLOSSARY

**AD** = axial diffusivity; **ART** = automatic real time; **DTI** = diffusion tensor imaging; **FT** = foveal thickness; **GCIPL** = ganglion cell and inner plexiform layer; **GEE** = general estimate equation; **HC** = healthy control; **LETM** = longitudinally extensive transverse myelitis; **LGN** = lateral geniculate nucleus; **LPA** = lesion prediction algorithm; **LST** = Lesion Segmentation Toolbox; **MD** = mean diffusivity; **NMOSD** = neuromyelitis optica spectrum disorder; **OCT** = optical coherence tomography; **ON** = optic neuritis; **OR** = optic radiation; **pRNFL** = peripapillary retinal nerve fiber layer; **RD** = radial diffusivity; **ROI** = region of interest; **VA** = visual acuity.

Neuromyelitis optica spectrum disorders (NMOSDs) are relapsing inflammatory conditions of the CNS presenting with optic neuritis (ON) and longitudinally extensive transverse myelitis (LETM) as key clinical features and less frequently brainstem and cerebral involvement.[1] NMOSD is associated with serum antibodies to the astrocytic water channel aquaporin-4 (AQP4), which can be detected in 60%–80% of patients.[2,3] The remainder may not only

**Table 1** Demographic data of HCs and patients with NMOSD (mean ± SD)

|  | HC | NMOSD-LETM | NMOSD-ON |
|---|---|---|---|
| Subject, n | 26 | 6 | 19 |
| Sex, female/male | 22/4 | 6/0 | 17/2 |
| Age, y | 43.6 ± 15.7 | 43.1 ± 9.83 | 43.7 ± 12.5 |
| Disease duration, y |  | 3.0 ± 3.7 | 9.5 ± 8.9 |
| EDSS, median (min–max) |  | 3.5 (1.5-6.5) | 4 (0-6) |

Abbreviations: EDSS = Expanded Disability Status Scale; HC = healthy control; LETM = longitudinally extensive transverse myelitis; NMOSD = neuromyelitis optica spectrum disorder; NMOSD-LETM = NMOSD patients with a history of LETM but no history of ON; NMOSD-ON = NMOSD patients with a history of ON; ON = optic neuritis.

comprise patients with false-negative AQP4-antibody tests but also true AQP4 seronegatives that may harbor other autoantibodies (e.g., myelin oligodendrocyte glycoprotein) and may thus suffer from distinct disease entities.[4,5]

In contrast to MS, patients with NMOSD virtually never present clinically with progressive disease.[6] However, advanced imaging and histopathologic studies have shown conflicting results as to whether covert tissue damage can occur independent of attack-associated lesions in patients with NMOSD.[7–9] One possible explanation for these discrepancies may be the heterogeneity of previously investigated cohorts comprising both AQP4-antibody (AQP4-ab) positive and negative patients. Also on clinical examination, it may be difficult to identify subtle findings beyond the overtly affected functional system (i.e., optic nerve or spinal cord).

Against this background, we investigated microstructural and lesion-independent CNS tissue changes in a homogeneous cohort of exclusively AQP4-ab–seropositive NMOSD patients. To exclude any focal attack-related damage, we limited our study to patients who were only presenting with LETM but were otherwise asymptomatic. We used 2 imaging techniques: optical coherence tomography (OCT) to measure retinal thickness and diffusion tensor imaging (DTI)-based probabilistic tractography to analyze the optic radiation (OR).

**METHODS Patients.** We screened 51 patients with NMOSD participating in an ongoing prospective observational cohort study at the NeuroCure Clinical Research Center at the Charité—Universitätsmedizin Berlin. Six patients with a history of LETM but no other attack (i.e., history of ON) (NMOSD-LETM), 19 NMOSD-ON, and 26 age- and sex-matched healthy controls (HCs) were enrolled (table 1). In a previous study including nineteen (76%) of the 25 patients with NMOSD, normal subcortical gray matter volumes and microstructural changes were found.[10] Inclusion criteria were a minimum age of 18 years and a definite diagnosis of AQP4-ab–seropositive NMOSD according to the 2015 International Consensus Diagnostic Criteria.[11] AQP4-ab were determined by a cell-based assay (Euroimmun, Lübeck, Germany). Patients exhibiting ophthalmologic (e.g., glaucoma, myopia >5 dpt) or systemic diseases (e.g., systemic lupus erythematosus), which can potentially influence OCT or DTI results, were excluded from the study (figure 1). Visual function was tested monocularly with habitual correction and under photopic conditions. For high-contrast visual acuity (VA), Early Treatment in Diabetes

**Figure 1** Flowchart of cohort selection
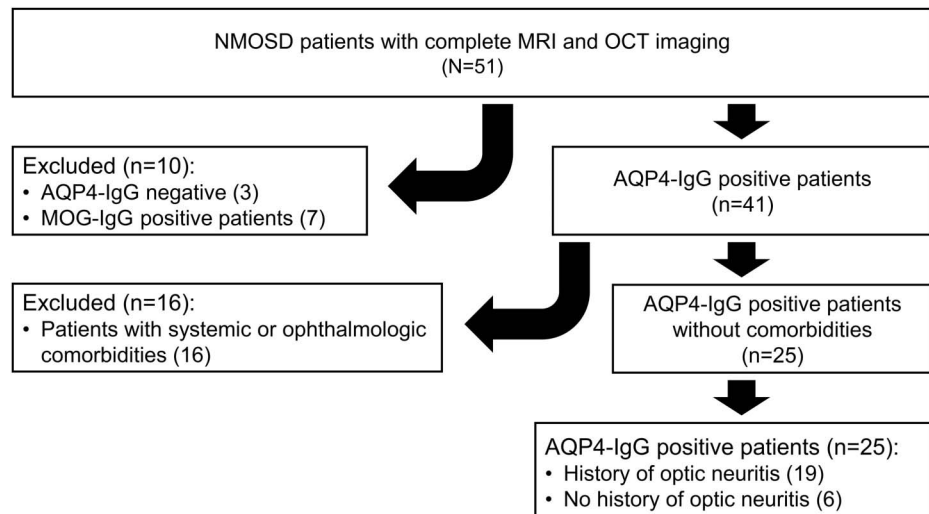


AQP4 = aquaporin-4; MOG = myelin oligodendrocyte glycoprotein; NMOSD = neuromyelitis optica spectrum disorder; OCT = optical coherence tomography.

Retinopathy Study charts were used at a 20-ft distance with an Optec 6500 P system (Stereo Optical, Chicago, IL).[12]

We additionally included a confirmatory OCT cohort consisting of 3 patients with AQP4-ab–seropositive NMOSD-LETM (women/men: 3/0; age: 41.3 ± 10.7 years; disease duration: 2.8 ± 2.1 years), 3 patients with AQP4-ab–seropositive NMOSD-ON (women/men: 3/0; age: 44.0 ± 1.0 years; disease duration: 2.9 ± 0.8 years), and 8 HCs (women/men: 8/0; age: 42.3 ± 1.7 years) following the same inclusion and exclusion criteria from a longitudinal prospective observational cohort study at the Department of Neurology, Klinikum rechts der Isar at the Technical University of Munich, Germany.

**Ethics statement.** The local ethics committee of the Charité—Universitätsmedizin Berlin approved this study (EA1/131/09). OCT data from the confirmatory cohort were collected under an ethics vote from the ethics committee at the Technical University of Munich (166/16S). The study was conducted in accordance with the Declaration of Helsinki in its currently applicable version and the applicable German laws. All patients provided written informed consent.

**Optical coherence tomography.** All retinal examinations were performed using a Heidelberg Engineering Spectralis spectral domain OCT (Heidelberg Engineering, Heidelberg, Germany) with automatic real-time (ART) function for image averaging. The peripapillary retinal nerve fiber layer (pRNFL) was measured with activated eye tracker using 3.4-mm ring scans around the optic nerve head (12°, 1,536 A-scans 16 ≤ ART ≤ 100). The combined ganglion cell and inner plexiform layer (GCIPL) volume was measured using a 6-mm diameter cylinder around the fovea from a macular volume scan (25° × 30°, 61 vertical B-scans, 768 A-scans per B-scan, ART = 15).[13] Segmentation of pRNFL and GCIPL was performed semiautomatically using software provided by the OCT manufacturer (Eye Explorer 1.9.10.0 with viewing module 6.0.9.0; Heidelberg Engineering). All measurements were checked for segmentation errors and corrected if necessary by an experienced rater. Foveal thickness (FT) was measured as the mean thickness of a 1-mm diameter cylinder around the fovea from each collected macular scan. We report our quantitative OCT data in line with the APOSTEL recommendations.[14]

**Magnet resonance imaging.** All MRI data were acquired on the same 3T scanner (MAGNETOM Trio Siemens, Erlangen, Germany) using a single-shot echo planar, DTI sequence (repetition time [TR]/echo time [TE] = 7,500/86 ms; field-of-view [FOV] = 240 × 240 mm²; matrix 96 × 96, slice thickness 2.3 mm, 64 noncollinear directions, b-value = 1,000 s/mm²), as well as a volumetric high-resolution fluid-attenuated inversion recovery sequence (3D FLAIR) (TR/TE/TI = 6,000/388/2,100 ms; FOV = 256 × 256 mm², slice thickness 1.0 mm). 3D FLAIR images of patients with NMOSD-LETM were checked and verified for OR lesions by a board-certified radiologist. Whole-brain segmentation and quantification of lesions of FLAIR images were performed using lesion prediction algorithm in the Lesion Segmentation Toolbox (LST) for MATLAB 2013a (MathWorks, Inc., Natick, MA).[15]

**Probabilistic tractography.** Diffusion tensors on the DTI images were fitted by a linear-least square approach. MRtrix package 0.2 (J-D Tournier; Brain Research Institute, Melbourne, Australia) was used to perform probabilistic tractography from seed to target mask.[16] Fiber orientation distribution was estimated with constrained spherical deconvolution and mapped with a maximum harmonic order of 6. The OR reconstruction pipeline was modified from the Martinez-Heras et al.[17] and Lim et al.[18] pipeline. The Juelich probabilistic atlas was used to generate binary masks of lateral geniculate nucleus (LGN) as the seed region of interest (ROI) and primary visual cortex (V1) as the target ROI. For binary exclusion masks, a midline sagittal exclusion plane, a termination coronal plane 20 mm posterior to the temporal pole, and a gray matter segmentation mask were created in the 3D coordinate system of the Montreal Neurological Institute (MNI-152). These were subsequently registered to individual DTI space, serving as a binary exclusion ROI for tractography. Ten thousand

**Table 2** OCT and DTI results from HC and NMOSD subgroups (mean ± SD)

| | HCs | NMOSD-LETM | NMOSD-ON | NMOSD-LETM vs HC | | | NMOSD-ON vs LETM | | | NMOSD-ON vs HC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | B | SE | p Value | B | SE | p Value | B | SE | p Value |
| FT, μm | 280 ± 21 | 260 ± 18 | 262 ± 18 | −20.38 | 8.233 | $1.5e^{-2}$ | 0.952 | 7.890 | $9.0e^{-1}$ | −20.32 | 5.540 | $2.4e^{-4}$ |
| pRNFL, μm | 97.1 ± 7.4 | 105.0 ± 6.9 | 71.7 ± 22.8 | −8.28 | 2.968 | $5.3e^{-3}$ | −33.03 | 5.066 | $7.0e^{-11}$ | −25.6 | 4.045 | $2.4e^{-10}$ |
| GCIPL, mm³ | 1.87 ± 0.15 | 1.93 ± 0.11 | 1.54 ± 0.30 | 0.061 | 0.049 | $2.1e^{-1}$ | −0.389 | 0.071 | $3.9e^{-8}$ | −0.333 | 0.062 | $8.3e^{-8}$ |
| FA | 0.57 ± 0.04 | 0.54 ± 0.03 | 0.53 ± 0.04 | −0.029 | 0.015 | $4.6e^{-2}$ | −0.014 | 0.015 | $3.2e^{-1}$ | −0.046 | 0.011 | $1.5e^{-5}$ |
| MD | 0.83 ± 0.07 | 0.90 ± 0.06 | 0.87 ± 0.05 | 0.050 | 0.032 | $1.2e^{-1}$ | −0.020 | 0.026 | $4.5e^{-1}$ | 0.003 | 0.016 | $3.7e^{-2}$ |
| AD | 1.43 ± 0.08 | 1.49 ± 0.09 | 1.43 ± 0.06 | 0.044 | 0.040 | $2.7e^{-1}$ | −0.048 | 0.036 | $1.8e^{-1}$ | −0.003 | 0.020 | $8.7e^{-1}$ |
| RD | 0.53 ± 0.08 | 0.61 ± 0.06 | 0.59 ± 0.06 | 0.054 | 0.031 | $8.3e^{-2}$ | −0.006 | 0.026 | $8.2e^{-1}$ | 0.053 | 0.018 | $2.7e^{-3}$ |
| Confirmatory cohort | | | | | | | | | | | | |
| FT, μm | 286 ± 10 | 257 ± 4 | 246 ± 4 | −27.89 | 3.72 | $6.6e^{-14}$ | −11.36 | 2.62 | $1.4e^{-5}$ | −40.62 | 4.60 | $<2.0e^{-16}$ |
| pRNFL, μm | 98.2 ± 4.6 | 114.0 ± 7.2 | 66.70 ± 14.9 | 15.68 | 2.77 | $1.5e^{-8}$ | −46.51 | 5.15 | $<2.0e^{-16}$ | −32.04 | 4.98 | $1.3e^{-10}$ |
| GCIPL, mm³ | 2.04 ± 0.09 | 2.07 ± 0.07 | 1.37 ± 0.14 | 0.04 | 0.05 | $5.1e^{-1}$ | −0.70 | 0.05 | $<2.0e^{-16}$ | −0.69 | 0.03 | $<2.0e^{-16}$ |

Abbreviations: AD = axial diffusivity; B = estimate; FA = fractional anisotropy; FT = foveal thickness; GCIPL = ganglion cell and inner plexiform layer volume; HC = healthy control; LETM = longitudinally extensive transverse myelitis; MD = mean diffusivity; NMOSD = neuromyelitis optica spectrum disorder; NMOSD-LETM = NMOSD patients with a history of LETM but no history of ON; NMOSD-ON = NMOSD patients with a history of ON; OCT = optical coherence tomography; ON = optic neuritis; pRNFL = peripapillary retinal nerve fiber layer thickness; RD = radial diffusivity.

unidirectional streamlines from the LGN to V1 were generated (fractional anisotropy (FA) threshold: 0.1; curvature threshold: 25%; step size: 0.2 mm) for each OR. Streamlines were thresholded for 25% of the maximum value. Resulting fibers were transferred to the Vistalab environment (vistalab.stanford.edu/, Vistalab, Stanford University, Stanford, CA) to compute tract profiles of weighted mean DTI values of FA, mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity at 50 equally spaced positions. We used the middle 30 of the 50 positions for statistical analysis for the exclusion of potential confounders from the LGN to V1 and to have a pure OR volume only.

**Statistical analysis.** Group differences were tested with a $\chi^2$ test for sex and a Wilcoxon-Mann-Whitney $U$ test for age. Group differences in OCT, DTI, and VA were evaluated by general estimate equation (GEE) models accounting for within-subject intereye dependencies and correcting for age and sex. Relationships between structural and functional parameters were analyzed using GEE models and correcting for age and

sex. Combined $p$ values of exploratory and confirmatory cohort results were calculated by Fisher combined probability test. All tests were performed with R version 3.1.2 with packages psych, geepack, and ggplot2. Graphical representations were created with R and Graphpad Prism 6.0 (Graphpad Software, San Diego, CA). For all calculations, statistical significance was established at $p < 0.05$.

**RESULTS OCT analysis.** The fovea is a region rich in AQP4-positive Müller cells, and foveal thinning has previously been reported in eyes from patients with NMOSD without ON.[19] We found that FT in eyes from patients with NMOSD-LETM was lower than that in HC, as was FT in patients with NMOSD-ON patients. Remarkably, FT in eyes from patients with NMOSD-LETM never experiencing visual symptoms was comparable to FT in eyes from patients with NMOSD-ON (table 2 and figure 2).

**Figure 2    OCT results**
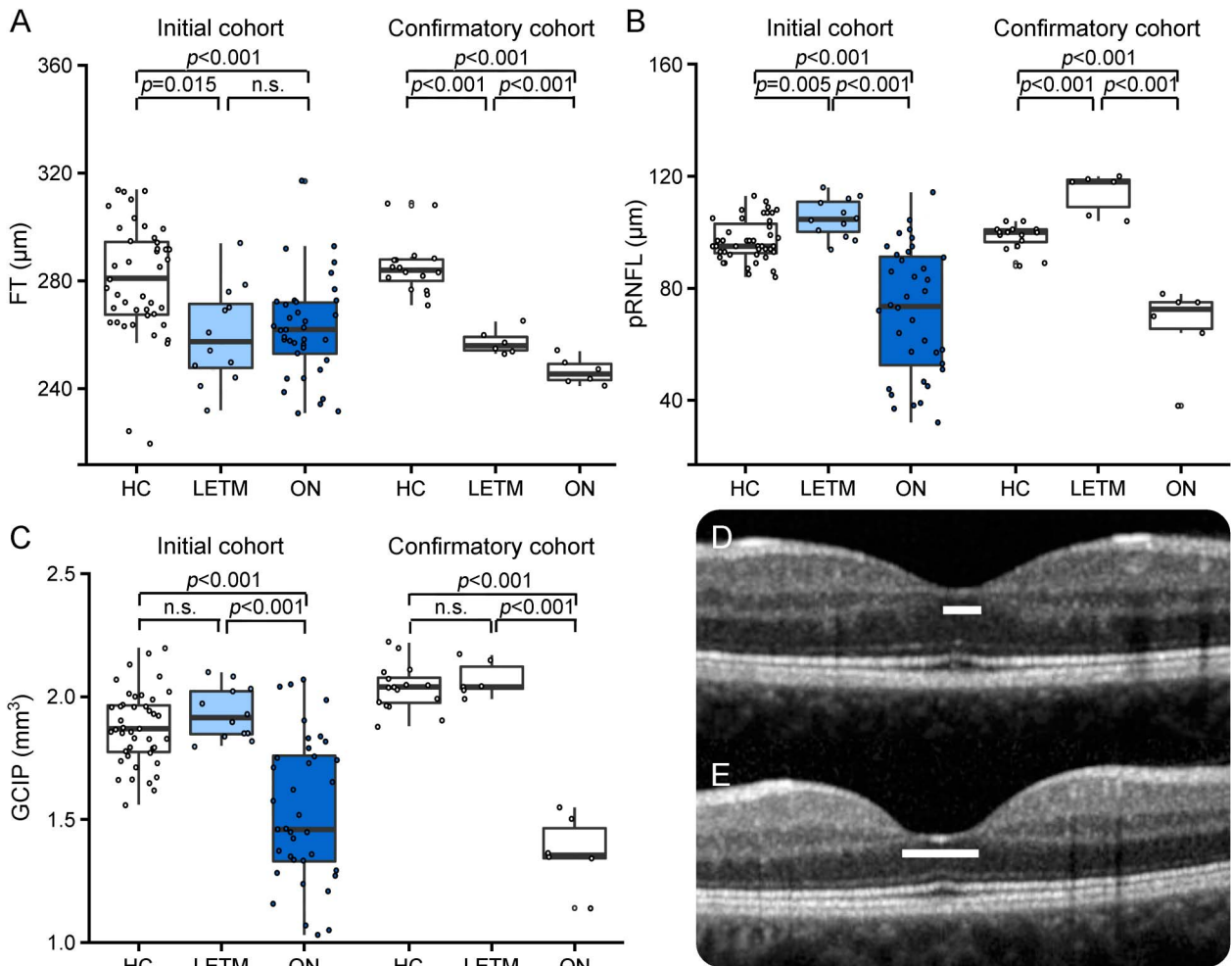


Boxplots of mean OCT values with values of individual eyes (jitter) in HC (left, white), NMOSD-LETM (middle, light blue), NMOSD-ON (right, dark blue), and for each confirmatory cohort (without color) for (A) FT values (μm); (B) pRNFL thickness (μm); (C) GCIPL volume (mm³); (D) FT in a representative macular scan of right eye from an HC; (E) FT changes in a representative macular scan of right eye from a patient with NMOSD-LETM. FT = foveal thickness; GCIPL = combined ganglion cell and inner plexiform layer volume; HC = healthy control; LETM = longitudinally extensive transverse myelitis; NMOSD-LETM = NMOSD patients with a history of LETM but no history of ON; NMOSD-ON = NMOSD patients with a history of ON; OCT = optical coherence tomography; ON = optic neuritis; pRNFL = peripapillary retinal nerve fiber layer thickness.

The FT reduction reflected a change in foveal shape, from an open V-shape in eyes from HCs to a wide U-shape in eyes from patients with NMOSD (figure 2, D–E).

In eyes from patients with NMOSD-LETM, pRNFL and GCIPL as markers of retinal neuroaxonal degeneration were not reduced but pRNFL instead increased in comparison with HC (table 1 and figure 2). By contrast and as expected, eyes with previous ON in the NMOSD-ON group presented with severe pRNFL and GCIPL loss, indicating ON-dependent neuroaxonal damage.[20,21] All OCT results were confirmed in a second independent cohort (figure 2). Statistical combination of $p$ values from the initial and confirmatory cohorts produced immense FT and pRNFL differences between NMOSD-LETM and HC (FT $p = 3.52e^{-14}$, pRNFL $p = 1.93e^{-9}$, and GCIP n.s.) as well as NMOSD-ON and HC (FT $p = 1.24e^{-16}$, pRNFL $p = 1.43e^{-18}$, and GCIP $p = 8.87e^{-22}$), supporting a high

likelihood of true-positive results, despite the low sample size in either cohort.

**MRI analysis.** Microstructural white matter changes in the OR were analyzed using DTI-based probabilistic tractography. Patients with NMOSD-LETM presented with FA reduction in comparison with HC ($p = 0.046$), which suggests structural changes in the OR of patients with NMOSD-LETM (table 2 and figures 3 and 4). Patients with NMOSD-ON expectedly showed pathologic changes in comparison with HCs (FA: $p = 1.5e^{-5}$; MD: $p = 0.037$; and RD: $p = 0.003$).

To ascertain that patients with NMOSD-LETM were indeed asymptomatic with respect to their visual system, we analyzed lesion distribution and volume on brain MRI. Whole-brain lesion volume did not differ between NMOSD-ON ($0.95 \pm 1.23$ mL) and NMOSD-LETM ($0.95 \pm 1.30$ mL; $p > 0.999$). Two patients with NMOSD-LETM had
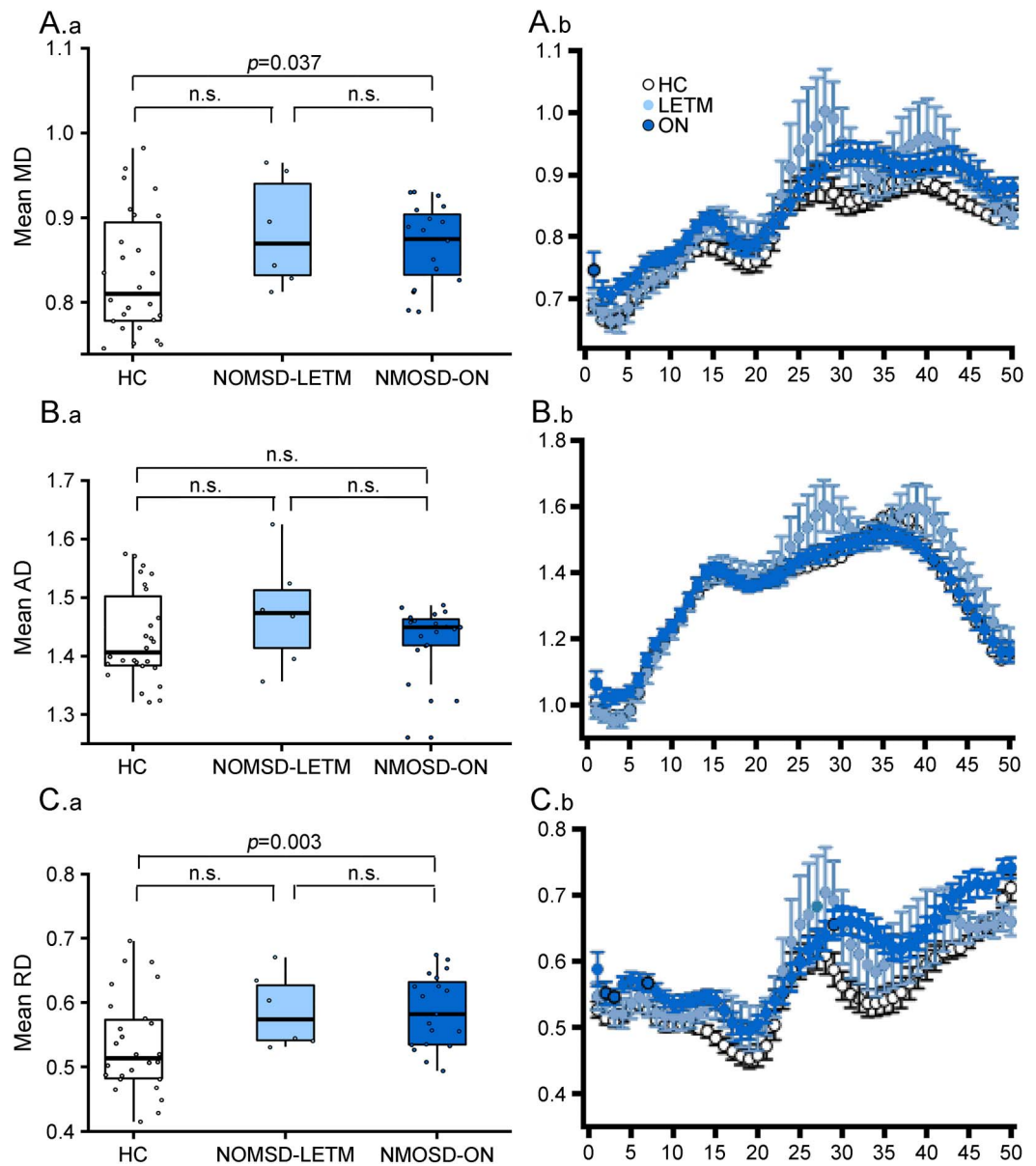
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**Figure 3    DTI results 1**
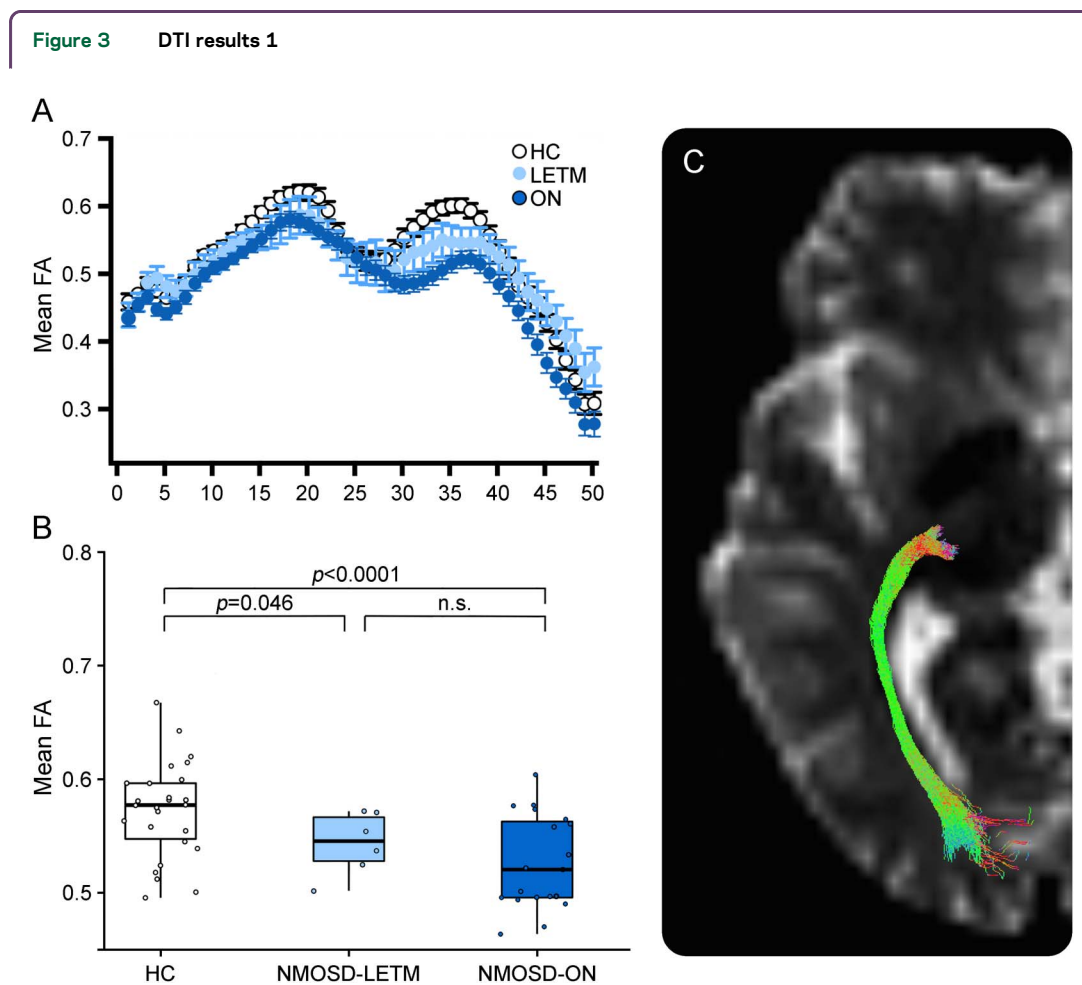


(A) Tract presentation of OR from LGN to V1 for averaged weight-mean DTI values of 50 segments in HC (white), NMOSD-LETM (light blue), and NMOSD-ON (dark blue) for FA (mean ± SEM). (B) Boxplot of mean FA values for middle 3/5 of the OR in HC (left, white), NMOSD-LETM (middle, light blue), and NMOSD-ON (right, dark blue). (C) Example of resulting fibers from tractography analysis. DTI = diffusion tensor imaging; FA = fractional anisotropy; HC = healthy control; LETM = longitudinally extensive transverse myelitis; LGN = lateral geniculate nucleus; NMOSD = neuromyelitis optica spectrum disorder; NMOSD-LETM = NMOSD patients with a history of LETM but no history of ON; NMOSD-ON = NMOSD patients with a history of ON; ON = optic neuritis; OR = optic radiation; V1 = primary visual cortex.

unspecific small dot-like lesions in the OR unilaterally. All confirmatory patients with NMOSD-LETM presented without any lesions in the OR. In patients with NMOSD-LETM, OR FA did not correlate with FT ($r = 0.066$, $p = 0.800$), pRNFL ($r = -0.204$, $p = 0.500$), or GCIPL ($r = 0.261$, $p = 0.400$), suggesting a structurally independent alteration without dependency on the observed foveal changes or covert retinal neuroaxonal damage. In patients with NMOSD-ON, reduced OR FA correlated with

reduced GCIP ($r = 0.361$, $p = 0.030$), but not with FT ($r = 0.210$, $p = 0.200$).

**Functional measurements.** VA ([logMAR]: $-0.02 \pm 0.10$) was normal in all patients with NMOSD-LETM. As expected, patients with NMOSD-ON had worse mean VA of all eyes ([logMAR]: $0.22 \pm 0.37$; $p = 0.002$). In patients with NMOSD-LETM and NMOSD-ON, VA did not correlate with FT (NMOSD-LETM: $r = -0.312$, $p = 0.300$;



**Figure 4    DTI results 2**

(A.a–C.a) Boxplots of mean DTI values for middle 3/5 of the OR and (A.b–C.b) Tract presentation of OR from the LGN to V1 for averaged weight-mean DTI values of 50 segments in HC (white), NMOSD-LETM (light blue), and NMOSD-ON (dark blue) for (A) MD, (B) AD, and (C) RD (mean ± SEM for all). AD = axial diffusivity; DTI = diffusion tensor imaging; FA = fractional anisotropy; HC = healthy control; LETM = longitudinally extensive transverse myelitis; LGN = lateral geniculate nucleus; MD = mean diffusivity; NMOSD = neuromyelitis optica spectrum disorder; NMOSD-LETM = NMOSD patients with a history of LETM but no history of ON; NMOSD-ON = NMOSD patients with a history of ON; ON = optic neuritis; OR = optic radiation; RD = radial diffusivity; V1 = primary visual cortex.

NMOSD-ON: $r = 0.082$, $p = 0.700$) and OR FA (NMOSD-LETM: VA: $r = -0.445$, $p = 0.100$; NMOSD-ON: $r = 0.073$, $p = 0.700$).

**DISCUSSION** Patients with AQP4-ab–positive NMOSD without a history of ON and with normal visual function and otherwise normal neuroaxonal retinal measurements (pRNFL, GCIPL) have foveal thinning and reduced OR fractional anisotropy, suggesting microstructural changes in the afferent visual pathway in the absence of clinical attacks of ON.

In NMOSD, 55% of all first clinical events are ONs,[22] which in conjunction with subsequent attacks cause damage to the optic nerve with resultant visual impairment.[20,21,23–25] However, subclinical tissue alterations in NMOSD affecting the afferent visual system have been controversially discussed.[19–21] For example, while one study reported axonal damage in eyes that never experienced ON,[19] another study did not find any signs of neuroaxonal damage in eyes without ON in patients with NMOSD.[21]

Our study now clearly demonstrates structural retinal and OR changes outside attack-related lesions.[26] The parafoveal area is characterized by a high density of retinal astrocytic Müller cells, which express AQP4 and may thus serve as retinal targets in NMOSD.[19,27–29] Müller cells regulate the retinal water balance and have a relevant role in neurotransmitter and photopigment recycling, as well as in energy and lipid metabolism.[27] Müller cell dysfunction or degeneration could thus lead to impaired retinal function including changes in water homeostasis. Of interest, both the initial cohort and the confirmatory cohort showed a mild increase of pRNFL thickness, which could indicate tissue swelling. These findings are supported by animal studies showing retraction of astrocytic end feet in some and astrocyte death in other cases, suggesting a primary astrocytopathy in NMOSD also outside acute lesions.[30–32] The changes we identified in the OR in this study furthermore indicate that a presumptive astrocytopathy may not be confined to the retina.[10,23,25] This is in line with astrocytic end feet changes reported in biopsies from LETM spinal cord lesions and spinal cord atrophy in AQP4-ab–positive patients without previous myelitis.[9,33] Whether these changes lead to subtle clinical manifestations should be further investigated using more sensitive functional measures such as visual evoked potentials or low-contrast VA. If confirmed, this would be in line with a preferential affection of the visual system, even without apparent clinical symptoms in NMOSD.

Reduction of FT in patients with NMOSD without overt clinical evidence of optic nerve involvement (normal VA, normal pRNFL, and GCIPL values) was comparable with that of patients with previous ON. To assure that we were only detecting AQP4-ab–associated pathologies, we rigorously excluded potential confounders. Most importantly, we only included a homogeneous group of AQP4-ab–seropositive patients who are expected to display a well-defined astrocytopathy phenotype.[34] Patients were only eligible if they presented with LETM and no history of ON, visual symptoms, or other typical NMOSD-associated bouts. Since our patients with NMOSD-LETM did not show pRNFL and GCIPL thinning, a previous subclinical ON is highly unlikely. However, a potential pRNFL swelling might have masked a mild subclinical neurodegeneration, but the effects would likely be small and would not be able to explain the observed changes, which are comparable to eyes after severe ON.[21] In light of a recent animal study,[32] it is conceivable that AQP4-specific T cells also contribute to foveal astrocytopathy. However, disease-independent factors in NMOSD, such as prematurity and environmental conditions,[35] may also play a role in foveal thinning.

Previous studies investigating retinal changes in patients with NMOSD regularly included measurements from unaffected fellow eyes from patients with unilateral ON. This is problematic since ON in NMOSD often involves the optic chiasm, and carry-over effects by chiasmic involvement of symptomatically unilateral ON have been reported in up to 64% of patients with AQP4-ab–positive NMOSD.[22] This sets our study apart from a previous study reporting FT reduction in eyes without previous ON in a cohort of patients with NMOSD, which could have been alternatively explained by both non-AQP4 pathologies and chiasmic carry-over effects.[19] Furthermore, none of the patients with NMOSD-LETM had NMOSD-related attacks other than LETM, minimizing the potential of attack- or lesion-related tissue alteration as the cause of the observed changes. Attack-related tissue alteration could have been the case in a recent study reporting spinal cord atrophy in AQP4-ab–positive NMOSD patients with ON.[9] Of interest, despite all patients in the NMOSD-LETM group reporting and showing no symptoms of visual dysfunction, a few patients showed small lesions near the OR. Measurements from these patients were not outliers but well positioned within the data distribution of the whole cohort (not shown).

One important limitation of our study, which we share with the majority of other studies published in NMOSD, is the small sample size. We were able to confirm our results, however, in a second independent cohort. Furthermore, our study cannot answer whether the reported changes are attack related or attack independent (e.g., due to circulating

antibodies). That at least some occult changes might be caused during acute attacks was suggested by a study reporting a correlation of brain volumes and perfusion change with the number of ON attacks in patients with NMOSD.[36]

We found microstructural changes in the afferent visual system in visually asymptomatic patients with AQP4-ab–positive NMOSD-LETM, which were most apparent in the fovea, a region rich in AQP-expressing Müller cells. Localization and extent of these changes are suggestive of an astrocytopathy without apparent neuroaxonal damage. Identifying occult brain changes in patients with NMOSD is important for a number of reasons. These occult changes could be relevant for symptoms that are not directly related to attacks, e.g., cognitive dysfunction, fatigue, and depression[37–39] and could predispose to full attacks causing severe astrocytic damage, demyelination, and neuroaxonal damage. As such, occult CNS including retinal changes in NMOSD may be an important diagnostic and target. Retinal imaging of NMOSD-specific changes could aid in early differential diagnosis of NMOSD and help to identify patients in need of an NMOSD-specific therapy. Although highly specific, antibody testing takes too much time during an initial attack of a de novo NMOSD patient, making acute attack-related therapeutic diversification currently difficult. Future research should thus focus on the sensitivity and specificity of the retinal findings in NMOSD also in contrast to relevant differential diagnoses such as myelin oligodendrocyte glycoprotein antibody (MOG-ab)-associated encephalomyelopathy or MS.[40] Finally, retinal assessment could aid as therapy response marker during novel drug development.

## AUTHOR CONTRIBUTIONS

F.C.O. and J.K.: data collection and analysis. F.C.O.: writing of the manuscript. H.Z.: OCT and data analysis. C.C.: lesion segmentation and data analysis. F.S. and J.B.-S.: study coordination and data acquisition. B.K. and T.K.: data collection and analysis. M.S.: data analysis, lesion segmentation, and tractography. A.K.: tractography. K.R. and F.P.: study coordination. A.U.B.: study concept, design, coordination, data analysis, and writing of the manuscript. All authors revised the manuscript for intellectual content and read and approved the final manuscript.

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## DISCLOSURE

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Frederike C. Oertel*
Hanna Zimmermann, MEng*
Janine Mikolajczak, MSc
Maria Weinhold, BSc
Ella Maria Kadas, PhD
Timm Oberwahrenbrock, PhD
Florence Pache, MD
Judith Bellmann-Strobl, MD
Klemens Ruprecht, MD
Friedemann Paul, MD
Alexander U. Brandt, MD

## CONTRIBUTION OF BLOOD VESSELS TO RETINAL NERVE FIBER LAYER THICKNESS IN NMOSD

OPEN

Neuromyelitis optica spectrum disorders (NMOSDs) are relapsing inflammatory demyelinating disorders with optic neuritis (ON) as the hallmark. ON causes neuroaxonal damage to the optic nerve and retina, regularly leading to severely impaired visual acuity (VA).[1]

Peripapillary retinal nerve fiber layer (pRNFL) thickness measured by optical coherence tomography (OCT) has been increasingly recognized as a marker for neuroaxonal damage and correlate of visual dysfunction.[1] As such, pRNFL is implemented as an outcome in clinical trials of ON-associated disorders. Blood vessels (BVs) running within the pRNFL contribute approximately 13% to an average RNFL thickness[2] and could present an important confounder when tracking small pRNFL changes or in diseases with severe thinning such as NMOSD.[1] Against this background, the objective of this study was to investigate the influence of retinal BVs on pRNFL measurements in an NMOSD cohort.

**Methods.** Forty patients from a prospective observational cohort study at the NCRC at Charité–Universitätsmedizin Berlin were enrolled (women/men: 39/1, age: 44.7 ± 15.4 years, 42 ON eyes). Inclusion criteria were a minimum age of 18 years and diagnosis of NMOSD according to the 2015 IPND criteria[3] (n = 37, aquaporin-4 antibody seropositive n = 28) or myelin oligodendrocyte glycoprotein-IgG–associated encephalomyelitis (n = 3).[4] Exclusion criteria were any other diseases which could influence OCT results.

All patients were examined with a Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) using automatic real time (ART) function for image averaging. pRNFL was measured with a 3.4 mm ring scan around the optic nerve head (12°, 1536 A scans 16 ≤ ART ≤ 100) and segmented semiautomatically (Eye Explorer 1.9.10.0 with viewing module 6.0.9.0) and manually corrected by an experienced grader. BV positions were automatically detected by OCTSEG[5] (figure, A and B) and manually corrected. Three eyes were excluded because of insufficient image quality based on OSCAR-IB criteria.

High-contrast VA was examined monocularly under habitual correction and photopic conditions with ETDRS charts at a simulated 20 ft distance using the Optec 6500 P System (Stereo Optical, Chicago, IL).

pRNFL without vessels was calculated as mean thickness of all ring scan positions not marked as part of vessels and was compared with pRNFL with vessels using paired $t$ tests. We performed Pearson correlation analyses for evaluation of the relationship between pRNFL and VA and Fisher z test for correlation comparison. The relative BV contribution in percentage was calculated as ([pRNFL$_{with\ vessels}$ − pRNFL$_{without\ vessels}$]/pRNFL$_{with\ vessels}$ × 100%). All statistical tests were performed using R 3.1 with significance established at $p < 0.05$. The study was approved by the local Ethics Committee at Charité–Universitätsmedizin Berlin and was conducted in accordance with the Declaration of Helsinki.

**Results.** pRNFL measurements were thinner without including BVs (76.1 ± 26.6 μm with, 68.3 ± 26.2 μm without, $p < 2e^{-16}$; figure, C). Relative BV contribution increased with lower pRNFL ($r = -0.700$, $p = 1e^{-12}$) (figure, D). When only considering eyes with pRNFL thickness below 60 μm, the mean relative BV contribution was significantly higher with 16% ± 5% compared with 9% ± 3% in eyes with RNFL >60 μm ($p = 8e^{-8}$).

VA (36 ± 19 ETDRS letters) was associated with pRNFL including BV ($r = 0.621$, $p = 2e^{-9}$) and without BV ($r = 0.618$, $p = 2e^{-9}$). In eyes with pRNFL measurements below 60 μm, pRNFL-VA correlation was numerically higher for pRNFL excluding BV ($r = 0.495$, $p = 0.007$) than pRNFL including BV ($r = 0.482$, $p = 0.009$), but the difference was not significant ($p = 0.476$). There were no influences of antibody status, disease duration and therapy on pRNFL, relative BV contribution, or enlargement of BV areas with pRNFL thinning (data not shown).[6]

**Discussion.** BV contribution to average pRNFL measurements is higher in thin compared with normal/high pRNFL measurements.

**Figure**    Contribution of blood vessels to retinal nerve fiber layer thickness
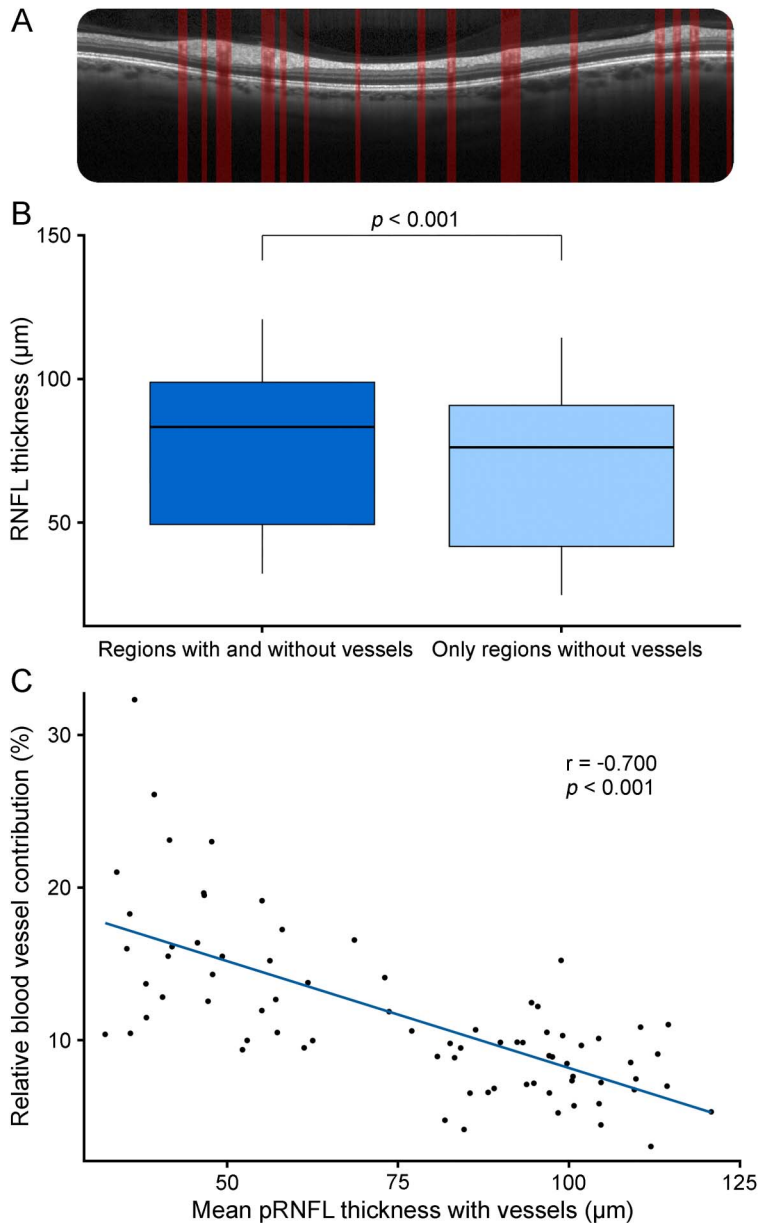


(A) pRNFL scanned by optical coherence tomography, segmented with vessel detection. (B) Mean pRNFL thickness in μm; all regions including vessels and only regions without vessels. (C) Correlation of the relative blood vessel contribution to the pRNFL including vessels. pRNFL = peripapillary retinal nerve fiber layer.

A previous study reported an average BV contribution of 13% to pRNFL measurements.[2] Our study expands these findings by showing that BV contribution is increased in low pRNFL measurements like the ones regularly found in NMOSD patients with severe ON.

A relevant contribution of BV artifacts to measurement noise has been reported.[7] Although our results did not show a structure-function correlation improvement for vessel-corrected measurements, they suggest a downgrade in pRNFL measurement sensitivity. In NMOSD cohorts, a wide range of pRNFL thickness measurements are seen, including those lower than 60 μm.[1] Typically, pRNFL differences of only a few micrometers are used to evaluate drug efficacy in ON trials.[8] Thus, in longitudinal studies, vessel artifacts potentially interfere with the comparability of an absolute thickness change because the relative vessel contribution increases with thinner pRNFL.

We propose analyzing OCT data in studies including NMOSD and other conditions with low pRNFL measurements in addition to vessel correction. Further studies of retrospective and prospective data and larger cohorts are required to confirm and specify BV influence and to identify reliable surrogates for tracking ON-related damage in NMOSD.

*Correspondence to Dr. Brandt: alexander.brandt@charite.de*

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# MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin

Sven Jarius[1*], Klemens Ruprecht[2], Ingo Kleiter[3], Nadja Borisow[4,5], Nasrin Asgari[6], Kalliopi Pitarokoili[3], Florence Pache[4,5], Oliver Stich[7], Lena-Alexandra Beume[7], Martin W. Hümmert[8], Corinna Trebst[8], Marius Ringelstein[9], Orhan Aktas[9], Alexander Winkelmann[10], Mathias Buttmann[11], Alexander Schwarz[1], Hanna Zimmermann[2], Alexander U. Brandt[2], Diego Franciotta[12], Marco Capobianco[13], Joseph Kuchling[2], Jürgen Haas[1], Mirjam Korporal-Kuhnke[1], Soeren Thue Lillevang[14], Kai Fechner[15], Kathrin Schanda[16], Friedemann Paul[4,5†], Brigitte Wildemann[1†], and Markus Reindl[16†]; in cooperation with the Neuromyelitis Optica Study Group (NEMOS)

## Abstract

**Background:** Antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG) have been suggested to play a role in a subset of patients with neuromyelitis optica and related disorders.

**Objective:** To assess (i) the frequency of MOG-IgG in a large and predominantly Caucasian cohort of patients with optic neuritis (ON) and/or myelitis; (ii) the frequency of MOG-IgG among AQP4-IgG-positive patients and vice versa; (iii) the origin and frequency of MOG-IgG in the cerebrospinal fluid (CSF); (iv) the presence of MOG-IgG at disease onset; and (v) the influence of disease activity and treatment status on MOG-IgG titers.

**Methods:** 614 serum samples from patients with ON and/or myelitis and from controls, including 92 follow-up samples from 55 subjects, and 18 CSF samples were tested for MOG-IgG using a live cell-based assay (CBA) employing full-length human MOG-transfected HEK293A cells.

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* Correspondence: sven.jarius@med.uni-heidelberg.de
†Equal contributors
Brigitte Wildemann, Markus Reindl and Friedemann Paul are equally contributing senior authors.
[1]Molecular Neuroimmunology Group, Otto Meyerhof Center, Department of Neurology, University Hospital Heidelberg, Im Neuenheimer Feld 350, 69120 Heidelberg, Germany
Full list of author information is available at the end of the article

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**Results:** MOG-IgG was detected in 95 sera from 50 patients with ON and/or myelitis, including 22/54 (40.7%) patients with a history of both ON and myelitis, 22/103 (21.4%) with a history of ON but no myelitis and 6/45 (13.3%) with a history of longitudinally extensive transverse myelitis but no ON, and in 1 control patient with encephalitis and a connective tissue disorder, all of whom were negative for AQP4-IgG. MOG-IgG was absent in 221 further controls, including 83 patients with AQP4-IgG-seropositive neuromyelitis optica spectrum disorders and 85 with multiple sclerosis (MS). MOG-IgG was found in 12/18 (67%) CSF samples from MOG-IgG-seropositive patients; the MOG-IgG-specific antibody index was negative in all cases, indicating a predominantly peripheral origin of CSF MOG-IgG. Serum and CSF MOG-IgG belonged to the complement-activating IgG1 subclass. MOG-IgG was present already at disease onset. The antibodies remained detectable in 40/45 (89%) follow-up samples obtained over a median period of 16.5 months (range 0–123). Serum titers were higher during attacks than during remission ($p < 0.0001$), highest during attacks of simultaneous myelitis and ON, lowest during acute isolated ON, and declined following treatment.

**Conclusions:** To date, this is the largest cohort studied for IgG to human full-length MOG by means of an up-to-date CBA. MOG-IgG is present in a substantial subset of patients with ON and/or myelitis, but not in classical MS. Co-existence of MOG-IgG and AQP4-IgG is highly uncommon. CSF MOG-IgG is of extrathecal origin. Serum MOG-IgG is present already at disease onset and remains detectable in the long-term course. Serum titers depend on disease activity and treatment status.

**Keywords:** Neuromyelitis optica (NMO), Devic's syndrome, Optic neuritis, Transverse Myelitis, Longitudinally extensive transverse myelitis (LETM), Neuromyelitis optica spectrum disorders (NMOSD), Multiple sclerosis, Autoantibodies, Myelin oligodendrocyte glycoprotein antibodies (MOG-IgG), Neuromyelitis optica antibodies (NMO-IgG), Aquaporin-4 antibodies (AQP4-IgG), Cell-based assays, Cerebrospinal fluid, Antibody index

## Background

Neuromyelitis optica (NMO) is a severely disabling auto-immune disorder of the CNS. In the majority of cases, NMO is caused by autoantibodies to aquaporin-4 (AQP4-IgG) [1–6]; however, 10–20% of patients with NMO are negative for AQP4-IgG [7–11]. Back in 2007, based on preliminary results, we and others suggested a potential role for IgG antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG) in AQP4-IgG-seronegative NMO. At that time, however, MOG-IgG were still detected by enzyme-linked immunosorbent assays (ELISA) or immunoprecipitation assays, methods that were not always reliable [12], and skepticism prevailed. The following years saw the rise of so-called cell-based assays (CBA) for the detection of auto-antibodies. CBA have shown excellent sensitivity and specificity in many applications, including AQP4-IgG testing [8, 9]. Briefly, cultured human cells (mostly HEK293 cells) are transfected with the antigen of interest not constitutively expressed in those cells and used as antigenic substrate in an indirect immunofluorescence assay; mock-transfected cells are used as internal controls. According to a recent consensus statement, CBA are currently considered the best method for detecting AQP4-IgG in NMO [9, 13]. Moreover, assays for detecting conformation-sensitive antibodies to MOG were devised. By the use of such assays, several groups have demonstrated antibodies to MOG in mostly pediatric patients with ADEM or MS-like disease [14–16].

Later on, in a study published in this journal in 2011, some of us demonstrated antibodies to full-length MOG in patients with NMO for the first time by means of a CBA [17]. In the meantime, several studies by us and others have confirmed the association of MOG-IgG with NMO and with related disorders such as isolated optic neuritis (ON) or myelitis [18–28]. Most studies have found MOG-IgG exclusively in patients with ON and/or myelitis who are negative for AQP4-IgG, suggesting that MOG-IgG may denote a disease entity in its own right. The latter notion is further supported by recent in vitro and in vivo studies suggesting a direct pathogenic role of MOG-IgG [17, 29] and by studies demonstrating substantial differences in the histopathology of AQP4-IgG- and MOG-IgG-associated CNS lesions [30–33].

However, there were some obvious limitations: First, many of the previously investigated cohorts were relatively small. Second, long-term data were often absent, with follow-up samples not being available. Third, some cohorts included no Caucasian patients or were genetically mixed, which may be of relevance since genetic factors are thought to play a role in NMO [34]. Fourth, some cohorts were preselected according to AQP4-IgG serostatus. Fifth, control groups in some previous studies were formally too small to assess the specificity of antibody results in a reliable way. Sixth, these last two limitations prompt uncertainty about the prevalence of the rare so-called 'double-positive' samples, i.e., samples positive for both NMO-IgG and AQP4-IgG, that have been reported in a few studies [17, 22, 35]. Finally, most previous investigations have focused on serum and included no or only few cerebrospinal fluid (CSF) samples.

In the present study we assessed the frequency of MOG-IgG as assessed by means of a live-cell CBA [17]

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(i) in a large series of samples from predominantly Caucasian patients sent in for AQP4-IgG and MOG-IgG testing and (ii) in a well-defined cohort of Caucasian control patients with multiple sclerosis (MS) and other inflammatory CNS disorders as well as in healthy controls ($N = 614$). In addition, we evaluated (iii) the prevalence of MOG-IgG and AQP4-IgG double positivity based on a very large number of samples ($N = 459$); (iv) the presence of MOG-IgG at disease onset; (v) the long-term persistence of MOG-IgG in individual patients; (vi) the influence of disease activity and treatment status on MOG-IgG titers; and (vii) the frequency and origin of MOG-IgG antibodies in the CSF.

This study is part of an article series on MOG-IgG in CNS inflammation. In part 2, we systematically evaluate the clinical and paraclinical features present in MOG-IgG-positive ON and/or myelitis as well as treatment responses and long-term outcome [36]. In part 3, we analyze the clinical and radiological features, course, and prognosis of patients with MOG-IgG-associated brainstem encephalitis [37]. In part 4, we report on the frequency and severity of afferent visual nerve damage in MOG-IgG-associated ON as detected by retinal optical coherence tomography (OCT) [38].

## Methods

In total, 614 serum samples and 18 CSF samples from 522 subjects were tested for MOG-IgG. Group I comprised 386 serum samples from 300 patients referred for routine MOG-IgG testing by 11 European academic centers, including the departments of neurology at the University of Heidelberg, the Charité-University Medicine Berlin, the University of Düsseldorf, the University of Bochum, Hannover Medical School, the University of Würzburg, the University of Rostock, the University of Freiburg, all in Germany; the University of Southern Denmark, Denmark; the MS Center at the Azienda Ospedaliero Universitaria San Luigi Gonzaga, Orbassano, Italy; and the IRCCS, C. Mondino National Neurological Institute, Pavia, Italy; eight of which are members of the German Neuromyelitis optica Study Group (NEMOS). Samples were taken for routine clinical assessment. Diagnoses at the time of blood sampling as reported by the referring centers, all of which were tertiary care university hospitals with specialized neuroimmunological departments, included "ON and myelitis" in 54 patients (1 × AQP4-IgG-positive; 79 serum samples available for testing), "monophasic ON" in 66 (69 samples), "recurrent ON" in 37 (median number of ON attacks 4, range 2–15; 76 samples), "longitudinally extensive transverse myelitis" in 45 (57 samples), "relapsing remitting MS" (RRMS) in 50 (54 samples), "secondary progressive MS" (SPMS) in 2 (2 samples), "primary progressive MS" (PPMS) in 2 (2 samples), and "other neurological disorder" (OND) in 44 (47 samples).

Group II consisted of 89 anonymized serum samples from 83 control patients with AQP4-IgG-positive ON and/or myelitis. Of those, 56 had a history of ON and myelitis, 22 of myelitis but no ON, and 5 of ON but no myelitis. AQP4-IgG had been previously detected by use of a commercial CBA (Euroimmun, Lübeck, Germany) in these patients [8] and by means of an ELISA (RSR, Cardiff, UK) [10].

Group III was made up of 85 anonymized serum samples from 85 control patients with MS according to the McDonald criteria (RRMS in 73, SPMS in 9, PPMS in 3).

Group IV comprised 54 anonymized samples from 9 control patients with OND (including 8 with connective tissue disorders and brain involvement [39]) and from 45 healthy controls.

Ninety-two follow-up samples (86 × group I, 6 × controls) from 55 subjects were tested. The sex ratios (m:f) were 1:2.4 in group I and 1:3 in the control groups II–IV. The median age was 39 years in group I and 38 years among the control patients (groups II–IV). See Table 1 for additional demographic data. 516/522 (98.9%) tested subjects were of Caucasian descent, including 298/300 (99.3%) in group I.

All sera were tested using a live-cell CBA employing HEK293A cells transfected with full-length human MOG as previously described [17]. Screening of serum samples was performed at dilutions of 1:20 and 1:40, and antibody titers of positive serum samples were determined by serial dilutions. MOG-antibody titers of ≥1:160 were classified as seropositive [17]. If samples were tested more than once, the highest titer obtained with each sample was used for analysis in all control groups to ensure that data on assay specificity were as conservative as possible. Low-titer results (1:160–1:320) were confirmed in a second, methodologically independent CBA employing formalin-fixed HEK293 cells transfected with full-length human MOG (Euroimmun). CSF samples were screened undiluted, and antibody titers of positive samples were determined by serial dilutions (1:2, 1:4, etc.). The control samples were tested with MOG-IgG-positive serum samples interspersed. MOG-IgG serostatus and titers were determined by two independent investigators blinded to all clinical data (M.R., K.S.). To assess the origin of CSF MOG-IgG, the MOG-specific antibody index ($AI_{MOG}$) was determined. Calculation of AIs allows quantification of antigen-specific intrathecal antibody synthesis [40–43]. Briefly, $AI_{MOG}$ values were calculated as the ratio between the CSF/serum quotient for MOG-IgG, $Q_{MOG-IgG}$, and the CSF/serum quotient for total IgG, $Q_{IgG(total)}$, or $Q_{lim}$, if $Q_{IgG(total)}$ exceeded $Q_{lim}$; i.e., $AI_{MOG} = Q_{MOG-IgG}/Q_{IgG(total)}$, if $Q_{IgG(total)} < Q_{lim}$, and $AI_{MOG} = Q_{MOG-IgG}/Q_{lim}$, if $Q_{IgG(total)} > Q_{lim}$. CSF and serum samples were obtained at the same time. Usually, values >1.5 are considered as evidence of intrathecal specific antibody synthesis [40, 41]. However, if titers instead of concentrations are used to calculate the AI, a

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**Table 1** Demographic and serological findings from 522 subjects and 614 serum samples tested for MOG-IgG

| Diagnostic categories | Sample numbers | Patient numbers | Sex ratio (m:f) | Age (ys), median | MOG-IgG+, samples | MOG-IgG+, patients | MOG-IgG+, median[§] | AQP4-IgG +, MOG-IgG + patients |
|---|---|---|---|---|---|---|---|---|
| Group I | 386 | 300 | 1:2.4 | 39 | 95/386 (24.6%) | 50/300 (16.7%) | 1:640 | 0/50 (0%) |
| "ON and/or MY"[a] | 281 | 202 | | | 95/281 (33.8%) | 50/202 (24.8%) | 1:640 | 0/50 (0%) |
| "ON and MY"[a] | 79 | 54 | | | 39/79 (49.4%) | 22/54 (40.7%) | 1:1280 | 0/22 (0%) |
| "mON/rON"[a] | 145 | 103 | | | 47/145 (32.4%) | 22/103 (21.4%) | 1:640 | 0/22 (0%) |
| "mON"[a] | 69 | 66 | | | 10/69 (14.5%) | 9/66 (13.6%) | 1:800 | 0/9 (0%) |
| "rON"[a] | 76 | 37 | | | 37/76 (48.7%) | 13/37 (35.1%) | 1:640 | 0/13 (0%) |
| "MY" (all LETM)[a] | 57 | 45 | | | 9/57 (15.8%) | 6/45 (13.3%) | 1:2560 | 0/6 (0%) |
| "MS"[a] | 58 | 54 | | | 0/58 (0%) | 0/54 (0%) | N.a. | N.a. |
| "OND"[a] | 47 | 44 | | | 0/47 (0%) | 0/44 (0%) | N.a. | N.a. |
| Group II | 89 | 83 | 1:1.9 | 46 | 0/89 (0%) | 0/83 (0%) | N.a. | 89/89 (100%) |
| AQP4+ NMO | 59 | 56 | | | 0/59 (0%) | 0/56 (0%) | N.a. | 59/59 (100%) |
| AQP4+ rON | 5 | 5 | | | 0/25 (0%) | 0/22 (0%) | N.a. | 25/25 (100%) |
| AQP4+ LETM | 25 | 22 | | | 0/5 (0%) | 0/5 (0%) | N.a. | 5/5 (100%) |
| Group III | 85 | 85 | 1:3 | 38 | 0/85 (0%) | 0/85 (0%) | N.a. | N.a. |
| RRMS | 73 | 73 | | | 0/73 (0%) | 0/73 (0%) | N.a. | N.a. |
| SPMS | 9 | 9 | | | 0/9 (0%) | 0/9 (0%) | N.a. | N.a. |
| PPMS | 3 | 3 | | | 0/3 (0%) | 0/3 (0%) | N.a. | N.a. |
| Group IV | 54 | 54 | 1:1.3 | 38 | 1/54 (1.9%) | 1/54 (1.9%) | 1:320* | 0/1 (0%) |
| OND | 9 | 9 | | | 1/9 (11.1%) | 1/9 (11.1%) | 1:320* | 0/1 (0%) |
| HC | 45 | 45 | | | 0/45 (0%) | 0/45 (0%) | N.a. | N.a. |
| Group II–IV | 228 | 222 | 1:1.9 | 38 | 1/228 (0.5%) | 1/222 (0.5%) | 1:320* | 0/1 (0%) |
| Total | 614 | 522 | 1:2.6 | 38 | 96/614 (15.6%) | 51/522 (9.8%) | 1:640 | 0/51 (0%) |

*N.a* not applicable, *ON* optic neuritis, *mON* monophasic ON, *rON* recurrent ON, *MY* myelitis, *LETM* longitudinally extensive transverse myelitis, *MS* multiple sclerosis, *OND* other neurological disorders, *RRMS* relapsing remitting MS, *SPMS* secondary progressive MS, *PPMS* primary progressive MS, *HC* healthy control. [a]Suspected diagnosis at the time of sample referral. [§]MOG-IgG-positive samples only. *Single patient

cut-off value of 4 has been recommended [44]. Reiber's empiric hyperbolic function $Q_{lim}$ was applied to control for possible underestimation of intrathecal specific synthesis due to disturbances of the blood-CSF barrier function and was calculated as follows [45]:

$$Q_{lim(IgG)} = 0.93\sqrt{(Q_{Alb})^2 + 6 \times 10^{-6}} - 1.7 \times 10^{-3}$$

The study was approved by the institutional review boards of the participating centers and patients gave their informed consent for publication of clinical data. The control samples were tested in anonymized fashion as requested by the institutional review board of the University of Heidelberg. The Mann-Whitney $U$ test was used to compare antibody titers between groups, and the Kruskal-Wallis test with Dunn's post test to compare more than two groups. Differences with $P$ values <0.05 were considered statistically significant.

## Results

### Frequency of serum MOG-IgG and syndrome specificity

Overall, 96/614 (15.6%) samples and 51/522 (9.8%) subjects were positive for MOG-IgG (Figs. 1 and 2). In group I (samples sent in for routine assessment of MOG-IgG), MOG-IgG was detected in 95/386 (24.6%) samples from 50/300 (16.7%) patients; if only patients with a diagnosis of ON and/or myelitis are considered, MOG-IgG was present in 95/281 (33.8%) samples from 50/202 (24.8%) patients. In group II (AQP4-IgG-positive controls), none of 89 samples from 83 patients was positive for MOG-IgG. MOG-IgG was also absent in 85 samples from 85 patients in group III (MS control samples). In group IV (OND and healthy controls), 1/54 (1.9%) samples from 1/54 (1.9%) patients was positive for MOG-IgG (Fig. 2). In total, MOG-IgG was present in 1 of 228 (0.4%) control samples or 1 of 222 (0.5%) control patients ($p < 0.0001$ for group I patients vs groups II–IV patients).

All MOG-IgG-positive patients in group I had a history of ON and/or myelitis (Table 1, Fig. 3); 22/50 (44%) had a history of both ON and myelitis; 22/50 (44%) had a history of ON but not of myelitis (recurrent in 13); and 6/50 (12%) had a history of longitudinally extensive myelitis (LETM) but not of ON. The relative frequencies of MOG-IgG in group I patients with a history of ON and myelitis, myelitis but not ON, and ON but not myelitis, respectively, were 22/54 (40.7%), 22/103 (21.4%), and 6/45 (13.3%). All of

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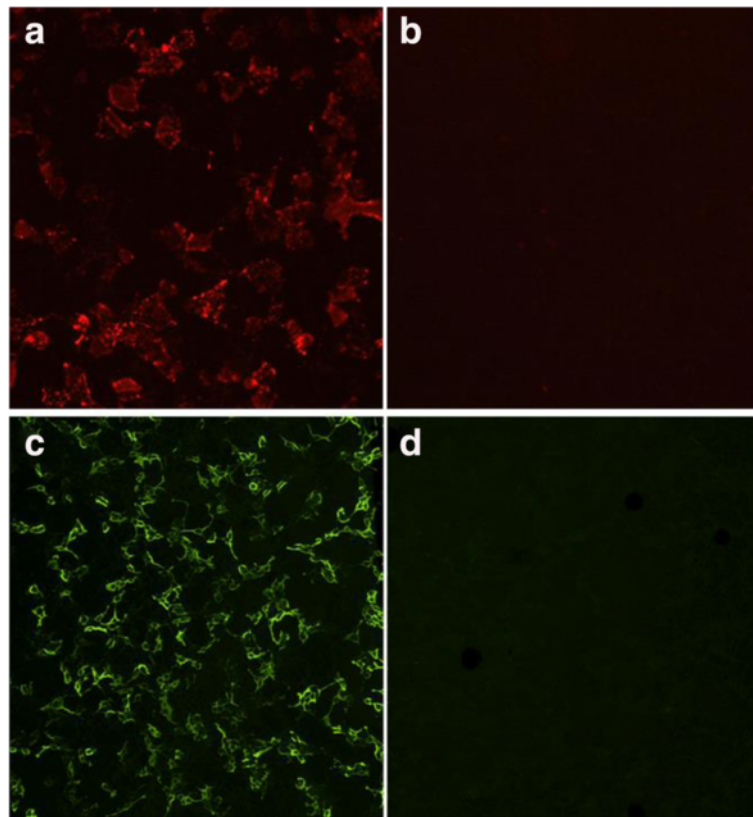


**Fig. 1** MOG-IgG as detected by two independent cell-based assays (CBA): typical findings. **a**, **b** Binding of serum IgG from a group I patient (**a**) but not from a control patient (**b**) to live HEK293A cells transfected with human full-length MOG. **c**, **d** Binding of serum IgG from a group I patient to formalin-fixed HEK293 cells transfected with full-length MOG (**c**) but not to their mock-transfected counterpart (**d**) in a commercial CBA. Bound MOG-IgG was visualized in the live-cell assay using a Cy3-conjugated goat anti-human IgG antibody and in the fixed-cell assay by use of a fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG antibody

the MOG-IgG-positive patients were negative for AQP4-IgG. Detailed clinical, radiological, electrophysiological, and laboratory data as well as data on treatment responses and outcome are reported in parts 2, 3 and 4 of this series [36–38]. Moreover, detailed case reports can be found in the *Appendix* sections of part 2 [36] and part 3 [37].

The only positive control sample was a low-titer sample (1 × 1:320, re-testing: 1 × 1:160) obtained from an OND patient from group IV originally diagnosed with systemic lupus erythematosus (American College of Rheumatology criteria met) and "leukoencephalitis of unknown origin". Symptoms included "scotoma", "seizures" and "depression"; the sample was negative when tested in the fixed-cell CBA used to confirm the other low titer samples, suggesting a possible false-positive result. As the control samples were analyzed in anonymized fashion, no more data were available on this case. By contrast, 11 further samples from 11 patients with CNS symptoms and systemic lupus erythematosus or other connective tissue disorders included in groups I and IV were negative for MOG-IgG. Follow-up samples were available from 6 MOG-IgG-negative control patients (groups II–IV), all of which were also negative for MOG-IgG.

### Co-existence of MOG-IgG and AQP4-IgG

None of the 51 MOG-IgG-positive group I and IV patients was positive for AQP4-IgG, and none of 84 AQP4-IgG-positive patients from groups II and I was positive for MOG-IgG (Table 1). AQP4-IgG was tested in MOG-IgG-positive patients using a standardized commercial CBA [8] in 48 (94%) and by ELISA [10] in 3 (6%). In addition, 226 patients from group I and 98 control patients from groups III and IV were negative both for MOG-IgG and for AQP4-IgG. Overall, 459 patients were tested for both MOG-IgG and AQP4-IgG.

### MOG-IgG serum titers

If all seropositive samples are considered, MOG-IgG titers in group I as determined in the live CBA ranged between 1:160 and 1:20480. If only the highest titer sample in each patient is considered, the median titer in group I was 1:1280 (range 160–20480; $N = 50$); maximum titres were higher in patients with a history of myelitis (median 1:2560, range 160-20480; $N=7$) or a history of both myelitis and ON (1:1280; range 160-10240; $N=22$) at last follow-up than in patients with a history of ON but no

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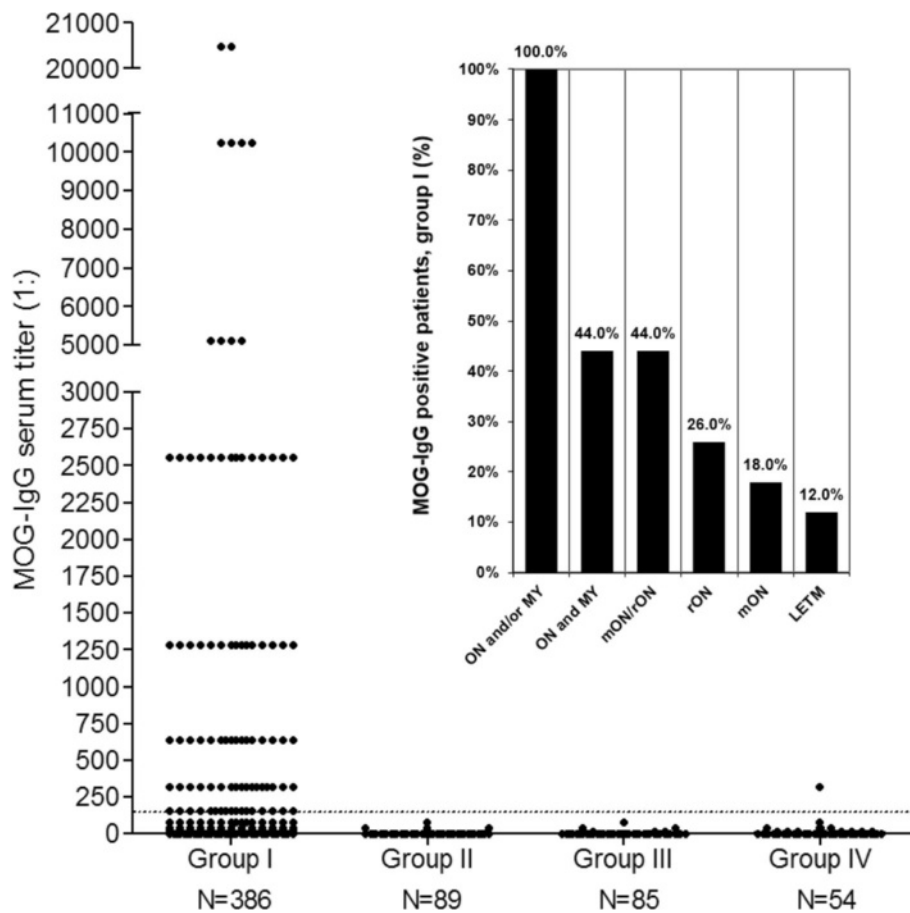


**Fig. 2** Frequency and titers of MOG-IgG in 614 serum samples from 522 subjects as detected using a live-cell CBA. MOG-IgG was detected in 95/386 (24.6%) samples in group I but was almost completely absent among 228 control samples (groups II–IV), including 89 samples from AQP4-IgG-positive patients, 85 samples from patients with MS according to the McDonald criteria (group III), and 54 samples from healthy controls and OND patients (group IV). While all low-titer samples (1:160–1:320) in group I were positive also in the fixed-cell CBA, the only positive control sample (from group IV) was negative in the fixed-cell CBA, suggesting a false-positive test result. The *horizontal dashed line* indicates the assay-specific cut-off (> = 1:160)

myelitis at last follow-up (1:640; range 160-20480; N=21), but the difference did not reach statistical significance.

**Presence of serum MOG-IgG at disease onset**
MOG-IgG was present already at disease onset in all patients with available data: 2 MOG-IgG positive sera were taken within the first week (at 2 and 4 days) after disease onset, 10 within the first month (median 10 days after onset, range 2–31), and 18 within the first 3 months (median 26 days after onset, range 2–85). The median MOG-IgG titer at disease onset was 1:2560 (range 160–20480; N = 18).

**Persistence of serum MOG-IgG in the long-term course**
In 18/22 (81.8%) patients with follow-up samples, all available samples were positive; in the remaining 4 patients, MOG-IgG turned negative at least once. Overall, 40 (89%) of 45 follow-up samples from MOG-IgG-

positive patients with ON and/or myelitis were positive after a median interval between first and last sampling of 16.5 months (range 0–123). 13/13 (100%) patients were still positive for MOG-IgG 1 year after the initial sample was taken, 8/8 (100%) 2 years after the initial sample, and 5/5 (100%) after 4 years. From three patients, stored samples obtained 6.5 years, 8.5 years, and more than 10 years before the last sample (and 13, 11, and 8.5 years after disease onset) were available for retrospective testing and were positive as well (first sample 1:1280 and last sample 1:640 in two patients; 1:320 and 1:160 in the third).

9/11 (81.8%) patients that were positive for MOG-IgG during an acute attack and had at least one available follow-up sample obtained during remission remained positive during remission. In one of these patients, MOG-IgG titers temporarily fell below the cut-off once during remission (1:80; cut-off 1:160); however, five additional follow-up samples from the same patient

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**Fig. 3** MOG-IgG serum titers in 386 samples from 300 patients included in group I. Diagnoses are given as provided by the referring centers. ON and MY = optic neuritis and myelitis; mON = monophasic optic neuritis; rON = recurrent ON; LETM = longitudinally extensive transverse myelitis; MS = multiple sclerosis; OND = other neurological disorders

obtained during remission were all positive. Similarly, titers were below the cut-off in two follow-up samples (2 × 1:80) taken during remission in another patient but were again positive (1:320) at last follow-up.

Follow-up samples obtained after plasma exchange (PEX) or immunoadsorption (IA) were available from two patients. In the first patient, titers declined from 1:10240 to 1:640 and subsequently disappeared completely after treatment with intravenous methylprednisolone (IVMP), PEX, intravenous immunoglobulins (IVIG), and oral steroids. In the second case, titers declined from 1:5120 to 1:20 after 5 cycles of IA.

Thirty-four follow-up samples were obtained from patients with ON and/or myelitis from group I who were negative at first testing; all of them were negative for MOG-IgG as well.

### Impact of disease activity on MOG-IgG serum titers
36/85 (42.4%) MOG-IgG positive samples with available data were taken within 60 days after an acute attack.

Median MOG-IgG titers were significantly higher (1:2560) in samples taken at the time of onset of an acute attack or shortly thereafter (median 14 days; $N = 33$) than in those taken during remission (>60 days since attack onset; 1:320; $N = 44$) ($p < 0.0001$) (Fig. 4a). MOG-IgG titers also differed significantly between acute attacks and remission in individual patients (Fig. 4b). However, titers observed during acute attacks varied both intra- and interindividually (interquartile range 1:1280–3200; absolute range 160–20480), and relatively high titers were also found in a few samples obtained during remission (interquartile range 1:160–640; absolute range 0–2560).

### Impact of clinical presentation on MOG-IgG serum titers during acute attacks
Median MOG-IgG titers were slightly higher during attacks involving acute myelitis (1:2560, range 320–20480; $N = 20$) than during attacks not involving acute myelitis (1:1280, range 160–5120; $N = 16$; $p < 0.007$) (Fig. 5a). Moreover, median titers were higher during attacks involving

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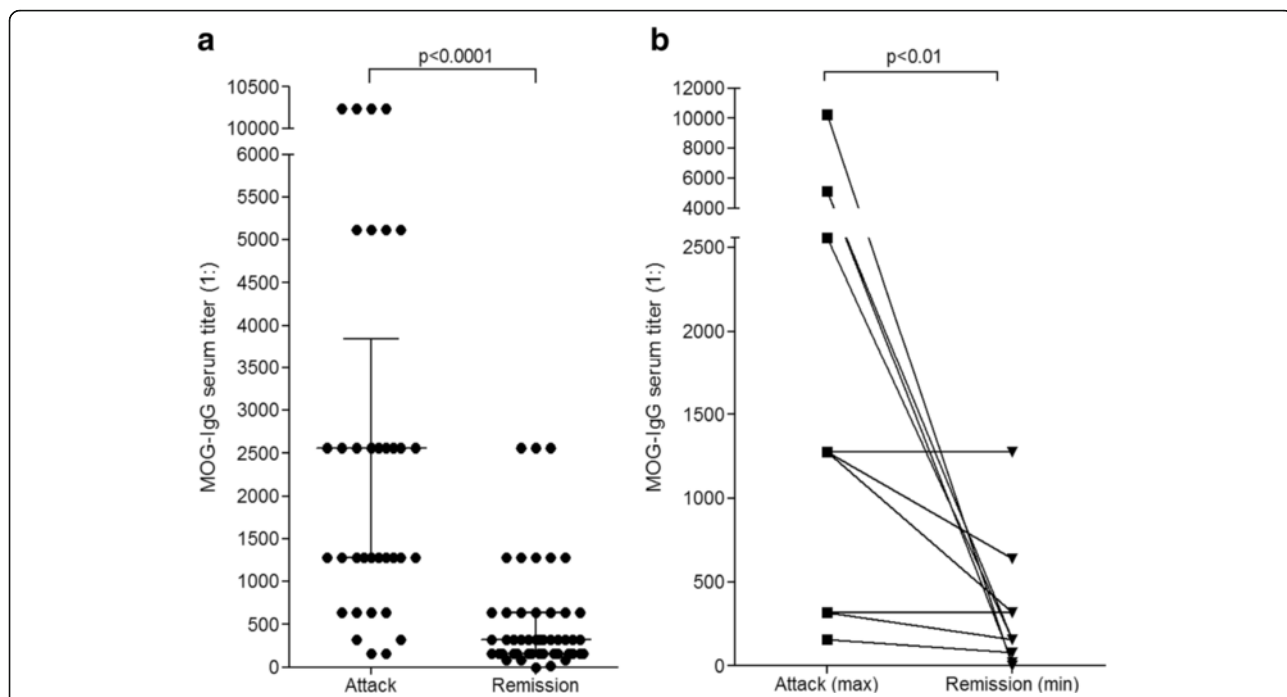


**Fig. 4** MOG-IgG titers and disease activity. Titers were significantly higher during acute attacks than during remission in the total cohort (**a**) as well as in individual patients with available follow-up sera (**b**). Horizontal lines and whiskers in panel **a** indicate median titers and interquartile ranges, respectively. The median interval between samples in the right panel was 16.5 months (range 2–103). Note that panel **b** shows maximum titers detected during acute attacks and minimum titers detected in follow-up sera. The difference was also significant if not the remission sample with the lowest titer but that with the longest time interval since attack onset was used (median 1:1280 vs. 1:320; *p* < 0.009; not shown)
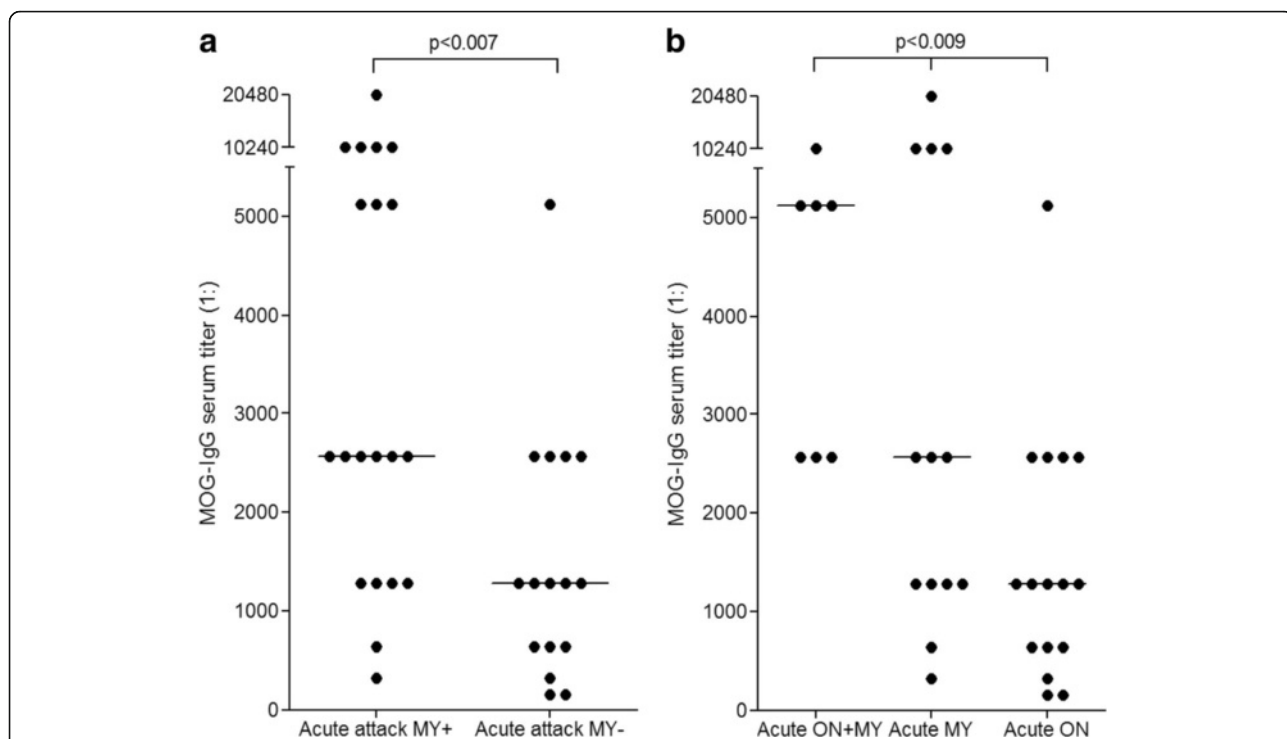


**Fig. 5** MOG-IgG titers and clinical presentation. Titers were higher during attacks involving myelitis than in attacks not involving myelitis (**a**), and higher during attacks involving simultaneous ON and myelitis than in attacks of isolated myelitis or isolated ON (**b**). The horizontal lines indicate median titers. ON = optic neuritis; MY = myelitis

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simultaneous myelitis and ON (1:5120, range 2560–10240; $N = 7$; additional brainstem encephalitis in three) than during attacks involving either ON but no myelitis or myelitis but no ON (1:1280 and 1:2560, respectively; Kruskal-Wallis $p < 0.009$; Dunn's post test $p < 0.05$ for ON + myelitis vs. ON) (Fig. 5b).

### Impact of treatment status on MOG-IgG serum titers

Precise data on the treatment status at the time of blood sampling were available for 76/84 (90.5%) MOG-IgG-positive samples. 28 samples were obtained during treatment with immunosuppressants (IS) or after PEX and 32 further samples were taken during or shortly after IVMP therapy ('treated subgroup'); another 31 samples were taken prior to immunotherapy or in treatment-free intervals ('untreated subgroup'). Treatments included IVMP, oral steroids, PEX, azathioprine, rituximab, methotrexate, mitoxantrone, natalizumab, and cyclosporine.

Median MOG-IgG serum titers differed significantly between relapse (1:2560, range 160–20480; $N = 23$) and remission (1:480, range 0–2560; $N = 23$) in patients treated with IS and/or PEX ($p < 0.0001$, Fig. 6a). However, a similar difference was present also in the untreated subgroup ($p = 0.0002$; Fig. 6b), suggesting that the decline in titers in the treated subgroup may have not been due only to treatment effects but may also reflect the natural disease course. In line with that notion, the median MOG-IgG titer in the treated subgroup did not differ significantly from that in the untreated subgroup, irrespective of whether all samples, only samples taken during relapse, or only samples obtained during remission are taken into account (data not shown).

Of note, 49/52 (94.2%) samples were positive despite treatment with IS/PEX and/or steroids (or 25/28 [89.3%] if only IS/PEX is considered). Of note, low MOG-IgG remained detectable in all four patients treated with rituximab at the time of blood sampling (titers 1:160–1:640). In a further patient treated with rituximab, a MOG-IgG titer of 1:1280 was documented during a relapse and was associated with recurrence of B cells.

### Frequency of CSF MOG-IgG

In total, 17 CSF samples from 15 MOG-IgG-seropositive patients from group I were available for testing. All but one sample were taken during an acute attack (median time since attack onset 10 days). Ten of those 17 CSF samples were obtained within 30 days after disease onset and 7 - including two follow-up samples - later in the disease course (range 71–5406 days after onset).

Twelve out of 17 CSF samples (71%) were positive for MOG-IgG. The median CSF MOG-IgG titer was 1:4 (range 2–64). Individual CSF and serum results are shown in Table 3. Median titers did no differ between CSF samples taken at the time of the first attack (1:3)



**Fig. 6** MOG-IgG titers and treatment status. While median MOG-IgG titers were lower during remission than during acute attacks in the treated subgroup (**a**), a similarly significant difference was also observed in the untreated subgroup (**b**). By contrast, no significant difference in median titers was observed between treated and untreated patients, neither during acute attacks nor during remission (not shown)

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and CSF samples taken during an acute attack later in the disease course (1:3). Twelve out of 15 (80%) patients were positive for CSF MOG-IgG at least once. In two of the three CSF-negative patients, lumbar puncture (LP) was delayed (1.5, 2 and 3 weeks, respectively, after attack onset) and was performed after or during IVMP therapy, respectively; and in all three, LP was done for acute isolated ON, a manifestation that was also associated with lower serum titers (Fig. 5a). Among the CSF positives, median CSF MOG-IgG titers in the initial sample taken during an acute attack were slightly higher in patients with acute myelitis (with or without concomitant ON and/or brainstem encephalitis) than in patients with acute ON (1:4 [range 2–64] vs. 1:1 [range 0–4]). An additional CSF sample obtained from the only serum MOG-IgG-positive control patient was negative for MOG-IgG.

In addition, 17 CSF samples from 17 control patients with RRMS were tested. All of those were negative for CSF MOG-IgG.

### CSF MOG-IgG in the long-term course

Follow-up CSF samples were available from two patients. In both cases, MOG-IgG were detectable in the CSF a few days after disease onset, at titers of 1:64 and 1:4, respectively, but not at repeat LP 51 and 21 days, respectively, later. One patient had been treated with IVMP, oral steroids, ten plasma exchanges, and IVIG in the meantime, the other one with IVMP alone. The decline in CSF titers was paralleled by a drop in serum titers from 1:10240 to 1:1280 and from 1:2560 to 1:1280, respectively, in these two patients.

### Origin of CSF MOG-IgG

Seventeen paired CSF and serum samples were titrated to calculate the MOG-specific AI. Evidence for intrathecal IgG synthesis was present in none of these 17 samples: in 5 samples no MOG-specific IgG was detectable in the CSF, and in the remaining 12 samples the MOG-specific AI was <4 (Table 2, Fig. 7), indicating that MOG-IgG are produced mainly in the periphery and reach the CSF by passive diffusion or through a leaky blood-brain and/or blood-CSF barrier. In line with that finding, CSF-restricted total IgG oligoclonal bands were absent in 16/17 samples tested and $Q_{IgG(total)}$ was below $Q_{lim}$ in 16/17 cases, while $Q_{Alb}$ exceeded the age-specific reference range in 6/17 (35.3%) samples, indicating disruption of the blood-CSF barrier function.

### MOG immunoglobulin class and subclass analyses

Twenty serum samples, including 14 MOG-IgG-positive sera from 13 patients from group I (8 × relapse, 6 × remission) and 6 control sera from group III patients, were tested for MOG-IgG1 using the fixed-cell CBA (Euroimmun). All 14 group I samples were positive for MOG-IgG1; by contrast, none of 6 control sera contained MOG-IgG1

**Table 2** Lack of evidence for intrathecal IgG synthesis in 17 CSF samples from 15

| Sample no. | MOG-IgG titer, serum | MOG-IgG titer, CSF | MOG-IgG titer required for AI >4 | Evidence for intrathecal MOG-IgG synthesis |
|---|---|---|---|---|
| #1 | 1:10240 | 1:64 | 1:925.7 | No |
| #2 | 1:2560 | 1:4 | 1:25.6 | No |
| #3 | 1:320 | 1:2 | 1:2.3 | No |
| #4 | 1:10240 | 1:16 | 1:152.5 | No |
| #5 | 1:640 | 1:4 | 1:9 | No |
| #6 | 1:2560 | 1:4 | 1:30.7 | No |
| #7 | 1:10240 | 1:16 | 1:176.1 | No |
| #8 | 1:2560 | 1:2 | 1:19.5 | No |
| #9 | 1:320 | 1:2 | 1:3.6 | No |
| #10 | 1:1280 | 1:2 | 1:9.4 | No |
| #11 | 1:2560 | 1:4 | 1:25 | No |
| #12 | 1:320 | 1:4 | 1:6 | No |
| #13[a] | 1:1280 | NEG | 1:17.4 | No |
| #14 | 1:320 | NEG | 1:3.7 | No |
| #15 | 1:1280 | NEG | 1:10.2 | No |
| #16 | 1:160 | NEG | 1:2.1 | No |
| #17[b] | 1:1280 | NEG | 1:10.2 | No |

MOG-IgG seropositive patients with ON and/or myelitis. *NEG* negative.
[a]Follow-up to sample #1; [b] follow-up to sample #2

antibodies (Fig. 8). MOG-IgG1 was also present in the CSF in 3/3 MOG-IgG serum positive patients tested.

In addition, 20 MOG-IgG-positive samples from 15 patients of group I were tested for MOG-IgM and MOG-IgA using the fixed-cell assay. Of these, only 2 samples (from a patient with a history of ON and myelitis) were positive for MOG-IgM and none for MOG-IgA (Table 3).

### Discussion

In 2011, some of us reported for the first time on serum autoantibodies to full-length human MOG in patients with NMO and related disorders [17]. This finding was later independently confirmed by several groups [18–25, 27, 46]. However, some previous analyses were hampered by low patient numbers and short follow-up times, lack of CSF samples, and, in some cases, uncertainty regarding assay specificity due to low control sample numbers. Moreover, some studies included no Caucasian patients. Here, we report on serological findings from a large cohort of MOG-IgG-positive patients, almost all of Caucasian origin. Our study demonstrates (i) that MOG-IgG are associated with ON and myelitis in a substantial proportion of cases; (ii) that MOG-IgG and AQP4-IgG do not usually co-exist in patients with ON and/or myelitis, which is in support of the notion of MOG-IgG being denoting an entity distinct from AQP4-IgG-positive NMO spectrum disorder (NMOSD) [47];

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**Fig. 7** MOG-specific antibody index (AI). Calculation of the MOG-specific AI in 17 paired CSF/serum samples from 15 MOG-IgG-positive patients did not reveal evidence for intrathecal synthesis of MOG-IgG. The *dotted line* indicates the upper limit of the reference range (AI = 4). *Inset*: Reiber diagram [40] demonstrating absence of total IgG intrathecal synthesis in 16 samples from 14 patients and presence of blood-CSF barrier dysfunction in 6/17 samples. $Q_{IgG}$ = CSF/serum total IgG ratio; $Q_{MOG-IgG}$ = CSF/serum MOG-IgG ratio; $Q_{Alb}$ = CSF/serum albumin ratio; $Q_{lim}$ = upper reference range of $Q_{IgG}$ (see methods section for details)



**Fig. 8** MOG-IgG1 as detected in the fixed-cell CBA. **a**, **b** Binding of serum IgG1 antibodies (from a patient with recurrent optic neuritis) to HEK293 cells transfected with human full-length MOG (**a**), but not to mock-transfected HEK293 cells (**b**). **c** Negative control serum (from a patient with RRMS) binding neither to the MOG-transfected cells (*upper panel*) nor to the mock-transfected control cells (*lower panel*). Bound patient IgG1 was detected by successive incubation with an unlabeled sheep anti-human IgG1 secondary antibody and an AlexaFluore®568-labeled donkey anti-sheep IgG antibody (*red fluorescence*). Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (*blue fluorescence*)

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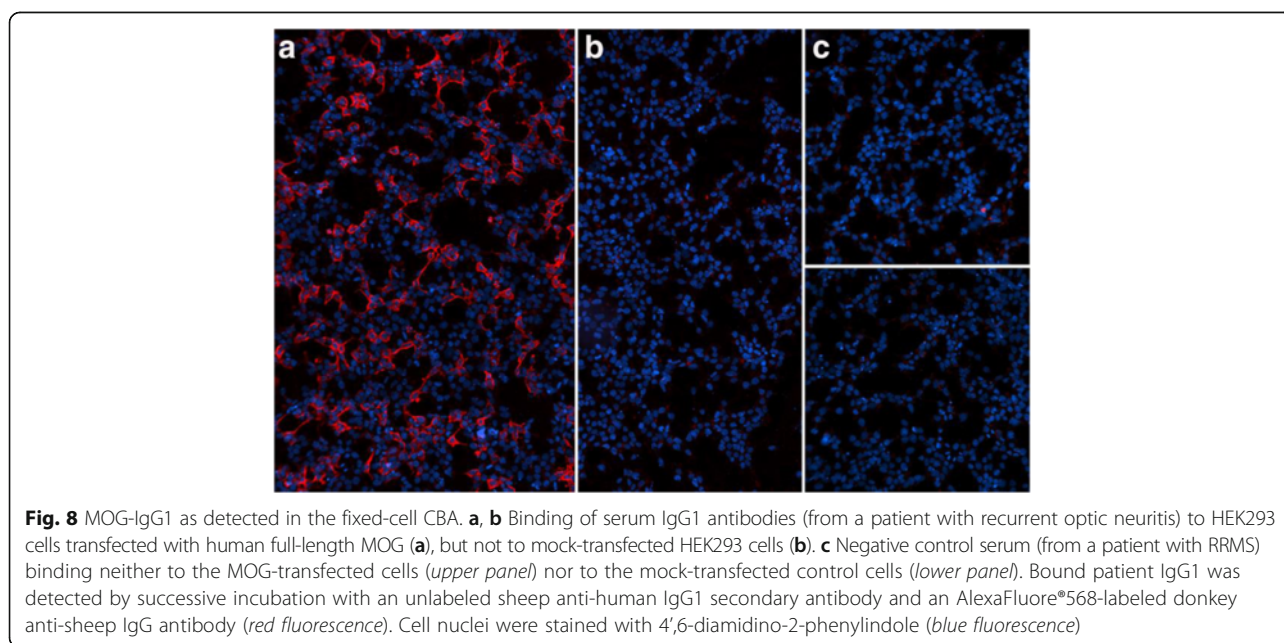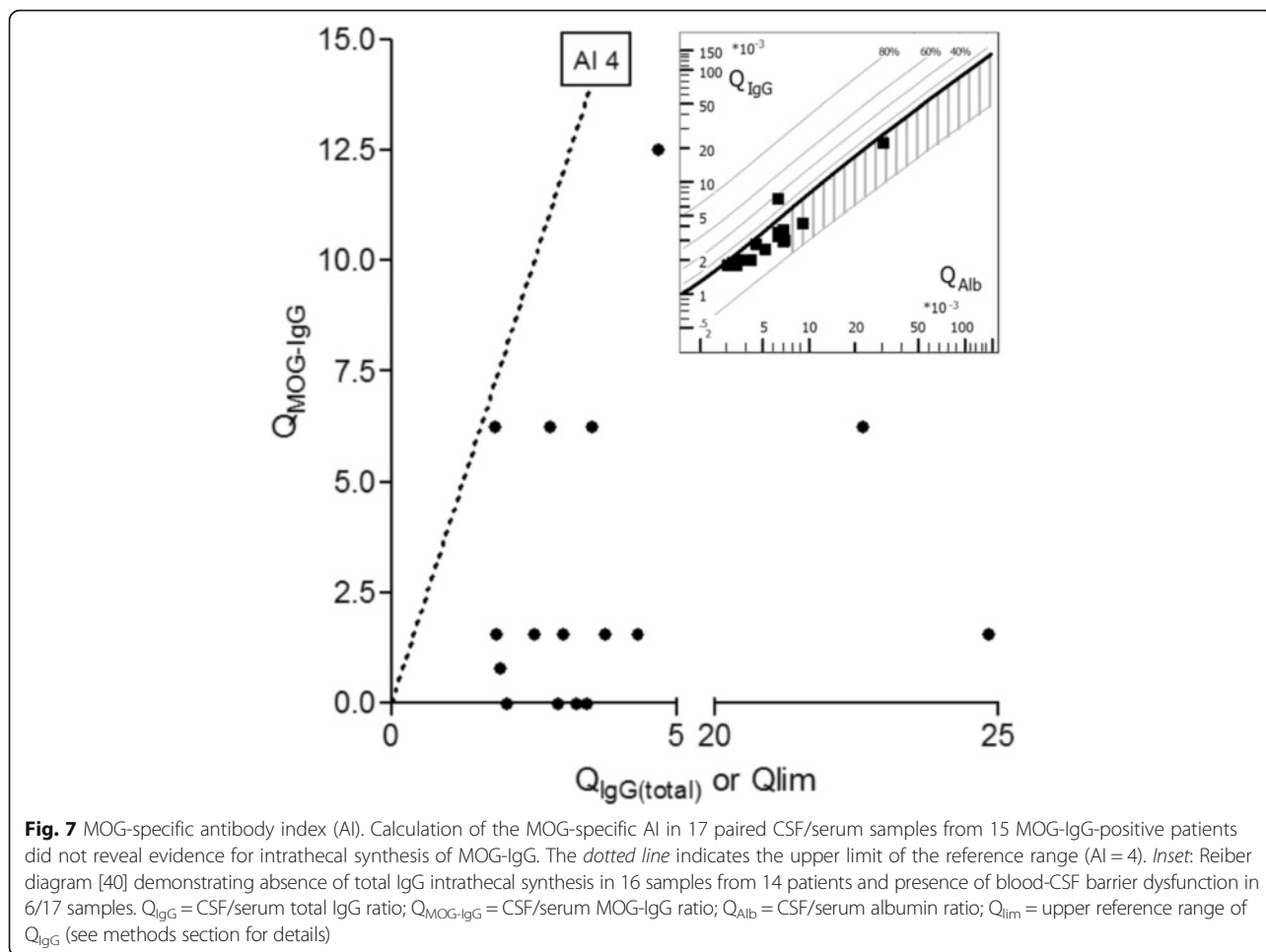**Table 3** MOG-IgG, MOG-IgG1, MOG-IgM, and MOG-IgA results from 21 samples

| No | MOG-IgG1 | MOG-IgG | MOG-IgM | MOG-IgA | Disease status |
|---|---|---|---|---|---|
| 1 | POS | POS | NEG | NEG | Relapse |
| 2 | POS | POS | NEG | NEG | Relapse |
| 3A | POS | POS | NEG | NEG | Relapse |
| 3B | POS | POS | NEG | NEG | Remission |
| 4 | POS | POS | NEG | NEG | Remission |
| 5 | POS | POS | NEG | NEG | Remission |
| 6 | POS | POS | NEG | NEG | Remission |
| 7 | POS | POS | NEG | NEG | Remission |
| 8 | POS | POS | NEG | NEG | Relapse |
| 9A | POS | POS | NEG | NEG | Relapse |
| 9B | n.d. | POS | NEG | NEG | Remission |
| 10A | POS | POS | NEG | NEG | Relapse |
| 10B | n.d. | POS | NEG | NEG | Remission |
| 11 | n.d. | POS | NEG | NEG | Relapse |
| 12 | n.d. | POS | NEG | NEG | Remission |
| 13 | n.d. | POS | NEG | NEG | Remission |
| 14A | n.d. | POS 1:1000 | POS 1:20 | NEG | Relapse |
| 14B | n.d. | POS 1:100 | POS 1:10 | NEG | Remission |
| 15A | n.d. | POS | NEG | NEG | Remission |
| 15B | n.d. | POS | NEG | NEG | Remission |
| 16 | POS | POS | n.d. | n.d. | Relapse |
| 17 | POS | POS | n.d. | n.d. | Relapse |
| 18 | POS | POS | n.d. | n.d. | Relapse |

MOG-IgG was determined using a commercial fixed CBA (cut-off 1:10). MOG-IgG1 was also present in 3/3 CSF samples from MOG-IgG1-seropositive patients (not shown). *POS* positive, *NEG* negative, *n.d* not done

(iii) that MOG-IgG are present already at the very onset of disease, which argues against MOG-IgG being a secondary epiphenomenon; (iv) that MOG-IgG remain detectable in the long-term course of the disease, indicating that the antibodies, if pathogenic, may not only trigger the disease but remain relevant in the long run; persisting MOG-IgG antibodies have also been described in pediatric patients diagnosed with relapsing demyelinating disease [26, 28]; (v) that MOG-IgG persist also during remission in the majority of patients, which is similar to what has been reported in AQP4-IgG-positive NMOSD [48], is important from a diagnostic point of view, and suggests that MOG-IgG alone is not sufficient to induce disease activity but other factors, such as an increase in titers, impaired blood-CSF barrier function (elevated $Q_{Alb}$ was indeed noted in 12/36 (32.4%) patients in the total cohort [36]) or T-cells, may be required; (vi) that, similar to AQP4-IgG [48], MOG-IgG serum titers depend on disease activity, with significantly higher median titers during acute attacks than during remission, both in treated and in untreated

patients, further supporting a potential pathogenic role of MOG-IgG; (vii) that absolute serum MOG-IgG titers vary substantially inter- and intraindividually, both during acute disease and during remission, with no clear cut-off for relapse induction; (viii) that MOG-IgG serum titers may also vary significantly with clinical presentation and, in some cases, treatment; (ix) that MOG-IgG (similar to AQP4-IgG [48]) may remain detectable even during treatment with rituximab, which suggests a role of long-lived plasma cells not affected by CD20-targeted immune therapy in the production of MOG-IgG and, given that no attacks occurred in the four patients tested in this study while on active treatment with that drug, that persistence of low-titer MOG-IgG does not per se argue against the efficacy of rituximab; (x) that MOG-IgG (like AQP4-IgG [42]) is detectable in the CSF in a substantial number of patients during acute attacks; this is in line with a small previous study by Dale et al., who found MOG-IgG in 2/4 patients positive for serum MOG-IgG [49]; (xi) that CSF MOG-IgG (just like CSF AQP4-IgG and in line with the lack of CSF-restricted oligoclonal bands (OCB) in most MOG-IgG-positive patients as shown in part 2 [36]) is mainly of extrathecal origin, i.e., enters the CNS from the systemic circulation, which may be of therapeutic relevance [42, 50–52]; (xii) that both serum and CSF MOG-IgG belong to the complement-activating IgG1 subclass (just as AQP4-IgG does [53, 54] and in agreement with the presence of complement deposits in CNS lesions in MOG-IgG-positive patients [31, 32]), again supporting the notion of MOG-IgG being of pathogenetic relevance; and, last but not least, (xiii) high specificity of the live CBA used in the present study [17] based on a very large series of control samples, which is important since it affirms the validity of results obtained in previous studies that have employed that assay [25, 26, 55, 56].

Our study features strengths and limitations. Among the strengths of the study we count (a) the high number of MOG-IgG-positive patients with ON and/or myelitis identified and analyzed ($N = 50$) compared with previous studies (median 9 patients in [17–25, 27, 46]); (b) the availability of a relevant number of follow-up or stored serum samples; (c) the availability of both samples taken at the very onset of the disease and samples taken more than a decade thereafter; (d) the availability of a substantial number ($N = 17$) of paired CSF and serum samples; (e) the inclusion of a relevant number of MOG-IgG-positive samples from untreated patients ($N = 31$); the fact (f) that virtually all patients were of Caucasian origin; (g) that the study was performed using a multicenter ($N = 11$) approach, thereby reducing potential center-specific selection biases; (h) that all MOG-IgG-positive patients were seen at university centers with specialized neuroimmunology departments, thereby potentially increasing

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diagnostic accuracy; (i) that detailed data on disease activity, clinical presentation, and treatment status at the time of blood sampling were available for most patients; (j) that both MOG-IgG and AQP4-IgG results were available from a relevant number of patients ($N = 459$); (k) that an already well-established CBA with published sensitivity and specificity [17] was used for MOG-IgG testing; (l) that a very large number of controls, interspersed in a random pattern, were included to re-validate the specificity of that assay ($N = 222$); (m) that all low-titer samples (1:160, 1:320) were confirmed using a second, methodologically independent CBA; and (n) that samples were evaluated by investigators not involved in patient recruitment and blinded to all clinical data.

The limitations include a potential referral bias due to the possibility that patients with ON and/or myelitis may have been preferentially referred for MOG-IgG testing as a consequence of the close association of MOG-IgG with these two conditions reported in the previous literature [18–28, 57]. However, MOG-IgG has also been reported in, mostly pediatric, patients with acute disseminated encephalomyelitis (ADEM). Although ADEM was considered as a differential diagnosis by the initially treating physicians in a few patients in our series (see part 2 [36] for details), our study did not specifically focus on children or on patients with a diagnosis of ADEM. Second, while the multicenter approach involving 11 specialized university departments is a potential strength as outlined above, it also carries the potential risk of a bias towards more severely affected patients. However, that risk is inherent to all tertiary care studies and cannot be completely avoided. It is important in this context that all centers involved in the present study also have specialized neuroinflammatory outpatient departments and that patients were recruited among both inpatients and outpatients. Finally, the threshold for admission is low in Germany, where public healthcare is free. In fact, a mild disease course was noted in a substantial proportion of patients (see part 2 [36] for details).

There is a discrepancy between the lack of MOG-IgG in the MS control group in this study and the fact that MS had been suspected by the then treating physicians at least once in 16/45 (35.6%) MOG-IgG positive patients, as outlined in part 2 of this series [36]. This discrepancy may highlight differences in diagnostic accuracy between carefully defined study cohorts comprising patients diagnosed at specialized centers and everyday clinical practice at primary or secondary care level. This notion is supported by the fact that MS had been initially considered in 11 of those 16 patients despite a lack of CSF-restricted OCB, a diagnostic hallmark of MS (see part 2 [36] for details). Similarly, 10 MOG-IgG-positive patients who formally met the 2010 McDonald criteria for MS had no OCBs. Moreover, 11 patients

with suspected MS had LETM lesions, which are usually absent in MS, and 11 did not meet Barkhof's MRI criteria for MS. Finally, 6 patients in whom MS had been previously suspected did not meet the 2010 McDonald criteria (see part 2 [36]). With the discovery of AQP4-IgG [1, 58–60], MOG-IgG [17], *N*-methyl-D-aspartate receptor-IgG [61], and a plethora of often non-paraneoplastic autoantibodies identified in acute CNS inflammation over the past decade [62–66], including in patients with primary or secondary demyelination, it becomes increasingly clear that not all patients presenting with relapsing CNS disease of putative autoimmune etiology have classical MS–even if they formally meet the 'positive' clinicoradiological criteria for MS [67]. In fact, 50% of the MOG-IgG-positive patients in this study had clinical or radiological involvement of the brain in addition to ON and/or myelitis and the Barkhof and McDonald criteria for multiple sclerosis (MS) were met by 15% and 33%, respectively, as shown in parts 2 and 3 of this series [36, 37]. MOG-IgG-positive patients, in whom the disease starts with isolated brain or brainstem involvement are particularly challenging [27, 36, 37, 68]. Thus more and more importance attaches to carefully considering the 'negative' criterion of ruling out other diagnoses ("no better explanation") included in the current diagnostic consensus criteria for MS [69]. It also suggests that re-including CSF analysis in the diagnostic criteria for MS, as previously recommended by us and others [70], might help to improve diagnostic accuracy in patients with suspected MS.

It is of clinical relevance that 15/28 (53.6%) of the MOG-IgG-positive patients with a history of myelitis identified in this study had recurrent attacks of myelitis [36]. If only patients with MOG-IgG-positive isolated myelitis are considered, 4/6 had recurrent myelitis and two had monophasic myelitis [36]. This suggests that MOG-IgG testing should be considered both in patients with monophasic and in patients with recurrent myelitis. Similarly, MOG-IgG was found both in patients with a single attack of ON and in patients with recurrent ON.

While treatment with IS was followed by a decline in relapse rate in individual patients, as outlined in part 2 of this series [36], no clear effect of IS on median MOG-IgG titers could be demonstrated in the present study. However, this is not totally surprising: while our study is among the largest in the field, patient numbers might still have been too low to detect such effects, especially when taking into account the large number of confounders such as disease activity, attack severity, clinical presentation, type and duration of treatment, drug-specific latency periods, and time since attack onset. Prospective studies with fixed sampling intervals and defined treatment regimens are highly warranted to assess the effect of immunotherapy on MOG-IgG titers and its impact on outcome and prognosis in a definite way.

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## Conclusion

In summary, our study provides evidence supporting a potential pathogenic role of MOG-IgG, and thus the notion of MOG-IgG denoting a disease entity in its own right, by demonstrating in the largest cohort of patients so far: (i) a close association of MOG-IgG with a specific clinical phenotype (i.e., ON and/or myelitis); (ii) an increase in serum MOG-IgG titers during acute attacks; (iii) the presence of MOG-IgG in the CSF in the early phase of acute attacks in untreated patients; (iv) the presence of complement-activating anti-MOG antibodies of the IgG1 subclass both in the serum and in the CSF; and (v) absence of AQP4-IgG, an already well-established cause of optic nerve and spinal cord damage, in MOG-IgG-positive patients. Detailed clinical and paraclinical data were available for all 50 MOG-IgG-positive patients with ON and/or myelitis identified in this study and are comprehensively analyzed in parts 2 [36], 3 [37] and 4 [38] of this series.

## Abbreviations
ADEM: acute disseminated encephalomyelitis; AI: antibody index; AQP4: aquaporin-4; CBA: cell-based assay; CSF: cerebrospinal fluid; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; IM: immunomodulatory; IS: immunosuppressive; IVIG: intravenous immunoglobulins; IVMP: intravenous methylprednisolone; LETM: longitudinally extensive transverse myelitis; LP: lumbar puncture; MOG: myelin oligodendrocyte glycoprotein; MS: multiple sclerosis; NMO: neuromyelitis optica; NMOSD: neuromyelitis optica spectrum disorder; OCB: oligoclonal bands; OCT: optical coherence tomography; ON: optic neuritis; PEX: plasma exchange; PPMS: primary progressive MS; $Q_{Alb}$: albumin CSF/serum quotient; $Q_{IgG}$: IgG CSF/serum quotient; RRMS: relapsing remitting MS; SPMS: secondary progressive MS

## Availability of data and materials
The datasets generated during and/or analysed during the current study are not publicly available due to local data protection requirements but are available from the corresponding author on reasonable request in an anonymized fashion.

## Consent for publication
Participants gave written informed consent for publication.

## Ethics approval and consent to participate
The study was approved by the ethical review boards of the participating centers and patients gave written informed consent.

## Author details
[1]Molecular Neuroimmunology Group, Otto Meyerhof Center, Department of Neurology, University Hospital Heidelberg, Im Neuenheimer Feld 350, 69120 Heidelberg, Germany. [2]Department of Neurology, Charité-University Medicine Berlin, Berlin, Germany. [3]Department of Neurology, Ruhr University Bochum, Bochum, Germany. [4]NeuroCure Clinical Research Center and Clinical and Experimental Multiple Sclerosis Research Center, Department of Neurology, Charité University Medicine, Berlin, Germany. [5]Experimental and Clinical Research Center, Max Delbrueck Center for Molecular Medicine, Charité University Medicine Berlin, Berlin, Germany. [6]Department of Neurology and Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark. [7]Department of Neurology, Albert Ludwigs University, Freiburg, Germany. [8]Department of Neurology, Hannover Medical School, Hannover, Germany. [9]Department of Neurology, Heinrich Heine University, Düsseldorf, Germany. [10]Department of Neurology, University of Rostock, Rostock, Germany. [11]Department of Neurology, Julius Maximilians University, Würzburg, Germany. [12]IRCCS, C. Mondino National Neurological Institute, Pavia, Italy. [13]Centro di Riferimento Regionale SM, Azienda Ospedaliero Universitaria San Luigi Gonzaga, Orbassano, Italy. [14]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark. [15]Institute of Experimental Immunology, affiliated to Euroimmun AG, Lübeck, Germany. [16]Clinical Department of Neurology, Medical University Innsbruck, Innsbruck, Austria.

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# MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome

Sven Jarius[1*], Klemens Ruprecht[2], Ingo Kleiter[3], Nadja Borisow[4,5], Nasrin Asgari[6], Kalliopi Pitarokoili[3], Florence Pache[4,5], Oliver Stich[7], Lena-Alexandra Beume[7], Martin W. Hümmert[8], Marius Ringelstein[9], Corinna Trebst[8], Alexander Winkelmann[10], Alexander Schwarz[1], Mathias Buttmann[11], Hanna Zimmermann[2], Joseph Kuchling[2], Diego Franciotta[12], Marco Capobianco[13], Eberhard Siebert[14], Carsten Lukas[15], Mirjam Korporal-Kuhnke[1], Jürgen Haas[1], Kai Fechner[16], Alexander U. Brandt[2], Kathrin Schanda[17], Orhan Aktas[8], Friedemann Paul[4,5†], Markus Reindl[17†], and Brigitte Wildemann[1†]; in cooperation with the Neuromyelitis Optica Study Group (NEMOS)

## Abstract

**Background:** A subset of patients with neuromyelitis optica spectrum disorders (NMOSD) has been shown to be seropositive for myelin oligodendrocyte glycoprotein antibodies (MOG-IgG).

**Objective:** To describe the epidemiological, clinical, radiological, cerebrospinal fluid (CSF), and electrophysiological features of a large cohort of MOG-IgG-positive patients with optic neuritis (ON) and/or myelitis ($n = 50$) as well as attack and long-term treatment outcomes.

**Methods:** Retrospective multicenter study.

(Continued on next page)

* Correspondence: sven.jarius@med.uni-heidelberg.de
†Equal contributors
Brigitte Wildemann, Markus Reindl, and Friedemann Paul are equally contributing senior authors.
[1]Molecular Neuroimmunology Group, Department of Neurology, University of Heidelberg, Heidelberg, Germany
Full list of author information is available at the end of the article

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**Results:** The sex ratio was 1:2.8 (m:f). Median age at onset was 31 years (range 6-70). The disease followed a multiphasic course in 80% (median time-to-first-relapse 5 months; annualized relapse rate 0.92) and resulted in significant disability in 40% (mean follow-up 75 ± 46.5 months), with severe visual impairment or functional blindness (36%) and markedly impaired ambulation due to paresis or ataxia (25%) as the most common long-term sequelae. Functional blindness in one or both eyes was noted during at least one ON attack in around 70%. Perioptic enhancement was present in several patients. Besides acute tetra-/paraparesis, dysesthesia and pain were common in acute myelitis (70%). Longitudinally extensive spinal cord lesions were frequent, but short lesions occurred at least once in 44%. Fourty-one percent had a history of simultaneous ON and myelitis. Clinical or radiological involvement of the brain, brainstem, or cerebellum was present in 50%; extra-opticospinal symptoms included intractable nausea and vomiting and respiratory insufficiency (fatal in one). CSF pleocytosis (partly neutrophilic) was present in 70%, oligoclonal bands in only 13%, and blood-CSF-barrier dysfunction in 32%. Intravenous methylprednisolone (IVMP) and long-term immunosuppression were often effective; however, treatment failure leading to rapid accumulation of disability was noted in many patients as well as flare-ups after steroid withdrawal. Full recovery was achieved by plasma exchange in some cases, including after IVMP failure. Breakthrough attacks under azathioprine were linked to the drug-specific latency period and a lack of cotreatment with oral steroids. Methotrexate was effective in 5/6 patients. Interferon-beta was associated with ongoing or increasing disease activity. Rituximab and ofatumumab were effective in some patients. However, treatment with rituximab was followed by early relapses in several cases; end-of-dose relapses occurred 9-12 months after the first infusion. Coexisting autoimmunity was rare (9%). Wingerchuk's 2006 and 2015 criteria for NMO(SD) and Barkhof and McDonald criteria for multiple sclerosis (MS) were met by 28%, 32%, 15%, 33%, respectively; MS had been suspected in 36%. Disease onset or relapses were preceded by infection, vaccination, or pregnancy/delivery in several cases.

**Conclusion:** Our findings from a predominantly Caucasian cohort strongly argue against the concept of MOG-IgG denoting a mild and usually monophasic variant of NMOSD. The predominantly relapsing and often severe disease course and the short median time to second attack support the use of prophylactic long-term treatments in patients with MOG-IgG-positive ON and/or myelitis.

**Keywords:** Myelin oligodendrocyte glycoprotein antibodies (MOG-IgG), Autoantibodies, Neuromyelitis optica spectrum disorders (NMOSD), Aquaporin-4 antibodies (AQP4-IgG, NMO-IgG), Optic neuritis, Transverse myelitis, Longitudinally extensive transverse myelitis, Magnetic resonance imaging, Cerebrospinal fluid, Oligoclonal bands, Electrophysiology, Evoked potentials, Treatment, Therapy, Methotrexate, Azathioprine, Rituximab, Ofatumumab, Interferon beta, Glatiramer acetate, Natalizumab, Outcome, Pregnancy, Infections, Vaccination, Multiple sclerosis, Barkhof criteria, McDonald criteria, Wingerchuk criteria 2006 and 2015, IPND criteria, International consensus diagnostic criteria for neuromyelitis optica spectrum disorders

## Background

The term 'neuromyelitis optica' (NMO) was coined in 1894 and has since been used to refer to the simultaneous or successive occurrence of optic nerve and spinal cord inflammation [1]. In the majority of cases, the syndrome is caused by autoantibodies to aquaporin-4, the most common water channel in the central nervous system (AQP4-IgG) [2–5]. However, 10-20% of patients with NMO are negative for AQP4-IgG [6–9]. Recent studies by us and others have demonstrated the presence of IgG antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG) in a subset of patients with NMO as well as in patients with isolated ON or longitudinally extensive transverse myelitis (LETM), syndromes that are often *formes frustes* of NMO [10–12].

Most studies to date have found MOG-IgG exclusively in AQP4-IgG-negative patients [11–17]. Moreover, the histopathology of brain and spinal cord lesions of MOG-IgG-positive patients has been shown to differ from that of AQP4-IgG-posititve patients [18–20]. Finally, evidence from immunological studies suggests a direct pathogenic role of MOG-IgG both in vitro and in vivo [10, 21]. Accordingly, MOG-IgG-related NMO is now considered by many as a disease entity in its own right, immunopathogenetically distinct from its AQP4-IgG-positive counterpart. However, the cohorts included in previous clinical studies were relatively small (median 9 patients in [10–17, 22–24]) and the observation periods often short (median 24 months in [11–13, 15–17, 23–26]). Moreover, some previous studies did not, or not predominantly, include Caucasian patients [12, 15, 26], which is potentially important since genetic factors are thought to play a role in NMO [27].

In the present study, we systematically evaluated the clinical and paraclinical features of a large cohort of 50 almost exclusively Caucasian patients with MOG-IgG-positive optic neuritis (ON) and/or LETM. We report on (i) epidemiological features; (ii) clinical presentation

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at onset; (iii) disease course; (iv) time to second attack; (v) type and frequency of clinical attacks; (vi) brain, optic nerve, and spinal cord magnetic resonance imaging (MRI) features; (vii) cerebrospinal fluid (CSF) findings; (viii) electrophysiological features (VEP, SSEP); (ix) type and frequency of coexisting autoimmunity; (x) type and frequency of preceding infections; (xi) association with neoplasms; (xii) association with pregnancy and delivery; (xiii) treatment and outcome of acute attacks; (xiv) response to long-term treatments; and (xv) the long-term prognosis. In addition, we evaluated whether and how many MOG-IgG-positive patients with ON and/or myelitis met Wingerchuk's revised 2006 diagnostic criteria for NMO [28], the new 2015 international diagnostic consensus criteria for NMO spectrum disorders (NMOSD) [29], Barkhof's MRI criteria for MS, and/or McDonald's clinicoradiological criteria for MS.

The present study forms part of a series of articles on MOG-IgG in NMO and related disorders. In part 1, we investigated the frequency and syndrome specificity of MOG-IgG among patients with ON and/or LETM, reported on MOG-IgG titers in the long-term course of disease, and analyzed the origin of CSF MOG-IgG [30]. In part 3, we describe in detail the clinical course and presentation of a subgroup of patients with brainstem encephalitis and MOG-IgG-associated ON and/or LETM, a so far under-recognized manifestation of MOG-related autoimmunity [31]. Part 4 is dedicated to the visual system in MOG-IgG-positive patients with ON and reports findings from optical coherence tomography (OCT) in this entity [32].

## Methods

Clinical and paraclinical data of 50 MOG-IgG-positive patients from 12 non-pediatric academic centers were retrospectively evaluated; eight of the participating centers are members of the German Neuromyelitis optica Study Group (NEMOS) [33–37]. MOG-IgG was detected using an in-house cell-based assay (CBA) employing HEK293A cells transfected with full-length human MOG as previously described [10] and confirmed by means of a commercial fixed-cell based assay employing HEK293 cells transfected with full-length human MOG (Euroimmun, Lübeck, Germany) (see part 1 of this article series for details [30]). The study was approved by the institutional review boards of the participating centers, and patients gave written informed consent. Averages are given as median and range or mean and standard deviation as indicated. Fisher's exact test was used to compare frequencies between groups and the Mann-Whitney U test to compare medians between groups. Due to the exploratory nature of this study no Bonferroni correction was performed. *P* values <0.05 were considered statistically significant.

## Case reports

As reliable cell-based assays for the detection of MOG-IgG have become available only recently, large and comprehensive case series illustrating the broad and heterogeneous spectrum of clinical manifestations, disease courses, and radiological presentations are lacking so far. We therefore decided to present, in addition to descriptive statistical data, detailed reports on all cases evaluated in order to draw for the first time a more vivid 'real-life' picture of this rare disorder than statistical analyses alone could provide. Moreover, only detailed case descriptions allow evaluation of treatment responses and outcomes in a meaningful way in a retrospective setting. This is important, since randomized treatment trials in MOG-IgG-positive ON or myelitis do not exist so far and will not be performed in the near future due to the rarity of the condition. The reports are to be found in the Appendix of this paper and in the *Case reports* section in part 3 of this article series [31].

## Results

### Epidemiological findings

Thirty-seven of the 50 MOG-IgG-positive patients were female, corresponding to a sex ratio of 1:2.8 (m:f) (Fig. 1a). Median age at onset was 31 years (35.5 years in patients presenting with isolated ON [$N = 32$] and 28.5 years in the remainder [$N = 18$]; $p < 0.04$) with a broad range of 6 to 70 years. 3 patients were > =60 years of age at onset, and 8 patients were under 18 at first attack (including $4 \leq$ 12 years) (Fig. 1b). Fourty-nine of the 50 patients (98%) were of Caucasian and 1 of Asian descent. Symptoms had started between Jul 1973 and Apr 2016. The mean observation period since disease onset was $75 \pm 46.5$ months (range 1-507 months). In line with the fact that many MOG-IgG-positive patients develop ON and myelitis only successively, the mean observation period was longer in patients with a history both of ON and of myelitis at last follow-up (88.6 months; $N = 22$) than in patients with a history of either ON but no myelitis or myelitis but not ON (64.6 months; $N = 28$).

### Disease course

Fourty of 50 MOG-IgG-positive patients (80%) had a relapsing disease course. In the remaining 10 cases only a single attack had occurred at last follow-up. The proportion of patients with a monophasic course declined with increasing observation time (Fig. 2, *upper panel*). If only patients with a very long observation period (≥8 years) are considered, 93% (13/14) had a recurrent course (Fig. 2, *lower panel*). In line with this finding, the median observation time was shorter in the 'monophasic' than in the relapsing cases (26 vs. 52.5 months). The proportion of patients with a relapsing disease

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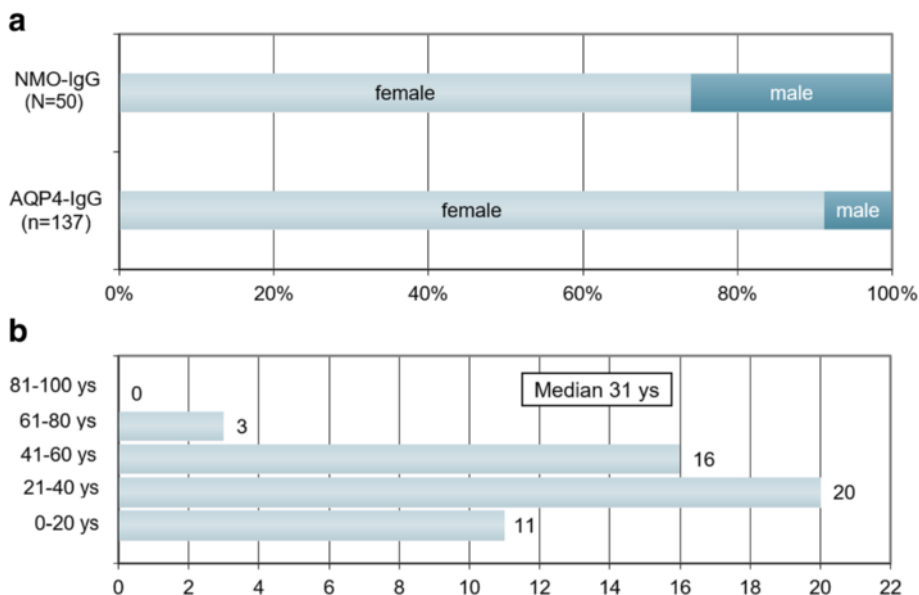


**Fig. 1** Sex ratio and age distribution. **a** Sex ratio in MOG-IgG-positive patients with ON and/or LETM compared with AQP4-IgG-positive ON and/or LETM (the latter data are taken from ref. [34]). **b** Age distribution at disease onset in 50 MOG-IgG-positive patients with ON and/or myelitis
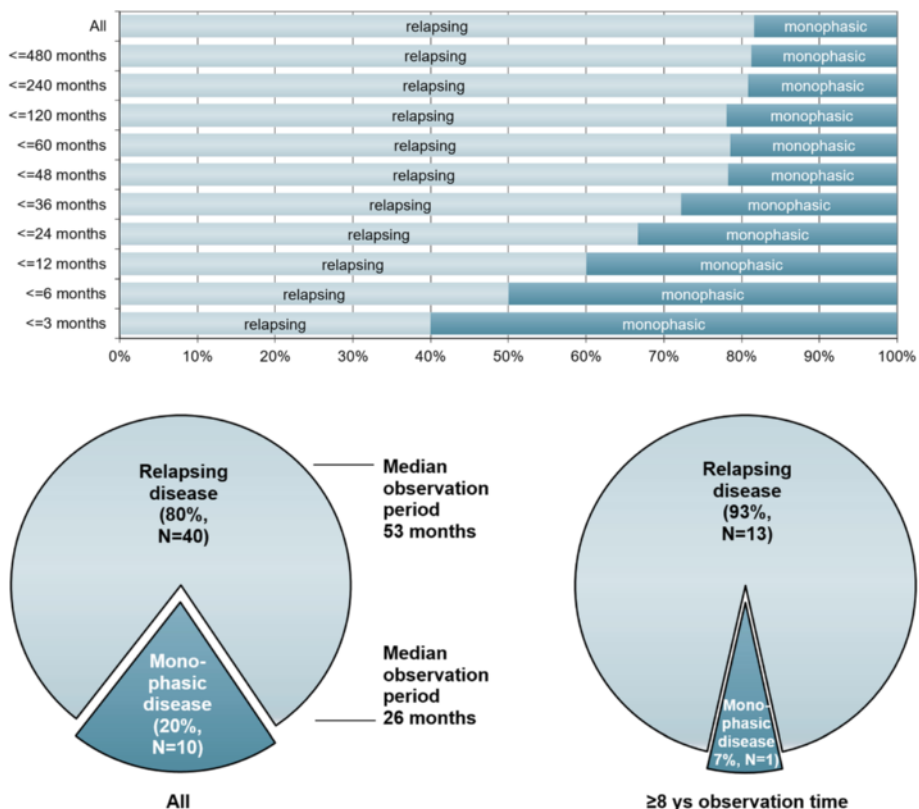


**Fig. 2** Disease course in relation to observation time in 50 MOG-IgG-positive patients with ON and/or myelitis. Upper panel: Note the decrease in the proportion of monophasic cases with increasing observation time; however, in some patients no relapse has occurred more than 10 years after the initial attack. Lower panel: Note the shorter observation time in the 'monophasic' group (*left lower panel*) and the lower percentage of non-relapsing cases among patients with a long observation period (≥8 years; *right lower panel*)

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course did not differ significantly between female (83.8% [31/37]) and male (69.2% [9/13]) patients.

Symptoms developed acutely or subacutely in the vast majority of cases; progressive deterioration of symptoms was very rare (at least once in 3/46 or 7%) and reported only in patients with myelitis.

### Clinical presentation during acute attacks

Overall, 276 clinically apparent attacks in 50 patients were documented. 205 attacks clinically affected the optic nerve, 73 the spinal cord, 20 the brainstem, 3 the cerebellum, and 9 the supratentorial brain. 44/50 (88%) patients developed at least once acute ON, 28/50 (56%) at least once acute myelitis, 12/50 (24%) at least once a brainstem attack, 2/50 (4%) acute cerebellitis, and 7/50 (14%) acute supratentorial encephalitis (Fig. 3, *upper panel*).

At last follow-up, 26/50 (52%) patients had developed at least two different clinical syndromes (i.e., combinations of ON, myelitis, brainstem encephalitis, cerebellitis, and/or supratentorial encephalitis), either simultaneously or successively. Of these, 22 (84.6%) had experienced attacks both of ON and of myelitis at last follow-up (corresponding to 44% [22/50] of the total cohort). Another 22 (44%) had a history of ON but not of myelitis (recurrent in 15 or 68.2%), and 6 (12%) had a history of myelitis but not ON (recurrent in 4; LETM in all) at last follow-up (Fig. 3, *lower panel*).

Myelitis and ON had occurred simultaneously (with and without additional brainstem or brain involvement) at least once in 9/22 (40.9%) patients with a history of both ON and myelitis at last follow-up (and in 18% or 9/50 in the total cohort).

Overall, 16/50 (32%) patients presented at least once with more than one syndrome during a single attack (more than once in 10/16). While 15 attacks of myelitis (without ON) in 11 patients were associated with clinical signs and symptoms of simultaneous brain or brainstem involvement, only 1 attack of ON (without myelitis) in 1 patient had this association. Clinically inapparent spinal cord, brain, or brainstem involvement was detected in further patients by MRI (see Brain MRI findings below and part 3 of this article series [31] for details).

### Symptoms associated with acute myelitis

Symptoms present at least once during attacks of myelitis included tetraparesis in 8/29 (27.6%) patients, paraparesis in 14/29 (48.3%), hemiparesis in 2/29 (6.9%), and monoparesis in 2/29 (6.9%). Paresis was severe (BMRC grades ≤2) at least once in 6/29 (20.7%) patients. Attacks included at least once pain and dysesthesia in 19/28 (67.9%) patients and were purely sensory in 15/29 (51.7%). Sensory symptoms included also Lhermitte's sign. Bladder and/or bowel and/or
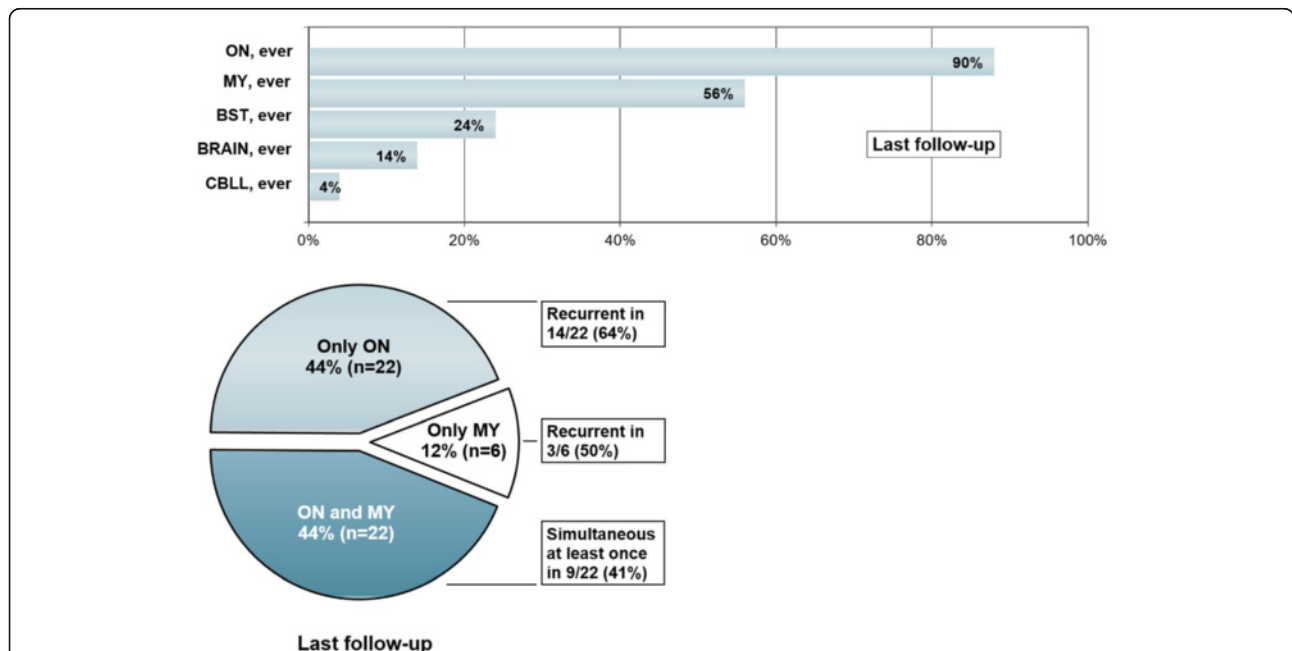


Fig. 3 Attack history at last follow-up. *Upper panel:* Frequencies of MOG-IgG-positive patients (*N* = 50) with a history of clinically manifest acute optic neuritis (ON), myelitis (MY), brainstem encephalitis (BST), supratentorial encephalitis (BRAIN), and cerebellitis (CBLL) at last follow-up. *Lower panel:* Frequencies of MOG-IgG patients with a history of optic neuritis (ON) and myelitis, ON but not myelitis, and myelitis (LETM in all cases) but not ON, respectively, at last follow-up (*n* = 50)

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erectile dysfunction occurred at least once in 20/29 (69%) patients (Fig. 4).

### Symptoms associated with acute ON

In 36/39 (92.3%) patients ON was associated with reduced high-contrast visual acuity (VA) as determined using a Snellen chart. In one patient, low-contrast but not high-contrast VA was reduced; in another patient with hazy vision but normal high-contrast VA, low-contrast VA was not tested. In a third patient, impaired color perception and papilledema were the only clinical symptoms.

Most patients with ON reported retrobulbar pain and/ or pain on eye movement. Disturbed color vision including color desaturation was reported in some patients, but was not systematically examined in all patients.

Attack-related functional blindness (defined as VA ≤0.1) in one or both eyes occurred at least once in 27/39 (69.2%) patients and VA ≤0.5 was present at least once in 33/39 (84.6%) during acute ON attacks (Fig. 5). Both eyes were affected simultaneously ('bilateral ON') at least once in 22/43 (51.2%) patients, and scotoma was noted at least once in 23/35 (65.7%) with available data.

### Other symptoms

Brainstem symptoms occurred in 12 MOG-IgG-positive patients. A detailed analysis can be found in part 3 of this article series [31]. Respiratory insufficiency due to brainstem encephalitis (2 ×) or myelitis (1 ×) occurred at least once in 3/48 (6.3%) patients with available data (median observation time 50.5 months; range 1-507) and was fatal in one of these two cases. Two patients had clinical signs and symptoms indicating cerebellar involvement. These included limb, gait, and stance ataxia with or without accompanying dysarthria. Sensory ataxia was noted in others.

Supratentorial brain lesions were symptomatic in 7 patients. These patients showed (sometimes severe)

headache, fatigue, psychomotor slowing, disorientation, impaired consciousness/somnolence, hemihypesthesia, meningism, and photophobia.

Of note, several further patients had brainstem, cerebellar, and/or supratentorial brain lesions (see section Brain MRI findings below and Appendix as well as part 3 of this article series [31]) but no clinical symptoms attributable to those lesions.

### Presentation at onset

ON was clearly the most common manifestation at disease onset (present in 37/50 [74%] patients), followed by myelitis (17/50 [34%]), brainstem encephalitis (4/50 [8%]) and symptoms attributable to brain (3/50 [6%]) or cerebellar lesions (1/50 [2%]). While in some patients only one site was clinically affected, multiple manifestations were noted in others: thirty-two of 50 patients (64%) initially presented with isolated ON; 9 (18%) with isolated myelitis; 5 (10%) with simultaneous ON and myelitis (additional brainstem involvement in 2); 1 (2%) with simultaneous myelitis, rhombencephalitis, and supratentorial encephalitis; 2 (4%) with myelitis and supratentorial encephalitis; and 1 (2%) with isolated brainstem encephalitis (Fig. 6). Accordingly, clinical evidence for dissemination in space (here understood as involvement of more than one of the following anatomical sites: optic nerve, spinal cord, prosencephalon, brainstem, and/or cerebellum) was present at onset in 8/50 (16%) patients (compared to 16/50 (32%) if the entire observation period is considered).

In the subgroup of patients with multiple manifestations at follow-up (including NMO and any other combinations of ON, myelitis, brainstem encephalitis, cerebellitis, and/or supratentorial encephalitis) (N = 26), disease had started with an isolated syndrome in 17 (65.4%) (isolated ON in 12 [46.2%] and isolated myelitis in 5 [36.4%]); with simultaneous ON and myelitis in 4 (15.4%); with simultaneous



**Fig. 4** Symptoms present during attacks involving acute myelitis (N = 28 patients). BB = bladder and/or bowel.

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**Fig. 5** High-contrast visual acuity (VA) loss during acute ON (N = 39 patients). Blind: complete or functional blindness (VA ≤0.1) in one or both eyes at least once; severe: VA ≤0.5; moderate: VA ≤0.75; mild: ≤1.0; none: high-contrast VA not affected, but low-contrast visual loss, color desaturation, and/or scotoma present

ON, myelitis, and brainstem encephalitis in 1 (3.8%); with simultaneous myelitis, rhombencephalitis and supratentorial encephalitis in 1 (3.8%); and with simultaneous myelitis and supratentorial encephalitis in 2 (7.7%).

In the subgroup of patients meeting Wingerchuk's 2006 criteria at last follow-up, 3/14 (21.4%) had simultaneous ON and myelitis at onset (exclusively or in combination with brain, brainstem or cerebellar symptoms) and 3/8 (37.5%) of those with ON at disease onset, including 2 of the 3 cases with simultaneous ON and myelitis – presented with bilateral ON.

The initial attack affected both eyes in 15/37 (40.5%) of all patients with ON at onset and in 11/32 (34.4%) of all patients with isolated ON at onset; overall, 15/50 (30%) patients had bilateral ON at onset (partly in combination with other manifestations).

The first attack of myelitis was clinically characterized by tetraparesis in 5 patients and by paraparesis in 6; in 5 patients, myelitis was associated with purely sensory and/or autonomous symptoms at onset. In 2 patients, respiratory dysfunction was among the presenting symptoms.



**Fig. 6** Presentation at onset. ON = optic neuritis, MY = myelitis, LETM = longitudinally extensive transverse myelitis, BST = brainstem encephalitis, BRAIN = supratentorial encephalitis, CBLL = cerebellitis. [§] Includes two cases of simultaneous ON, myelitis and brainstem encephalitis at onset. [*]Other presentations included simultaneous myelitis, rhombencephalitis and supratentorial encephalitis; simultaneous myelitis and supratentorial encephalitis (2 ×); and isolated brainstem encephalitis. No data on spinal cord lesion length at disease onset were available from 1 patient

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### Time to second attack

Among the MOG-IgG-positive patients with more than one documented attack and available data, the median time between the first and the second attack was just 5 months (range, 1-492; N = 38) (Fig. 7). There was no significant difference between patients with ON at onset (median of 6 months to next relapse; range 1-492) and patients with myelitis at onset (median 4 months; range 1-23). The median interval between first and second attack was slightly longer among patients with full recovery from the first attack (n = 17) than in the remaining patients (6 vs. 3.5 months; p = n.s.).

### Presentation at second attack

The most common manifestation (isolated [N = 22] or in combination with other syndromes) at second attack was ON (21/23 [91.3%]), which was mostly unilateral (21/23 [91.3%]; no data in one case). Other presentations at second attack included isolated myelitis (N = 12), isolated supratentorial encephalitis (N = 1), myelitis with brain or brainstem involvement (N = 2), and simultaneous ON and myelitis with brain involvement.

The initial presentation had high predictive value for the second attack: in 18 of 25 patients (72%) initially presenting with isolated ON, the second event was isolated ON again (and in 19/25 or 76% patients, ON was among the presenting manifestations); similarly, in 6/8 (75%) patients with isolated myelitis the second event was also isolated myelitis. Overall, at least one manifestation present at onset (ON, myelitis, brainstem encephalitis, cerebellitis, supratentorial encephalitis) was present also at the second attack in 31/40 (78%) patients with a recurrent disease course.

Of note, both optic nerves were affected clinically early in the disease course: in 6/10 (60%) patients with available data who experienced a unilateral ON at disease onset and ON at first relapse, the second attack affected the previously unaffected eye (or both eyes). Overall, 21/34 (62%) patients

had a history of ON in both optic nerves (simultaneously or subsequently) already after the second event.

### Annualized relapse rate

If all patients with an observation time of ≥12 months are considered, the median annualized relapse rate (ARR) was 0.83 (range 0.05-6.92) in the total group (n = 39) and 0.92 (range 0.05-6.92) among patients with a recurrent disease course (n = 34). It was higher among female than among male patients both in the total cohort (0.92 vs. 0.535; N = 29 and 10, respectively) and in the relapsing subgroup (0.92 vs. 0.83; N = 27 and 7, respectively), but the differences were not statistically significant.

The median ARR was highest (1.17; range 0.05-4.2; N = 19) in relapsing patients with a history of both ON and myelitis (n = 21), compared with 0.8 (range 0.5-6.92) among patients with recurrent isolated ON but no myelitis (n = 12) and 0.57 and 0.83 in the two only patients with recurrent isolated LETM but no ON and an observation time ≥12 months.

### Brain MRI findings

Supratentorial MRI abnormalities were present at onset in 17/48 (35.4%) MOG-IgG-positive patients and infratentorial MRI lesions in 7/48 (14.6%). Supratentorial MRI lesions at onset included periventricular lesions; lesions in the corpus callosum (some of them confluent); frontal, parietal, temporoparietal, and occipital deep white matter lesions; subcortical or juxtacortical lesions (including insular lesions); and, in one case, lesions in the thalamus (pulvinar) and in the basal ganglia (putamen) (Fig. 8). In one patient leptomeningeal enhancement was noted at onset (Fig. 8, panel d), and in one both optic tracts were affected (Fig. 8, panel c).

Infratentorial lesions at onset included lesions in the cerebral peduncles, the pons (including tegmentum),
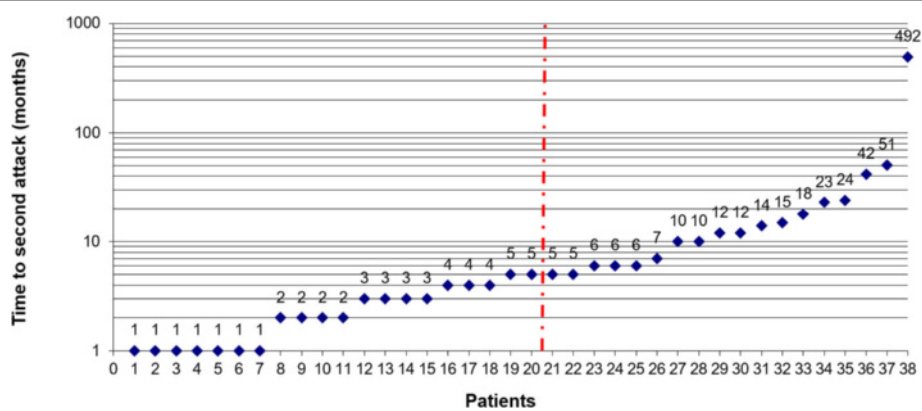


**Fig. 7** Time to first relapse in months. The *red line* indicates the median. The first relapse was defined as a new clinical attack occurring more than 30 days after onset of the initial attack. No exact data was available in two cases

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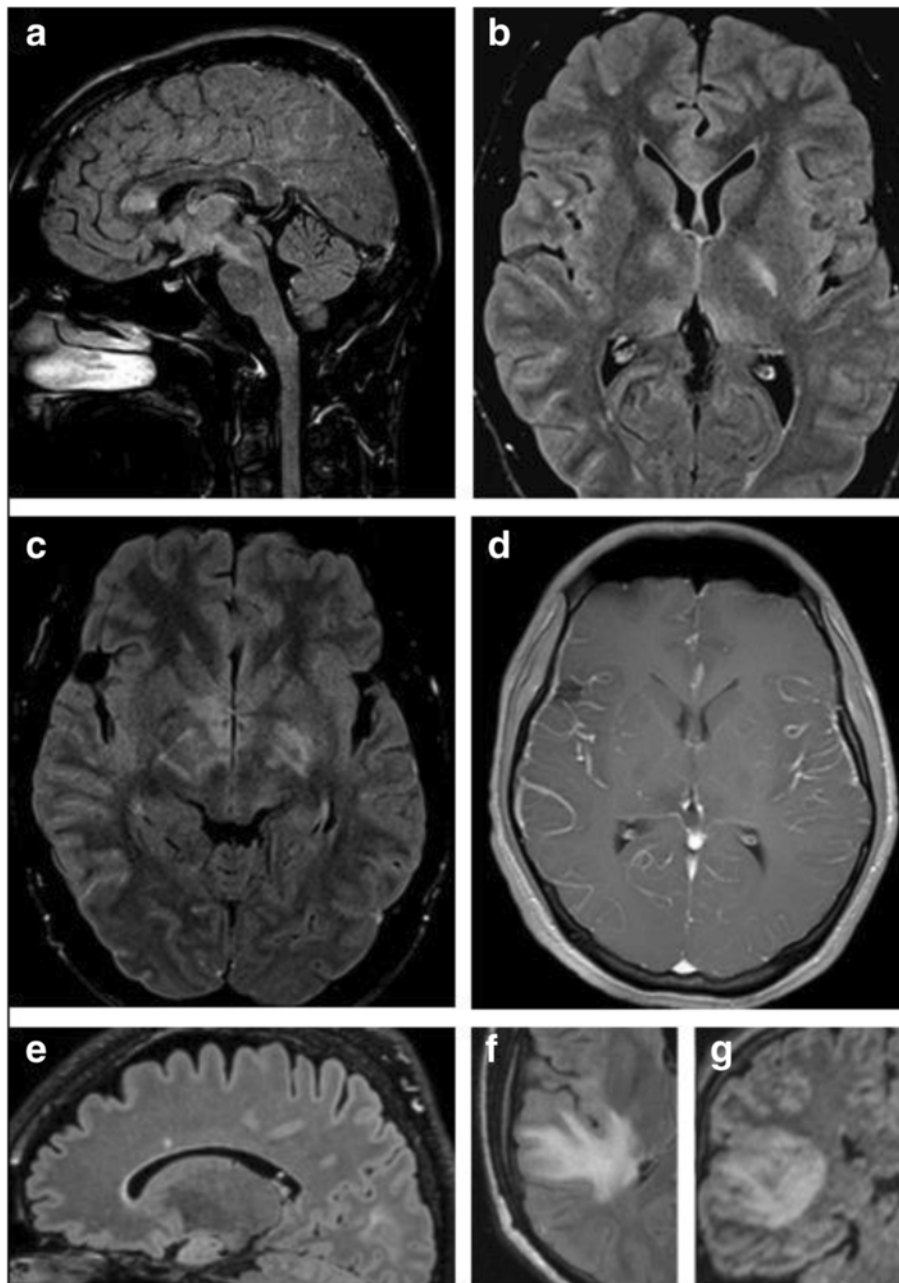


**Fig. 8** Examples of brain lesions detected by MRI. **a** Sagittal FLAIR image showing callosal lesions as well as lesions extending from the diencephalon to the pons (see case 8 in part 3 of this article series [31] for details). **b** Axial FLAIR MRI demonstrating lesions in the basal ganglia, juxtacortically on the right side, und in the genu corporis callosi in the same patient. **c** Axial FLAIR image at the diencephalic level revealing periependymal lesions (in addition to basal ganglia lesions). **d** Axial T1-weighted image with Gd demonstrating leptomeningeal enhancement (see case 8 in part 3 [31]). E: Sagittal MRI showing a callosal lesion (see case 10 in the Appendix for details). **f**, **g** Axial T2-weighted (**f**) and coronal FLAIR (**g**) images showing large, confluent T2 hyperintense lesions in the right temporal lobe (see case 7 in part 3 [31])

medulla oblongata, cerebellar hemispheres, and cerebellar peduncles (see part 3 of this series [31] for details).

Taking not only the first but all MRIs into account, 22/47 (46.8%) patients had supratentorial brain lesions at least once; brainstem lesions occurred at least once in 14/48 (29.2%); and cerebellar lesions were noted at least

once in 6/48 (12.5%) (see part 3 of this series for details [31]). Lesions affected the periventricular white matter, deep white matter (in some cases large and confluent) and corona radiata, sub- or juxtacortical white matter, corpus callosum, thalamus (pulvinar), basal ganglia, cerebral peduncles, pons (ventral, median, tegmentum), medulla

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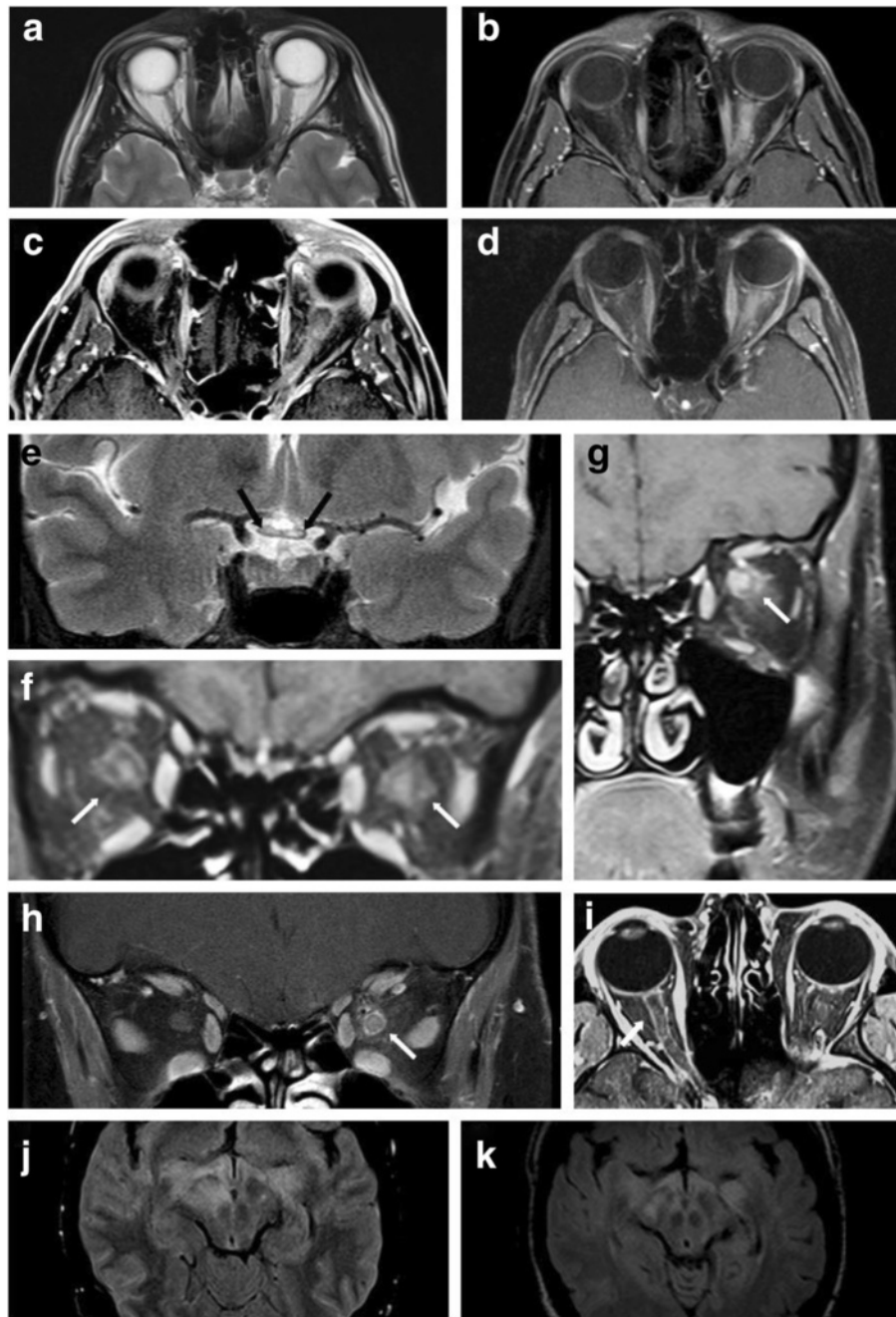


**Fig. 9** Examples of optic nerve lesions detected by MRI. **a**, **b** T2-weighted (**a**) and T1-weighted (B, with Gd) MRI reveals swelling and Gd enhancement of the left optic nerve. **c**, **d** (fat-suppressed): Longitudinal extensive Gd enhancement of the optic nerve (see cases 9 and 12 in part 3 [31] for details). **e** Longitudinally extensive bilateral optic neuritis extending from the chiasm (E, *black arrows*) into the orbits, affecting the left more than the right optic nerve. **f-h** Coronal T1-weighted MRIs display marked contrast enhancement of the intraorbital optic nerve as well as concurrent enhancement of the perioptic nerve sheath, partly extending in the surrounding orbital fat, in patients with acute ON (cases 11, 29 and 19). I: Axial T1-weighted MRI shows Gd enhancement along the right optic nerve in another patient (see case 13 in part 3 of this article series [31]). **j**, **k** Axial FLAIR imaging demonstrates bilateral lesions in the optic tract (see case 8 in part 3 [31] for details) (**j** MRI at attack onset; **k** follow-up MRI 1 month later)

oblongata (including the area postrema and the peria-queductal gray), cerebellar hemispheres, and cerebellar peduncles and were partly Gd-enhancing. Lesions were found in the frontal, parietal, temporal, and occipital lobes and in the insula. Taking the entire course of disease into account, callosal lesions were present at least once in 8/48 (16.7%) patients and periventricular lesions in 12/47 (25.5%). Callosal lesions were

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longitudinally extensive (more than half the length of the corpus callosum), as considered typical for AQP4-IgG-positive NMOSD [29], in 1/8 (12.5%).

### Optic nerve MRI findings

MRI signs of ON were present in at least 24/44 (54.5%) patients with available data, all of whom had a history of clinical ON (Fig. 9). Intraorbital swelling of the optic nerve was noted at least once in 13/21 (61.9%) patients, and contrast enhancement in 20/21 (95.2%). A longitudinally extensive optic nerve lesion (more than half the length of the nerve) ($n = 6$) and/or involvement of the optic chiasm ($n = 4$), two findings previously considered typical for AQP4-IgG-positive NMO [29], were present during acute ON in 8/26 (30.8%) cases with available data. Signs of optic nerve atrophy were noted in at least 5 patients and involved the optic chiasm in at least one of them. However, post-chiasmatic parts of the optic pathway were also affected in individual patients: as mentioned above, one patient had optic tract lesions, and occipital lobe white matter lesions were documented in four cases.

Of particular note, in 11/28 (39.3%) patients with available data, perioptic contrast enhancement, i.e. gadolinium enhancement within the nerve sheath and the immediately surrounding orbital tissues, was present during acute ON (Fig. 9). The remaining patients had either no history of ON or no or no suitable post-contrast orbital MRI was performed or retrospectively available for re-analysis and the presence of absence of perioptic enhancement was not mentioned in their MRI reports.

### Spinal cord MRI findings

MRI signs of spinal cord inflammation were present in 29/44 (65.9%) patients with available data, including 27/28 (96.4%) with a history of clinical myelitis (Fig. 10).

Spinal MRI was performed also in 16 patients without a history of clinically apparent myelitis and showed a spinal cord lesion extending over 2 segments in 2 of them.

In 20 out of the 28 (71.4%) patients with a history of clinical myelitis and available data, two or more lesions were present simultaneously (i.e., in the same MRI) at least once.

Spinal cord lesions on MRI extending over three or more vertebral segments (VS), i.e., so-called LETM lesions, were documented in 21/29 (72.4%) patients at least once. LETM lesions were present during the first attack in 11/17 (64.7%) patients initially presenting with acute myelits.

By contrast, in 8 patients exclusively short lesions (<3 VS), i.e., so-called non-longitudinally extensive transverse myelitis (NETM) lesions, were documented over the entire observation period. Of potential differential diagnostic importance, spinal cord MRI showed one or more NETM lesions but no LETM lesions at disease onset in 6/17 (35.3%) patients initially presenting with acute myelitis (alone or in combination with other syndromes). If all available MRIs are considered, MRI lesions extended over
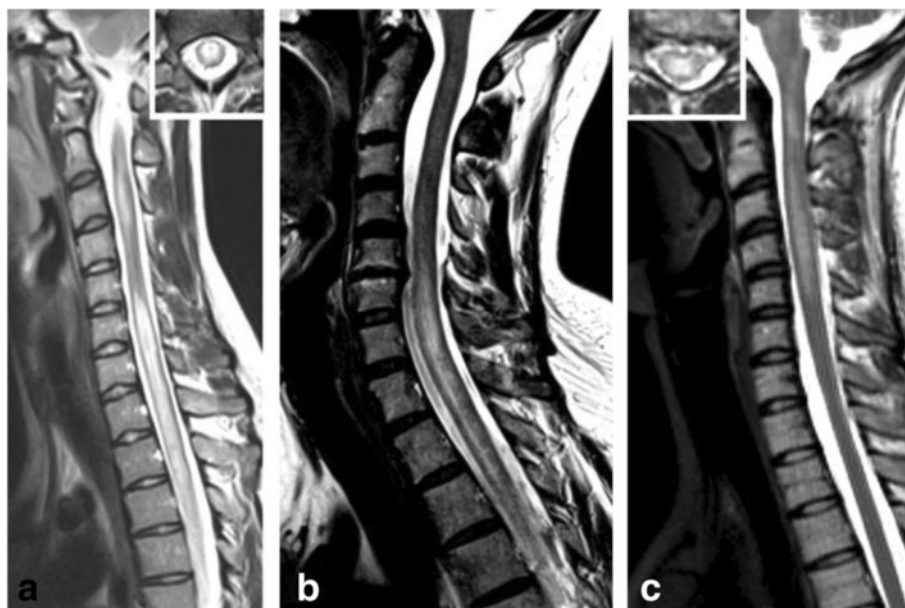


**Fig. 10** Examples of spinal cord MRI findings. **a** Sagittal T2-weighted spinal MRI performed at disease onset revealed a large longitudinal centrally located lesion extending over the entire spinal cord as well as swelling of the cord. **b** Longitudinal extensive central spinal cord T2 lesion in another patient. **c** T2-hyperintense lesions extending from the pontomedullary junction throughout the cervical cord to C5 in a third patient. The *insets* in A and C show axial sections of the thoracic cord at lesion level

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fewer than three segments during acute attacks of myelitis in 12/27 (44.4%) patients.

The median length of all documented LETM lesions ($n = 32$) was 4 VS (range 3-20) and that of all documented NETM lesions ($n = 44$) was 1.5 VS (range 1-2). If all spinal cord lesions with available data are considered ($n = 76$), i.e., both LETM and NETM lesions (including NETM lesions present in addition to LETM lesions in the same MRI), the median longitudinal extension was 2 VS (range 1-20). Finally, the median length of the longest spinal cord lesion (LETM or NETM) ever observed in each patient was 5 VS (range, 1-20; $N = 27$) if all patients with available MRI data were considered and 5 (range, 1-20; $N = 26$) if only patients with clinical evidence for myelitis were considered.

Swelling of the spinal cord was noted at least once in 19/27 (70.4%) patients and contrast enhancement in 19/28 (67.9%). Signs of necrosis of the spinal cord were noted in 0/23 (0%) patients with available data.

Spinal cord lesions were located in the cervical spinal cord at least once in 23/28 (82.1%) patients and in the thoracic spinal cord at least once in 21/28 (75%). Lumbar and conus lesions were documented only in 3/27 (11.1%) and 3/27 (11.1%) patients, respectively. Taking all available spinal cord MRIs into account, cervical lesions were present in 44/81 (54.3%) MRIs, thoracic lesions in 31/81 (38.3%), lumbar lesions in 4/81 (4.9%), and the conus was affected in 3/81 (3.7%). However, as a limitation, not all MRIs performed showed the entire spinal cord, and spinal cord MRI data were absent for 18 myelitis attacks in 8 patients.

Information on intramedullary lesion location was available for 34 lesions in 20 MOG-IgG-positive patients. Lesions were located predominantly in the central portion of the spinal cord in 17 MRIs and predominantly in the peripheral portion in another 17 MRIs.

The spinal cord MRI was normal during 2 attacks; in both cases, symptoms were purely sensory (paresthesia and hyp- and dysesthesia, respectively). Of note, a total of five asymptomatic spinal cord lesions were noted in two patients (in addition to brainstem lesions in one) with a history of ON but no clinical evidence of myelitis over the course of disease.

### Evaluation of Barkhof's and Paty's MRI criteria for MS

Seven of 46 (15.2%) MOG-IgG-positive patients with a history of myelitis and/or ON and 7/26 (26.9%) of those with brain lesions met Barkhof's MRI criteria for MS at least once [38]. However, at least 2 of the 7 patients meeting Barkhof's criteria also had one or more NMOSD-typical lesions at least once.

The revised 2006 diagnostic criteria for NMO [28] required a brain MRI at disease onset that does not meet Paty's MRI criteria for MS [39] if either no LETM lesion is present or NMO-IgG is negative. Accordingly, Paty's criteria were evaluated only at disease onset. In the present cohort, the initial MRI of 12 out of 48 (25%) MOG-IgG-positive patients with available data met Paty's criteria.

### Intrathecal IgG synthesis

Data on CSF-restricted oligoclonal IgG bands (OCB) were available from 45/50 (90%) MOG-IgG-positive patients. Pattern 2 or 3 OCB [40] indicative of intrathecal IgG synthesis were positive at least once only in 6/45 (13.3%). A second lumbar puncture was performed in 2 out of the 6 OCB-positive patients, in both of whom OCB remained positive.

Patients with classical MS display a polyspecific, intrathecal humoral immune response to neurotropic viruses such as measles, rubella, and varicella zoster virus (the so-called MRZ reaction, MRZR) [41–44]. MRZR was tested in 11 MOG-IgG-positive patients (2 x ON + myelitis; 1 x ON + myelitis + brainstem encephalitis; 1 x myelitis + brainstem encephalitis; 3 x LETM; 5 x ON) and was negative in all of them.

### CSF white cell counts

White cell counts (WCC) in the CSF were documented at least once in 46 MOG-IgG-positive patients and were elevated (>5/μl) in 32 (69.6%). In those patients with pleocytosis, WCC ranged between 6 and 306 cells/μl (median 33; quartile range 13-125). WCC ≥100 cell/μl were present at least once in 9/32 (28.1%) patients. Neutrophil granulocytes were present at least once in 9/14 (64.3%) patients with pleocytosis and available data (median 22% of all white cells; range 3-69%).

### Blood-CSF barrier function

An increased albumin CSF/serum ratio (QAlb) reflects a disturbed blood-CSF barrier (BCSFB) function caused by structural damage and/or a reduced CSF flow rate [45]. QAlb was determined in 37 MOG-IgG-positive patients and was elevated in 12 (32.4%). Blood-CSF barrier dysfunction was present both among patients with a history of isolated ON (2/15; 13.3%) and, more frequently, in patients with a history of spinal cord and/or brain/brainstem involvement (10/21; 47.6%).

### Visual evoked potentials

Data on visual evoked potentials (VEP) were available from 47 MOG-IgG-positive patients. A delayed P100 latency was noted at least once in 34 (72.3%); in another 6 (12.8%) patients latencies could not be determined since potentials were lost due to severe optic nerve damage.

Only 41 (78.7%) of the 47 patients examined had a history of clinically manifest ON; in 31 of these 41 patients (75.6%) P100 latency was delayed, and in 6 further patients (14.6%) latencies could not be determined.

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The remaining 6 patients had a history of myelitis (LETM in all cases) but no history of clinically manifest ON. 3 of those 6 had delayed P100 latencies in at least one eye, indicating that subclinical optic nerve damage might be relatively frequent in MOG-IgG-positive patients with myelitis.

In 23/41 (56.1%) patients, all of whom had a history of clinical ON, VEP amplitudes were reduced ($n = 16$) or lost ($n = 7$) at least once. In all but one patient with reduced amplitudes, P100 latencies were also delayed at some point in time, but not vice versa.

### Somatosensory evoked potentials

Data on somatosensory evoked potentials (SSEP) were available from 39 MOG-IgG-positive patients, including 24 with a history of clinically manifest myelitis. SSEP were delayed, reduced in amplitude, or lost in 19/39 (46.2%), including in 16/24 (66.7%) with a history of clinical myelitis and available data. Of note, 3 patients with no clinical history of myelitis had SSEP abnormalities suggestive of subclinical spinal cord damage (none of them displayed unequivocal spinal cord MRI abnormalities).

### Ophthalmoscopic findings

Fundoscopy revealed uni- or bilateral papillitis or papilledema in at least 15 patients with acute ON, suggesting inflammation of the anterior part of the optic nerve. The true prevalence of papillitis could be higher, however, since ophthalmoscopic data were not available from all patients. In case 6 (see Appendix), papilledema was described as marked (3 dpt) at first ON and as mild at second and third ON, while later on the optic disk was described as atrophic and pale. Optic atrophy as detected by fundoscopy was noted at last follow-up in 13/22 (59.1%) patients with available data.

### Evaluation of the 2010 McDonald criteria for MS

If MOG-IgG seropositivity is not considered to constitute per se a "better explanation" [46], i.e., based solely on clinicoradiological criteria, 15/46 or 33% of the patients with available data met the most current diagnostic criteria for MS [46] (Table 1). Taking only MOG-IgG-positive patients with a history of both ON and myelitis into account, 10/20 or 50% with available data fulfilled those criteria, compared with 7/31 or 23% with a history of ON but not myelitis or of myelitis but not ON at last follow-up. If only patients with a relapsing disease course are taken into account, 44% (15/34) met the 2010 McDonald criteria.

### Evaluation of the 2006 criteria for NMO

63.6% (14/22) of all MOG-IgG-positive patients with a history of both ON and myelitis met Wingerchuk's 2006 revised diagnostic criteria for NMO [28] (Table 1). Of the 8 patients with ON and myelitis who did not meet

**Table 1** Patient numbers and diagnoses

| Diagnostic categories | N (%) |
|---|---|
| History of ON and/or MY | 50/50 (100%) |
| History of ON | 44/50 (88%) |
| History of myelitis | 28/50 (56%) |
| Meeting Wingerchuk's 2006 criteria for NMO[a] | 14/50 (28%) |
| Meeting 2015 consensus criteria for NMOSD[b] | 16/50 (32%) |
| Meeting 2010 McDonald criteria for MS[c] | 15/46 (33%) |
| History of ON and of myelitis | 22/50 (44%) |
| Meeting Wingerchuk's 2006 criteria for NMO[a] | 14/22 (63.6%) |
| Meeting 2015 consensus criteria for NMOSD[b] | 15/22 (68.2%) |
| History of ON but not of myelitis | 22 (44%) |
| Meeting Wingerchuk's 2006 criteria for NMO[a] | 0/22 (0%) |
| Meeting 2015 consensus criteria for NMOSD[b] | 1/22 (4.5%) |
| History of myelitis but not of ON | 6/50 (12%) |
| Meeting Wingerchuk's 2006 criteria for NMO[a] | 0/6 (0%) |
| Meeting 2015 consensus criteria for NMOSD[b] | 0/6 (0%) |

*MS* multiple sclerosis, *NMO* neuromyelitis optica, *NMOSD* NMO spectrum disorder, *ON* optic neuritis. [a]see ref. [28], [b]see ref. [29], [c]see ref. [46]

Wingerchuk's 2006 criteria, two had an LETM lesion but the first brain MRI met Paty's criteria for MS; five did not meet Paty's criteria at onset but spinal cord lesions extended over fewer than three vertebral segments; and one met Paty's criteria at onset and had no LETM lesion.

Twenty eight patients had a history of ON but not myelitis or a history of myelitis but not ON (both with and without brain involvement) and did therefore not meet the 2006 diagnostic criteria. Taking the total cohort into account, 28% (14/50) of all patients met the 2006 criteria for NMO. Seven out of 43 (16%) patients with available data fulfilled both the clinicoradiological 2006 criteria for NMO [28] and the clinicoradiological 2010 McDonald criteria for MS [46].

### Evaluation of the 2015 criteria for NMOSD

On the understanding that MOG-IgG seropositivity does not per se constitute an "alternative diagnosis", i.e., based solely on clinical and radiological criteria, 16/50 (32%) patients met the 2015 international consensus criteria for NMOSD [29] (Table 1). Of those, 15 had a history of both ON and myelitis and 1 a history of ON but not of myelitis (this patient fulfilled the criteria despite the lack of myelitis due to the presence of brainstem encephalitis with periependymal lesions around the fourth ventricle and of symptomatic, extensive white matter lesions); none had a history of myelitis but not of ON. Of those patients who met the 2006 criteria, 12 (85.7%) also met the 2015 criteria. Conversely, 12 (75%) of those who met the 2015 criteria also met the 2006 criteria. 8 out of 43 (19%) patients with available data fulfilled both the clinicoradiological 2015 criteria for NMOSD and the clinicoradiological 2010 McDonald

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criteria for MS. If only patients with a relapsing course of disease are considered, 16/40 (40%) met Wingerchuk's 2015 criteria.

### Previous diagnoses

As reliable tests for MOG-IgG became available only relatively recently, most of the patients initially received diagnoses other than MOG-IgG-positive encephalomyelitis (EM). In 16/45 (35.6%) patients with available data, a diagnosis of MS was suspected at least once. Other suspected diagnoses included acute disseminated EM (ADEM), multiphasic disseminated EM, AQP4-IgG-negative NMO according to Wingerchuk's 2006 criteria [28], AQP4-IgG-negative NMOSD according to the 2015 international diagnostic consensus criteria [29], viral encephalitis, bacterial encephalitis, paraneoplastic encephalitis, isolated vasculitis of the CNS, chronic relapsing inflammatory optic neuropathy (CRION), CNS lymphoma, sarcoidosis, spinal stenosis, "spinal tumor of unknown dignity", suspected spinal ischemia, para- or postinfectious ON, and myelitis; some patients were diagnosed with ON, rON, (longitudinally extensive transverse) myelitis, brainstem encephalitis or EM "of unknown origin".

### Coexisting autoimmunity

Coexisting autoantibodies were present in 19/45 (42.2%) MOG-IgG-positive patients. These included antinuclear antibodies (ANA) in at least 14 patients and cardiolipin antibodies or phospholipid/glycoprotein beta-2 antibodies (2 ×), anti-tissue transglutaminase IgA (1 ×), rheumatoid factor (1 ×), anti-thyroid peroxidase (2 ×), anti-thyreoglobulin (1 ×), anti-thyroid-simulating hormone receptor (1 ×), perinuclear anti-neutrophil cytoplasmic antibodies (ANCA) (1×). None of the 50 patients was positive for AQP4-IgG [30].

Concomitant autoimmune disorders were present only in 4/47 (8.5%) patients and included rheumatoid arthritis (RA) (2 ×), Hashimoto thyroiditis (1 ×), Grave's disease (1 ×). A further patients had atopic dermatitis and asthma bronchiale.

### Preceding infections

Disease onset was preceded by infection in at least 11 patients. Diagnoses included common cold, sore throat, tonsillitis, sinusitis, bronchitis, "respiratory infection", "feverish infection", and, in one case, a gastrointestinal infection with positive *Yersinia* serology (species not determined).

Taking not only the first but all attacks into account, attacks were preceded by infection at least once in at least 15/37 (40.5%) patients; the infections included, in addition to those already mentioned above, "mycoplasma pneumonia," one case each of a non-specified "respiratory" or "bronchopulmonary" infection, a "feverish common cold", "fever and fatigue", and a non-specified

"feverish infection". In at least one patient, both the first and the second attack were preceded by infection.

One further patient reported a history of two episodes of "borreliosis with meningitis" 20 and 19 years before onset.

### Preceding vaccinations

Disease onset was preceded by revaccination against diphtheria, tetanus, pertussis, polio, and influenza 2 weeks prior to symptom onset in one patient (for details of this case see part 3 of this series [31]), and by vaccination against diphtheria, tetanus, and pertussis 13 days prior to symptom onset in a second case; the latter patient developed fever 2 - 3 days before symptoms started. Both patients (1 × male, 1 × female) were vaccinated at adult age (19 and 47 years) and both developed recurrent disease. While the first patient experienced seven relapses involving the the optic nerves (4 ×), spinal cord (5 ×), brain (2 ×), and brainstem (1 ×) within 20 months, which fully responded to IVMP or combined IVMP and plasma exchange (PEX), the second patient developed three attacks (2 × ON, 1 × myelitis and ON) within 6 months, which only partially responded to IVMP, PEX and IA and resulted in an EDSS of 8 at discharge; two of the attacks occurred despite treatment with rituximab.

### Pregnancy-associated attacks

Seventeen percent (5/30) of all female patients aged ≥15 years at last follow-up experienced at least one attack of ON or myelitis during pregnancy or post partum. This corresponded to 50% (5/10) of all patients with a documented pregnancy (no data in 9) and, importantly, included all 5/5 women of reproductive age with available data who were pregnant shortly before (i.e., within the last 18 months), at, or after disease onset. Of a total of seven attacks, three had occurred during pregnancy and four post partum. These included the first attack ever in 3 patients: Disease started with simultaneous ON and LETM and accompanying brainstem and brain lesions occurring just 6 weeks after the delivery of the first child in one case; with an attack of unilateral ON 3 months post partum and during breast-feeding in a second patient; and with an attack of bilateral ON 8 months after delivery and while still breast-feeding in a third (as a limitation, however, ON was also preceded by a common cold with mild fever in this last case). In a fourth patient, an attack of LETM occurred during week 6 of pregnancy and an attack of bilateral ON a few weeks after delivery; however, the disease had started 8 years earlier in this patient and several ON attacks had occurred in the meanwhile. A fifth patient experienced at least two attacks of ON during pregnancy, which responded well to IVMP; disease had started 2 years before. While 3 patients had a relapsing course, 2 have not developed further attacks so far, although the follow-up time is short (6 and 3 months, respectively). Overall, 7/23 attacks in the 3 relapsing patients were associated with pregnancy or delivery, while the majority of attacks were not.

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## Tumor associations

In a single patient presenting with post-infectious whole-spine myelitis and severe brainstem and brain inflammation, a mature cystic ovarian teratoma had been removed 2 months before onset of the neurological symptoms, but no signs of malignancy had been found; NMDAR antibodies were negative. In the same patient, a ganglioneuroma was found and resected at a later date. MOG-IgG were not associated with malign tumors also in all other patients studied.

## Treatments for acute attacks

Acute attacks were treated with high-dose IVMP at least once in 47/48 (97.9%) MOG-IgG-positive patients, with PEX at least once in 19/48 (39.6%), and with immunoadsorption (IA) in two. Other treatments included oral steroids or dexamethasone i.v. followed by oral steroids in single patients as well as acyclovir and/or antibiotics for pragmatic treatment of initially suspected CNS infection.

Overall, 136 documented attacks were treated with IVMP, 15 with PEX, and 25 with both IVMP and PEX or – in five of them - IA; 18 were not treated at all. PEX or IA were used to treat 20 ON attacks, 16 myelitis attacks (with or without brain and/or brainstem and/or cerebellum involvement), 3 attack of simultaneous ON and myelitis (with or without additional clinical brain involvement), and 1 pure brainstem attack.

## Overall outcome of acute attacks

Outcome data were available for 134 ON attacks in 39 MOG-IgG-positive patients and for 46 myelitis attacks in 23 MOG-IgG-positive patients. Complete or almost complete recovery from acute ON was noted after 70 (52.2%) ON attacks, partial recovery after 54 (40.3%), and no or almost no recovery after 10 (Fig. 11b). Complete or almost complete recovery from acute myelitis was noted in 16 (34.8%) attacks, partial recovery in 30 (65.2%), and no or almost no recovery in none (Fig. 11a).

At last follow-up, 38/48 (79.2%) patients had experienced complete or almost complete recovery from at least one attack. In contrast, 22/48 (45.8%) had experienced at least one attack that was followed by no or almost no recovery. While 62.2% (28/45) of the patients' initial attacks remitted completely or almost



**Fig. 11** Outcome after acute attacks in MOG-IgG-positive patients compared with a previously published AQP4-IgG-positive cohort. **a** Outcome after acute myelitis in MOG-IgG-positive (46 evaluable attacks) and in AQP4-IgG-positive patients (298 evaluable attacks [34]). **b** Outcome after acute ON in MOG-IgG-positive (134 evaluable attacks) and in AQP4-IgG-positive patients (205 evaluable attacks; see ref. [34]). Note that 'complete recovery' includes 'almost complete recovery' in the left graph (no such distinction was made in the AQP4-IgG-positive cohort)

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completely, the proportion was lower for all subsequent attacks (40.6% or 69/170) and dropped to 26.4% or 19/72 after the fifth relapse.

## Outcome of attacks treated with IVMP

Outcome data were available for 122 attacks treated with IVMP but not PEX (including attacks of ON; myelitis; brainstem encephalitis; cerebellitis; supratentorial encephalitis; simultaneous ON and myelitis; simultaneous ON, myelitis, and brainstem encephalitis; simultaneous ON, myelitis, and supratentorial encephalitis; simultaneous myelitis and brainstem encephalitis; simultaneous myelitis and supratentorial encephalitis; and simultaneous myelitis, brainstem, and brain inflammation). In 61 (50%) of those relapses, IVMP treatment was followed by complete or almost complete recovery, in 54 (44.3%) by partial recovery, and in 7 (5.7%) by no or almost no recovery.

Of particular note, symptoms flared up after withdrawal or tapering of steroids at least once in 21/47 (44.7%) patients (see Appendix and Discussion for details). To control symptoms, IVMP was combined with or escalated to PEX or IA in 17/48 (35.4%) patients at least once and for 9.1% (25/276) of all documented attacks. If those attacks that were subsequently treated with PEX are also taken into account and on the understanding that the use of PEX after IVMP implies partial or full IVMP failure, 86/147 (58.5%) attacks initially treated with IVMP responded only partially or not at all to IVMP, while IVMP was followed by complete or almost complete recovery in 41.5%.

## Outcome of attacks treated with PEX or IA

Outcome data were available for 40 attacks treated either with PEX/IA alone or with both IVMP and PEX/IA; IA instead of PEX was used to treat five of those attacks.

Stand-alone PEX/IA was used for treating attacks (*N* = 15) of ON and/or myelitis with and without brain or brainstem involvement and attacks of isolated brainstem encephalitis. The median number of PEX/IA cycles used per attack was 5 (range, 3-11). In 3 (20%) of those 15 attacks, PEX treatment was followed by complete or almost complete recovery, in 11 (73.3%) by partial recovery, and in 1 by no or almost no recovery.

In addition, 25 attacks of ON and/or myelitis (with and without brain and/or brainstem involvement) were treated with both IVMP and, subsequently, PEX/IA. In 10 (40%) of these attacks, PEX/IA treatment was followed by complete or almost complete recovery, in 14 (56%) by partial recovery, and in 1 by no or almost no recovery.

If all attacks treated with PEX/IA (with or without IVMP) are considered, PEX/IA treatment was followed by complete or almost complete recovery in 13 (32.5%) attacks, by partial recovery in 25 (62.5%), and in 2 (5%) by no or almost no recovery.

IA was used instead of PEX for two attacks of ON in case 11. While treatment with four courses of IA was followed by almost complete recovery from an ON attack that had responded only transiently to a first IVMP cycle (and not at all to a second one) and by a relapse-free period of 3 years, the next ON attack responded only partially to IVMP and four courses of IA. The reason for the differential response to IA during those two relapses is unknown, but, as with PEX, differences in antibody titers as well as timing issues might have played a role. In case 28, IA resulted only in partial recovery when used after IVMP to treat three attacks of isolated myelitis, simultaneous ON and myelitis, and of isolated ON, respectively.

## Outcome of untreated attacks

Only 14 attacks in 11 patients were not treated with steroids or PEX/IA. Among those attacks, no or almost no recovery was noted in 2 cases (acute ON and brainstem encephalitis in one patient, ON in a second), one of which was fatal, partial recovery in 3 (acute ON in all), and full or almost full recovery in 9 (acute ON in 7, acute encephalitis/brainstem encephalitis in 2 ). The reasons for not treating patients for acute attacks were not specified in all cases. IVMP treatment was declined by at least two patients once each (no recovery in one and full recovery in the other one), and a decision in favor of palliative care had been previously made in another patient; in at least one case, ON was considered mild and therefore left untreated.

## Long-term treatments

Long-term immunosuppressive (IS) or immunomodulatory (IM) treatments were used at least once in 35/49 (71.4%) patients and included azathioprine (AZA) in 18, methotrexate (MTX) in 8, rituximab in 16, glatiramer acetate (GLAT) in 5, interferon-beta (IFN-beta) in 4, natalizumab (NAT) in 3, ofatumumab in 1, intravenous immunoglobulins (IVIG) in 1, mitoxantrone in 2, ciclosporin in 1, mycophenolate mofetil in 1, and oral steroids in 5; 14 patients (including 8 with a so far monophasic disease course) never received any IS/IM treatment.

Breakthrough attacks were noted in 21/31 (67.7%) patients treated with IS/IM at least once.

## Response to AZA treatment

Data on acute attacks during AZA therapy were available from 17/18 patients treated. The median treatment period was 10 months (range 2-101). Of these 17 patients, 14 (82.4%) experienced at least one attack under treatment with AZA. In total, 34 attacks occurred under AZA over a cumulative treatment period of 412 months (cumulative ARR 0.99) with a median of 1 attack/patient (range 0-6) in the total AZA group and of 1.5 attacks/patients (range 1-6) in those who had breakthrough relapses.

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Of particular note, 14 of the 34 attacks (41%) took place during the first 6 months, i.e., during the drug-specific latency period. Of these, 11 attacks developed during the first 3 months and only 3 during months 4-6. If all patients are taken into account, the median of all individual ARRs was 2 during the 6-month AZA latency period and 0.92 after the latency period.

Cotreatment with oral steroids or, in a single case, regular PEX was administered only in 9/23 (39.1%) patients (no data in 2), either for 3 or for 6 months or for the entire treatment period. Importantly, most attacks (12/14) observed during the AZA latency period occurred in patients who were not cotreated. Relapses occurred in only 1 of 14 cotreated patients during the latency period, but in 6/9 patients who were not cotreated. Similarly, 4/5 patients who developed relapses after the latency period were not cotreated, and 14/17 attacks occurring during that period affected non-cotreated patients. Taking the total treatment period into account, 10/12 patients with relapses under AZA were not cotreated at the time of the attack and 26/31 attacks occurred in non-cotreated patients.

### Response to MTX treatment

Data on acute attacks before and during MTX therapy were available from six patients. In case 13 (see Appendix for case reports), a single (though severe and non-remitting) relapse occurred under MTX within 134 months compared with 3 attacks in an 11-month period including 9 months of combined treatment with AZA and oral steroids. As a possible limitation, it remains unclear whether further attacks in the affected right eye went unrecognized due to the pre-existing severe visual deficit. Patient 3 experienced two attacks (both with complete recovery) within a period of 5.5 years of MTX treatment. Of note, however, this included the patient's first attack ever, which occurred under active MTX treatment for pre-existing RA. MTX was used as treatment for RA also in patient 6 described in part 3 of this article series [31]; in that patient, temporary discontinuation of MTX after 5 years due to severe infection was followed by the first relapse for 40 years. MTX was continued and no further attack occurred over the following 12 months. Similarly, patient 12 in part 3 of this series [31] suffered no attacks during 21 months of MTX treatment, although, three attacks had occurred within 7 months prior to commencement of MTX. Finally, combined treatment with MTX and oral steroids (plus ciclosporin A during the initial 7 months) resulted in disease stabilization in case 6, with only two relapses (with only partial recovery though) in almost 7 years; by contrast, 14 attacks had occurred in the preceding 5 years in this patient (including during treatment with IFN-beta, GLAT, AZA, or rituximab).

Overall, 5 attacks took place in 22.5 years in these patients under treatment with MTX. This corresponds to a cumulative ARR of 0.22, which is lower than the cumulative ARR of 0.95 found among all patients (*n* = 34) with a relapsing disease course. Patient 1, in whom three breakthrough attacks occurred within 8 months of MTX therapy, was the only patient with apparent MTX failure.

### Response to IFN-beta treatment

No decrease in relapse rate was observed under treatment with various IFN-beta preparations, which were given for suspected MS. In case 6, commencement of therapy with i.m. IFN-beta-1a (Avonex®) was followed by two ON relapses 1 and 4 months later. Similarly, s.c. IFN-beta-1a (Rebif®) was followed by an ON relapse less than 2 months after treatment was started. Finally, treatment with s.c. IFN-beta-1b (Betaferon®) was associated with another ON relapse after 2 months. Overall, four relapses occurred within around 16 months of IFN-beta treatment (ARR 3.0). This is in strong contrast to just two ON relapses within 71 months under therapy with MTX and oral steroids in that patient (ARR 0.33). Of interest, both IFN-beta-1a and -1b led to leukopenia. In another patient (see case 5 in part 3 of this article series [31] for details), further relapses occurred and marked disease exacerbation on MRI was noted after the initiation of i.m. IFN-beta-1a treatment, with new spinal and brainstem lesions. In a third patient (case 12), two relapses occurred within 11 months and led to discontinuation of s.c. IFN-beta-1a therapy. A fourth patient experienced an attack of mild ON and myelitis after 8 months of IFN-beta (Rebif®) therapy. When the same patient was again treated with IFN-beta 5 years later (now with Avonex®), an attack of severe unilateral ON occurred two months after treatment initiation and an attack of ON in the opposite eye with simultaneous myelitis after a further 2 months. In total, she experienced three attacks during a total IFN-beta treatment period of 19 months.

### Response to GLAT treatment

Five patients were treated with GLAT for suspected MS. In case 6, no relapse occurred over a period of 6 months; by contrast, four relapses had occurred under IFN-beta over a period of 16 months in the same patient. However, considering the GLAT-specific latency period of 3-6 months observed in MS it remains uncertain whether that decline in relapse rate was due to GLAT treatment or to discontinuation of (potentially disease-exacerbating) IFN-beta treatment. GLAT treatment had to be stopped due to leukopenia in that patient. In case 8, no relapses occurred over a period of 36 months on therapy with GLAT and remission of spinal cord lesions was detected by MRI. However, this patient had previously experienced a relapse-free interval of more than 5 years, rendering it uncertain also in this case whether GLAT was effective. A third patient (see case 1 in part 3 of this article series [31] for details) was relapse-free for almost a year under GLAT, but

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experienced two relapses (1 x ON, 1 x myelitis) 11 and 13 months after initiation of therapy, leading to discontinuation of GLAT. Previously, one to two relapses per year had occurred over a period of around 6 years, and three relapses within the last 10 months prior to GLAT. A fourth patient (case 14 in part 3 [31]) experienced three ON attacks during 8 months of GLAT treatment; moreover, a further relapse of severe ON leading to transient unilateral blindness occurred a few weeks after GLAT therapy was discontinued. In a further patient (case 13 in part 3 [31]), two attacks occurred during 7 months of treatment with GLAT (3 and 7 months after the first injection). When treated a second time with GLAT more than 3 years later, she experienced a protracted attack of myelitis with paresis, impaired coordination, and impaired ambulation 1 months after commencement of therapy (and thus during the drug's latency period), which lasted over 2 months and required a total of three cycles of high-dose IVMP therapy.

**Response to NAT treatment**
Three patients were treated with NAT for suspected MS. In one of them (see case 1 in [31]), two infusions of NAT were followed by three relapses by 2, 3 and 5 months, which only partially responded to PEX. Treatment with NAT was not continued after the second infusion due to recurrent headache. In the second patient (see case 5 in part 3 [31]) an attack of brainstem encephalitis occurred and MRI showed a new LETM lesion 9 months after commencement of NAT therapy. The third patient (case 13 in part 3 [31]) experienced two myelitis attacks 1 and 4 months after initiation of NAT treatment, followed by a relapse-free interval of 21 months. When NAT was re-initiated 11 months later, she developed two further attacks of myelitis after 4 and 5 months, followed by a relapse-free interval of 9 months; treatment was discontinued due to John Cunningham virus (JCV) seroconversion. In total, four attacks occurred during 29 months of NAT treatment.

**Response to rituximab and ofatumumab**
Of 16 patients treated with rituximab at least once, observation periods under rituximab therapy were sufficiently long to allow meaningful analyses of the drug's efficacy only in 9 patients.

Treatment with rituximab was followed by a decline in relapse rate in 3/9: In one patient (see case 18 in the Appendix), no relapse occurred in 12 months under rituximab compared with four relapses of ON within 6 months beforehand. In another patient (see case 7 in part 3 of this series [31]) one minor relapse with spontaneous remission took place in 28 months, compared with three attacks within the previous 4 months). Finally, in case 12, no relapses occurred during 8 months of rituximab treatment compared with three relapses in the preceding 14 months (two of which, however, took place under treatment with

IFN-beta, which was reported to cause disease exacerbation in NMO and which was associated with ongoing or increasing disease activity also in our patients).

Of note, in the other six patients one or more attacks were noted during therapy with rituximab, most of which occurred shortly after rituximab infusion. This is reminiscent of early attacks observed in AQP4-IgG-positive NMO patients treated with rituximab. Two relapses of ON occurred 3 and 7 weeks after the first rituximab infusion (2 × 1000 mg i.v., days 1 and 15) in case 6 (see Appendix). Similarly, patient 1 in part 3 of this series [31] developed severe clinical and radiological deterioration 4 weeks after the first and 2 weeks after the second infusion of rituximab. The latter patient had been treated with PEX 1 month before rituximab was started, indicating that even pretreatment with PEX may not be sufficient in all cases to prevent the risk of rituximab-related attacks. A further patient developed two relapses of ON one months after the first and 2 months after the second infusion, respectively. The fourth patient (case 11 in part 3 [31]) developed severe bilateral ON three months after the second infusion (i.e. four months after the first infusion) of rituximab. A fifth patient developed two attacks of myelitis and of ON 2 months after the first and three months after the second infusion. Finally, one patient who was treated with rituximab for a first attack of myelitis, developed ON just five months after the first infusion of 1000 mg rituximab. By contrast, no early relapses were noted in ten cases.

Of note, two end-of-dose relapses in rituximab-treated patients were documented. One patient (see case 7 in part 3 [31]) relapsed immediately after reappearance of B cells 9 months after the first infusion. Similarly, a relapse occurred in case 6 12 months after the first rituximab infusion. By contrast, CD19 cells were still undetectable and no new relapse has occurred 14 months after onset in case 12.

In one patient (case 13 in part 3 [31]), therapy with rituximab had to be discontinued due to an allergic exanthema.

A single patient was treated with ofatumumab (18 months, four cycles to date). While eight attacks of ON and three attacks of myelitis (one with accompanying brainstem encephalitis) had taken place over a period of 63 months under various previous therapies (ARR 2.1), only a single attack of ON occurred during 18 months (ARR 0.66) of ofatumumab treatment in this patient.

**Response to mitoxantrone and other rare therapies**
In the only patient with available data (case 1 in part 3 of this article series [31]), three infusions of mitoxantrone ($1 \times 12$ mg/m$^2$ and $2 \times 8$ mg/m$^2$) did not prevent three relapses of myelitis and two of ON within around 5 months, with some of the relapses occurring just a few weeks after infusion. A further patient (case 13 in part 3 [31]) experienced a relapse of sensory myelitis 1 month after initiation of fingolimod. Discontinuation of fingolimod after 3 months due to lymphopenia was immediately followed by a relapse of

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myelitis with impaired ambulation, paresthesia and dysesthesia below T5, and two flare-ups over the next 2 months, requiring a total of three cycles of (escalating) high dose IVMP therapy.

Ciclosporin was used in combination with MTX and oral steroids in a single patient (see case 6 in the Appendix) for a period of 6 months; no relapses occurred under this regimen. One patient (see case 13 in part 3 [31]) was treated for 4 months with dimethylfumarate. While no relapses occurred during that period, treatment had to be discontinued due to reflux, pharyngitis and laryngitis.

Another patient (case 2 in part 3 [31]) was treated with IVIG over 11 months (and tapering of oral steroids during the initial 3 months). No new relapses occurred during that period and IVIG treatment was temporally associated with clinical improvement and resolution of MRI lesions; the patient was still relapse-free 12 months after discontinuation of IVIG.

### Long-term outcome

At last follow-up, VA was impaired in at least one eye in 21/38 (55.3%) patients with a history of clinical ON (median observation time 53.5 months, range 1-507) and around one third (14/38; 36.8%) of all patients were either functionally blind at last follow-up in one eye or both or had a severe visual impairment (VA >0.1 and ≤0.5). Functional blindness (VA ≤0.1) in at least one eye was noted in 10/38 (26.3%) patients, severe visual impairment but no functional blindness (VA >0.1 and ≤0.5) in 4/38 (10.5%), moderate impairment (VA >0.5 and ≤0.75) in 2/38 (5.3%), and mild impairment (VA >0.75 and <1.0) in 5/38 (13.2%). Both optic nerves had been affected at least once at last follow-up (median observation time 54 months, range 1-394), either clinically or subclinically (i.e., based on MRI, VEP, fundoscopy, and/or OCT findings only) and either simultaneously or successively, in 35/42 (83.3%) patients, while only one optic nerve had been affected in the remainder (median observation time 29.5 months, range 5-507).

Severe paresis was present at last follow-up in 1/28 (3.6%) patients with a history of clinical myelitis (median observation time 41.5 months, range 6-102),

moderate paresis in 4/28 (14.3%), and mild paresis in 6/28 (21.4%). Ambulation was impaired at last follow-up due to paresis and/or gait ataxia in 25%.

If the total cohort is considered, VA was reduced at last follow-up in 23/47 (48.9%) patients with available data (median observation time 49 months, range 1-507) and paresis was present in 14/48 (29.2%) (median observation time 50.5 months, range 1-507).

### EDSS at last follow-up

The expanded disability status scale (EDSS) was developed for use in classical MS and strongly focuses on ambulation deficits [47]. When interpreting EDSS results, it should be taken into consideration that complete bilateral visual loss corresponds to an EDSS score of just 4 and that patients with isolated ON can reach no higher scores. In accordance with that well-recognized underrepresentation of visual deficits – the main long-term sequelae in our patients (with functional blindness or severe visual loss present in 36%) – EDSS scores were nominally low at last follow-up in most cases (median 2.5 [range 0-10] in the total cohort, N = 47, and 3 [range 0-10] among patients with relapsing disease, N = 40). A median EDSS ≥3.5 was reached after more than 60 months (Fig. 12). The median EDSS was 3 (range 1-10) among patients with an observation period of ≥100 months (n = 12) and 3.25 [range 1.5-10] among patients with an observation period of ≥120 months (n = 8). A higher median EDSS at last follow-up was noted in women (3, range 0 -10; n = 35) than in men (1, range 0-6; n = 12; p < 0.05) despite a longer median observation period in the male subgroup (72 months [range 1-127] vs. 50.5 months [range 1-507]).

### Survival rate

After a median follow-up period of 52 months (range 1-507), 49/50 (98%) patients were still alive. One patient died from severe brainstem encephalitis leading to respiratory insufficiency 123 months after disease onset and after a total of 27 attacks, including attacks of ON, myelitis, encephalitis and/or brainstem encephalitis (see case 1 in part 3 of this article series [31]).
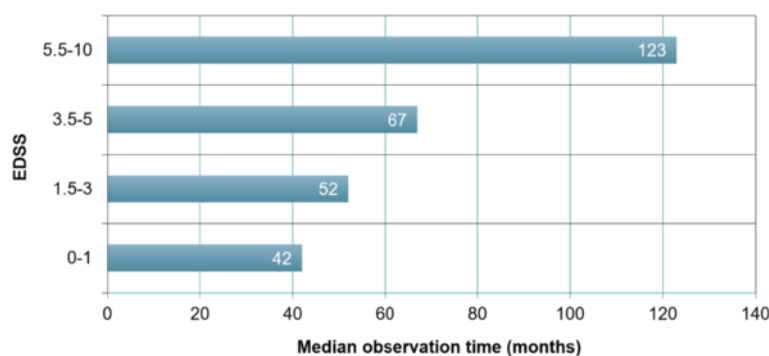


**Fig. 12** Increase in median EDSS scores with observation time in 47 MOG-IgG-positive patients

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## Discussion

MOG-IgG-positive ON and myelitis are increasingly recognized as important differential diagnoses of AQP4-IgG-positive NMOSD. Here, we comprehensively analyzed the clinical, laboratory, radiological, and electrophysiological features of one of the largest cohorts of MOG-IgG-positive patients reported to date, as well as treatment responses and long-term outcomes. In our cohort, which was characterized by the longest observation time so far (mean 75 ± 46.5 months since onset, median 52 [1-507] months), the disease took a relapsing course in most cases. Attacks were often severe and characterized by substantial visual loss or by paresis with longitudinally extensive spinal cord inflammation. Many patients had radiological and/or clinical signs of brain and brainstem involvement. While a relatively favorable long-term outcome was noted in the majority of cases, the disease caused persistent severe visual impairment including unilateral blindness in more than one third of all patients with a history of ON, persistent mild to severe paresis or gait ataxia in almost 50% of all myelitis patients, and was fatal in one patient due to recurrent brainstem attacks. Flare-ups after steroid treatment were noted in more than 40% of cases, and even PEX was not always effective. In around 70% of our patients, relapses occurred despite immunosuppressive therapy at least once. Given that genetic factors have been suggested to play a role in NMO, it is a potential strength of the present study that the cohort investigated here was genetically relatively homogeneous, with all patients except one being of Caucasian origin.

In this study, MOG-IgG were detected by means of new generation cell-based assays (CBA) employing recombinant full-length human MOG instead of enzyme-linked immunoassays, which are prone to both false-negative and false-positive results and which are no longer recommended for clinical routine diagnosis of MOG antibodies [10]; a CBA was also used for detection of AQP4-IgG [7, 8].

### Substantial phenotypic overlap with AQP4-IgG-positive NMOSD and MS

MOG-IgG-positive myelitis and ON showed a significant overlap with AQP4-IgG-positive NMOSD in clinical and radiological presentation, with more than 60% patients with a history of both ON and myelitis meeting Wingerchuk's 2006 criteria for NMO [28] and around a third of all patients fulfilling the revised 2015 criteria [29]. Even manifestations considered relatively typical for AQP4-IgG-positive NMOSD, such as medulla oblongata lesions and intractable nausea and vomiting or ON with involvement of the optic chiasm, were noted in some cases. Moreover, MS was initially suspected in more than a third of all patients, and every fourth patient with MOG-IgG-positive ON and/or myelitis presenting with brain and/or brainstem lesions met Barkhof's criteria for MS, demonstrating a substantial phenotypic overlap between these two conditions.

With the discovery of AQP4-IgG [1, 6, 48], MOG-IgG [10], N-methyl-D-aspartate receptor-IgG [49], and a plethora of often non-paraneoplastic autoantibodies identified in acute CNS inflammation over the past decade [50–54], including in patients with primary or secondary demyelination, it becomes increasingly clear that not all patients presenting with relapsing CNS disease of putative autoimmune etiology have classical MS – even if they formally meet the 'positive' clinicoradiological criteria for MS [46]. MOG-IgG-positive patients in whom the disease starts with isolated brain or brainstem involvement are particularly challenging. Thus more and more importance attaches to carefully considering the 'negative' criterion of ruling out other diagnoses ("no better explanation") included in the current diagnostic consensus criteria for MS [46].

Of note, 11 patients who met the clinicoradiological criteria for MS and 11/14 patients in whom a diagnosis of MS was initially suspected by their then treating physicians were negative for CSF-restricted OCB. Similarly, many patients with AQP4-IgG-positive NMO who were falsely diagnosed with MS in the past were negative for OCBs in a previous study [34]. This suggests that CSF analysis should be re-included in the diagnostic criteria for MS as an important tool to exclude alternative diagnoses, as previously recommended by us and others [55]. Moreover, 11/16 patients in whom MS had been initially suspected, later developed LETM lesions, which are not typically present in classical MS. In total, 15 out of the 16 patients were either negative for OCBs or had LETM lesions.

### Most patients have relapsing disease

The relatively long observation time is a particular strength of the present study, since it allows assessment of disease course and outcome in the long run. While previous studies with shorter observation periods (12 months in [13], 18 months in [11], 2 years in [12]) and smaller sample sizes (4 patients in [13], 9 in [11], 16 in [12]) suggested that MOG-IgG-positive patients might often have monophasic disease, our series demonstrates that most MOG-IgG-positive patients with ON or myelitis have a relapsing disease course. Moreover, a very short median time to first relapse of just 5 months was noted in this cohort, indicating an overall high risk of early relapse in MOG-IgG patients.

Given that (i) the observation period among 'monophasic' patients was significantly shorter than in the relapsing subgroup and below the median time to relapse in around one third of the 'monophasic' patients, (ii) the proportion of relapsing patients increased with observation time (Fig. 2), and (iii) the interval between the first and the second attack was long in some of the relapsing cases (>12 months in eight; up to 492 months), it is conceivable that some of the few 'monophasic' patients will develop

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further attacks in the future. A monophasic course of disease might thus be even less common than suggested here.

On the other hand, time since onset was >5 years at last follow-up in 3 patients in the monophasic group, all of whom were not treated with immunosuppressants, so the disease may in fact follow a monophasic course at least in small proportion of cases.

Similarly, the significantly shorter observation time since onset in patients with a history of ON but no myelitis or of LETM but no ON than in patients with a history of both ON and myelitis (i.e., NMO) suggests that the differences in presentation between these groups are probably an effect of observation time and that some of our patients with isolated ON may develop myelitis in the future and some of those with isolated LETM may develop attacks of ON. Indeed, disease had started with either isolated ON or isolated myelitis (rather than simultaneous ON and myelitis) in around three fourths of patients with a diagnosis of NMO at last follow-up. Importantly, myelitis occurred only after several ON attacks in some of these patients and ON only after several myelitis attacks in others. Similarly, disease starts with isolated ON or myelitis rather than simultaneous ON and myelitis in the vast majority of AQP4-IgG-positive patients [34].

These findings are highly important when it comes to deciding whether to treat MOG-IgG-positive patients or not. The frequently relapsing course observed in the present cohort indicates that prophylactic long-term immunotherapy should be considered in MOG-IgG-positive patients. Given that a relapsing course was also noted in 5/8 (63%) patients with onset under the age of 18, this might possibly hold true also for children and adolescents. Studies systematically investigating the efficiency of long-term immunosuppression and/or immunomodulation in MOG-IgG-positive ON and myelitis are therefore strongly warranted. Moreover, given the lack of systematic long-term treatment data in MOG-IgG-positive disease, currently planned or ongoing treatment trials in NMO that include AQP4-IgG-negative patients should consider testing for MOG-IgG to allow subgroup analyses.

### Severe attacks and unfavorable long-term outcome are relatively frequent

Severe attack-related disability was noted in many cases, including tetraparesis in around 30%, severe motor dysfunction with MRC grades ≤2 in around 25%, pain -and/or dysesthesia in around 70%, bladder and bowel disturbances in around 70%, functional blindness in almost 75%, bilateral optic nerve damage in 51%, scotomas in 66%, and brainstem encephalitis with, among other symptoms, ataxia, intractable nausea and vomiting or, of particular note, attack-related respiratory insufficiency in two patients, which was fatal in one. Importantly, long-term outcome was characterized by marked persisting visual impairment

or blindness and/or significantly impaired ambulation in 40%. Moreover, inflammatory damage was noted in the entire CNS, with the spinal cord, optic nerves, brainstem, diencephalon, cerebellum, and telencephalon affected in individual patients. These findings, together with the mostly relapsing course observed in our patients, underline that MOG-IgG-related CNS autoimmunity is a severe condition that requires consistent treatment and care.

Although a favorable outcome was noted in several patients, our findings do not support the notion that MOG-IgG seropositivity generally denotes a mild disease course [11, 13]. Again, previous studies reporting such findings may have been unintentionally biased by short observation periods and small sample size.

It should be taken into account in both epidemiological and therapeutic studies in the future that optic nerve damage is the leading manifestation of MOG-IgG-positive autoimmunity and that MOG-IgG-positive patients may present for many years or decades with isolated ON. The EDSS, which was developed for use in classical MS and which largely focuses on ambulation, may not sufficiently reflect the high degree of disability resulting from persisting visual loss in a substantial number of MOG-IgG-positive patients. Other scales of disability may need to be used in addition.

### IVMP was not always effective and flare-ups were frequent

In this context it is relevant that high-dose IVMP, though effective in many cases, was followed by only partial recovery or no recovery in 50% of all treated attacks. Moreover, IVMP lead to only temporary improvement in 44% of all patients at least once, resulting in flare-up of symptoms requiring repeat or ultra-high-dose IVMP therapy. In at least one case symptoms flared up not immediately after IVMP treatment but in a delayed fashion after tapering of subsequent oral steroid treatment. In some cases, even ultra-high-dose IVMP was ineffective or only transiently effective with a second flare-up occurring shortly after. Interestingly, in some patients IVMP was effective during initial attacks but not later in the disease course.

The occurrence of cerebral venous sinus thrombosis in one of our patients highlights the risks that repeat IVMP therapy and escalation to ultra-high-dose IVMP carry.

It is unknown why IVMP was effective during some attacks but not all. However, timing issues and differences in antibody titers, other immunological parameters (e.g., T cell activation), IVMP dosage, and previous or concomitant treatments might play a role.

Given the high frequency of flare-ups observed in our cohort, close clinical monitoring after acute attack therapy for MOG-IgG-positive ON and/or myelitis is recommended. Moreover, oral tapering of corticosteroid therapy as well as additional PEX treatment (see below) should be considered.

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### MOG autoimmunity may underlie CRION in a subset of patients

Given the high proportion of patients with flare-up of ON after steroid withdrawal, i.e., of steroid-dependent ON, we propose that a subset of patients previously diagnosed as having CRION [56] may in fact have MOG-IgG-positive ON. Indeed, at least 3 of our patients had received a diagnosis of CRION before MOG-IgG was detected. Testing of larger cohorts of patients with CRION for MOG-IgG is highly warranted.

### PEX treatment was often followed by full or partial recovery

PEX was used in most cases as rescue therapy if steroids did not result in complete recovery; only in four patients was PEX used as first-line treatment for acute attacks. Of note, PEX treatment (as stand-alone therapy or following IVMP) was followed by complete or almost complete recovery in a substantial number of attacks (around 40%). For example, in case 2 ON symptoms flared up twice after high-dose and subsequent ultra-high-dose IVMP therapy; only PEX ended the attack and was followed by complete recovery. The efficacy of PEX in this and other cases of MOG-IgG-positive ON and/or myelitis has potentially important pathophysiological implications, since it suggests a direct pathogenic role of the antibody. Interestingly, PEX treatment stopped the progression of dysesthesia in case 9, one of only 3 cases in which a slowly progressive (yet also relapsing) course of disease was noted.

However, as a limitation, it should not be overlooked that in almost 60% of attacks treated with PEX, and thus in the majority of cases, only partial recovery was achieved, and in 2 cases there was no response to PEX. The variability in response to PEX may be linked to differences in PEX timing; MOG-IgG titers; intensity, extension, and site (e.g., ON vs. myelitis as seen in AQP4-IgG-positive NMOSD [35]) of inflammation; and, importantly, the number of PEX courses applied, which varied between 3 and 11 in the present cohort. Preliminary findings from our laboratory (S.J., unpublished data) show that AQP4-IgG and MOG-IgG may remain detectable even after five to seven plasma exchanges, raising the question of whether PEX treatment is discontinued too early in some cases. This is also supported by the early reoccurrence of attacks in cases 1 and 9 in part 3 of this series [31], just 1, 2 and 3 months after PEX. Alternatively, T cell-mediated mechanisms might play a more important role than antibody-mediated mechanisms in patients who do not sufficiently respond to PEX.

On the understanding that the use of PEX after IVMP implies previous IVMP failure, only incomplete recovery or no recovery at all was achieved in around 60% of all attacks treated with IVMP ($N = 147$). Twenty-five of those attacks were subsequently treated with PEX, and full recovery was achieved in 40% of them. This would suggest a beneficial role of PEX in MOG-IgG-positive patients with IVMP failure, similar to what has been observed in AQP4-IgG-positive NMOSD [35]).

The overall good response to escalatory PEX therapy, together with the risks associated with extensive cortisone pulse therapy as highlighted by the occurrence of sinus thrombosis with brain edema and seizures in case 2 might suggest that PEX treatment should be considered more often in patients with MOG-IgG-positive ON and/or myelitis. PEX may be considered as a substitute for escalatory ultra-high-dose IVMP therapy for severe attacks, particularly in patients who have responded well to PEX in the past. However, the observation of urosepsis in case 1 of part 3 [31] after several cycles of PEX illustrates that attention must be paid also to risks associated with PEX and IA, especially if those treatments are applied repeatedly and in combination with IVMP or IS treatment.

### Breakthrough attacks despite long-term immunotherapy

Similarly, long-term IS and IM treatments were not always effective in preventing further relapses. Almost 70% of all patients treated with IS or IM drugs developed at least one attack during therapy. This included patients receiving AZA, MTX, NAT, IFN-beta, GLAT, rituximab, ofatumumab, and mitoxantrone. In case 6 at least 12 attacks of ON and myelitis occurred under various immunotherapies, and as many as 15 attacks occurred in case 1 in part 3 of this article series [31].

Complications of IS/IM therapy were rare in this cohort and included condylomata acuminata requiring surgical treatment, elevated liver enzymes under AZA treatment, and an allergic reaction to rituximab.

### AZA failure was associated with latency period and lack of cotreatment

AZA, which has been previously reported to be partially effective in NMO [57–59], including in AQP4-IgG-positive NMOSD [60], was the most commonly applied IS therapy in our cohort. However, more than 80% of all AZA-treated patients experienced at least one attack while under therapy. As AZA has a latency period of 3-6 months during which cotreatment with oral steroids has been recommended [33], we analyzed the temporal pattern of AZA failure. Of 34 attacks during AZA treatment, 14 (41.2%) took place during the first 6 months (11 during months 1-3 and 3 during months 4-6). Furthermore, 12 of those 14 attacks (85.7%) occurred in patients ($n = 6$) not cotreated with oral steroids, PEX, or other immunosuppressants during that period. This suggests that AZA failure in MOG-IgG-positive patients may be caused in a substantial proportion of cases by the drug's well-known latency in efficacy. Moreover, it may indicate that

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cotreatment with oral steroids during the initial 6 months of AZA treatment should not be abandoned in MOG-IgG-positive patients, provided contraindications have been excluded. However, it must be mentioned as a potential limitation that AZA was discontinued early in some patients after breakthrough attacks occurred, which may have introduced a considerable bias towards a higher proportion of attacks in the first 6 months. Larger studies are therefore needed before any treatment recommendations can be made.

Future studies on the efficacy of AZA and oral steroids as well as on that of oral steroids as stand-alone therapy in MOG autoimmunity should take into account that a recent retrospective analysis suggested a better response rate to high-dose azathioprine (2.5-3 mg/kg) than to standard treatment (1-1.5 mg/kg) in AQP4-IgG-positive NMOSD [58]. Whether such a high-dose regimen is also required in MOG-IgG-positive patients is currently unknown.

### Low relapse rate under MTX in most but not all cases

A recent study suggested that MTX might be effective in patients with AQP4-IgG-positive NMOSD [33, 61]. In our cohort, eight MOG-IgG-positive patients with ON and/or myelitis were treated with MTX, and exact data on attack dates were available from six. A lower relapse rate than in the total cohort and long attack-free intervals were observed in most MTX-treated patients. Based on these preliminary yet promising results, further retrospective studies seem warranted to assess the efficacy of MTX in MOG-IgG-positive ON and/or myelitis.

### Attacks related to initial rituximab infusion and reappearance of B cells

Rituximab treatment was followed by a clear reduction in relapse rate in three out of nine patients. In the six remaining patients, relapses occurred 2, 3, 4, 4, 7, 8, 8, 12 and 20 weeks after the first or second infusion (in one case despite PEX treatment 1 month earlier). This is reminiscent of the transient deterioration reported in some patients with AQP4-IgG-positive NMO after commencement of rituximab, which is associated with an temporary increase in BAFF and autoantibody levels [62, 63]. Another MOG-IgG-positive patient who experienced postinduction relapses (three within 3 months) has recently been described [63]. Whether cotreatment with steroids can prevent such events still needs to be explored.

Of note, one patient relapsed immediately after reappearance of B cells. This is similar to what has been observed in AQP4-IgG-positive NMO patients [64, 65] and suggests that (i) B cells should be closely monitored in MOG-IgG-positive patients treated with rituximab and (ii) treatment intervals should be short and doses

high enough to prevent B cell reappearance. Rituximab has also been found to be effective in AQP4-IgG-positive NMOSD in some studies [65, 66], though not in all [67].

Ofatumumab is a fully human anti-CD20 monoclonal antibody which targets an epitope distinct from that of rituximab [68]. The marked reduction in relapse rate in the single patient treated with ofatumumab in this study is promising. However, more data are needed before any recommendations can be made. To the best of our knowledge this is the first report on ofatumumab both in MOG-IgG- and in AQP4-IgG-associated EM.

### Ongoing or increasing disease activity under IFN-beta

In the present cohort, 4 patients were treated with IFN-beta. All 4 showed ongoing or increasing disease activity. Although preliminary, these data suggest that IFN-beta, which has already been shown to be ineffective and to cause disease exacerbation in AQP4-IgG-positive NMOSD [69–72], may also be ineffective or even detrimental in MOG-IgG-positive patients. Given the substantial clinical overlap between MOG-EM and conventional MS, a condition often treated with IFN-beta, this would be of high clinical relevance. Larger retrospective studies evaluating the efficacy of IFN-beta in MOG-IgG-positive EM are therefore highly warranted.

### Preliminary data do not support use of GLAT or NAT

Like IFN-beta, GLAT is frequently used to treat patients with conventional MS. With the efficacy of GLAT being equivocal in two patients and eight breakthrough attacks having occurred in another three, the use of GLAT cannot currently be recommended in MOG-IgG-positive EM. Of note, GLAT has also been suggested to be of no clear benefit in patients with AQP4-IgG-associated NMOSD [36].

NAT, another drug shown to be beneficial in MS, could not prevent relapses in three MOG-IgG-positive patients in our cohort. While these preliminary data are not supportive of the use of NAT in MOG-IgG-positive ON or myelitis, systematic studies are certainly needed before definite conclusions can be drawn. NAT has also been found to be ineffective or even detrimental in patients with AQP4-IgG-positive NMOSD in recent studies [73–75]. Numerous relapses also occurred in a patient treated with mitoxantrone, another agent considered effective in conventional MS.

The failure of the MS therapeutics IFN-beta, GLAT and NAT in many of our patients supports the view that classical MS and AQP4- or MOG-IgG-associated disorders differ in terms of immunopathogenesis. This stance

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is further supported by the lack of OCB, a hallmark of conventional MS, in most of our MOG-IgG-positive patients as well as in most patients with AQP4-IgG-positive NMOSD [34, 76].

### MOG-IgG needs to be considered in children as well as in elderly patients

The median age at onset was around 30 years, which is similar to MS but differs from that in AQP4-IgG-positive NMOSD (~39) [34] by almost 10 years. However, the youngest patient in this cohort was just 6 years of age at onset and the oldest experienced his first attack at age 70, suggesting that MOG-IgG-positive ON and/or myelitis – just like AQP4-IgG-positive NMOSD [34] – can occur irrespective of age and need to be considered also in children and in the elderly.

All four patients with onset during childhood (at the ages of 6, 10, 12, and 12 years) initially presented with ON (bilateral in three), accompanied by myelitis in only one of them. Similarly, disease started with ON in the four oldest patients (onset at 58, 64, 66 and 77 years). Of note, 8/11 (73%) patients with onset at age <20 had a recurrent disease course at last follow-up, which led to relevant disability in 5 of them (EDSS 3.5, 3.5, 6.0, permanent unilateral blindness, and VA of 0.2, respectively, at last follow-up), and in one of the two remaining patients observation time was too short to rule out relapsing disease. This would argue against the notion that MOG-IgG-positive ON in young patients is generally a monophasic disease and suggests that long-term immunotherapy (e.g., with IVIG if immunosuppressants are to be avoided) should be considered also in children and adolescents; however, larger studies are certainly needed. A recurrent course was also present in three of the four oldest patients in this cohort.

Interestingly, the time between onset and first relapse was extraordinarily long in one of our patients (see case 6 in part 3 of this series [31]), who had suffered from a first attack of ON at age 12, followed by an LETM attack 41 years later. Of note, two further patients reported events during childhood that are compatible with a first attack of MOG-IgG-positive EM (two episodes of bulbar movement pain, diplopia, and headache at ages 10 and 11 in case 23, and "neurogenic diabetes insipidus" at age 7 in case 22). As it remains unclear whether those early events were caused by the same disorder as the patients' more recent complaints, which started 18.5 and 22 years later, they were not considered for statistical analysis.

With an age at onset of 70 years, patient 20 is, to the best of our knowledge, the oldest Caucasian MOG-IgG patient reported to date. The youngest patient described in the previous literature was just 1 year of age [77] and the oldest, a Japanese patient, 70 years [12] at onset.

Similarly, AQP4-IgG-positive NMOSD has been described both in children and in elderly patients [78–81]. Accordingly, MOG-IgG-positive EM is an important differential diagnosis of AQP4-IgG-positive NMOSD irrespective of age.

### Women are more often affected than men

Women outnumbered men by a factor of around 2.8 in this study. Female gender has also been identified as a risk factor for AQP4-IgG-positive NMOSD [82, 83]. However, a significantly higher preponderance of women (a male to female ratio of around 1:9) has been found in the latter condition in Caucasian patients [34]. The ratio found among MOG-IgG-positive patients in the present and previous cohort is more similar to that found in AQP4-IgG-negative NMOSD [34] and in classical MS [84]. Women might possibly be affected more severely than men as indicated by a higher median EDSS at last follow-up despite shorter median disease duration; however, confirmatory studies are needed to verify this finding.

### Attacks may occur during pregnancy and post partum

The effect of pregnancy on MOG-IgG-positive EM has not yet been systematically investigated. A recent study (n = 16) indicated that pregnancy may negatively influence the disease course of NMO; however, no data on the patients' AQP4-IgG or MOG-IgG status were given [85]. The authors found a significantly higher attack rate in the first trimester after pregnancy and greater disability progression 1 year after delivery [85]. In another cohort [86], 14/40 AQP4-IgG-positive patients developed the first symptoms of NMOSD either during pregnancy (n = 3) or within a year after delivery or abortion (n = 11). While the ARR during pregnancy did not differ from that before pregnancy, it increased significantly during the first and second trimesters after delivery; moreover, 77% of all deliveries were associated with post-partum relapses. In MS, pregnancy is thought to reduce the number of MS relapses, especially in the second and third trimesters; although attack rates tend to rise in the first 3-6 months post partum, no increased long-term disability has been found. In the present cohort, around a quarter of MOG-IgG-positive women experienced one or more attacks during pregnancy or post partum. Of special note, the disease started post partum in three of these patients. This could indicate that pregnancy- and/or delivery-related immunological changes may play a role both in triggering attacks and, possibly, in disease induction. However, given that most attacks in these patients as well as in the total female subgroup occurred irrespective of pregnancy and delivery, other risk factors may be more important. Based on these data,

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systematic prospective studies on the role of pregnancy and delivery in MOG-IgG-positive EM are warranted.

**Attacks may follow infection or vaccination**

Attacks were preceded by infection in around 40% of patients at least once, and disease started shortly after an infection in at least 11 cases and after vaccination in two cases. This is similar to AQP4-IgG-positive NMOSD, which has been reported to be preceded by infection in 20-30% of cases [34, 87]. Acute infections are also thought to trigger clinical attacks in classic MS. However, the exact relationship between infection or vaccination and MOG-IgG-positive EM is unknown. While there is no evidence yet for molecular mimicry, it is conceivable that infection-associated immunological changes and/or blood-brain barrier disruption could promote CNS lesion formation. In AQP4-IgG-positive NMOSD, acute relapses are indeed associated with an elevated QAlb, which can be caused by structural barrier damage [76, 88]. QAlb was also elevated in around one third of MOG-IgG-positive patients in the present study. As a limitation, QAlb likely also reflect changes in the CSF flow rate [45]. It is of potential interest and deserves further investigation that the two post-vaccinal cases both occurred after vaccination against tetanus, diphtheria and pertussis. Of note, both patients developed relapsing disease. This is different from conventional postvaccinal ADEM, which is usually monophasic.

**CSF findings differ from MS but mimic AQP4-IgG-positive NMO**

Examination of CSF harbors important potential for differentiating classical MS and MOG-IgG-positive EM but not MOG-IgG-positive EM and AQP4-IgG-positive EM: As in AQP4-IgG-positive patients [76], OCB and a positive IgG CSF/serum ratio, which are present in most patients with MS and which are thus considered a diagnostic hallmark of that disease, were missing in around 90% of our MOG-IgG-positive patients. Moreover, OCB disappeared later in 2 out of the 6 only OCB-positive patients; by contrast, OCB are considered to remain stable for decades in MS [89]. Finally, neutrophil granulocytes, which are also present in AQP4-IgG-positive NMO [34, 76, 90] (as well as in bacterial meningoencephalitis [91]), were found in the CSF at least once in 64% of cases, but are absent in classical MS.

Missing OCB or granulocytic pleocytosis should thus prompt physicians to challenge the diagnosis in patients with suspected MS and to consider MOG-IgG- or AQP4-IgG-positive encephalomyelitis.

**Subclinical evidence for dissemination in space**

Electrophysiological evidence for optic nerve damage was present in at least 3 patients with no history of clinically apparent ON, and for spinal cord damage in at least 3 patients with no history of clinically apparent myelitis in our cohort. Similarly, supratentorial, brainstem, or cerebellar MRI lesions were present in 21 patients who had never shown clinical signs of encephalitis or cerebellitis but only of ON and/or myelitis. Finally, spinal cord MRI lesions were detected in 2 patients with ON but no history of clinical myelitis. This indicates that subclinical inflammation occurs in some cases and that clinical examination needs to be complemented by electrophysiology and MRI to assess the real extent of CNS inflammation in MOG-IgG-positive patients.

If not only clinical attacks are taken into account but also clinically silent lesions as detected electrophysiologically or by MRI, evidence for dissemination in space (defined as involvement of more than one of the following structures: optic nerves, spinal cord, supratentorial brain, brainstem, cerebellum) was present at last follow-up in 37/50 or 74% of patients, compared with 26/50 or 52% based solely on clinical grounds.

VEP and SSEP were not considered in the 1999 and 2006 diagnostic criteria for NMO, which required clinically apparent attacks of myelitis and ON, and are still not considered in the 2015 criteria for NMOSD [29]. Systematic studies on the potential prognostic, diagnostic, and therapeutic implications of pathological EP and MRI findings suggesting dissemination in space in patients with MOG-IgG-positive isolated ON or isolated myelitis are warranted. Evidence for subclinical optic nerve damage has also been reported in AQP4-IgG-positive NMOSD [92].

**Bilateral ON and simultaneous ON and myelitis are common at onset**

More than 40% of all patients with a history of both ON and myelitis at last follow-up presented with simultaneous myelitis and ON at least once, which is not different from what has been described in AQP4-IgG-positive NMO (42% according to [34]). However, the frequency of simultaneous myelitis and ON at disease onset, i.e., as the initial presentation, was much higher in MOG-IgG-positive patients (23% of all patients with a history of ON and myelitis) than in AQP4-IgG-positive NMOSD patients (6.7% according to [34]; $p < 0.03$). Similarly, bilateral ON at onset was more frequent in MOG-IgG-positive patients with a history of ON (35%) than in AQP4-IgG-positive NMOSD patients with a history of ON (14.3% [34]; $p < 0.04$). Simultaneous ON and myelitis as well as bilateral ON at onset may thus be of diagnostic value and should prompt physicians to consider MOG-IgG testing.

**Short spinal cord lesions do not preclude MOG-IgG positivity**

Spinal cord MRI lesions extending over three or more vertebral segments (so-called LETM) are considered a hallmark of AQP4-IgG-positive NMOSD, but are usually

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not found in classical MS. The presence of an LETM lesion in addition to clinical myelitis was also listed as a supportive criterion in the 1999 diagnostic criteria for NMO and one of three minor characteristics, two of which had to be present in addition to a history of ON and myelitis before a diagnosis of NMO could be made, in the 2006 criteria [28]. The association of MOG-IgG with LETM found in this and in previous studies is therefore of differential diagnostic importance.

However, two recent studies could demonstrate that up to 15% of all MRIs of AQP4-IgG-positive patients show non-longitudinally extensive lesions [34, 93]. Similarly, lesions never exceeded two vertebral segments in 8 of our MOG-IgG-positive patients; in another 10 patients, at least one MRI showed only a non-LETM lesion but longitudinally extensive lesions were present in previous or later MRI examinations. The presence or absence of 'short' lesions in patients with AQP4-IgG- or MOG-IgG-positive myelitis is thought to depend, among other factors, on timing issues [94]. If MRI is carried out very early in the attack course or long time after an acute attack, lesions may be still evolving or be already in the process of resolution, respectively.

Similar to AQP4-IgG-positive myelitis, more than one lesion in the same MRI and swelling of the spinal cord were detected in many patients at least once. By contrast, necrotic lesions leading to spinal cord cavitation, as sometimes noted in AQP4-IgG-positive myelitis, were not reported in any of our MOG-IgG-positive patients.

### Lesions may affect the entire visual pathway

While retrobulbar optic neuritis was highly common among our MOG-IgG-positive patients, lesions affecting other parts of the optic pathway should be taken into consideration as well in MOG-IgG-positive patients presenting with visual symptoms. Many patients had signs of papillitis as detected fundoscopically; evidence for inflammation of the anterior part of the optic nerve was also found by MRI (Fig. 9). However, some patients presented with lesions in the chiasm (Fig. 9) and/or with longitudinally extensive ON (LEON) affecting both the anterior and the posterior portion of the optic nerve. Both LEON lesions and chiasmatic lesions were previously thought to be indicative of (AQP4-IgG-positive) NMOSD [29]. Our findings are in line with a recent Australian study that reported greater optic nerve lesion lengths in MOG-IgG-associated ON and AQP4-IgG-associated ON than in MS-related ON [95]. In a single patient, visual disturbances were associated with lesions within the optic tract (Fig. 9). Finally, some patients had occipital white matter lesions.

### Perioptic contrast enhancement warrants further investigation

As shown in Fig. 9, contrast enhancement was not only seen within the optic nerve but also in the perioptic nerve sheath and the immediately surrounding orbital tissue. This imaging pattern is of potential differential diagnostic relevance and, thus, deserves to be further investigated. In accordance with this finding, Kim et al. in a very recent study found perineural enhancement in 6 of 18 MOG-IgG-positive patients [96]. As is the case with other MRI features, it is likely that the presence or absence of that phenomenon depends on disease and treatment status: more than one third of all MRIs without perioptic enhancement in our cohort were performed during remission, and some of the remaining patients had been treated with high-dose IVMP before the MRI was performed.

### Coexisting autoimmunity is rare in MOG-IgG-positive patients

Coexisting autoimmune disorders are present in more than one third of AQP4-IgG-positive NMOSD patients [34]. By contrast, only around 9% of our MOG-IgG-positive patients had a coexisting autoimmune disorder (2 × RA, 1 × Hashimoto thyroiditis, 1 × Grave's disease). Systemic lupus erythematosus, Sjögren syndrome, and myasthenia gravis, which are common in AQP4-IgG-positive NMOSD [97–102], were absent in all of our MOG-IgG-positive patients. This is in line with a previous study that reported a lower frequency of concomitant autoimmune disorders in 'AQP4-IgG-seronegative' NMOSD patients [34]. Interestingly, first symptoms of anti-TPO-, anti-thyreoglobulin-, anti-TSH receptor-associated hyperthyreosis appeared just seven weeks after the first attack of MOG-IgG-positive myelitis in one of our patients, suggesting that MOG autoimmunity might have been part of a broader immune dysregulation in this case.

### Nosological issues

The 2015 diagnostic consensus criteria for NMOSD demand that "alternative diagnoses" should be excluded [29]. However, it remains unclear whether MOG-IgG-positive ON or myelitis should be considered an "alternative diagnosis" or not [103–105]. AQP4-IgG-positive and MOG-IgG-positive EM differ in terms of target structures (astrocytes vs. oligodendrocytes) and, accordingly, immunohistopathology [18–20], but MOG-IgG is not explicitly mentioned as an exclusion criterion. This mainly reflects the fact that at the time the criteria were developed, data on MOG-IgG-positive patients were still scarce.

In the present study, only around one third of all MOG-IgG-positive patients met the clinical and radiological criteria for "NMOSD without AQP4-IgG" [29]. If MOG-IgG is not considered an exclusion criterion for

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NMOSD, this would result in a subset of MOG-IgG-positive patients being considered eligible for clinical studies and treatment trials, while others would be excluded based solely on phenotypic presentation and despite all of these patients belonging to the same immunopathogenetically defined disease spectrum. This could introduce a relevant inclusion bias given that almost all of the patients who met the criteria had a history of ON and LETM (15/16 or 94%), while most of those who did not meet the criteria had isolated ON or isolated LETM at last follow-up (27/34 or 79%). Conversely, inclusion of MOG-IgG-positive patients in NMOSD cohorts (which are predominantly AQP4-IgG-positive) would introduce bias as well.

We therefore believe that confirmed MOG-IgG seropositivity should be considered an exclusion criterion for NMOSD and that the term 'NMOSD' should be restricted to AQP4-IgG-positive patients and, possibly, double-seronegative patients meeting the criteria for 'AQP4-IgG-negative NMOSD' [29].

There are two potential limitations to such an approach. First, using MOG-IgG seropositivity as an exclusion criterion for NMOSD would require providing a reference assay for MOG-IgG testing with excellent specificity as established in an appropriately controlled multicenter setting. Alternatively, however, diagnostic criteria for MOG-IgG-positive NMOSD based both on serological and on supporting clinicoradiological criteria could be established in analogy to the current consensus criteria for NMOSD. Second, so-called 'double-positive' patients, i.e., patients positive for both AQP4-IgG and MOG-IgG, would pose a diagnostic dilemma. However, such patients should in any case be excluded from clinical trials as they may have two immunopathophysiologically distinct diseases. Moreover, 'double-positive' patients seem to be extremely rare (see part 1 of this article series [30] and Table 4 in [17]) and the few cases reported so far worldwide have not been independently confirmed.

### Limitations

We acknowledge some obvious limitations of our study. First, the study design was retrospective, as in all previous studies in the field, and a high number of neurological centers were involved. However, due to the low prevalence of the condition, prospective single-center studies including sufficiently large numbers of patients are impracticable. Moreover, the multicenter design of this study, which included 11 academic centers, reduces the risk of referral bias, which was acknowledged as a possible limitation by the authors of previous large single-center studies in the field of NMO [28, 106]. Moreover, reliable assays for detecting MOG-IgG have only recently been developed; accordingly, only retrospective long-term data are currently available. Second, patients with a benign or monophasic long-term course

are less likely to be admitted to hospital and might thus be under-represented in the present cohort. However, this type of potential bias is inherent in hospital-based studies and cannot be completely avoided. It is important in this context that all centers involved in the present study also have specialized outpatient clinics for patients with neuroinflammatory conditions and that participants were recruited among both inpatients and outpatients. Moreover, the threshold for admission is low in Germany and Italy, where public healthcare is free. Third, MOG-IgG has also been reported in patients with conditions classified as 'ADEM' based on clinical and radiological features, especially in children [107]. Such cases were not systematically included in the present cohort, which focused on patients with ON and/or myelitis. Given that our study found a relapsing disease course in most patients with MOG-IgG-positive CNS inflammation and that attacks did not develop until years after initial presentation in some cases, systematic follow-up studies on patients previously diagnosed with MOG-IgG-positive 'ADEM' seem warranted to confirm that rare association.

### Conclusions and outlook

In summary, our study demonstrates that MOG-IgG-associated ON and myelitis frequently follow a relapsing course and result in severe and/or persisting disability in a substantial number of cases. Functional blindness due to optic nerve damage is the most common disabling sequela. In addition to tetra- or paraparesis, dysesthesia and pain are common symptoms in patients with myelitis. Some patients experience mild attacks with purely sensory symptoms that may not be accompanied by marked MRI or electrophysiological changes. Although in our cohort most patients with MOG-IgG-positive myelitis had LETM, non-longitudinally extensive lesions were found on a number of MRI examinations and thus do not preclude the diagnosis. Coexisting clinical or radiological evidence for brain, brainstem, or cerebellar involvement is frequent and may be extensive in some cases. Brainstem symptoms may include intractable nausea and vomiting as well as life-threatening or fatal respiratory complications. As in AQP4-IgG-positive NMOSD, CSF examination reveals mostly mild pleocytosis (partly with neutrophils) and, in contrast with MS, no evidence of intrathecal IgG synthesis in the vast majority of cases. Treatment of acute attacks with IVMP and PEX was effective in many patients, and immunosuppressive therapy was often followed by relapse-free intervals; however, failure of acute and long-term treatment and, subsequently, rapid accumulation of disability was noted in several cases. Of particular note, flare-up of symptoms after discontinuation of IVMP for treatment of an acute attack is frequent in MOG-IgG-positive patients. Full recovery was achieved by PEX in some cases,

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including patients showing IVMP failure. Breakthrough attacks in AZA-treated patients occurred particularly during the latency period of AZA and in patients not cotreated with oral steroids. MTX was identified as a potentially effective treatment in MOG-IgG-positive ON and/or myelitis. IFN-beta was used in rare patients misdiagnosed with classical MS and was associated with an increase in disease activity. Rituximab was effective in some patients, but new attacks occurred within a few weeks after the first infusion in a subset of cases, similar to what has been reported in AQP4-IgG-positive NMOSD. Our series, which includes some of the youngest as well as the oldest Caucasian MOG-IgG-positive cases, demonstrates that MOG-IgG positivity should be considered in patients presenting with ON or myelitis of unknown origin irrespective of age. Women are affected more often – and possibly more seriously – than men. Coexisting autoimmunity in MOG-IgG-positive NMOSD seems to be rare compared with AQP4-IgG-positive NMOSD. A substantial overlap in clinicoradiological presentation both with AQP4-IgG-positive NMOSD and with classical MS was found, and many patients were initially diagnosed with MS. While some patients with MOG-IgG-positive ON and/or myelitis meet the 2015 international diagnostic criteria for NMOSD, others do not; this is problematic from a nosological point of view, assuming that the same immunopathogenesis underlies all MOG-IgG positive cases. Several clinical and radiological features hitherto thought to be typical for AQP4-IgG-positive NMO, such as longitudinally extensive spinal cord lesions, lesion location in the central portion of the spinal cord, longitudinally extensive optic nerve lesions, lesions involving the optic chiasm, area postrema lesions, intractable nausea and vomiting, and thalamic lesions, or for MS, such as INO or periventricular, subcortical, juxtacortical, and callosal white matter lesions, were present in some of our MOG-IgG-positive patients. Similar to AQP4-IgG-positive NMOSD and to MS, disease onset or relapse was preceded by infection or vaccination in several cases. Around 30% of all the women in our cohort who gave birth at least once developed attacks during pregnancy or post partum.

Our findings from a predominantly Caucasian cohort strongly argue against the notion that MOG-IgG denotes a milder and usually monophasic variant of NMOSD, as suggested by previous, smaller cross-sectional studies with shorter observation periods. Given the relapsing and often severe disease course of MOG-IgG-positive ON and myelitis, the use of long-term immunosuppressive treatments in this condition should be considered. Prospective multicenter studies and treatment trials in MOG-IgG-positive EM will be difficult to perform due to the rarity of the condition but are highly warranted.

## Appendix

Due to the novelty and rarity of the disorder, large and comprehensive case series illustrating the broad and heterogeneous spectrum of clinical manifestations, disease courses, and radiological presentations in MOG-IgG-positive encephalomyelitis are lacking. In this appendix, we provide detailed reports on 28 cases of MOG-IgG-positive ON and/or myelitis. We believe that case reports can draw a more vivid 'real-life' picture of this rare disorder than statistical analyses alone. For the reader's convenience, the most important findings are briefly summarized and discussed in a comment at the end of each report. Additional reports are to be found in the "Case reports" section in part 3 of this article series [31].

## Contents

### I. MOG-IgG-positive NMO without brain involvement

*Case 1 – Recurrent LETM and ON resulting in persisting unilateral functional blindness and optic nerve atrophy.* This Caucasian woman first presented with ON of the right eye in 08/2004 at age 40. Symptoms completely remitted after IVMP treatment. Since then, at least seven relapses of unilateral ON have occurred, which have successively affected both eyes and only partially responded to IVMP or IVMP and PEX. In addition, she experienced two attacks of myelitis, one with paraparesis and bladder and bowel dysfunction (04/2009) and the other with gait ataxia and sensorimotor paraparesis (08/2011); spinal MRI showed LETM lesions extending from C5 to Th2 (04/2009) and at T5 and T11 (08/2011), respectively, with Gd enhancement. Multiple brain MRI examinations were normal or showed only a few nonspecific lesions not meeting the Paty or Barkhof criteria for MS; in addition, atrophy of both optic nerves was noted. Electrophysiology revealed prolonged P100 latencies in both eyes; later potentials were lost. While CSF was negative for OCB, very mild pleocytosis (6 cells/μl) was noted. The patient was given AZA (150 mg/d) between 01/2010 and 08/2010, and MTX was commenced in 08/2010. During treatment with AZA, three ON attacks took place; one attack of myelitis and two of ON occurred within 8 months despite treatment with MTX. At last follow-up, the patient had VA of 0.1 in the left eye (and 1.0 in the right eye) but no paresis.

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Comment: This case – characterized by ON and LETM, mild pleocytosis, no OCB, and a normal MRI at onset – illustrates how similar the clinical presentation in MOG-IgG-positive patients can be to that of AQP4-IgG-positive NMO. In fact, a substantial proportion of patients in this cohort met Wingerchuk's 2006 criteria. Of further note, 10 attacks occurred within only 85 months in this patient. Overall, the disease took a relapsing course in 80% of our patients. Moreover, as observed in many cases, acute treatment with IVMP and PEX and long-term immunosuppressive treatment were both only partially effective, resulting in a poor clinical outcome with functional blindness in one eye at last follow-up.

*Case 2 – Recurring LETM and IVMP-refractory ON in a young girl, complete recovery after PEX.* This young Caucasian woman experienced a first attack of myelitis in 06/2012 at age 17 with hypesthesia ascending from both feet to level T4, followed by predominantly left-sided paraparesis and urinary incontinence. Spinal MRI in 08/2012 demonstrated an LETM lesion extending from T3 to T6 with Gd enhancement (no swelling or necrosis documented). CSF WCC, OCB, QIgG, and total protein were normal. After IVMP (5 × 1000 mg) symptoms remitted except for a mild foot flexor paresis. AQP4 antibodies were negative.

A first relapse of myelitis occurred 1 year later, leading to hemihypesthesia predominantly of the left arm and torso. No MRI was performed. OCB were again negative. 5 × 1000 mg IVMP resulted in only partial recovery (EDSS in remission 3.0). AQP4-IgG was again absent, but MOG-IgG, which had not been previously tested, were positive.

In 07/2014, she suffered a first episode of unilateral ON with painful eye movement, blurred vision, and a small scotoma. VEP demonstrated prolonged P100 latency (amplitudes not reported). IVMP (5 × 1000 mg) resulted in transient full recovery. However, after just 2 weeks symptoms flared up again, with similar VEP results but an enlarged scotoma. Again, symptoms remitted completely after treatment with IVMP (3 x 2000 mg), only to recur after another 2 weeks. In between the first and second re-flare, thrombosis in the patient's superior sagittal sinus occurred, most likely linked to extensive cortisone pulse therapy. This was associated with two symptomatic epileptic seizures probably due to focal edema. The second re-flare was therefore treated with five cycles of PEX, which resulted in complete remission. While VEP showed a prolonged P100 latency (140 ms) in the affected eye during relapse, VEP were normal at last follow-up in 03/2015.

Comment: As in patient 1, the disease took a severe, recurring course, though in this case final outcome was good. Of particular note, IVMP was effective during the first attack but not or only partially during subsequent attacks. Flare-ups after initial response to IVMP were noted in several other patients, too. In this case, only therapy escalation to PEX resolved the patient's attack-related symptoms. The occurrence of sinus thrombosis with brain edema and seizures illustrates the risks of extensive cortisone pulse therapy.

*Case 3 – Simultaneous bilateral ON and LETM in a patient with rheumatoid arthritis; recurrent myelitis; complete recovery.* A 48-year-old woman with an 18-month history of suspected seronegative RA, which had been treated with oral MTX 15 mg per week starting in March 2009, developed bilateral pain on eye movement and severe visual loss in March 2010. Her personal and family history was otherwise unremarkable. A few days later, she additionally developed bilateral weakness of her legs which progressed to severe tetraparesis. Furthermore, she developed numbness of her trunk and legs starting at level T3, severe disturbances of micturition (residual urine 400 mL) and defecation, and severe back pain which was pronounced on head movement. Spinal MRI in an external hospital showed an LETM lesion extending from C2 to T4. Cranial MRI showed no inflammatory lesions but an old right-sided anterior infarction. A detailed search for sources of embolism was negative. CSF analysis revealed 73 leukocytes/μl and moderate blood-CSF barrier dysfunction, while OCB were negative. Both vasculitis screening including antiphospholipid antibodies and AQP4 antibodies were repeatedly negative, as were extensive infectiological investigations. The symptoms gradually improved during initial intravenous treatment with ceftriaxone, ampicillin, and aciclovir. Subsequent high-dose intravenous steroid pulse therapy over 5 days accelerated the remission of symptoms. When the patient first presented to our department 3 weeks after her symptoms had started, vision had normalized and walking was already possible without assistance. VEP amplitudes were reduced bilaterally and the P100 latency was slightly prolonged on the left side (121 ms). Tibialis SSEP were normal and MEP to the tibial anterior muscles showed bilateral slight prolongation of the central conduction time. The patient continued MTX treatment. In August 2010, a detailed neurological examination was unremarkable apart from mild proximal paresis of her right leg, not to be explained by the old anterior infarction on the same side. VEP, SSEP, and MEP were normal. The patient was then seen at half-yearly, later yearly intervals without developing further symptoms until September 2013, when she presented with acute painful numbness of her legs and lower trunk starting at level T10 and a positive Lhermitte's sign. Cervicothoracic and cranial MRI did not show new or enhancing lesions. CSF analysis revealed 17/mm$^3$ leukocytes and no evidence of a disturbed blood-CSF barrier function; isoelectric focusing again did not show CSF-specific OCB. Somatosensory and visual evoked potentials

were normal. High-dose corticosteroid pulse therapy in-duced full remission of the symptoms. Cranial MRI in 07/2015 still did not show inflammatory lesions; cervicothor-acic MRI in 08/2015 was unchanged. The patient was last seen in 08/2015 without having developed further new symptoms. She still continues treatment with MTX.

Comment: This patient's initial presentation with sim-ultaneous LETM and bilateral ON was considered typ-ical of NMO for many decades. However, recent studies have demonstrated that AQP4-IgG-positive NMO starts with unilateral ON in the majority of cases; by contrast, simultaneous ON and myelitis as well as bilateral ON at onset have been found to be more common among 'seronegative' patients with NMO [34]. In the present study, the disease started with simultaneous ON and myelitis (with or without brainstem involvement) in 10%, and the initial attack affected both eyes in 41% of all patients with ON at onset, which corresponded to 30% of all patients. Both the severity of the patient's presenting symptoms and the promptness as well as the nearly complete remission observed in this case are remarkable. Of particular interest, the patient's first relapse occurred while on treatment wth MTX for RA. In total, two attacks occurred within 6 years of MTX treatment. Relatively low relapse rates under treatment with MTX were also ob-served in cases 3, 6, and 13 described here as well as in cases 6 and 12 in part 3 of this series [31].

*Case 4 – Simultaneous myelitis and bilateral ON with complete recovery in a child.* This 10-year-old Caucasian girl experienced back pain and, within 10 days, developed intention tremor of the upper extremities and complete urinary retention. Neurological examination showed brisk knee jerks without Babinski's sign and urinary retention, but was otherwise unremarkable. While cranial MRI was normal, axial spinal cord imaging showed two small, non-contrast-enhancing T2-hyperintense lesions in the cervical cord with normal signal in the remaining spinal cord in-cluding the conus. Ophthalmological assessment revealed mild bilateral ON with impaired color perception and mild papilledema of the left eye and slightly delayed P100 laten-cies elicited from both eyes. CSF analysis disclosed 179 cells/µl with a high proportion of neutrophil (69%) and eo-sinophil granulocytes, mild blood-CSF barrier dysfunction, marginal intrathecal IgM synthesis, and CSF-restricted OCB. Serology and PCR testing ruled out infection with herpes simplex type 1, type 2, and type 6, varicella zoster virus, enterovirus, arbovirus, *Borrelia burgdorferi*, and *Treponema pallidum*. CSF cultures for bacterial microor-ganisms were also negative. The serum tested negative for AQP4-IgG, ANA, and ANCA. CRP was normal. MOG-IgG was present at a titer of Treatment included short-term antibiotics (ceftriaxone) followed by a 5-day pulse of

high-dose IVMP and prompted rapid resolution of symp-toms. At last follow-up 26 months after onset the patient did not report any residual symptoms; P100 latencies elic-ited from both eyes were slightly delayed.

Comment: This case – together with cases 8 and 12 as well as case 6 in part 3 of this series [31] – highlights that MOG-IgG-related NMO may begin as early as in child-hood or adolescence. As in case 3, the patient presented with bilateral ON at onset and with simultaneous myelitis and ON, two presentations that were previously shown to be more common in AQP4-IgG-negative patients with NMO [34]. Of note, the patient's spinal cord lesions did not extend over three or more vertebral segments. 'Non-longitudinally extensive' lesions have recently been described in two independent studies [34, 93] also in pa-tients with AQP4-IgG-positive NMO; accordingly, LETM lesions are no longer listed among the new, revised 2015 criteria for AQP4-IgG-positive NMOSD [29]. It is of inter-est that mixed but predominantly neutrophilic pleocytosis was present in this patient; CSF neutrophils were also noted in 21 other patients with CSF pleocytosis and avail-able data. Neutrophil granulocytes were shown to be present also in around 50% of CSF samples in AQP4-IgG-positive NMOSD during acute attacks [76].

*Case 5 – Recurrent ON and purely sensory myelitis; good recovery.* This Caucasian man developed unilateral ON at age 22 with complete recovery after high-dose IVMP therapy. Lumbar puncture (LP) revealed CSF pleocytosis (128 white cells/µl) and blood-CSF barrier dysfunction. Brain MRI was normal except for optic nerve swelling and Gd enhancement. VEP demonstrated a delayed P100 latency but normal amplitudes. A second ON attack occurred 10 months later but symptoms again remitted completely following IVMP therapy. Over the following 117 months, two more attacks of unilateral ON with complete or almost complete recovery after IVMP occurred, and one attack of myelitis with purely sensory symptoms (including pain/dysesthesia) and blad-der bowel dysfunction. Spinal cord MRI showed a short lesion (one vertebral segment) without swelling or Gd enhancement. IVMP treatment resulted in partial recov-ery. Serum AQP4-IgG was negative, but MOG-IgG was de-tected at a serum titer of 1:1280. At last follow-up, an EDSS of 1.5 and normal VA was documented. No relapses have occurred so far under treatment with rituximab, which was commenced 2 months before the time of writing.

Comment: This case again illustrates that lesions in MOG-IgG-positive myelitis are not always longitudinally extensive and often exclusively cause sensory symptoms, including pain and dysesthesia. Of note, the latter symp-toms were the most common manifestations of myelitis

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in this cohort, having being present in around 70% of all patients at least once.

## II. MOG-IgG-positive NMO with brain involvement

*Case 6 – LETM and 13 attacks of ON resulting in unilateral blindness; white matter and callosal lesions.* This Caucasian woman had a first, left-sided attack of ON at age 47, with visual loss and retro-orbital pain. MRI showed a longitudinally extensive Gd-enhancing optic nerve lesion on the left side and a clinically inapparent short, Gd-enhancing lesion in the posterior part of the prechiasmatic segment of the optic nerve which extended into the optic chiasm. Symptoms completely remitted following high-dose IVMP treatment. Within 4 months she developed transverse myelitis with thoracic pain, sensory impairment of the abdomen and legs, and urinary incontinence along with blurred vision affecting the other, previously clinically unaffected eye, yet retained high-contrast VA. Spinal MRI revealed two T2 hyperintensities in the thoracic cord; one of these lesions extended over three spinal segments and was accompanied by marked cord swelling and intense contrast enhancement. Cranial MRI showed several non-Gd-enhancing T2-hyperintense lesions in the subcortical supratentorial white matter not involving the corpus callosum. On neurophysiological testing, tibial nerve SSEPs had normal latencies, VEP amplitudes were bilaterally reduced, and P100 latencies were delayed on the left side. The CSF disclosed 14 cells/μl (mostly lymphocytes with a small number of neutrophils) and a normal protein profile without blood-CSF barrier dysfunction and no CSF-restricted oligoclonal IgG bands. The serum tested negative for ANA, anti-ds-DNA-Ab, extractable nuclear antigens (ENA), ANCA, and anti-phospholipid Ab; complement components C3 and C4 were also not altered. High-dose IVMP treatment followed by oral tapering of corticosteroids prompted almost complete recovery of clinical symptoms with mild dysesthesia and restlessness of the lower extremities.

Over the following 12 years, the patient experienced 12 further attacks of mostly unilateral ON, which emerged at a frequency of once or twice per year and were not affected by various long-term immune therapies: 2 of the 12 relapses occurred under treatment with i.m. IFN-beta-1a (1 and 4 months after first injection), 1 under s.c. IFN-beta-1a (less than 2 months after first injection), 1 under s.c. IFN-beta-1b (after 2 months), none under GLAT (6 months), and 1 during a short course of AZA (4 months; no cotreatment with oral steroids). Three PEX cycles (3-5 exchanges each) could also not prevent bilateral deterioration of VA due to recurrent ON with almost complete unilateral blindness and severe contralateral visual loss (0.4 on last follow-up). Of note, two relapses of ON occurred a few weeks after the first and the second infusion of rituximab, respectively.

A further relapse of ON took place 12 months after initiation of rituximab therapy and thus probably after reconstitution of B cells. Treatment with IFN-beta-1a, IFN-beta-1b, and GLAT had to be stopped prematurely because of leukopenia; AZA was stopped due to elevated liver enzymes.

Some stabilization of ON frequency was achieved following prolonged therapy with MTX and long-term maintenance treatment with oral corticosteroids (combined with ciclosporin for a period of 7 months); the patient experienced only two further attacks of ON within almost 7 years after implementation of these regimens.

Repeat MRI 2 years after the initial ON revealed numerically stable brain lesions and complete regression of cord hyperintensities; however, after another 2 years, the brain lesion load had increased, with some lesions now involving the corpus callosum and infratentorial white matter tracts. Follow-up laboratory analysis revealed a normal CSF cell count with mildly elevated neutrophils (6%) and protein profile with negative OCB. Serology for AQP4-Ab was negative.

Comment: This case once more illustrates that MOG-IgG-positive NMO is not always a mild and monophasic disease but can take a relapsing course with poor long-term outcome. Here, the disease caused functional blindness in one eye and marked visual loss in the other eye despite IVMP therapy for acute attacks and various immunotherapies. Of particular note and similar to case 12 described below and case 5 in part 3 of this series [31], INF-beta treatment was paralled by several relapses. By contrast, long-term treatment with MTX in combination with oral steroids was followed by significant stabilization.

*Case 7 – Recurrent ON and pregnancy-related LETM with permanent functional blindness in one eye; white matter lesions.* This female Caucasian patient developed a first episode of unilateral ON in 2005 at age 31 with painful eye movement and blurred vision making reading impossible. Several relapses of unilateral ON occurred up to 2013, all of which responded well to IVMP (5 × 1000 mg) with good partial or full remission of VA. In 04/2013, at which time she was 6 weeks pregnant, she developed numbness of both hands, later accompanied by intense pain of her right arm and shoulder. MRI in 05/2013 revealed an LETM lesion in the cervical spinal cord extending over five vertebral segments. In 12/2013, i.e., only a few weeks after delivery, severe bilateral ON occurred, starting in the left eye and shortly afterwards affecting the right eye. Treatment with IVMP (3 × 1000 mg) and therapy escalation with 2000 mg IVMP 2 weeks later did not result in any significant improvement of the patient's VA (left eye 0.025 in 02/2014). Following the start of rituximab in 02/2014, very slow

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subjective improvement of visual function was noted. AQP4-IgG and a broad panel of other anti-neural auto-antibodies were negative. MOG-IgG were positive in two samples. Brain MRI was normal at onset but showed inflammatory white matter lesions later. LP revealed pleocytosis (37 cells/μl) but no OCB.

Comment: Of particular note, two attacks in this patient occurred during pregnancy or shortly after delivery, respectively. Similarly, attacks occurred 1.5, 3 and 8 months after delivery in case 12 in part 3 of this series [31] and in cases 23 and 19 described below, respectively. One further patient experienced at least two attacks during pregnancy (see case 13 in part 3 [31]). An increase in relapses post partum has been reported in MS [108], and a negative influence of pregnancy on the disease course has been suggested in NMO as well [85, 86]. On the other hand, disease onset preceded pregnancy by more than 8 years in our patient and several relapses had occurred before the first pregnancy-related attack. A simple coincidence thus cannot be ruled out. Note the poor outcome in this patient: While IVMP was initially effective, both high- and ultra-high-dose IVMP therapy failed to restore vision later on, resulting in permanent visual loss in the left eye.

*Case 8 – LETM and recurrent bilateral ON with transient blindness but full recovery in a young boy, ventricular lesion.* This young Caucasian male patient first presented in September 2006 at age 12 with an attack of bilateral ON with blurred vision. The symptoms remitted partially after IVMP pulse therapy.

Six weeks later, he developed headaches and tetraparesis. MRI showed a supratentorial lesion at the anterior horn of the right lateral ventricle and a cervical lesion extending from C2 to C6 level with Gd enhancement and swelling. ADEM was suspected. Extensive laboratory examinations did not reveal any infectious or rheumatological cause. Family history was negative for relevant autoimmune diseases. LP showed a normal CSF WCC, normal CSF protein, glucose, and lactate levels, and negative OCB. After IVMP therapy, vision and muscle strength returned to normal.

The third attack occurred in 03/2012 with almost complete loss of vision of the right eye. LP revealed a normal CSF WCC, normal CSF protein concentration, and negative OCB. AQP4 antibodies were negative. Cerebral MRI showed Gd enhancement of the optic chiasm. VEP revealed bilaterally delayed P100 latencies with normal amplitudes. OCT demonstrated bilateral temporal thinning of the retinal nerve fiber layer (RFNL). No clinical signs or symptoms of myelitis were present, but MRI showed a T2 lesion at C3, spanning around one and a half vertebral segments, as well as diffuse lesions

extending from level C4 to C7 and from level T7 to T9 with Gd enhancement and swelling. After treatment with IVMP, symptoms remitted completely. MS with an opticospinal focus or NMO was suspected and treatment with GLAT was initiated.

Spinal cord MRI in 09/2013 showed remission of the cervical lesions with a remaining small lesion at C2. At last follow-up in 06/2015, after 36 months of treatment with GLAT, the patient did not report any further clinical relapses and had only mild clinical deficits (EDSS 1).

Comment: While bilateral ON and myelitis did not occur strictly simultaneously in this patient, the interval between the two events was just 6 weeks. Similarly, a very short median time to first relapse (5 months) was found in the total cohort. This suggests that patients presenting with a first attack of MOG-IgG-positive ON or myelitis should be closely monitored and treated early. The severe symptoms noted during acute ON (almost complete unilateral blindness) contrast remarkably with the complete remission achieved after IVMP in this case. Note the young age at onset in this and in three other patients (cases 4 and 12 here and case 6 in part 3 of this article series [31].

*Case 9 – Bilateral ON and subsequent LETM with late onset and only partial recovery, callosal lesions on MRI.* This Caucasian woman developed bilateral optic neuritis in 11/2009 at age 64 with spontaneous complete remission within 6 weeks but subsequently prolonged P100 latencies. From 01/2011, she complained of increasing dysesthesia in both forearms and impaired ambulation. Spinal MRI in 05/2011 showed two cervical contrast-enhancing lesions. LP revealed identical OCB in CSF and serum (mirror pattern) and a normal cell count. AQP4 antibodies were negative. Except for ANA (1:160), no other concomitant autoantibodies were detected. Treatment with IVMP in 05/2011, 07/2011, and 09/2011 did not result in significant improvement of the still increasing dysesthesia. In 10/2011, spinal MRI showed diffuse hyperintensity ranging from C2 to C6 with Gd enhancement. Cranial MRI revealed numerous nonspecific T2 hyperintensities considered to reflect microvascular lesions due to a long history of hypertension. In 12/2011 MOG-IgG was tested for the first time and was positive at a titer of 1:1280. After 11 courses of PEX (12/2011 to 08/2013) and 29 months of treatment with AZA (2 x 50 mg/day, from 01/2012) dysesthesia was still present but not increasing anymore and no further relapses had occurred at last follow-up (06/2014). Follow-up MRI in 02/2013 showed, in addition to the vascular lesions, callosal lesions compatible with focal demyelination and residual discrete T2 hyperintensities in the cervical spinal cord.

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Comment: As in 14 further cases, disease started with bilateral ON in this patient. While bilateral ON had traditionally been considered typical for NMO, it was recently shown to be in fact more frequent in AQP4-IgG-negative than in AQP4-IgG-positive patients with NMOSD at disease onset [34]. Of particular interest, LETM was purely sensory, characterized by excruciating dysesthesia, took a protracted or even progressive course, and did not respond to repeat IVMP treatments over a period of 12 months; only PEX and subsequent immunosuppression brought some relief. Surprisingly, 15/19 (78.9%) patients in this cohort reported at least one occurrence of dysesthesia or pain during acute myelitis attacks. Dysesthesia and pain are also common symptoms in AQP4-IgG-positive NMO and are the only symptom in some patients [34]. Both MOG-IgG- and AQP4-IgG-related EM should be considered in the differential diagnosis of patients presenting with purely sensory symptoms compatible with myelitis.

*Case 10 – Recurrent attacks of ON and myelitis with good partial recovery; subcortical and callosal lesions.* This Asian male patient developed bilateral optic neuritis (VA not documented) in 12/2006 at the age of 35. CSF examination showed a normal cell count and normal protein, glucose, and lactate levels with negative OCB. Brain MRI showed Gd enhancement of both optic nerves, one subcortical lesion in the left cerebral hemisphere, and one lesion in the corpus callosum. Spinal cord MRI was not performed at that time. After IVMP pulse therapy complete remission was documented. Extensive laboratory work-up did not reveal any infectious or rheumatological cause of the symptoms.

A second attack occurred in 10/2007 with clinical symptoms of myelitis (paraparesis, urinary retention, and tingling sensation in the lower extremities). LP revealed an increased cell count (197 cells/μl) with a normal protein level and negative OCB. Spinal MRI showed multifocal myelitis of the upper cervical and thoracic segments (C3-C4, T3-T4) with patchy gadolinium enhancement. After IVMP pulse therapy there was only partial remission and slight unsteadiness of gait remained. MS with an opticospinal focus or neuromyelitis optica was suspected. Treatment with AZA 2 mg/kg (150 mg/day) was initiated and was continued until 07/2008 when treatment was stopped due to clinical stabilization.

The third attack occurred in 03/2010 with unilateral optic neuritis. MRI showed Gd enhancement of the right optic nerve and the previously known T2 lesions with patchy gadolinium enhancement. AQP4 antibodies were tested 4 times during this period and were always negative. Complete remission after IVMP pulse treatment was achieved.

After a fourth attack in 08/2010 with tingling sensation of the lower extremities which was treated with 5 g IVMP with complete remission, treatment with AZA was reinitiated directly after discontinuation of IVMP.

Beginning in 01/2011 (i.e., still within the latency period of AZA) another relapse of myelitis with tingling sensation of the lower extremities and of the right hand (EDSS 2) was treated successfully with IVMP. A VEP examination performed at that time revealed delayed P100 latencies bilaterally with normal amplitudes. OCT showed bilateral thinning of the RFNL. Cerebral and spinal MRI did not show any disease activity. AZA was increased to 200 mg/day and later continued at 150 mg/day. The last MRI of the head and spinal cord was performed in 04/2014 and showed no disease activity. The patient was still relapse-free at last follow-up in 04/2015 with an EDSS of 2.5. In total, this patient has experienced five attacks, comprising two attacks of ON (1 × bilateral) and three of myelitis.

Comment: First, the presence of callosal brain lesions noted in this patient and in others in our study (see cases 6 and 9 here and cases 3 and 8 in part 3 [31]) renders the radiological differential diagnosis of MOG-IgG-positive EM and classical MS more challenging. Callosal lesions have also been observed in AQP4-IgG-positive NMO, where they are typically long (half of the length of the corpus callosum or greater), diffuse, heterogeneous, or edematous [29, 109]. In the present cohort, a longitudinally extensive callosal lesion was noted only in a single patient (see case 3 in part 3 of this series [31]). Second, as a result of ON, RFNL thinning as detected by OCT was present in this as well as in several other patients. Similar findings have previously been reported in AQP4-IgG-positive patients [110, 111]. A detailed OCT analysis of 16 patients with MOG-IgG-positive ON can be found in part 4 of this article series [32].

### III. MOG-IgG-positive recurrent ON

*Case 11 – Three attacks of ON; no brain lesions; functional blindness in the left eye.* A 53-year-old woman first noted reduced vision and pain on moving the right eye in 05/2011. Clinical examination demonstrated a VA of 0.1 in the right eye and 1.0 in the left eye, as well as color desaturation and a relative afferent pupillary defect of the right eye. Cranial MRI showed right optic neuritis, but no parenchymal brain lesions. VEP were slightly delayed in the right eye, but amplitudes were preserved. CSF analysis was normal; in particular, there were no CSF-specific OCB. The patient was treated with high-dose IVMP (2 g/d for 3 days), which resulted in improvement of symptoms. However, about 3 weeks later vision in the right eye deteriorated again.

In 11/2011, she also noted reduced vision in the left eye. She was again administered several courses of IVMP,

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which did not improve VA, so that she was subsequently treated with four courses of IA. This was associated with almost complete recovery over the next 3 years.

At the end of 11/2014 she again noted reduced VA in the left eye. A cranial MRI performed in 12/2014 showed a left optic neuritis with contrast enhancement as well as hyperintensities in the right optic nerve without contrast enhancement, but no further cerebral lesions. She was again treated with IVMP and four courses of IA, which resulted in only incomplete recovery. In 03/2015, when her VA was finger counting in the left and 0.2 in the right eye, she was admitted to our department. She was tested for serum antibodies to MOG, which were positive both in a live-cell and in a fixed-cell CBA (determined after IA). She was treated with IVMP, five courses of PEX, and rituximab (2 × 1000 mg). However, VA recovered only slightly. At the last follow-up in 05/2015 her vision was 0.2-0.3 in the right and 0.02 in the left eye.

Comment: This case, together with cases 12-18, demonstrates that MOG-IgG-associated ON is not always a monophasic disease but frequently follows a relapsing course. In fact, recurrent ON was noted in 65% of all patients with isolated ON in the present study. Moreover, the disease took a very severe course with partly therapy-refractory attacks, leading to permanent functional blindness in one eye and substantial loss of VA in the other eye. As in other patients in this series, high-dose and even ultra-high-dose IVMP treatment improved the symptoms only transiently or not at all. Although IA was initially effective, it could not restore vision when used to treat a subsequent relapse. While the reason for that discrepancy is unknown, the relatively long time (6 weeks) between attack onset and IA might have played a role. Alternatively, IA as well as PEX might not result in a sufficient decrease in MOG-IgG titers in some patients if not repeated long enough; finally, other pathomechanisms than antibody-related ones, e.g., T cells, might have been involved.

*Case 12 – Recurrent ON in a child; no brain lesions; severe permanent visual loss.* This Caucasian girl experienced a first episode of ON in 04/2001 at age 6; this attack was bilateral (OD> > OS) with papilledema. VEP showed prolonged P100 latencies. LP demonstrated mild CSF pleocytosis (7 leukocytes/μl). Brain MRI was normal. Symptoms remitted spontaneously. Two years later (04/2003) a second attack of ON occurred, affecting the right eye. Symptoms improved with IVMP. A third attack of ON, again of the right eye, developed 11 months later in 03/2004. CSF was normal. A serum sample drawn in 2004 that was later retrospectively tested was positive for MOG-IgG. When right-sided ON recurred in 08/2008, temporal pallor was noted on fundoscopy

bilaterally. In 2009, ocular myositis was suspected, but no additional information is available. In 01/2013 another attack of unilateral ON occurred, associated with contrast enhancement of the right optic nerve; symptoms improved with IVMP. In 04/2013, the patient was started on IFN beta-1a s.c. However, she experienced two more attacks of right-sided ON in 10/2013 and 03/2014, leading to the discontinuation of IFN-beta treatment; both relapses improved after IVMP. MRI still did not show any brain lesions in 10/2013, i.e., 12 years after onset. VA was 0.4 in the right and 0.8 in the left eye at that time. AQP4-IgG antibodies were negative. An OCT examination in 04/2014 showed massive thinning of the RNFL in both eyes (OD > OS). In 07/2014 and again in 01/2015, the patient received two doses of rituximab (2 × 1000 mg, 2 weeks apart). At last follow-up in 03/2015 no further relapses had occurred.

Comment: This is one of the youngest Caucasian patients with MOG-IgG positive ON reported thus far. MOG-IgG autoimmunity is an important differential diagnosis in pediatric patients presenting with NMOSD and other forms of CNS inflammation of unknown cause [107, 112, 113]. The patient's relapsing disease course confirms that MOG-IgG in pediatric patients with ON is not, as originally thought, limited to monophasic cases [11–13]. Of particular note, the disease has been restricted to the optic nerves in this patient for more than a decade, both clinically and radiologically. As in two other patients (case 6 here and case 4 in part 3 of this series [31]), treatment with IFN-beta was not effective in preventing relapses.

*Case 13 – Recurrent ON with late onset; no inflammatory brain lesions; permanent unilateral blindness.* This Caucasian woman developed severe bilateral optic neuritis in 12/2003 at age 66 following a gastrointestinal infection with positive *Yersinia* serology (species not specified). VA at nadir was 0.1 in both eyes. VEP showed no response of the right eye and was delayed on the left side. Symptoms partly responded to IVMP and oral prednisolone. Brain MRI was normal except for numerous vasculopathic lesions due to decades of hypertension. LP showed CSF pleocytosis (80 cells/μl) but no OCB.

The patient was treated with AZA (150 mg/d) and oral steroids from 03/2004 to 11/2004. However, one ON relapse in the right eye occurred at the end of 04/2004 (with a flare-up in mid-05/2004) and a second in 10/2004 after tapering prednisolone to below 10 mg/day. Symptoms did not substantially improve despite several courses of IVMP (VA 1/35). A follow-up CSF examination performed at that time was normal.

In 11/2004, treatment was switched to MTX (15 mg/week). In 01/2006, the patient experienced another

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attack of unilateral ON, which led to blindness of the right eye. The patient declined IVMP, and symptoms did not improve spontaneously.

No further relapses have occurred under treatment with MTX since then. In 06/2013 VA of 0 on the right and 1.0 on the left side was documented. At last follow-up, in January 2016, the patient was still on MTX (2.5 mg/day). She never experienced any signs or symptoms of myelitis; spinal MRI (11/2004) was normal. Slightly elevated ANA (1:80) were documented on one occasion, but she has no coexisting autoimmune disorders.

Comment: This case is interesting for several reasons: First, disease started after an acute infection, as also seen in at least 10 other patients in our series. Infections have also been reported as a trigger of acute attacks in AQP4-IgG-positive NMOSD [34] and in classic MS [114]. Second, tapering of steroids resulted in recurrence of the patient's symptoms. Similarly, withdrawal of steroids or reductions in steroid dosage resulted in flare-ups in 20 further patients in our series. Third, disease resulted in permanent blindness in one eye in this patient. Similarly, a decline in VA ≤0.5 occurred in 85% of our patients during relapse and severe visual impairment or functional blindness was present in 37% at last follow-up. This underlines that the notion of MOG-IgG-associated ON being generally mild is incorrect. Fourth, while two attacks occurred under treatment with AZA (and cotreatment with oral steroids) within a period of 9 months in this patient, only one – albeit very severe – relapse occurred under MTX over a period of 10 years. The favorable disease course observed in this and in other MTX-treated patients in this study (see cases 3, 6, and 13 here and cases 6 and 12 in part 3 of this article series [31]) warrants further investigations into the potential efficacy of MTX in MOG-IgG-related autoimmunity.

*Case 14 – Recurrent ON starting at age 28; no brain lesions; permanent functional blindness of the left eye.* This patient presented with unilateral ON (VA OD 1/35) in 05/2009 at age 28. Symptoms improved following high-dose IVMP (3 × 1 g), but recurred shortly thereafter (VA OD 1/35) and only partly responded to a second IVMP (3 × 1 g) cycle (VA 0.2). Brain MRI was normal.

Five months after onset, the patient developed left-sided ON, which fully responded to IVMP. Brain MRI was again normal, except for enhancement of the left optic nerve and edematous thickening of the right optic nerve. VEP examination revealed bilaterally prolonged P100 latencies (OD > OS) and reduced amplitudes.

Another attack of ON, on the left side, occurred 2 months later with severe visual loss (VA 0.1). Fundoscopy revealed mild papilledema. IVMP and 10 plasma exchanges resulted in partial improvement. The patient was

started on AZA 100 mg/day in 10/2009 with 6 months cotreatment with oral steroids. In 02/2012, she experienced another relapse of ON, which partially responded to IVMP. The patient had no other immune disorders except for pollinosis. LP had revealed mild pleocytosis (14 cells/μl) but no CSF-restricted OCB and no blood-CSF barrier dysfunction. A serum sample obtained 2 weeks after IVMP therapy was positive for MOG-IgG at a titer of 1:160 in the live-cell assay. MOG-IgG-seropositivity was confirmed in the fixed-cell assay. AQP4-IgG was negative. At last follow-up VA of 0.1 in the left eye was documented.

Comment: As in many patients in this series, the first high-dose IVMP cycle led only to transient remission of symptoms in this case; moreover, only partial recovery was achieved after a second cycle applied for the same attack as well as during two later attacks. The observation of many cases of partial or complete IVMP failure in this series suggests that additional treatment options should be considered in MOG-IgG-positive patients presenting with acute ON or myelitis. It is unknown why IVMP was effective during some attacks, but not all, in this and other patients. However, timing issues and differences in antibody titers, other immunological parameters (e.g., T cell activation), IVMP dosage, and previous or concomitant treatments might all play a role. Note the poor outcome in this case. Functional blindness in at least one eye was also noted in 9 additional patients at last follow-up.

*Case 15 – Three attacks of ON; no brain lesions; full recovery.* This 25-year-old woman presented in 10/2010 with bilateral ON accompanied by visual loss and retro-orbital pain on eye movement. Fundoscopy revealed optic papillitis in both eyes. Except for a marginal difference in biceps tendon reflexes the neurological examination was normal. LP showed normal intracranial pressure, slight pleocytosis (6 cells/μl), normal CSF total protein, glucose, and lactate levels, and negative OCB. VEP were lost in both eyes. Cerebral MRI was normal including the optic nerves. Spinal MRI was not performed at that time. AQP4 antibodies were negative as were extensive infectious and rheumatological laboratory diagnostics. After IVMP therapy (1 g/d for 5 days), the patient completely recovered within 2 weeks.

In 11/2010, 4 weeks after onset of the first symptoms, a second attack occurred with complete visual loss in the right eye and a decrease in VA to 60% in the left eye. Ophthalmological examination revealed bilateral optic papillitis. VEP in both eyes could not be elicited. IVMP pulse therapy (2 g for 5 days), followed by oral reduction over 4 weeks, led to rapid and complete recovery.

The patient was clinically stable without any maintenance therapy until 11/2014, when she developed a third attack of ON with a decrease in VA of the right eye to 40%

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and papillitis of the right optic nerve. The neurological examination was normal. Two cycles of IVMP pulse therapy (1 g/d) for 3 and 5 days, respectively, resulted in complete remission after 2 weeks. In 12/2014 a second brain MRI and a first spinal cord MRI were performed, each with normal findings. SSEP were normal as well.

MOG antibodies were first determined in 03/2015 and were positive. AQP4 antibodies were still negative. At that point the patient was free of symptoms and VEP were normal in both eyes. The patient rejected the idea of commencing any long-term immunosuppressive treatment.

Comment: Despite recurrent disease and severe acute visual impairment with transient blindness and loss of VEP, this patient recovered completely from all attacks following high-dose IVMP (and subsequent oral tapering for one attack). Remarkably, there were no clinical, radiological, or electrophysiological signs of myelitis, brain, brainstem, or cerebellar involvement 3 years after onset; similarly, there was no evidence for spatial dissemination at last follow-up in around a quarter of all MOG-IgG-positive patients in this study.

*Case 16 – Recurrent ON; no brain lesions; significant visual loss in both eyes.* This patient had a first attack of ON at age 58. At last follow-up, 86 months after onset, eight unilateral ON attacks alternately affecting the left and the right eye (never simultaneously bilateral) had occurred but no brain or spinal cord involvement was noted. MOG-IgG were detected retrospectively in a stored sample taken under treatment with oral steroids. VA was 0.4 in the left and 0.5 in the right eye at the last follow-up visit (during remission). There was no relevant comorbidity, including no concomitant autoimmune diseases, and no autoantibodies other than MOG-IgG were detected.

Comment: This case, which is characterized by high disease activity (ARR 1.2) and poor long-term outcome, again illustrates that MOG-IgG-positive ON is not always a monophasic and mild disease. Disease activity varied substantially among untreated patients with isolated ON. While this patient experienced eight ON attacks within just 86 months, no relapse occurred within 72 months in case 22. This renders decisions about long-term treatment difficult, all the more as reliable long-term prognostic markers are lacking. However, with more than 60% of all patients with isolated ON having developed relapses and around two thirds of those with relapses having been functionally blind or otherwise severely impaired in at least one eye at last follow-up, long-term treatment should be considered in most cases.

*Case 17 - Recurrent bilateral ON; transient unilateral blindness; full recovery.* This Caucasian woman developed a first episode of bilateral ON at the age of 50, presenting with bi-frontal headache, painful eye movements, and blurred vision. VA was 0.5 in the right and 0.8 in the left eye at first clinical presentation. VEP displayed a prolonged P100 latency (right > left). Brain MRI showed contrast agent enhancement of both optic nerves but was otherwise normal, as was spinal cord MRI. CSF revealed mild pleocytosis (21/μl, lympho-monocytic), elevated total protein (929 mg/l), no OCB, and a normal IgG CSF/serum ratio. No other laboratory abnormalities were noted (ANA, ANCA, cardiolipin, beta2-glycoprotein, ACE, soluble IL2R, borreliosis, syphilis). The patient's medical history was unremarkable except for arterial hypertension. Following high-dose IVMP, VA initially improved to 0.7 right and 1.0 left; however, a bilateral flare-up of ON occurred within 30 days, resulting in a drop of VA to light perception on the right and 0.5 on the left. The patient received five plasma exchanges which resulted in full recovery.

Three months later another bilateral ON attack with a large scotoma occurred. Full remission (bilateral VA 1.0) was achieved with high-dose IVMP. A further 2.5 years later (10/2012) the patient experienced a mild ON attack with blurred vision but without a drop in VA in the right eye. Following the patient's preferences, no relapse treatment was given and no preventive immunosuppressive treatment was initiated. Re-testing for serum autoantibodies was unremarkable (NMDA-IgA/IgG, amphiphysin, CV2/CRMP5, Ma2/Ta, Ri, Yo, Hu, LGI1, CASPR2, GABA-B receptors, AMPA receptors, GAD, MAG, c-ANCA, p-ANCA, rheumatoid factor, ganglioside antibodies) except for low-titer ANA (1:160). At last follow-up (11/2015) VA was 1.0 in both eyes and no further relapses had occurred.

Comment: Disease again affected exclusively the optic nerves. The excellent long-term outcome despite large scotoma and near-blindness during acute attacks is remarkable. As in around 44% of all patients, IVMP was only transiently effective when used to treat acute attacks; PEX was required to achieve full remission but, of particular note, could prevent further relapses only for 3 months.

*Case 18 – Recurrent ON with late onset; permanent functional blindness; stabilization under rituximab.* A 58-year-old woman with a history of hepatitis A two decades previously developed amaurosis in the right eye and headache in 04/2014. Clinical examination revealed a VA of 1/100 in the right eye with global alteration of right visual field and delayed and reduced amplitude of P100 wave at visual evoked potential (VEP). Brain MRI showed T2/FLAIR hyperintensity of the right optic nerve with spotty

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post-contrast enhancement and non-specific subcortical frontal hyperintense lesions. Spinal MRI and CSF were normal. Routine laboratory examinations were normal except for low-titer ANA (1:80). Despite treatment with high-dose IVMP, almost no improvement was achieved (VA 1/10). Two months later (06/2014) the patient developed left-sided ON. Treatment with oral steroids resulted in only mild improvement (5-6/10). Two and four months later (08/2014 and 10/2014), respectively, further ON attacks affecting the left eye occurred; high-dose IVMP was followed by almost full recovery of VA in this eye. At that time, serum positivity to anti-MOG antibodies was detected. In 10/2014 treatment with rituximab (2 × 1000 mg, 2 weeks apart) and oral steroids was started and was followed by further improvement of VA in the left but not in the right eye. At a follow-up visit 1 year after the last attack, VA was 9/10 on the left (with normal VEP) and 1/10 on the right. Of note, CD19 cells were still undetectable 14 months after the first rituximab infusion in this patient.

Comment: While this patient almost completely recovered from three ON attacks in the left eye, no improvement had been achieved after the initial attack, which had affected the right eye and had left the patient functionally blind. This illustrates that the severity of MOG-IgG-associated attacks varies substantially not only between patients but also intraindividually. Studies investigating risk factors for poor attack outcome in MOG-IgG-positive patients are highly warranted.

### IV. MOG-IgG-positive monophasic ON

*Case 19 – Single episode of post-infectious bilateral ON 8 months post partum; no brain lesions; partial recovery.* A 30-year-old woman who was breastfeeding her 8-month-old healthy daughter noticed bilateral blurred vision, pain when moving the eyes, and moderate frontal headaches in February 2011. During the weeks before symptom onset, she had had common colds with mild fever. Her past medical history was otherwise unremarkable. Ophthalmological examination demonstrated reduced VA of the right (1/25) more than of the left eye (0.5), reduced color vision in the right eye, and papillitis of the right more than of the left eye. Visual field examination revealed bilateral large centrocecal scotomas, again more prominent in the right eye. The patient was admitted to our department, where the rest of the neurological examination was normal. Cranial and orbital MRI demonstrated contrast enhancement in both optic nerves, compatible with bilateral optic neuritis, but no lesions in the brain parenchyma. Spinal MRI and chest radiography were unremarkable. CSF analysis revealed a mildly elevated total cell count of 12 white blood cells per μl (reference range, <5/μl) with 88% lymphocytes and 12% monocytes. CSF protein and lactate were normal and there

were no CSF-specific OCB. A complete blood count and C-reactive protein were normal, as were microbiological (*Borrelia, Treponema pallidum, Toxoplasma*) and virological tests (herpes simplex virus type 1 and 2, varicella zoster virus). ANA were detectable at a titer of 1:320. Screening for ENA, ANCA, and AQP4-IgG was negative. However, antibodies to MOG were detected in the patient's serum at a titer of 1:1280. The patient was treated with IVMP (1 g/day for 5 days). An ophthalmological follow-up examination in August 2011 showed markedly improved VA of the right (0.7-0.8) and left (0.7) eye. The visual field defects had almost completely resolved. Funduscopy revealed bilateral mild temporal disk pallor consistent with mild partial optic atrophy.

Comment: This patient developed her first relapse in the third trimester post partum. The first 9 months after pregnancy have been previously identified as a risk period for relapses both in AQP4-IgG-positive NMO [85, 86, 115] and in MS [108]. However, systematic studies comparing ARRs before, during, and after pregnancy in MOG-IgG-positive patients are still lacking. Of note, the temporal association between the two events could still be coincidental in the present case, all the more as disease onset was also preceded by feverish infection. Remarkably, almost complete recovery was achieved despite substantial visual loss during an acute attack.

*Case 20 – Protracted single episode of bilateral ON; delayed SSP; no brain lesions; complete recovery.* This Caucasian man developed a first, bilateral ON at age 70 with severe vision loss (0.5 OD, 0.4 OS), papilledema, scotoma, and delayed P100 latencies (but no or only marginally reduced P100 amplitudes). Treatment with high-dose IV prednisolone (1000 mg 3d) with oral tapering resulted in marked but incomplete short-term recovery with persisting contrast sensitivity impairment and hazy vision. Within 4 weeks from onset of symptoms bilateral visual loss (0.5 OD and OS) and scotoma recurred and improved gradually after a second course of high-dose i.v. prednisolone (1000 mg 3 d) with oral tapering and additional intravenous antibiotics (sobelin, ceftriaxone). Brain MRI revealed slight perineural contrast enhancement around the left optic nerve and signs of ethmoidal cell sinusitis, but was otherwise unremarkable. While spinal MRI did not unequivocally reveal any lesions, tibial nerve SSEP were bilaterally delayed (69 ms on the right; only late components obtainable on the left), suggesting possible subclinical myelitis. Median nerve SSEP were normal except for a side difference in latencies. Transcortical magnetic stimulation to the legs was normal as well. CSF assessment revealed a normal cell profile, mild blood-CSF barrier dysfunction, and no OCB. AQP4-IgG-testing was negative. The patient had

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been diagnosed with hepatitis B more than 20 years before onset of symptoms; however, Hbs, HBc-IgG, HCV-IgG, and HBE were all negative at the time of ON, as were ANA, ANCA, CRP and rheumatoid factor. Chest radiography did not reveal evidence of sarcoidosis, and serum ACE was normal. At repeated long-term follow-up visits (most recently in 10/2010) complete recovery was confirmed with normal VA and no evidence of scotoma.

Comment: This case, alongside the pediatric cases reported here, illustrates the broad variability in age of onset in MOG-IgG-related disorders. A late onset (>60 years) was also observed in two further patients (66 years in case 13; 64 years in case 9). While MOG-IgG were originally described in pediatric patients with ADEM, these cases demonstrate that MOG-IgG need to be considered also in elderly patients presenting with a first attack of optic neuritis or myelitis. Moreover, this case is one of the few monophasic ones, with no relapse more than 6 years after onset. By contrast, disease followed a relapsing course in 80% of all MOG-IgG-positive patients in this cohort, irrespective of clinical presentation.

*Case 21 – Single episode of unilateral ON; no brain lesions; complete recovery.* This man presented at age 53 with unilateral retrobulbar ON of the left eye (VA 0/10, peripheral scotoma, intraorbital swelling, and Gd enhancement of the optic nerve with contrast enhancement, VEP delayed and amplitudes reduced; right eye normal). LP was unremarkable with 2 cells/µl, no OCB, and normal CSF/serum ratios of IgG and albumin. MRI revealed no extra-optic nerve brain lesions. MOG-IgG were positive at a titer of 1:1280 prior to treatment. Treatment with high-dose steroids resulted in marked improvement (1 mg/d for 6 days) with complete recovery after some months (VA 10/10). Electrophysiological control examinations 7 and 18 months after onset showed only a residual borderline delay in P100 latency on the left side; brain MRI was still normal 25 months after onset; and at last follow-up at month 75 no new symptoms had occurred. The patient had a history of poliomyelitis during childhood and of brucellosis 12 years prior to ON. Serum AQP4-IgG was absent, but MOG-IgG was present at a titer of 1:1280.

Comment: The favorable attack outcome – with complete recovery following IVMP treatment – and the monophasic disease course – with no new symptoms 75 months after onset – once more illustrate the broad variability in prognosis in MOG-IgG-positive ON.

*Case 22 – Single episode of unilateral ON; no brain lesions; persisting visual deficit.* A 29-year-old man developed sudden loss of vision and reduced visual field in the right eye. VA was initially 20/200 in the affected eye.

Unilateral ON was diagnosed. Brain MRI and intracranial pressure were normal. CSF showed normal leukocyte count, IgG index, and protein level, and no OCB. AQP4-IgG was negative. At follow-up, persisting visual loss (20/60) of the right eye was apparent. So far, no further attacks have occurred. Of note, this patient had psychiatric difficulties in the past (classified as ADHD) and a previous history of central diabetes insipidus of unknown cause with onset at age 7 years.

Comment: It remains unknown whether the patient's diabetes insipidus and psychiatric symptoms were caused by CNS autoimmunity. Of note, however, psychiatric symptoms (psychomotor slowing, disorientation, and impaired consciousness) were present in two further patients in this series (described in detail in part 3 [31]) and occasionally occur also in AQP4-IgG-positive patients [116–118]. Central diabetes insipidus, which is characterized by a lack of antidiuretic hormone (ADH) in the brain, has been previously reported in a patient with NMO but unknown AQP4-IgG and MOG-IgG serostatus [119], and several AQP4-IgG-positive NMOSD patients with Schwartz-Bartter syndrome (also termed syndrome of inappropriate ADH secretion) due to hypothalamic lesions have been described over the past few years [120–122].

*Case 23 – Post-partum episode of ON; possible onset of disease already at age 10; low- rather than high-contrast VA affected.* In 1995, at the age of 10 years, this Caucasian patient had suffered from a self-reported "*Borrelia*-induced meningitis" with headache, diplopia, and bulbar movement pain, with one recurrence 1.5 years later. More than 18 years later, in 07/2015, and 3 months after delivery of her first child, the then 30-year-old woman developed subacute retrobulbar pain and frontal headache on the left side, as well as "focusing deficits" of her left eye. A neurological examination performed 2 weeks after symptom onset was normal including high-contrast VA (1.25 on both sides), but refined vision tests revealed a bilateral reduction of the low-contrast VA (right eye: 0.8; left eye: 0.6). Brain MRI showed a T2-hyperintense left optic nerve lesion and a few bi-frontal non-specific white matter lesions that did not fulfill the diagnostic criteria for MS. MRI of the cervicothoracic spinal cord was unremarkable. LP, performed 3 weeks after symptom onset, revealed slight pleocytosis (9 cells/µl) but was otherwise normal; in particular, OCB were negative. VEP showed normal amplitudes and delayed P100 latencies in the left eye, but only when directly comparing the two eyes with each other (right: 104 ms; left: 112 ms). By contrast, spectral-domain OCT revealed severe bilateral thinning of the RNFL, most prominent in the temporal sectors of both eyes (mean RNFL right: 75 µm, mean RNFL left: 68 µm). Motor,

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somatosensory, and acoustic evoked potentials were unremarkable. A broad laboratory work-up including ANA, cANCA, pANCA, and AQP4-IgG, was negative. Anti-MOG-IgG antibodies were positive at 1:320. MOG-IgG seropositivity was confirmed by a fixed-cell CBA. While maintaining breastfeeding the patient was treated with IVMP for 5 days (1000 mg once daily), which led to a reduction of the left-sided retrobulbar pain up to the time of the last follow-up in 10/2015. At that time, the patient still refused any long-term immunosuppressant treatment.

Comment: This case is interesting for several reasons. First, the patient presented with unilateral retrobulbar pain and headache but normal VA as detected by Snellen chart and near-normal VEPs. Only additional tests (MRI, OCT, and low-contrast VA testing) revealed marked bilateral optic nerve damage. Similarly, no impairment of high-contrast VA but pathological VEPs were found in cases 25 and 27 here as well as in case 2 in part 3 of this series [31]. These cases indicate that subclinical ON needs to be taken into account also in MOG-IgG-positive patients with apparently normal VA as routinely detected by a Snellen score. Second, symptoms compatible with previous episodes of ON (retrobulbar pain) headache and brainstem encephalitis (diplopia) had occurred 20 and 18.5 years before the present attack in this patient, though it remains unknown whether she was already positive for MOG-IgG at that time. Similarly, disease started with an attack of unilateral ON at age 13 in another patient in this cohort (see case 6 in part 3 of this series [31]), which was followed by a first attack of myelitis only several decades later. Long intervals between first and second attack (up to 17 years) have also been described in AQP4-IgG-positive patients [34]. Third, only OCT, not MRI or VEP, was able to demonstrate damage also of the right optic nerve. Fourth, as in cases 7 and 18 here and in case 12 in part 3 [31], symptoms developed post partum, a period associated with an increased risk for relapse also in MS [108] and in AQP4-IgG-positive NMOSD [115].

*Case 24 – Fifteen attacks of ON with poor response to treatment and unfavorable bilateral functional visual outcome.* In 2013, this 42-year-old female Caucasian patient experienced for the first time typical clinical signs of left-sided ON, with reduced vision, red color desaturation, and eye pain. Fundoscopic examination showed a hyperemic and swollen papilla of the left eye. The VEP amplitudes were reduced on the left side. Further diagnostic work-up including anti-AQP4 antibodies, onconeural antibodies, and immunological screening was negative. The CSF was normal; in particular, there was no pleocytosis, no OCB, and no disruption of the blood-CSF barrier function. MRI of the brain showed an intense, long-segment gadolinium (Gd) enhancement of

the left optic nerve, but only few non-MS-specific T2-hyperintense white matter lesions without subclinical progression or Gd enhancement in follow-up scans. The brainstem was never involved clinically or radiologically. Although the patient reported fluctuating paresthesia of both legs and left arm, there was no further clinical, radiological, or electrophysiological evidence for myelitis.

Due to repeated relapses of isolated ON predominately affecting the left eye and partial response to steroid treatment, diagnosis of a chronic relapsing inflammatory optic neuropathy (CRION) was suggested. After 26 months the patient had experienced altogether 15 relapses of ON, consecutively affecting both eyes, but never with simultaneous bilateral involvement.

Except for two attacks (including the initial one), which both remitted completely, she responded only partially to high-dose IVMP treatment. One relapse was treated with PEX, but to no avail. Successive immunosuppressive treatment with AZA (up to 200 mg per day over 9 months and cotreatment with oral steroids), MTX, mycophenolate, and continuous prednisone failed to prevent further attacks of ON. At the time of the last follow-up, 26 months after onset, she had a residual VA of < 0.2 in both eyes.

Comment: The poor visual outcome after just 26 months and the high number of relapses despite regular IVMP treatment for acute relapses and various IS therapies illustrate that MOG-IgG-positive ON may take a severe, relapsing, and sometimes therapy-refractory course. Testing for MOG-IgG should be considered in all patients with suspected CRION.

### V. MOG-IgG-positive recurrent LETM

*Case 25 – Recurrent LETM; slightly delayed VEP; no brain lesions; almost complete recovery.* This 22-year-old Caucasian woman presented with bilateral dys- and hypesthesia of the lower limbs in 11/2011. The neurological examination additionally revealed very mild foot flexor and extensor paresis bilaterally (5-/5) and saddle anesthesia. MRI of the complete neuroaxis (including brain, cervical, thoracic, and lumbar spine) was unremarkable. CSF showed pleocytosis (58 cells/µl) and slightly elevated protein levels (48.9 mg/dl). After negative viral and microbiological diagnostics, initial treatment with acyclovir and ceftriaxone was changed to 4 × 1 g IVMP and, subsequently, oral steroids with tapered dose reduction. Clinical symptoms receded to slight plantar dysesthesia with good response to pregabalin.

In 03/2012 a relapse with bilateral dys- and hypesthesia occurred, this time affecting the upper limbs (fingertips bilaterally), lower limbs (thighs), and trunk (T4). Cerebral MRI again showed no pathologies; spinal MRI, however, revealed two LETM lesions, one stretching from C1 to C4,

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the other from C7 to T9; both showed swelling, the latter also Gd enhancement. Diagnostic workup again revealed pleocytosis in the CSF (25 cells/μl) and elevated CSF protein levels (53.2 mg/dl). OCB and AQP4-IgG were negative. Serologically, TPO antibodies were positive, but otherwise there was no indication for another autoimmune disease or vasculitis. Electrophysiology showed marginal P100 delay (117 – 118 ms bilaterally) in VEP and reduced amplitudes in SSEP (pronounced on the right). Again, 4 × 1 g IVMP with tapered dose reduction was applied. With the suspected diagnosis of AQP4-IgG-negative NMO, AZA was started at the end of 03/2012. The neurological symptoms improved under this therapy but the patient developed recurrent genital condyloma, necessitating operative removal.

The patient presented at our hospital for the first time in 11/2014. She had symptoms of slight gait ataxia, mainly at night, slight hypesthesia (inner thighs), and complained of severe fatigue, partly accentuated due to the recurrent operative procedures during the past few weeks. Thus the decision was made to switch the medication to rituximab, and AZA treatment was ended in 12/2014. During the drug-free interval of 3 months, the patient noted a remarkable clinical amelioration of the neurological and neuropsychological symptoms and finally came to be very reserved concerning the initiation of rituximab. Due to the patient´s concerns, it was decided to continue with close clinical and MRI follow-up. At the last two follow-up visits (03/2015 and 06/2015) the patient showed continuous recovery with only slight residual nocturnal gait ataxia (EDSS 1). MRI showed no new lesions and no Gd enhancement. At the first follow-up visit, MOG-IgG were found using a commercial CBA (Euroimmun) and were confirmed in the live-cell CBA.

Comment: The good long-term outcome (EDSS 1.0 at 3.5 years after onset) despite three myelitis attacks and despite the presence of extensive inflammation affecting the spinal cord over a length of 14 vertebral segments is remarkable. MOG-IgG-positive myelitis was longitudinally extensive in most of our patients and involved large parts of or even the entire spinal cord in some cases. Purely sensory attacks, as observed here, occurred in 13 other cases and, as said before, dysesthesia and pain were common symptoms in this cohort. Of note, good response to pregabalin was noted in this patient.

Case 26 – Recurrent LETM; spinal cord biopsy; partial recovery. This Caucasian man developed a first attack of myelitis in 12/2013 at age 41, with tetraparesis. Spinal cord MRI revealed an LETM lesion extending from C3 to C5. Brain MRI showed a right-sided Gd-enhancing T2 lesion adjacent to the posterior horn of the right ventricle. LP demonstrated mild pleocytosis (23 cells/μl) but no OCB. A decompression operation was performed in 01/2014 due to a suspected diagnosis of cervical myelopathy. However, symptoms worsened again in 04/2014 and a biopsy sample was analyzed to rule out neoplasm and vasculitis. Neuropathology revealed T cell infiltration but there was no specific staining for IgG and complement deposition. Follow-up MRI examinations over a period of 1 year persistently showed contrast enhancement in the cervical spinal cord. Two cycles of high-dose IVMP in 10/2014 and 11/2014 with subsequent oral steroid therapy resulted in only transient improvement. Several tests for AQP4-IgG were negative. In 02/2015 MOG-IgG was tested for the first time and was positive at low titer (1:160) in a live-cell assay; the result was confirmed in a commercial fixed-cell assay for MOG-IgG (Euroimmun). Broad differential diagnosis for infectious, (para)neoplastic, and autoimmune conditions was unremarkable. At follow-up in 02/2015 residual paresis (EDSS 4) was present; MOG-IgG were again positive at a titer of 1:160 and were confirmed in a second, commercial fixed-cell CBA (Euroimmun). There was no clinical, MRI, or electrophysiological (normal VEP 03/2015) evidence of optic nerve involvement. In September 2015, just five months after the first infusion of rituximab, the patient developed a relapse of acute myelitis with severe paresis. PEX resulted in only partial recovery. At last follow-up in March 2016, an EDSS of 6 was noted.

Comment: The differential diagnosis of LETM lesions include, among others, tumors, lymphoma, and spinal cord compression. Accordingly, reports on (unnecessary) neurosurgical procedures, including biopsies, exist also in AQP4-IgG-positive LETM patients [123–126]. Except in the case of emergency, AQP4-IgG and MOG-IgG should be excluded using at least two sensitive assays, at least one of which should be a cell-based assay [8, 29, 124], before any such procedure is considered.

### VI. MOG-IgG-positive monophasic LETM

Case 27 – Single episode of LETM; no brain lesions; partial recovery at discharge. A 23-year-old Caucasian man presented with a sensory level at T4, local dysesthesia, mildly positive Babinski reaction, and bladder and erectile dysfunction shortly after an unspecified infection. MRI showed an LETM lesion extending from C3 to C7 with swelling. Brain MRI was normal. LP revealed lymphomonocytic pleocytosis (59 cell/μl) and mild blood-CSF barrier dysfunction (QAlb 6.96), but no intrathecal IgG synthesis (no OCB, QIgG normal). Further examinations for infection (*Borrelia burgdorferi*, *Treponema pallidum*, HAV, HBV, HCV, HIV, CMV, EBV, HSV1, HSV2, FSME, *Mycoplasma pneumoniae*) or common autoimmune disorders (ANA, ANCA, rheumatoid factor, CRP, C3d) were negative except for slightly elevated phospholipid/glycoprotein beta2 IgG antibodies (IgM negative). VEP were bilaterally delayed, indicating a

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history of subclinical ON; MRI of the orbit was unrevealing. MOG-IgG were positive at a titer of 1:10,240. Treatment with rocephine, acyclovir and, subsequently, high-dose steroids and oral tapering was followed by marked improvement. At discharge, residual mild and circumscript paresthesia as well as bladder dysfunction (requiring urinary catheterization) was present.

Comment: In this and two other cases without a history of clinically apparent ON (case 25 here and case 2 in part 3 of this series [31]), delayed P100 latencies were noted, suggesting subclinical optic nerve inflammation. The predictive value of a positive VEP for a future clinical ON relapse in patients with MOG-IgG-positive myelitis is so far unknown. Of note, the current diagnostic criteria for AQP4-IgG-negative NMOSD do not take into account VEP results but only clinical episodes of ON [29].

### VII. Postvaccinal ON and myelitis

*Case 28 – Recurrent myelitis and ON after vaccination against tetanus, diphtheria and pertussis resulting in functional blindness and tetraparesis; poor outcome.* A 47-year-old Caucasian woman presented with acute sensorimotor tetraparesis (upper limbs: BMRC grade 4; lower limbs: BMRC grade 0), transient somnolence, and respiratory distress. Symptoms had started 12 days after vaccination against tetanus, diphtheria, and pertussis (Boostrix®) and had been preceded by a 2- to 3-day episode of fever prior to symptom onset. MRI showed a single parieto-occipital lesion and a longitudinally extensive spinal cord lesion extending over 15 vertebral segments (C2 to T9). CSF examination revealed moderate pleocytosis (210 white cells/µl) and disturbed blood-CSF barrier function but no CSF-restricted OCB. The symptoms responded only partially to IVMP and IA. As MOG-IgG was positive, treatment with rituximab was started. By 11 days after the first infusion, spinal cord T2 hyperintensities had resolved almost completely. However, just 7 weeks after the second infusion of rituximab and 3 months after onset of the first attack, the patient developed an episode of simultaneous myelitis and unilateral optic neuritis leading to severe loss of vision in the right eye. P100 latencies were delayed in both eyes and amplitudes reduced in the right eye. Brain MRI demonstrated an increase in size of the parieto-occipital lesion. Spinal MRI showed T2 hyperintensities from C7 to T8, predominantly in the posterior columns. Treatment with IVMP and IA was followed by incomplete remission of the symptoms. Just 48 days after onset of the second attack, the patient was readmitted with a new attack of unilateral ON in the left eye resulting in almost complete visual loss (VA 0.05). Treatment with IVMP, IA, and cyclophosphamide led only to partial recovery (VA 0.16 at discharge). At last follow-up, severe spastic paralysis of the lower limbs and an EDSS score of 8 was documented.

Comment: Disease onset in this patient followed vaccination with a polyvalent vaccine against tetanus, diphtheria, and pertussis. Although a causal link between the two events cannot be proved, the close temporal association is highly suggestive of vaccine-mediated immune activation. Of particular note, symptoms also started within 2 weeks after a polyvalent vaccination against tetanus, diphtheria, and pertussis (as well as polio and influenza virus) in a second MOG-IgG-positive patient of this cohort (see case 8 in part 3 [120]). Whether molecular mimicry between vaccine epitopes and neural antigens played a role or whether vaccination only indirectly triggered or promoted the immune reaction against MOG is currently unknown but certainly warrants further investigation.

### Authors' contributions
SJ, BW, MRe, and FrP conceived the study. SJ designed the study, collected data, created the database and database software, analysed the data, and wrote the manuscript. MRe and KS performed the live-cell CBA. SJ and KF performed

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the fixed-cell CBA. All other authors collected clinical and paraclinical data, were involved in patient care, and/or have contributed case reports. All authors were involved in revising the manuscript for intellectual content. All authors read and approved the final draft before submission.

## Author details

[1]Molecular Neuroimmunology Group, Department of Neurology, University of Heidelberg, Heidelberg, Germany. [2]Department of Neurology, Charité University Medicine Berlin, Berlin, Germany. [3]Department of Neurology, Ruhr University Bochum, Bochum, Germany. [4]NeuroCure Clinical Research Center and Clinical and Experimental Multiple Sclerosis Research Center, Department of Neurology, Charité University Medicine, Berlin, Germany. [5]Experimental and Clinical Research Center, Max Delbrueck Center for Molecular Medicine and Charité University Medicine Berlin, Berlin, Germany. [6]Department of Neurology and Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark. [7]Department of Neurology, Albert Ludwigs University, Freiburg, Germany. [8]Department of Neurology, Hannover Medical School, Hannover, Germany. [9]Department of Neurology, Heinrich Heine University, Düsseldorf, Germany. [10]Department of Neurology, University of Rostock, Rostock, Germany. [11]Department of Neurology, Julius Maximilians University, Würzburg, Germany. [12]IRCCS, C. Mondino National Neurological Institute, Pavia, Italy. [13]Centro di Riferimento Regionale SM, Azienda Ospedaliero Universitaria San Luigi Gonzaga, Orbassano, Italy. [14]Department of Neuroradiology, Charité University Medicine – Berlin, Berlin, Germany. [15]Department of Neuroradiology, Ruhr University Bochum, Bochum, Germany. [16]Institute of Experimental Immunolog, affiliated to Euroimmun AG, Lübeck, Germany. [17]Department of Neurology, Medical University Innsbruck, Innsbruck, Austria.

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## RESEARCH

CrossMark

# MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 3: Brainstem involvement - frequency, presentation and outcome

Sven Jarius[1*], Ingo Kleiter[2], Klemens Ruprecht[3], Nasrin Asgari[4], Kalliopi Pitarokoili[2], Nadja Borisow[6,7], Martin W. Hümmert[5], Corinna Trebst[5], Florence Pache[6,7], Alexander Winkelmann[8], Lena-Alexandra Beume[9], Marius Ringelstein[10], Oliver Stich[9], Orhan Aktas[10], Mirjam Korporal-Kuhnke[1], Alexander Schwarz[1], Carsten Lukas[11], Jürgen Haas[1], Kai Fechner[12], Mathias Buttmann[13], Judith Bellmann-Strobl[6,7], Hanna Zimmermann[3], Alexander U. Brandt[3], Diego Franciotta[14], Kathrin Schanda[15], Friedemann Paul[6,7†], Markus Reindl[15†], and Brigitte Wildemann[1†]; in cooperation with the Neuromyelitis Optica Study Group (NEMOS)

## Abstract

**Background:** Myelin oligodendrocyte glycoprotein antibodies (MOG-IgG) are present in a subset of aquaporin-4 (AQP4)-IgG-negative patients with optic neuritis (ON) and/or myelitis. Little is known so far about brainstem involvement in MOG-IgG-positive patients.

**Objective:** To investigate the frequency, clinical and paraclinical features, course, outcome, and prognostic implications of brainstem involvement in MOG-IgG-positive ON and/or myelitis.

**Methods:** Retrospective case study.

**Results:** Among 50 patients with MOG-IgG-positive ON and/or myelitis, 15 (30 %) with a history of brainstem encephalitis were identified. All were negative for AQP4-IgG. Symptoms included respiratory insufficiency, intractable nausea and vomiting (INV), dysarthria, dysphagia, impaired cough reflex, oculomotor nerve palsy and diplopia, nystagmus, internuclear ophthalmoplegia (INO), facial nerve paresis, trigeminal hypesthesia/dysesthesia, vertigo, hearing loss, balance difficulties, and gait and limb ataxia; brainstem involvement was asymptomatic in three cases. Brainstem inflammation was already present at or very shortly after disease onset in 7/15 (47 %) patients. 16/21 (76.2 %) brainstem attacks were accompanied by acute myelitis and/or ON. Lesions were located in the pons (11/13), medulla oblongata (8/14), mesencephalon (cerebral peduncles; 2/14), and cerebellar peduncles (5/14), were adjacent to the fourth ventricle in 2/12, and periaqueductal in 1/12; some had concomitant diencephalic (2/13) or cerebellar lesions (1/14). MRI or laboratory signs of blood-brain barrier damage were present in 5/12. Cerebrospinal fluid pleocytosis was found in 11/14 cases, with neutrophils in 7/11 (3-34 % of all CSF white blood cells), and oligoclonal bands in 4/14. Attacks were preceded by acute infection or vaccination in 5/15 (33.3 %). A history of teratoma was noted in one case. The disease followed a relapsing course in 13/15 (87 %); the brainstem was involved more than once in 6. Immunosuppression was not always effective in
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* Correspondence: sven.jarius@med.uni-heidelberg.de
†Equal contributors
Brigitte Wildemann, Markus Reindl and Friedemann Paul are equally contributing senior authors.
[1]Molecular Neuroimmunology Group, Department of Neurology, University Hospital Heidelberg, Otto Meyerhof Center, Im Neuenheimer Feld 350, 69120 Heidelberg, Germany
Full list of author information is available at the end of the article

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preventing relapses. Interferon-beta was followed by new attacks in two patients. While one patient died from central hypoventilation, partial or complete recovery was achieved in the remainder following treatment with high-dose steroids and/or plasma exchange. Brainstem involvement was associated with a more aggressive general disease course (higher relapse rate, more myelitis attacks, more frequently supratentorial brain lesions, worse EDSS at last follow-up).

**Conclusions:** Brainstem involvement is present in around one third of MOG-IgG-positive patients with ON and/or myelitis. Clinical manifestations are diverse and may include symptoms typically seen in AQP4-IgG-positive neuromyelitis optica, such as INV and respiratory insufficiency, or in multiple sclerosis, such as INO. As MOG-IgG-positive brainstem encephalitis may take a serious or even fatal course, particular attention should be paid to signs or symptoms of additional brainstem involvement in patients presenting with MOG-IgG-positive ON and/or myelitis.

**Keywords:** Myelin oligodendrocyte glycoprotein (MOG) antibodies, MOG-IgG, Neuromyelitis optica spectrum disorders (NMOSD), Brainstem encephalitis, Rhombencephalitis, Optic neuritis, Myelitis, Longitudinally extensive transverse myelitis (LETM), Cerebellitis, Ataxia, Respiratory insufficiency, Intractable nausea and vomiting, Facial nerve palsy, Diplopia Internuclear ophthalmoplegia (INO), Hearing loss, Aquaporin-4 antibodies (AQP4-IgG, NMO-IgG)

## Background

Over the past few years, a new diagnostic role has been found for antibodies to myelin oligodendrocyte glycoprotein (MOG-IgG) in adults [1]. While MOG-IgG had initially been thought to play a role in classical multiple sclerosis (MS), recent studies have demonstrated that MOG-IgG are in fact a marker of autoimmune optic neuritis (ON) and (often longitudinally extensive) transverse myelitis [1, 2]. Based on the fact that aquaporin-4 (AQP4)-IgG is usually absent in MOG-IgG-positive patients [3–10], that the histopathology of inflammatory CNS lesions differs between MOG-IgG- and AQP4-IgG-positive patients [11–13], and that MOG-IgG are pathogenic both in vitro and in vivo [2, 14], MOG-IgG-related autoimmunity is now considered by many a disease entity in its own right, distinct both from classical MS and from AQP4-IgG-mediated neuromyelitis optica spectrum disorders (NMOSD) [15, 16].

While the association of MOG-IgG with ON and myelitis is now well established [2–4, 6, 17], less is known about extra-opticospinal manifestations in MOG-IgG-related autoimmunity. Here, we report the largest series of Caucasian patients with MOG-IgG-positive brainstem encephalitis so far. Brainstem involvement was severe in some of the cases and was fatal in one patient. Clinical, laboratory, and radiologic findings are reported in addition to treatment outcomes.

This article is the third of a four-part series on the clinical, laboratory, magnetic resonance imaging (MRI), electrophysiological, and optical coherence tomography features of patients with MOG-IgG-related CNS autoimmunity [3, 17, 18].

## Methods

All 15 patients were identified from a large European cohort of almost exclusively Caucasian patients with MOG-IgG-associated ON and/or myelitis ($n = 50$) from 12 European academic centers, eight of which are members of the German Neuromyelitis optica study group (NEMOS) [19–23]; this cohort is described in parts 1 and 2 of this series [3, 17]. MOG-IgG had been tested for clinical purposes in all cases and was detected using a live-cell-based assay (CBA) [2] and a commercial fixed-cell CBA (Euroimmun, Luebeck, Germany), both of which employ recombinant human full-length MOG as antigenic substrate. AQP4-IgG was tested using a commercial CBA (Euroimmun) employing recombinant human full-length AQP4 [24–26] and was negative in all cases [3]. The study was approved by the institutional review boards of the participating centers. Patients gave their informed consent for publication. The median number of documented clinically apparent brainstem attacks per patient was 1 (range 1-5); brainstem encephalitis was asymptomatic in three patients. In total, 27 brainstem events were analyzed, including 21 symptomatic brainstem attacks and 6 ON and/or myelitis attacks that were associated with MRI evidence for concomitant subclinical brainstem encephalitis. In 6 patients, the brainstem was involved more than once. The patients' median disease duration at last follow-up was 54 months (range 10-507; over >24 months in 12/15 cases).

## Case reports

As reliable tests for MOG-IgG have only recently become available, comprehensive case series illustrating the broad and heterogeneous spectrum of clinical manifestations, disease courses, and radiologic presentations in MOG-IgG-positive patients are lacking so far. In particular, there are almost no detailed reports on patients with brainstem encephalitis. To paint for the first time a vivid 'real-life' picture of the disorder, which statistical analyses alone cannot achieve, we decided to provide detailed reports on all patients included in this study. Cases

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1, 2 and 3 are described below; reports on the remaining 12 cases are to be found in the Appendix. For a comprehensive summary of the patients' clinical, radiological and laboratory features see the Table 1. A selection of illustrative MR images demonstrating brainstem damage in MOG-IgG-positive patients is shown in Figs. 1 and 2.

### Case 1 – Fatal rhombencephalitis in a patient with recurrent ON and LETM

A previously healthy 44-year-old Caucasian woman first developed right-sided ON in November 2003. Lumbar puncture (LP) demonstrated a normal CSF white cell count as well as normal CSF protein, glucose, and lactate levels and negative oligoclonal bands (OCB). Brain MRI revealed asymptomatic lesions in the right cerebellar hemisphere and in the cerebral peduncle of the midbrain. Magnetic resonance imaging (MRI) of the spinal cord was not performed. After treatment with intravenous methylprednisolone (IVMP), vision returned to normal. Extensive laboratory tests did not reveal any infectious or rheumatologic cause of the patient's symptoms.

Up to 2010 ON attacks occurred once to twice annually, but vision always returned to normal after IVMP therapy. However, after another attack of ON in August 2010, vision in the right eye dropped permanently to 0.85 on the Snellen chart despite IVMP therapy.

In January 2011, the patient developed a first attack of myelitis with accompanying brainstem encephalitis. Symptoms included right hemiparesis, dysarthria, and dysphagia. MRI showed no supratentorial brain lesions but a T2 lesion in the medulla oblongata and a cervical longitudinally extensive transverse myelitis (LETM) lesion extending from C2 to C4 with gadolinium (Gd) enhancement. LP again demonstrated a normal CSF white cell count, normal CSF protein level, and negative OCB. Treatment with IVMP was followed by complete remission. In June 2011, after another attack of myelitis with paraparesis, treatment of which with IVMP had resulted in incomplete remission of the symptoms, treatment with glatiramer acetate (GLAT) for suspected MS was initiated.

After a relapse-free period of 1 year, two further relapses (ON in May 2012 and myelitis with paraparesis in July 2012) followed. While treatment with IVMP led only to partial recovery, plasma exchange (PEX) treatment (five exchanges) was followed by complete clinical remission after both relapses. After the second relapse, GLAT was stopped (July 2012) and natalizumab started. However, treatment with natalizumab was discontinued after two infusions due to recurrent headaches.

In September, October, and December 2012 three further relapses of myelitis with paraparesis occurred. PEX (five exchanges; no IVMP) resulted in partial recovery in all cases.

In January 2013, the patient received a first infusion of mitoxantrone (12 mg/m$^2$, nadir 3000/µl), and after a further myelitis relapse with paraparesis in February 2013, a second mitoxantrone infusion was given (8 mg/m$^2$, nadir 2800/µl). Complete recovery was achieved following PEX (five exchanges; no IVMP). After a further flare-up of myelitis later the same month with paraparesis and Gd enhancement at levels T2, T3, T5, and T9, treated with a cycle of five plasmaphereses with incomplete remission in February 2013, a third infusion of mitoxantrone with 8 mg/m$^2$ was given. A serum sample taken at that time later tested positive for MOG-IgG in a live-cell CBA [2] (1:1280). Early in 2013, two new ON relapses occurred and were treated with a cycle of PEX (five exchanges), again with incomplete remission. MRI now showed Gd enhancement in large parts of the thoracic spinal cord. An expanded disability status scale (EDSS) score of 6 was documented at that time. MOG-IgG were retrospectively positive at a titer of 1:640.

Rituximab (500 mg) was infused for the first time in May 2013, followed 2 weeks later by a second infusion of 500 mg rituximab. Fifteen days after the second infusion the patient's paraparesis worsened (EDSS 8) and dysarthria re-occurred. Cerebral and spinal MRI showed active lesions with Gd enhancement at the C4/C5 level, in the pons, and in the medulla oblongata. After PEX (five exchanges; no IVMP), the patient was able to walk again and her dysarthria completely remitted. Two new myelitis relapses in July and August 2013 with paraparesis (EDSS 7.5) and dysarthria were again treated with PEX (five exchanges; no IVMP), which was followed by partial recovery. MRI showed a new inactive lesion in the pons in July 2013 and in August 2013 Gd enhancement extended from C2 to C5. After a second PEX cycle, urosepsis occurred and was treated successfully with antibiotics. The next relapse occurred in November 2013 with a left ON (visual acuity of 0.05), which remitted incompletely after one cycle of PEX (five exchanges) (EDSS 7.5).

In January 2014, massive deterioration of the brainstem symptoms occurred, with dysarthria, dysphagia, left oculomotor and facial nerve palsy, and new T2-hyperintense lesions in the pons, pedunculus cerebellaris, cerebral crus, and medulla oblongata. No supratentorial lesions were seen on brain MRI. Spinal MRI demonstrated a diffuse, asymptomatic T2-hyperintense lesion from C2 to C4 as well as patchy Gd enhancement in the pons, medulla oblongata, and the entire cervical spinal cord. The patient was treated with two courses of five PEX each (no IVMP) and initially improved. In the following month, however, clinical symptoms deteriorated again and the patient developed bilateral blindness and central hypoventilation. She was transferred to palliative care and died in May 2014.

The total number of attacks in this patient was 25, 15 of which occurred under various immunomodulatory

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**Table 1** Clinical, radiological and laboratory findings in 15 MOG-IgG-positive patients with a history of brainstem involvement

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 |
|---|---|---|---|---|---|---|---|---|
| Sex | f | f | f | f | f | f | m | m |
| Ethnicity | Cauc | Cauc | Cauc | Cauc | Cauc | Cauc | Cauc | Cauc |
| BSTI at onset | y | y | n | n | n | n | n | n |
| Age at first evidence for BSTI (years) | 53 | 18 | 45 | 35 | 31 | 53 | 19 | 19 |
| Time to first evidence for BSTI (years) | 0 | 0 | 18 | 0.75 | 0.25 | 41 | 0.3 | 0.17 |
| No of clinical BST attacks | 5 | 1 | 2 | 3 | 1 | 0 | 1 | 1 |
| No of attacks with subclinical BSTI | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Clinical BST findings | Central hypoventilation, dysphagia, dysarthria, CN III and VII paresis | Respiratory impairment, difficulties coughing, dysphagia, dysarthria, diplopia | Cerebellar gait and upper limb ataxia | Impaired balance, vertigo | Intractable nausea and vomiting | None, subclinical BST involvement | Double vision and gait ataxia | Hearing loss |
| Infratentorial MRI findings | MRI1: Cerebral peduncle of the midbrain, MO, pons, MRI2: MO, new T2 lesions, Gd+, MRI3: pedunculus cerebellaris, crus cerebri, patchy Gd + pons, MO | Pontine tegmentum and cerebellar peduncles | Crus cerebri and entire pons, around the 4th ventricle, extending into the left cerebellar hemisphere | Pons and medulla oblongata | Right and left dorsal MO, adjacent to the 4th ventricle, incl. the area postrema | MO with patchy Gd enhancement | Lesions in the peri-aqueductal gray, ventral pons | T2 lesion in the pons |
| Cerebral peduncles | y | n | y | n | n | n | n | n |
| Pons | y | y | y | y | n.d. | n | y | y |
| Cerebellar peduncles | y | y | n | n | n | n | n | n |
| Cerebellum | n | n | y | n | n | n | n | n |
| Medulla oblongata | y | n | y | y | y | y | n | n |
| Bulbo-spinal lesion, ever | y | n | n | y | y | y | n | n |
| Gd+, ever | y | n | y | y | y | y | y | y |

**Table 1** Clinical, radiological and laboratory findings in 15 MOG-IgG-positive patients with a history of brainstem involvement (Continued)

| Supratentorial MRI findings | Normal | T2-hyperintense lesions in the frontal and parietal subcortical white matter | Crus cerebri, left subcort. white matter (adjacent to the temporal horn), corpus callosum, juxtacortical regions of parietal lobes | Lateral ventricular lesions | Normal | Normal | Confluent T2 hyperintense lesions in the right temporal lobe, pulvinar bilaterally | T2 lesions in basal ganglia, corpus callosum, periventr., pulvinar thalami, rostral putamen; leptomeningeal contrast enhancement |
|---|---|---|---|---|---|---|---|---|
| Postinfectious/postvaccinal | n | y | n | n | n | y | n | y |
| Simultaneous ON and BSTI | y | n | n | n | n | n | n | y |
| Simultaneous MY and BSTI | y | y | n | y | y | y | y | y |
| History of both ON and MY | y | n | n | y | y | y | y | y |
| Recurrent disease | y | n | y | y | y | y | y | y |
| NMOSD 2015 | n | n | y | y | y | n | y | y |
| CSF-restr. OCB | n | n | n | y | n.d. | n | y | y |
| CSF WCC | normal | 360 | 50 | normal | 122 | 30 | 22 | 60 |
| CSF neutrohils | n.a. | 7 % | 6 % | n.a. | 3 % | n.d. | 6 % | 26 % |
| QAlb elevated | n.d. | y | n.d. | n | n | y | n | y |
| Last EDSS | 10 | 1 | 7.5 | 3 | 3 | 4.5 | 1 | 0 |

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**Table 1** Clinical, radiological and laboratory findings in 15 MOG-IgG-positive patients with a history of brainstem involvement *(Continued)*

| | #9 | #10 | #11 | #12 | #13 | #14 | #15 |
|---|---|---|---|---|---|---|---|
| Sex | m | m | f | f | f | f | f |
| Ethnicity | Cauc | Cauc | Cauc | Cauc | Cauc | Cauc | Cauc |
| BSTI at onset | y | y | y | y | n | y | n |
| Age at first evidence for BSTI (years) | 50 | 37 | 44 | 27 | 26 | 25 | 22 |
| Time to first evidence for BSTI (years) | 0 | 0 | 0 | 0 | 1.7 | 0 | 0 |
| No of clinical BST attacks | 0 | 1 | 1 | 1 | 1 | 2 | 1 |
| No of attacks with subclinical BSTI | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Clinical BST findings | None, subclinical BST involvement | Trigeminal hypesthesia | None, subclinical BST involvement | Trigeminal hyp- and paresthesia, diplopia, nystagmus, unsteady gait | Hypesthesia tongue and face, impaired smooth pursuit | Hemihypesthesia including the face | INO |
| Infratentorial MRI findings | Median pontine lesion | N.d. | Cerebellar peduncle, single lesion | T2 lesions extending from the ponto-med. junction, throughout the MO to C5, incl. around the 4th ventricle | T2 lesions in the MO and pons, detectable over a period of at least 12 months | Bilateral pontine lesions and bilateral cerebellar peduncle lesions | Large, Gd + lesion: pons bilat, both pedunculi cerebelli; paramedian ponto-medullary junction |
| Cerebral peduncles | n | n.d. | n | n | n | n | n |
| Pons | y | n.d. | n | y | y | y | y |
| Cerebellar peduncles | n | n.d. | y | n | n | y | y |
| Cerebellum | n | n.d. | n | n | n | n | n |
| Medulla oblongata | n | n.d. | n | y | y | n | y |
| Bulbo-spinal lesion, ever | n | n.d. | n | y | n | n | n |
| Gd+, ever | y | n.d. | n.d. | n | n | n.d. | y |
| Supratentorial MRI findings | Normal | Normal | Single frontal lobe lesion | Juxta-cortical T2 lesion, insular region | Callosal, periventr., juxtacortical, deep white matter | Single small lesion directly adjacent to the left lateral ventricle | Peritrigonally and corona radiate, Gd + |
| Postinfectious/postvaccinal | y | n | n.d. | n | n | y | n |
| Simultaneous ON and BSTI | y | y | n | y | n | n | n |
| Simultaneous MY and BSTI | y | y | y | y | y | n | y |

**Table 1** Clinical, radiological and laboratory findings in 15 MOG-IgG-positive patients with a history of brainstem involvement *(Continued)*

| | | | | | | |
|---|---|---|---|---|---|---|
| History of both ON and MY | y | y | y | y | n | y |
| Recurrent disease | y | n | y | y | y | y |
| NMOSD 2015 | n | n | y | y | n | y |
| CSF-restr. OCB | n | n | n | y | n | n |
| CSF WCC | normal | 8 | n.d. | 59 | 150 | 8 |
| CSF neutrohils | n.a. | 34 % | n.d. | n.a. | 26 % | n.d. |
| QAlb elevated | n | n | n.d. | n | y | y |
| Last EDSS | 1 | 0 | 2.5 | 0 | 3 | 0.5 |

*Abbreviations: BSTI* brainstem involvement, *y* yes, *n* no, *n.a.* not applicable, *n.d.* no data, *f* female, *m* male, *Cauc* Caucasian, *MO* medulla oblongata, *Gd* + gadolinum enhancing, *CN* cranial nerve, *MRI* magnetic resonance imaging, *MY* myelitis, *ON* optic neuritis, *NMOSD 2015* neuromyelitis optica spectrum disorder according to Wingerchuk et al. (2015), *CSF* cerebrospinal fluid, *OCB* oligoclonal bands, *QAlb* albumin CSF/serum ratio, *EDSS* extended disability status scale, *INO* internuclear ophthalmoplegia

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and immunosuppressive therapies. These included around 13 relapses of ON and 12 of myelitis; the brainstem was clinically affected during 5 attacks. This corresponds to an annualized relapse rate of 2.38, though 15/25 relapses occurred during the last 24 months before the patient's death.

### Case 2 – Single episode of post-infectious whole-spine myelitis with severe brainstem and brain involvement yet complete recovery

An 18-year-old Caucasian woman had purulent tonsillitis in June 2010 and was treated with amoxicillin for 5 days. About 1 week later she developed intermittent fever (up to 39 °C), general malaise, headaches, meningism, fluctuating diplopia on right gaze, and subtle psychomotor slowing, for which she was admitted to the Department of Neurology, Charité – University Medicine Berlin. The day after admission, her condition deteriorated and she additionally noted weakness of her legs, urinary incontinence, respiratory impairment with difficulty in coughing, and mild dysphagia. On neurologic examination the patient was awake and oriented. Cranial nerve examination showed no abnormalities, but she had marked dysarthria and reported fluctuating horizontal diplopia. Her legs were plegic and she had reduced pain and touch sensation below T10. There was also British Medical Research Council (BMRC) grade 4 distal weakness in both arms. Deep tendon reflexes were preserved throughout. Except for a mature cystic ovarian teratoma without signs of malignancy, which had been removed 2 months before, her past medical history was unremarkable. N-Methyl-D-aspartate receptor (NMDAR) antibodies were negative. Spinal MRI demonstrated a prominent, longitudinally extensive, T2-hyperintense and centrally located lesion without contrast enhancement extending over almost the entire spinal cord, as well as swelling of the cord (Fig. 2). Cranial MRI showed T2-hyperintense lesions in the frontal and parietal subcortical white matter, the pontine tegmentum, and the cerebellar peduncles without contrast enhancement (Fig. 1). Thoracic and abdominal MRI revealed a right retroperitoneal mass with a diameter of 5.6 cm, which was resected 5 months later and found to be a ganglioneuroma. CSF analysis on the day of admission demonstrated an elevated total cell count with 306 white blood cells/µl (reference range <5/µl) with 80.2 % lymphocytes, 8.2 % monocytes, 7 % neutrophils, 2.4 % eosinophils, and 2.2 % activated lymphocytes, but no plasma cells or tumor cells. CSF lactate was elevated at 28.4 mg/dl (reference range <20 mg/dl). There was moderate blood–CSF barrier (BCSFB) dysfunction with an albumin CSF/serum ratio (QAlb) of $30.6 \times 10^{-3}$ (age-adjusted upper limit of normal $5.2 \times 10^{-3}$). Accordingly, total CSF protein was elevated (213 mg/dl; reference range <45 mg/dl). She had no local IgG synthesis and no CSF-specific OCB.

Routine serum chemistry including C-reactive protein (CRP) showed normal results. A complete blood count revealed mild leukocytosis (12.44/nl; reference range <11/nl). Extensive microbiological (herpes simplex virus type 1 and 2, varicella zoster virus, Epstein–Barr virus, cytomegalovirus, human herpes virus 6, enteroviruses, influenza A virus, adenovirus, tick-borne encephalitis virus, human immunodeficiency virus, human T-cell leukemia virus type 1, *Borrelia*, *Treponema pallidum*, *Brucella*, *Bartonella*, *Mycoplasma*, *Chlamydia*, *Mycobacterium tuberculosis*, *Aspergillus*) work-up detected no signs of acute CNS infection.

Antinuclear antibodies were detectable at a titer of 1:1280, but antibodies to double-stranded DNA, extractable nuclear antigens, onconeuronal antigens (Hu, Yo, Ri, CV2/CRMP5, Ma2/Ta, amphiphysin), cardiolipin, β2-glycoprotein, phosphatidyl serine, gangliosides (GD1a, GD1b GM1, GM2, GM3, GQ1b, GT1b [IgG and IgM in each case]), and aquaporin-4 were negative, as were anti-neutrophil cytoplasmic antibodies. Screening of serum and CSF for antibodies against NMDA receptors (IgG, IgA, IgM), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, glycine receptor, myelin-associated antigen, and glutamic acid decarboxylase was likewise negative.

However, testing of a serum sample obtained on the day of admission in a live-cell CBA demonstrated high-titer serum IgG antibodies against MOG (1:10240). Anti-MOG antibodies were also detectable in the CSF (1:64), but there was no evidence of intrathecal production of anti-MOG antibodies, with an anti-MOG antibody index (AI) of 0.3 (reference range <4) [3].

The patient was initially treated with ceftriaxone and ampicillin, which was discontinued after a bacterial infection was ruled out, as well as with IVMP (1 g/day for 6 days). Corticosteroids were thereafter administered orally (prednisolone 100 mg/d), slowly tapered, and eventually stopped in October 2010. Starting on the day after admission, she was additionally treated with a total of 10 PEX. MOG-IgG was retrospectively detectable also in PEX plasma (titer 1:320 after three exchanges). Thereafter, therapy with intravenous immunoglobulins (IVIG, 2 g/kg body weight) was initiated in July 2010. IVIG therapy was continued at 5-week intervals until June 2011, with dose reduction to 1 g/kg body weight from January 2011 onwards. The patient gradually recovered and was able to walk with a walking frame by August 2010. Follow-up spinal MRI in August 2010 demonstrated resolution of the longitudinal extensive lesion, but there were several residual patchy hyperintense lesions within the spinal cord. In a serum sample obtained at the same time, the anti-MOG antibody titer was clearly lower (1:640) than the initial titer, and anti-MOG antibodies were no longer detectable in CSF.

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The patient did not receive any further immunomodulatory or immunosuppressive therapy. At the last follow-up examination, 28 months after disease onset and 16 months after discontinuation of IVIG, she reported no further relapses and had no impairments in activities of daily living except for residual unsteadiness when walking for a longer time, difficulties on running for more than 5 min, and slightly increased urinary frequency (EDSS 1). Neurologic examination showed a very mild residual right-sided pyramidal syndrome with brisk tendon reflexes, but was otherwise unremarkable.

### Case 3 – Recurrent ON with rhombencephalitis and extensive brain involvement with poor outcome

A Caucasian woman experienced a first episode of bilateral ON in 1985 at age 28 and had three further attacks of bilateral and two of unilateral ON up to 1999, leaving her with residual visual acuity of <0.2 in both eyes. CSF and brain MRI was normal. In October 2003, the patient presented with intractable headaches lasting for several days, followed by mild impairment of consciousness. Cerebral computed tomography showed no abnormalities, while CSF analysis disclosed mixed lymphocytic and neutrophilic pleocytosis (1024 cells/μl) along with increased total protein (1350 mg/l) and mildly elevated lactate (3.6 mmol/l). CRP and leukocytes were elevated at 18 mg/l and 20.9 Gpt/l, respectively. There was no evidence of an infectious etiology, and symptoms resolved after combined treatment with dexamethasone 10 mg 4× per day for 4 days and ceftriaxone 4 g daily for 10 days. However, within 2 weeks the patient developed bilateral intention tremor of the upper extremities, marked gait ataxia, and right-sided visual worsening. Cranial MRI revealed extensive T2 hyperintense lesions in the brainstem, including around the fourth ventricle and in the supratentorial white matter, the corpus callosum, and, to some extent, the cerebral cortex. None of these abnormalities showed Gd enhancement. The CSF had 50 white cells/μl, mostly lymphocytes with some neutrophils (6 %) and occasional eosinophils (2 %). Treatment with IVMP (1 g/d for 5 days) followed by oral tapering of steroids for 6 weeks resulted in marked improvement of symptoms.

Six months later, the patient noted visual worsening in the left eye and recurrence of gait ataxia. Repeat cranial MRI depicted progressive T2-hyperintense lesions in the entire pons, especially around the fourth ventricle and the foramina of Luschka, extending bilaterally into the white matter of the left cerebellar hemisphere and into the crus cerebri with Gd enhancement. There was also involvement of the left subcortical white matter (adjacent to the temporal horn), the corpus callosum, and the juxtacortical regions of both parietal lobes, along with contrast enhancement in some of these lesions.

Cerebral angiography showed no abnormalities. CSF analysis showed 5 cells/μl with a predominance of lymphocytes and some neutrophils and eosinophils. There was no evidence of intrathecal immunoglobulin synthesis. Anti-Hu and anti-Yo antibodies were negative. The patient received 1000 mg methylprednisolone i.v. daily for 5 days and subsequently long-term oral steroids, and this coincided with partial amelioration of both clinical symptoms and radiologic findings. Nightmares and visual hallucinations resolved along with rapid tapering of steroid doses. For long-term immunosuppression, overlapping treatment with azathioprine (AZA) (150 mg/day) was initiated in August 2004. However, five more relapses occurred over the following 101 months despite AZA treatment. At last follow-up, the EDSS of 7.5 and bilateral optic nerve atrophy (involving the chiasm) was documented. Retrospective testing of a serum sample taken in 2010 revealed high-titer MOG-IgG (1:20,480). MOG-IgG seropositivity was confirmed in a second sample obtained 22 months later.

## Discussion

We describe the largest series to date of MOG-IgG-positive patients with brainstem involvement, thereby expanding the clinical spectrum of MOG-IgG autoimmunity. The 15 cases presented here were identified as part of a large European cohort ($n = 50$) of MOG-IgG-positive patients reported in part 1 [3] and part 2 [17] of this series, suggesting a relatively high frequency (30 %) of brainstem involvement among patients with MOG-IgG-associated myelitis and/or ON. Similarly, AQP4-IgG-positive NMO, also initially thought to affect mainly the optic nerves and spinal cord, was later reported to involve the brainstem in up to one third of all cases [16, 20, 27, 28]. We detected MOG-IgG by means of new-generation cell-based assays (CBA) employing recombinant full-length human MOG instead of enzyme-linked immunoassays, which are prone to both false-negative and false-positive results and which are no longer recommended for routine clinical diagnosis of MOG antibodies [2]; CBAs were also used for detecting AQP4-IgG [24, 25]. It is a further potential strength of this study that all patients analyzed were of Caucasian descent. This may be important since genetic factors are thought to play a role in NMO and related disorders [29].

The cases described here underline our finding from part 2 [17] that MOG-IgG-related CNS autoimmunity is not mostly a mild and monophasic disease, as suggested by some earlier studies with smaller sample sizes and shorter observation periods [4, 6], but can in fact take a relapsing and severe disease course with potentially life-threatening complications: brainstem involvement caused respiratory impairment in two patients in our series and was fatal in one of them. Respiratory insufficiency due to brainstem inflammation has previously
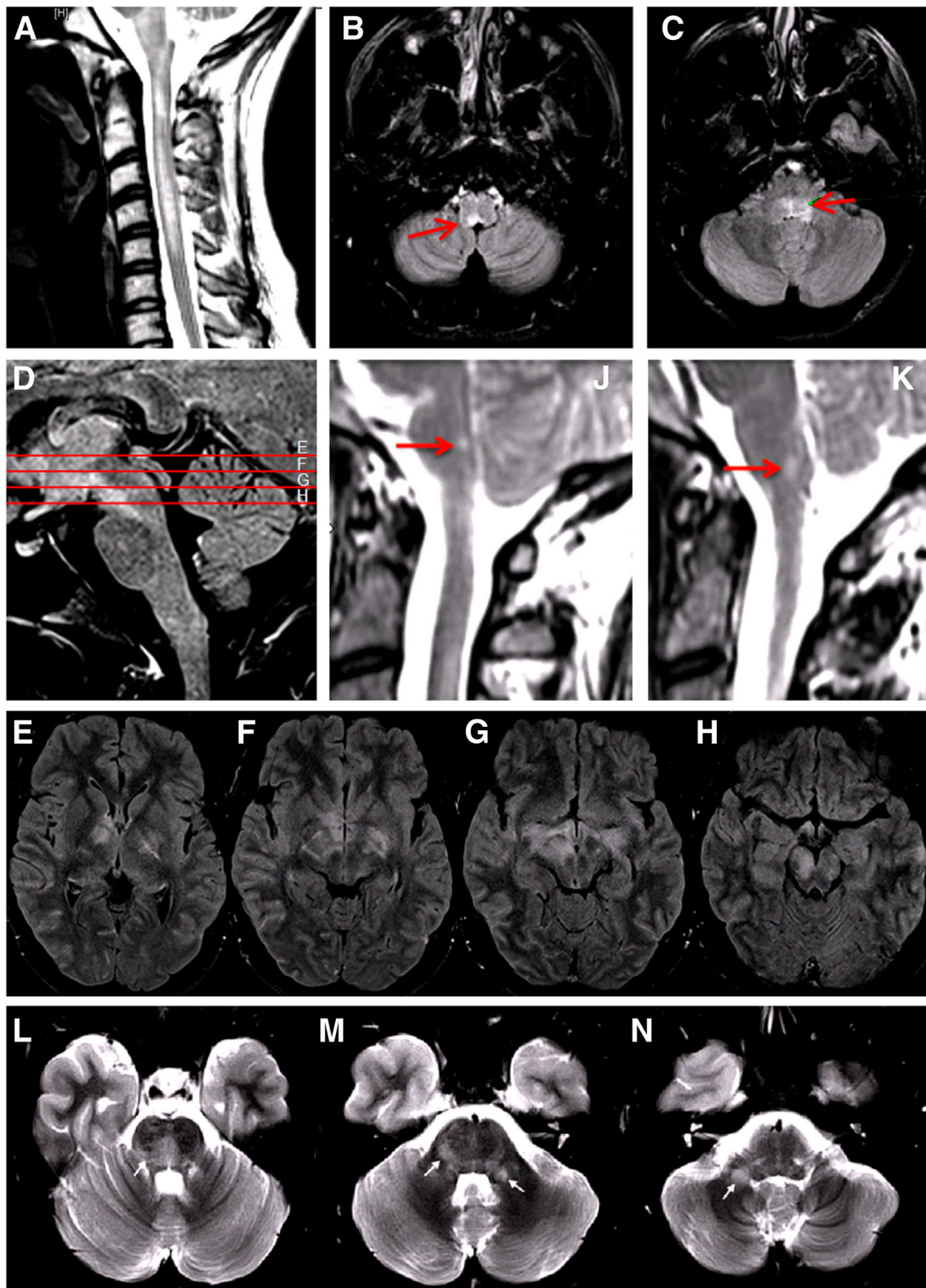
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**Fig. 1** Examples of the magnetic resonance imaging (MRI) findings in patients with MOG-IgG-positive brainstem encephalitis. **a-c** Patient 12: T2-hyperintense lesions extending from the pontomedullary junction throughout the cervical cord as far as C5; lesions included the dorsal medulla oblongata (B, *arrow*). **d-h** Patient 8: T2-hyperintense lesions in the pons, midbrain, thalamus, and basal ganglia; lesions involved the periependymal surfaces of the third ventricle. **j-k** Patient 4: T2-hyperintense lesions in the right (**j**) and left (**k**) half of the dorsal medulla oblongata including the area postrema (**j**). **l-n** Patient 2: T2-hyperintense lesions in the frontal and parietal subcortical white matter, the pontine tegmentum, and the cerebellar peduncles (*arrows*)

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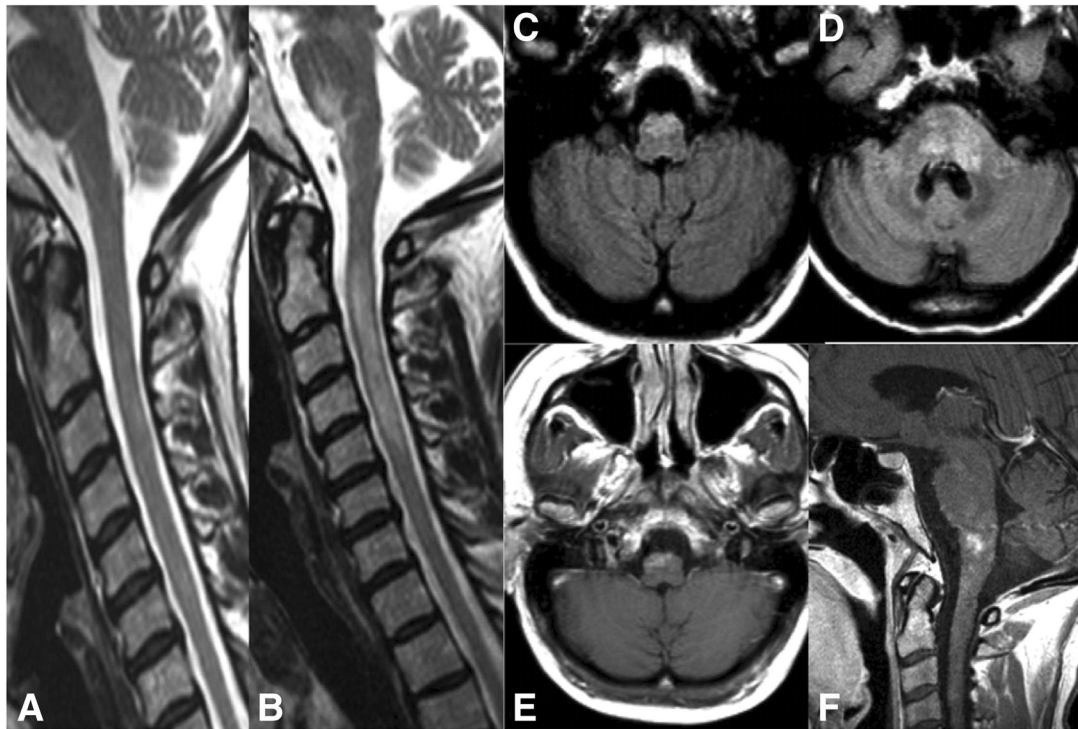


**Fig. 2** Serial MRI examination of patient 1. **a** Sagittal T2 weighted baseline MRI showing no involvement of the brainstem or of the spinal cord.
**b-e** Follow-up MRI 5 month later revealed a new spinal cord lesion extending over 4 vertebral segments with cord swelling on T2-weighted imaging as well as new lesion formation in the pons and medulla oblongata with gadolinium enhancement on T1-weighted imaging. Within the following 5 months clinical symptoms deteriorated further with infratentorial T2 lesion enlargement and new lesion formation in the pons, e.g. adjacent to the middle cerebellar peduncle, accompanied by gadolinium enhancement (**f**)

been identified as the main cause of death in AQP4-IgG-positive NMO [30].

Besides central hypoventilation, the patients' symptoms included dysarthria, dysphagia, impaired cough reflex, sensory impairment due to trigeminal nerve damage, facial nerve palsy, trigeminal hypesthesia, oculomotor nerve palsy and diplopia, nystagmus, internuclear ophthalmoplegia (INO), vertigo, hearing loss, balance difficulties, gait and limb ataxia, and, of particular note, intractable nausea and vomiting (INV).

### Brainstem symptoms included intractable nausea and vomiting

INV in patients with brainstem encephalitis is caused by lesions in the dorsal medulla oblongata (area postrema). While presence of an area postrema syndrome (APS) has hitherto been considered to have high predictive value for a diagnosis of AQP4-IgG-positive NMO [15, 31–35], our study demonstrates that area postrema lesions and INV also occur in MOG-IgG-positive patients. However, this is not totally unexpected. First, MOG is expressed throughout the entire CNS and, accordingly, inflammatory lesions were found in almost all areas of the CNS in our cohort [17]; there is *a priori* no reason why the area postrema in

particular should be spared. Second, it has been speculated that the circumventricular organs, including the area postrema, which lack a proper blood-brain barrier (BBB), may be sites prone to entry of AQP4-IgG into the CNS; assuming MOG-IgG are pathogenic (as suggested by recent studies [2, 14]), this would also be relevant for MOG-IgG.

### Inflammation occurred in all areas of the brainstem

Apart from the pons, which was affected in 11/13 (84.6 %) patients, MRI brainstem lesions were most commonly located in the medulla oblongata (8/14 or 57.1 %), in the mesencephalon (lesions in the cerebral peduncles in 2/14 or 14.3 %), and in the cerebellar peduncles (5/14 or 35.7 %).

### Additional involvement of the diencephalon and the cerebellum

In several cases brain involvement on MRI was not restricted to the brainstem. Concomitant diencephalic (pulvinar) and cerebellar lesions – and thus rhombencephalitis – were present in 2/13 (15.4 %) and 1/14 (7.1 %), respectively. In case 3, an additional lesion was present in the cerebellum which was accompanied by limb ataxia and marked gait ataxia. Cerebellar gait and stance ataxia was

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also noted in case 2 as well as cerebellar dysarthria. Similarly, patient 9 in part 2 [17] presented with a (clinically silent) lesion in the cerebellar white matter; however, that patient had no lesions in the brainstem. Cerebellar symptoms have very rarely been described in AQP4-IgG-positive patients, too [20]. However, not all patients with ataxia had cerebellar lesions; some patients in this and in the total cohort [17] presented with sensory ataxia and/or unsteady gait due to paresis caused by acute myelitis.

Supratentorial brain lesions were present in 10/15 (66.7 %) patients. Lesions were found in the deep (including the periventricular) white matter (including in the corona radiata), the sub-/juxtacortical white matter (including in the insula), and, in a single patient, to some extent the cerebral cortex; furthermore, in the corpus callosum and, in one case, the basal ganglia and thalami (see part 2 of this series for exemplary MRI images [17]). In patient 3, brain and brainstem lesions were extensive and even resulted in impaired consciousness. Similarly, extensive confluent supratentorial lesions were seen in patient 7. Barkhof's MRI criteria for MS were met in 4/15 (26.7 %) patients.

### Acute brainstem involvement was associated with ON and/or myelitis in most cases

Isolated attacks of brainstem encephalitis, i.e., attacks that were not accompanied by clinical symptoms of ON or myelitis, were very rare in the total cohort of 50 MOG-IgG-positive patients [17], accounting for only 5/276 (1.8 %) documented attacks. However, they were relatively common if only patients with a history of at least one brainstem attack are taken into account (5/21 [23.8 %] attacks, 4/15 [26.7 %] patients).

In most patients, however, brainstem attacks were at least once associated with clinical myelitis (13/15 [86.7 %] patients), ON (6/15 [40 %]) and/or supratentorial encephalitis. In 5 out of 14 patients (36 %) with a history of simultaneous brainstem encephalitis and myelitis and available MRI data, lesions contiguously stretched from the medulla oblongata into the cervical cord at least once, similar to what can be seen in AQP4-IgG-positive patients. In case 2, inflammation affected almost the entire neuraxis, including the lumbar, thoracic, and cervical spinal cord as well as areas in the brainstem and in the supraventricular white matter. This was associated with exceptionally high MOG-IgG serum titers (1:10,240) and presence of detectable MOG-IgG levels also in the CSF.

### Some patients met Wingerchuk's criteria for NMO(SD)

At last follow-up, 12/15 (80 %) patients with brainstem encephalitis had a history of both ON and myelitis. Two patients had a history of myelitis but not of ON, and one had a history of recurrent ON (rON) but not of

myelitis. All but 2 (86.7 %) had a relapsing disease course at last follow-up. Of those 12 patients, 8 (66.7 %) met the 2006 Wingerchuk criteria for NMO [36]; in the remaining 4 patients, the criteria were not fulfilled due to the presence of brain MRI lesions at onset meeting Paty's criteria and/or because criteria for LETM were not met. On the understanding that MOG-IgG-positive ON and/or myelitis are not considered "alternative diagnoses", i.e., based solely on clinicoradiologic findings, 9/15 (60 %) met the revised 2015 consensus criteria for NMOSD.

### Brainstem involvement may be asymptomatic

While overall there was a high degree of agreement between MRI findings and clinical presentation, in 5/15 (33.3 %) patients asymptomatic brainstem lesions were detected by MRI at least once, suggesting that subclinical brainstem encephalitis is not uncommon in MOG-IgG-related CNS autoimmunity. The true prevalence of brainstem involvement in MOG-IgG-positive patients may thus be higher than expected based solely on clinical presentation. Similarly, evidence for subclinical involvement of the brain, the spinal cord, or the optic nerves, as detected by MRI or electrophysiology, has been found in some of our MOG-IgG-positive patients (see part 2 of this series for details [17]).

### CSF and MRI findings may mimic infectious CNS disease

In one case, leptomeningeal contrast enhancement was noted at the time of disease onset. This is of particular interest in the light of recent studies indicating the existence of a lymphatic system of the CNS within the meninges [37]. Meningeal involvement has been reported in neuromyelitis optica [38–43], MS, and other disorders of putative autoimmune etiology and may indicate a new path for immune cell entry into the CNS. Moreover, this finding is of potential differential diagnostic relevance, since it may wrongly suggest acute infectious meningitis, all the more as CSF findings compatible with an infectious etiology were found in some patients: CSF pleocytosis was present in 11/14 (78.6 %) patients (50/μl, range 8-360) with available data and comprised neutrophil granulocytes in 7/11 (63.6 %) (accounting for 3-34 % of all white CSF cells) or eosinophil granulocytes in 2/6 (33.3 %), increased lactate levels in 2/3 (66.7 %) patients, BCSFB dysfunction in 5/12 [41.7 %], and CSF-restricted OCB, a mainstay of classical MS, were absent in 10/14 (71.4 %). Together with fever and other signs of systemic infection – disease onset was preceded by acute infections in at least three patients (purulent tonsillitis, ENT infection, and bronchopulmonary infection, respectively) – these CSF findings are compatible with early bacterial or viral meningoencephalitis and thus bear the risk of delayed diagnosis of autoimmune

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encephalitis, as was the case in one of the patients reported here. Neutrophilic and eosinophilic pleocytosis [20, 44, 45], elevated lactate levels [45, 46], and missing OCB [45, 47] are also features of AQP4-IgG-positive NMO. Elevated neutrophil counts have been previously reported also in MOG-IgG-positive pediatric patients [48]. Moreover, attacks are preceded by acute infection in 20-30 % of cases of AQP4-IgG-positive NMO [20, 49, 50], suggesting that infection-related immunologic changes or infection-related BBB disruption [45, 47] may trigger disease activity.

### Post-infectious onset and the role of BBB damage

In one of the three patients with post-infectious onset (case 6), interruption of long-term MTX treatment for RA due to infection was followed by the first attack a few weeks later, leaving the possibility that MOG-IgG were already present before clinical onset but were able to enter the CNS to a pathologically relevant degree only after infection-related BBB breakdown. In fact, a markedly elevated QAlb as well as Gd entry into the lesion was noted during acute brainstem encephalitis in that case. In AQP4-IgG-positive NMO, the autoantibody has indeed been retrospectively demonstrated in samples obtained months or years before disease onset [51–53]. Overall, 8/9 patients showed Gd enhancement during disease activity, and 3/7, including the single patient without Gd enhancement, had an increased QAlb, indicating possible BBB disruption. Primary or secondary impairment of the BBB function may be an important step in the pathogenesis of MOG-IgG-positive brainstem encephalitis, since it may allow MOG-IgG to enter the CNS.

In one patient, the first symptoms developed within 2 weeks after vaccination for diphtheria, tetanus, pertussis, polio, and influenza, and thus within a time window considered to be compatible with a post-vaccination reaction. Of note, we describe a second adult patient with disease onset shortly after vaccination against diphtheria, tetanus and pertussis in part 2 of this series [17]. The association of MOG-IgG seropositivity with infection and vaccination deserves to be investigated in more detail. MOG-IgG have also been reported in children with acute disseminated encephalomyelitis [1], a condition with suspected post-infectious or post-vaccinal etiology [54, 55]. Postvaccinal onset of NMO has been described also in a few AQP4-IgG-positive patients, although a causal link has not yet been proven [56, 57].

### MOG-IgG positivity associated with a mature teratoma

Both MOG-IgG-positive encephalomyelitis and AQP4-IgG-positive NMOSD are not usually found in a paraneoplastic context. It is therefore of potential interest that one of the patients described above had a history of mature teratoma

that was removed just 2 months before onset of her CNS disorder. In addition, a ganglioneuroma was detected after disease onset in the same patient. It remains unknown whether this patient's tumors and MOG-IgG seropositivity were related. Teratomas have been shown to play a role in the pathogenesis of NMDAR encephalitis, another autoantibody-related disorder of the CNS [58]. However, NMDAR antibodies were negative in our patient. While, to the best of our knowledge, the presence of MOG in teratomas has not been investigated so far, expression of CNPase, an oligodendrocyte marker, has been described in mature teratomas [59], and several reports on oligodendrogliomas arising in mature teratomas exist [60–63]. Ectopic expression of MOG by the patient's tumor therefore cannot be completely ruled out.

### Influence of age and sex

The median age at first clinical brainstem attack was 31 years (range 18-53 years) in the present, adult cohort. This did not differ from the age of disease onset in the total MOG cohort (see part 2 [17]) (31 years, range 6-70; $N = 50$). Remarkably, in 7/15 (46.7 %) cases brainstem inflammation (as evidenced clinically or by MRI) was already present at or very shortly after disease onset. In the remainder, the median time between disease onset and first clinically apparent brainstem encephalitis was just 0.8 years. However, this interval varied widely, with brainstem lesions occurring for the first time only 7, 17, and 41 years, respectively, after the first attack in three patients. Together, these findings suggest that the presence or absence of brainstem involvement is not an effect of age or disease duration.

The sex ratio in our cohort (1:2.8) did neither differ from that previously reported in AQP4-IgG-negative NMO patients with and without brainstem lesions (1:2; $n = 27$) [20] nor from that in the total MOG cohort (1:2.8; $n = 50$) [17] (but is in stark contrast to the sex ratio of ~1:9 reported in AQP4-IgG-positive patients [20]), indicating that female gender is probably not a risk factor for the development of brainstem involvement in patients with MOG-IgG-positive ON and/or myelitis.

### Good recovery from acute brainstem attacks after IVMP and/or PEX

While many patients seemed to benefit from immunotherapy, treatment responses and long-term outcomes differed markedly. Treatments applied during acute brainstem attacks (with and without concomitant ON and/or myelitis) included IVMP, oral steroids, and PEX. Overall, treatment outcome was available for 18 attacks with clinical brainstem involvement. Treatment with IVMP (with and without oral tapering) or with PEX (with or without additional IVMP) were followed by

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good partial recovery after 6 and 3 brainstem attacks, respectively, and by full recovery after 3 and 3 brainstem attacks, respectively. No treatment was given for the last (and subsequently fatal) attack in patient 1, which occurred during palliative care, for two brainstem attacks in case 5, which remitted spontaneously, and for the only attack in case 5 (initially considered to be of infectious origin due to granulocytic pleocytosis), which partially remitted.

PEX treatment was beneficial in patient 1 during most attacks, including three brainstem attacks and one attack in which IVMP had led only to partial recovery. Similarly, PEX also resulted in full or almost full remission when used to treat attacks with brainstem involvement in patients 6 and 8 (used in combination with IVMP), as well as in patient 9 after failure of high-dose IVMP therapy.

Importantly, however, PEX treatment could not prevent relapses 1-3 months later in case 1, including the fatal brainstem attack in that patient, as well as a relapse of ON within 2 months in patient 9. Preliminary findings from our laboratory (S.J., unpublished data) show that anti-neural autoantibodies may remain detectable or reappear soon after five to seven plasma exchanges, raising the question of whether PEX treatment may be discontinued too early in some cases. Alternatively, T cell-mediated mechanisms may play a more important role in patients who do not sufficiently respond to PEX.

Both IVMP and PEX were also not always effective when used to treat attacks other than brainstem encephalitis in this cohort and in the total cohort (see part 2 for detailed analysis [17]).

### Long-term immunotherapy did not prevent brainstem attacks in some patients

Immunosuppressive or immunomodulatory (IS/IM) drugs used in our patients included mitoxantrone, AZA, rituximab, natalizumab, IFN-beta, and IVIG. Treatment responses varied considerably inter- and intraindividually. Patient 2 suffered from serious neurologic impairment during the acute attack and during the following months, but immunotherapy with IVMP, oral steroids, PEX, and, for 12 months, IVIG was eventually followed by almost complete remission and no more attacks. Pathophysiologically, it is of interest that immunotherapy and clinical recovery were paralleled by a significant drop in MOG-IgG titers from 1:10,240 to 1:640 in this patient. By contrast, IS/IM therapy was not effective in preventing brainstem encephalitis in several other cases. Of particular note, rituximab was followed by severe clinical and radiologic deterioration with myelitis and active (yet asymptomatic) lesions in the pons and medulla oblongata within a few weeks after infusion in patient 1, which is reminiscent of the possibly BAFF-

mediated deterioration reported in some NMO patients after commencement of rituximab [64]. Moreover, a massive brainstem attack with dysarthria, dysphagia, left oculomotor and facial nerve palsy and new T2 lesions in the pons, pedunculus cerebellaris, cerebral crus and medulla oblongata occurred just 4 months after the last rituximab infusion. In case 5, one brainstem attack took place while the patient was being treated with AZA, one during treatment with IFN-beta, and one 9 months after commencement of natalizumab therapy. Patient 13 developed an attack of simultaneous myelitis and brainstem encephalitis four months after the first natalizumab infusion and another one (with lesions in the medulla oblongata) while on treatment with glatiramer acetate. In case 7, a severe attack involving the brainstem, supratentorial brain, and spinal cord occurred 4 weeks after commencement of AZA treatment; similarly, patient 8 experienced several relapses, including a brainstem attack, while on treatment with AZA. Of note, 14/34 relapes (in 10/17 AZA treated patients) were documented in the total cohort that took place during the latency period of AZA (months 1-6) (see part 2 of this series [17]). Of those, around 40 % occurred in patients not co-treated with oral steroids during that period. This suggests that co-treatment should be considered during the latency period of AZA treatment in MOG-IgG-positive patients, provided contraindications have been excluded.

### Disease exacerbation after IFN-beta

In common with other patients described in part 2 of this series [17], patient 4 was initially diagnosed with MS. Accordingly, she was treated with IFN-beta-1a i.m. However, commencement of IFN-beta treatment was associated with marked disease exacerbation, characterized by new brainstem and spinal cord lesions and a new clinical attack. Similarly, patient 13 developed three attacks of myelitis and/or optic neuritis while on treatment with IFN-beta 1a i.m. or, later on, IFN-beta 1a s.c. Disease exacerbation following IFN-beta administration has also been reported in AQP4-IgG-positive NMO [65–69] and likely reflects differences in the immunopathogenesis of MS and NMO. This observation is of high potential interest, since initial misdiagnosis as classical MS – and, in consequence, mistreatment with IFN-beta – might be even more common in MOG-IgG-positive patients than in AQP4-IgG-positive patients given the high rate of brain involvement in that condition [1]. Falsely classified AQP4-IgG- and, possibly, also MOG-IgG-positive patients might account for some of the occasional IFN-beta non-responders observed in MS studies. Larger studies on the efficacy of IFN-beta in MOG-IgG-positive patients treated with this substance

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in the past seem warranted, as does retrospective testing for MOG-IgG of samples from IFN-beta non-responders identified in past clinical trials.

### Long-term prognosis differed widely but did not depend on brainstem damage in most cases

Cases 1 and 2 illustrate that the prognosis differs widely among MOG-IgG-positive patients: while brainstem encephalitis led to respiratory insufficiency in both patients, it was fatal in the former case and remitted almost completely in the latter. Unexpectedly, residual neurologic impairment in our patients was mostly not related to brainstem damage. The median EDSS at last follow-up in patients with a disease duration of >24 months ($n = 12$) described here was 3 and ranged between 0 and 10; only 4 patients had an EDSS >3 at last follow-up (EDSS 4, 7.5, and 10 after 123, 225, and 507 months, respectively).

### Brainstem involvement was associated with a more aggressive disease course

However, the median EDSS at last follow-up in patients with brainstem involvement and a disease duration of >24 months was still higher (median 3, range 0-10, $n = 12$) than among all patients from the total cohort [17] who had no history of clinical or subclinical brainstem involvement at last follow-up and an observation time of ≥24 months (median EDSS 2; $n = 23$; $p < 0.04$), as were the total number of attacks at last follow-up (median 7.5, range 1-27, vs. median 3, range 1-28), the number of myelitis attacks (median 2, range 0-11, vs. median 0.5, range 0-3), the proportion of patients who had experienced both attacks of ON and attacks of myelitis at last follow up (75 % vs. 41.7 %), the median annualized relapse rate (1.32 vs. 0.59, $p < 0.03$), and the proportion of patients with additional supratentorial brain lesions (75 % vs. 30.4 %; $p < 0.02$). As observation times did not differ significantly between these two subgroups (median 69.5, range 34-507, vs. median 70, range 26-394), brainstem involvement seemed be a risk factor for a more severe disease course. This has potential therapeutic implications and should be addressed in future prospective studies.

### Limitations

We acknowledge some limitations of our study. Firstly, the retrospective design is a potential limitation. However, prospective studies would be difficult to perform due to the very low prevalence of the disease. Moreover, reliable tests have become available only recently; accordingly, only retrospective long-term data are currently available. Furthermore, the number of patients included and the number of items documented in the present study were high and data loss relatively low.

Secondly, the multicenter design, which was necessary given the low prevalence of the condition, could be a limitation. However, the study design also strongly reduced the risk of selection bias, which was acknowledged as a possible limitation by the authors of previous large single-center studies in the field of NMO [30, 36]. Moreover, all patients were documented at university centers providing a similar standard of tertiary care. Thirdly, we cannot fully exclude a potential referral bias, since MOG-IgG testing may have been ordered particularly in patients presenting with ON and/or myelitis based on the previous literature. It is therefore conceivable that MOG-IgG-positive patients with isolated brainstem and/or brain involvement are underrepresented in our study. Finally, from a pathophysiological point of view it is a possible limitation that we cannot formally prove that the antibody was already present at disease onset in all cases, since routine MOG-IgG testing was not available in the past. However, MOG-IgG was present already at disease onset in all patients with available data in the main cohort, as reported in part 1 of this series [3]: 2 MOG-IgG positive sera were taken within the first week (at 2 and 4 days) after disease onset, 10 within the first month (median 10 days, range 2-31), and 18 within the first 3 months (median 26 days, range 2-85). The median MOG-IgG titer at disease onset was 1:2560 (range 160-20480; $N = 18$).

### Conclusions

In summary, our study demonstrates that brainstem involvement is common in patients with MOG-IgG-related ON and/or myelitis. Our findings do not support the notion that MOG-IgG seropositivity generally denotes a milder and usually monophasic variant of NMOSD as suggested by earlier, smaller studies with shorter observation periods. Most patients have a relapsing general disease course, and serious and potentially life-threatening complications of brainstem encephalitis such as respiratory insufficiency may occur. This needs to be kept in mind when deciding on long-term treatment, and attention should be paid to signs or symptoms of additional brainstem involvement in patients with MOG-IgG-positive ON and/or myelitis. Clinical manifestations of MOG-IgG-positive brainstem encephalitis are diverse and, notably, may include symptoms previously thought to be typical for AQP4-IgG-positive NMOSD, such as APS and INV, or of MS, such as INO. In accordance with what was observed in the total cohort [17], treatment with IVMP and/or PEX was associated with good recovery in many cases. As most MOG-IgG-positive patients develop relapses and since brainstem involvement may indicate a more aggressive disease course, prophylactic long-term treatment should be considered in patients presenting

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with MOG-IgG-associated brainstem encephalitis. Larger studies, which given the conditions' relative rarity will require an international collaborative approach, are highly warranted to improve our understanding of the full clinical spectrum, acute and long-term treatment needs, and prognosis of MOG-IgG-related CNS disease and in particular MOG-IgG-associated brainstem encephalitis.

## Appendix

### Case 4 – Recurrent NMO starting with simultaneous bilateral ON and LETM followed by an area postrema syndrome; good partial recovery

In January 2012, a 30-year-old woman with no previous personal or family history of autoimmune disease developed sudden loss of vision and reduced visual field in the left eye and 2 days later in the right eye, followed by tetraparesis 2 weeks later. While brain MRI showed no abnormalities, spinal cord MRI revealed an LETM lesion extending from C2 to C7. The patient thus met Wingerchuk's 2006 criteria for NMO [35]. LP revealed CSF pleocytosis with 122 leukocytes/µl (97 % mononuclear cells, 3 % granulocytes); OCB were not determined. Treatment with IVMP was followed by partial recovery. Four months later, a relapse of myelitis occurred in the high cervical cord, now associated with brainstem encephalitis. Symptoms included tetraparesis and INV. MRI showed a brainstem lesion adjacent to the fourth ventricle and including the area postrema, which continued into the spinal cord. Brainstem symptoms completely remitted 2 months after IVMP treatment, and spinal symptoms partially remitted with residual paresthesia and mild paraplegia (BMRC 4+) after 3 months.

The patient subsequently had recurrent attacks of isolated ON (3 × full recovery after IVMP) or isolated myelitis (2 × full recovery after IVMP, 1 × partial recovery after IVMP). Six of the eight attacks in this patient occurred during 36 months of treatment with AZA, all of them after discontinuation of co-treatment with oral steroids (given for 3 months). At last follow-up, an EDSS of 3 was documented. MOG-IgG was detected retrospectively in a sample taken during remission by means of a live-cell CBA (1:640) and was confirmed by use of a commercial fixed-cell CBA (Euroimmun). AQP4-IgG was negative. The patient had no previous personal or family history of autoimmune disease.

### Case 5 – Recurrent ON, LETM and brainstem attacks, with exacerbation after initiation of interferon-beta treatment; high relapse rate

A 34-year-old Caucasian woman with a positive family history for type 1 diabetes mellitus first presented with paraparesis due to transverse myelitis in 10/2006, which partially remitted after treatment with IVMP. Brain MRI showed asymptomatic lesions adjacent to the lateral ventricles. Five months later a second attack of transverse myelitis with paraparesis occurred, again with partial recovery after IVMP treatment; MR images from that time are unavailable. Later the same year, the patient developed a first attack of unilateral ON. LP revealed CSF pleocytosis but no CSF-restricted OCB. Administration of IVMP was followed by partial recovery. MS was suspected and treatment with intramuscular interferon beta-1a (IFN-beta) was started. However, 3 months later she experienced a brainstem attack with balance difficulties and vertigo. MRI revealed disease exacerbation with new lesions in the pons and in the medulla oblongata as well as multiple cervical and thoracic spinal cord lesions. Symptoms remitted spontaneously. In 01/2009 IFN-beta was discontinued and treatment with natalizumab initiated. During the period of natalizumab treatment (03/2009 – 08/2010), one further attack of brainstem encephalitis with impaired balance and vertigo occurred. In 06/2010, follow-up spinal MRI performed during remission demonstrated an LETM lesion extending from T2 to T6. The findings on brain MRI still did not meet the diagnostic criteria for MS. A diagnosis of relapsing NMO according to Wingerchuk's 2006 criteria [35] was made. Over the following 75 months, the patient experienced eight further attacks of myelitis, one of ON, and one of brainstem encephalitis with balance difficulties and dizziness. Four of these episodes, including two of the myelitis attacks and the ON attack (each with full remission after high-dose steroid treatment) as well as the brainstem attack (complete spontaneous remission), occurred while the patient was being treated with AZA for 36 months. Using a live-cell CBA, low-titer MOG-IgG antibodies (1:160) were detected retrospectively in a sample taken during remission. MOG-IgG seropositivity was later confirmed in an independent commercial fixed-cell CBA (Euroimmun). In addition, low-titer serum IgA tissue transglutaminase antibodies and antinuclear antibodies (ANA) were found. Serum AQP4-IgG was negative. At last follow-up, an EDSS of 3 was documented.

### Case 6 – ON followed by post-infectious LETM and brainstem encephalitis 40 years later with poor outcome

At the age of 13 years this female patient had severe unilateral ON (visual acuity 10 %) with complete remission. No further neurologic symptoms occurred for 40 years. At age 53 she developed subacute tetraparesis and autonomic bladder and bowel dysfunction after a severe bronchopulmonary infection and a prodromal phase of diffuse tingling in all four extremities. She had been previously diagnosed with rheumatoid arthritis (RA) at the age of 43 years, and long-term treatment with weekly methotrexate (MTX) had been initiated at the age of 48 years. Due to the severe infection her

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immunosuppressive therapy had been stopped 4 weeks before onset of the neurologic symptoms. Initial MRI workup was inconclusive. Examination of CSF revealed mild pleocytosis with 30 cells/μl and a disturbed BCSFB function (QAlb 21.5) and no OCB. She was treated with empirical antibiotics and aciclovir. As the clinical presentation worsened, the patient became bedridden and lost bladder and bowel control. MRI was repeated and now revealed a longitudinally extensive spinal cord lesion extending centrally from the medulla oblongata to the mid-thoracic level with patchy Gd enhancement; brain MRI revealed no other abnormalities. No symptoms attributable to the medulla oblongata lesion were present. High-dose steroid treatment was initiated and was followed by 5 cycles of therapeutic PEX. She was restarted on her immunosuppressive treatment with MTX and recovered rapidly but only partially. At last follow-up, 1.5 years after this episode, she is ambulatory with no motor deficits but still reports painful dysesthesia and is restricted by severe sensory gait ataxia (EDSS 6.5). Anti-MOG antibodies were retrospectively assessed and found positive in a live-cell CBA of stored serum samples obtained between steroid treatment and the commencement of therapeutic PEX (1:2560) and at a follow-up visit (1:160); both samples were also positive in the fixed-cell CBA. AQP4-antibodies were negative in several samples.

### Case 7 – Recurrent LETM and subclinical ON with extensive brainstem and brain involvement; almost full recovery

A Caucasian man experienced a first attack of myelitis with marked paraparesis in February 2012 at the age of 19; this episode remitted fully after IVMP therapy. Spinal cord MRI showed a transverse inflammatory lesion at T9/10 with swelling and Gd enhancement. Brain MRI detected no abnormalities. LP revealed CSF-restricted OCB. Another attack of myelitis with hypesthesia of both legs occurred in May 2012. MRI showed enlargement of the known thoracic lesion, now spanning from T8 to T12, again with swelling and Gd enhancement. At that time, prolonged P100 latency (but with normal amplitudes) was noted on the left side (visual acuity [VA] 0.95 in both eyes). IVMP therapy was again followed by complete remission. AQP4 antibodies were negative, but NMO was suspected and, therefore, treatment with AZA started. A few weeks later, the patient developed severe headache, diplopia, and gait ataxia. MRI performed several times over a period of 4 weeks showed evolving confluent T2-hyperintense lesions in the right temporal lobe, lesions in the periaqueductal grey, the pulvinar bilaterally, and the ventral pons, as well as cervical lesions at C6/7 and an LETM lesion

extending from T8 to the conus with Gd enhancement. LP revealed an elevated cell count (22/μl), total CSF protein of 460 mg/l, and positive OCB. VEP showed increased P100 latency on both sides and normal amplitudes. The clinical deficits remitted only partially after one course of IVMP and after therapeutic PEX (five exchanges). B-cell-depleting therapy with rituximab was started in August 2012 and was followed by further slow resolution of symptoms. In May 2013, after repopulation of B cells, mild ON in the right eye occurred but remitted spontaneously. Rituximab therapy was resumed and the patient remained free of disease activity with minor deficits (EDSS of 1.0) until last follow-up in December 2014. MOG-IgG were retrospectively tested using a live-cell CBA and were positive at a titer of 1:1280.

### Case 8 – Post-vaccination myelitis and recurrent ON with brainstem and brain involvement; full recovery

A 19-year-old male patient presented with headache, meningism, and photophobia. The patient had received a vaccination (diphtheria, tetanus, pertussis, polio; and influenza) 2 weeks before symptom onset. Examination of the CSF revealed pleocytosis (43 cells/μl; predominantly lymphomonocytic, 3.1 % neutrophils). Brain MRI demonstrated multiple T2 hyperintensities in the pulvinar thalami bilaterally and in the rostral putamen as well as leptomeningeal contrast enhancement. Spinal MRI revealed a lesion at C1 and another one extending from T11 to T12. Pragmatic treatment with ceftriaxone, ampicillin, and acyclovir was started, together with IVMP. After clinical recovery, the patient was discharged to a rehabilitation facility with the diagnosis of suspected viral meningoencephalomyelitis of undetermined origin. However, 1 month later he was readmitted with disorientation, headache, meningism, fever, and fatigue. CSF analysis again revealed pleocytosis (60 white cells/μL; 26 % neutrophils). Brain MRI demonstrated T2-hyperintense lesions in the midbrain, pons, thalamus, basal ganglia, and corpus callosum. Lesions involved the periependymal surfaces of the third ventricle. Spinal cord MRI showed a contrast-enhancing lesion at C5. Shortly thereafter, the patient developed proximal spastic paraparesis, urinary retention, and bilateral visual deficits, accompanied by delayed P100 latencies, MRI-detectable lesions involving the optic chiasm and both optic tracts, and a new T2 hyperintensity at C3. MOG-IgG were positive at a titer of 1:2560. AQP4 antibodies were negative. Treatment with IVMP (1 g/d for 5 days) followed by 5 cycles of PEX led to full clinical and neuroradiologic recovery within 3 months. Long-term immunosuppressive treatment with AZA was initiated. Within the following 20 months, four further attacks of ON (ranging from mild to severe) and presumably one further attack of brainstem encephalitis with

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hearing loss occurred, with each showing full remission after high-dose steroid treatment (EDSS 0 at last follow-up).

### Case 9 – Recurrent ON with subclinical brainstem and spinal cord involvement; almost full recovery

In December 2014, 4 weeks after an ENT infection, a 50-year-old Caucasian man developed a first, left-sided ON (blurred vision, retro-orbital pain, VA OS 30 %, P100 OS delayed) which remitted almost completely after treatment with IVMP. Neurologic examination was otherwise unrevealing except for slightly increased tendon reflexes, in particular of the lower extremities. Brain MRI showed an asymptomatic, median pontine lesion without Gd enhancement; in addition, there was inflammation of the entire optic nerve and parts of its sheath, with extensive Gd enhancement and subtle swelling compared with the other side. Spinal cord MRI demonstrated a possible lesion at C6/7. LP was unrevealing (negative OCB, no pleocytosis, no BCSFB dysfunction). Infectious causes of ON were excluded. Serologically, there was no indication of vasculitis. MOG-IgG antibodies were found to be positive at that time. Due to flaring up of the ON shortly after, the pulse therapy was repeated with $5 \times 2$ g IVMP, which had no effect. By contrast, PEX treatment (8 cycles) starting 10 days after the last IVMP dose resulted in almost complete recovery.

However, 2 months later a relapse of unilateral ON of the left eye occurred with complete visual loss (VA OS 0 %; Snellen chart), impaired color vision, and retro-orbital pain. Brain MRI showed inflammation of the posterior (pre-chiasmal) part of the optic nerve with Gd enhancement but no significant swelling. Standard VEP findings had worsened (P100 not detectable in left eye); flash VEP revealed a marked P100 prolongation and reduced amplitudes in the left eye. While the pontine lesion had markedly decreased in size, several new dorsolateral and central spinal cord lesions without Gd enhancement had developed (T7/8, T8/9 and T11/12). MOG-IgG were positive at a titer of 1:10,240. PEX treatment (8 cycles) led to almost full clinical recovery, with discretely disturbed color vision and an increased sensitivity to glare as residual symptoms. Standard VEP also improved following PEX treatment, but still showed a delay in latency (159 ms) and reduced amplitudes compared with the right eye. Treatment with rituximab was commenced without complications. At last follow-up (May 2015) the patient had almost fully recovered (VA OS 0.9; EDSS 1.5).

### Case 10 – Protracted and recurrent ON with complete visual loss and signs of mild myelitis and brainstem encephalitis; complete recovery

A previously healthy 37-year-old man presented with a 4-day history of bifrontal headache, pain upon eye movement, and bilateral complete visual loss and color desaturation. Ophthalmoscopy revealed bilateral papilledema. Markedly prolonged P100 latencies were noted in both eyes, suggestive of demyelination. Brain MRI showed a bilateral optic nerve lesion with swelling and Gd enhancement but did not reveal any brain lesions. Spinal cord MRI was normal. Serum AQP4-IgG was negative, as were CRP, ANA, ANCA, rheumatoid factor, lupus coagulant, anti-gliadin and anti-transglutaminase, HIV, HTLV-1, and vitamin B12. CSF examination demonstrated mild pleocytosis (5 cells/µl; including 34 % neutrophils and 6 % eosinophils) and identical OCB in CSF and serum but was otherwise normal (including a negative MRZ reaction). Treatment with IVMP (1 g/d for 5 days) was followed by marked improvement in VA. However, only 4 days later the patient was readmitted with new visual impairment; scotoma; hypesthesia of the right lower face and nose, the right hand, and the lateral parts of the right arm; paresthesia in the fingers of both hands and in both legs; and headache. No repeat MRI was performed. VEP and SSEP were normal. AQP4-IgG was still negative. Repeat LP revealed a mild lymphomonocytic pleocytosis (8 cells/µl). The patient's visual deficits increased (0.6 OS, 0.8 OD) despite a second, prolonged IVMP cycle (1 g/d for 7 days); symptoms stabilized (but did not improve) after PEX (five exchanges). Two days after discharge the patient was again readmitted with further decrease in VA in the left eye (0.3), marked bilateral scotoma (OS > > OD), headache (NAS 3), and vertigo ("like in an elevator moving down"). VEP showed a further increase in P100 latency in the left eye. Treatment with IVMP was followed by complete remission (VA 1.0 in both eyes at discharge). However, follow-up VEP still showed markedly delayed, albeit slightly improved, P100 latencies in both eyes (154 ms compared to 178 ms at previous examination). To prevent further flare-ups after steroid withdrawal, treatment with oral steroids (100 mg/d; tapering to 10 mg/day) was initiated and, taking into consideration the patient's positive MOG-IgG serostatus, long-term treatment with AZA started. At follow-up 63 days after onset, the patient reported persisting color desaturation and impaired contrast perception, together with paresthesia in the left leg. The patient discontinued AZA after two months.

Six months after onset and one month after the first infusion of rituximab (1000 mg), he developed a relapse of ON, which fully responded to high-dose IVMP treatment, another infusion of rituximab (1000 mg) and subsequent oral steroid treatment. Another two months later, shortly after oral steroids were tapered out, a third attack of ON occurred, which was associated with mild hemiparesis and hemihypesthesia, which fully remitted following treatment with IVMP and, subsequently, oral steroids.

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### Case 11 – Myelitis and recurrent ON with lesions in the cerebellar peduncle and the frontal lobe; partial remission

A previously healthy 44-year-old Caucasian woman presented in February 2011 with sensorimotor paraparesis and urinary incontinence. Spinal MRI showed a cervicothoracic, longitudinally extensive spinal cord lesion with patchy Gd enhancement and further short, patchy spinal cord lesions. Brain MRI revealed a lesion in the cerebellar peduncle and one small cerebral lesion located in the left frontal lobe with slight Gd enhancement. The myelitis symptoms partially recovered under IVMP (1 g/d for 5 days) with residual detrusor sphincter dyssynergy and slight gait ataxia. Subsequently, the patient suffered recurrent urinary tract infections, which were effectively controlled by use of methionine (3 × 500 mg/d). In May 2011 she developed vision loss in the right eye (VA 0.2) with prolonged P100 latency and reduced P100 amplitudes. VA recovered to 0.75 after IVMP therapy within two months. Two months later, the patient experienced an attack of ON in the previously unaffected left eye (VA reduced to light perception). Partial remission was achieved after 5 PEX cycles; previous treatment with IVMP for 5 days had not resulted in any improvement. In August 2011, immunosuppressive treatment with mitoxantrone was started (10 mg/m$^2$; 4 cycles at 6-week intervals; no relapses). In January 2012, therapy was switched to AZA (2 mg/kg). After at least three attacks of unilateral ON under AZA, the patient's treatment was changed to rituximab (1000 mg i.v. in February 2015 and March 2015, respectively). Complete B cell suppression was noted 3 months after treatment. In June 2015 she developed bilateral ON with VA of 0.2 in the right eye and 0.05 in the left eye. After IVMP (5 g) and five cycles of PEX, VA improved. At the time of her last follow-up visit in August 2015, VA was 0.8 in both eyes and an EDSS of 2.5 was documented.

### Case 12 – Simultaneous ON and LETM with post-partum onset and pontomedullary brainstem encephalitis; full recovery

A 27-year-old woman developed a first, painful attack of ON in July 2012, just 6 weeks after the delivery of her first child. Ophthalmologic assessment revealed VA of 0.8, papilledema, and delayed VEP latencies (but normal amplitudes) in the left eye. Cranial MRI showed swelling as well as intraneural and perineural contrast enhancement extending over the anterior two thirds of the left optic nerve but was otherwise unremarkable. The CSF had normal cell and protein profiles with negative OCB. High-dose intravenous pulse therapy with IVMP (1000 mg/d for 3 days) was followed by complete resolution of symptoms. However, a few days later (and 2 weeks after attack onset) the patient noticed right-sided ocular pain upon eye movements, diplopia, paresthesia around her mouth and her waist, and urge incontinence.

On neurologic examination she had a left-sided sixth nerve palsy, gaze-directed horizontal and vertical nystagmus, and a mildly unsteady gait. VA was normal. MRI of the brain and spinal cord now revealed T2-hyperintense lesions extending from the pontomedullary junction throughout the cervical cord as far as C5 as well as an additional T2 hyperintensity located in the juxtacortical insular region on the left side. None of these abnormalities showed Gd enhancement. Repeat lumbar puncture disclosed lymphocytic pleocytosis (59 cells/μl) in the presence of normal lactate and protein levels, including negative OCB. Antineuronal and AQP4 antibodies were negative and there was no evidence of an infectious etiology. The patient was treated with IVMP (1000 mg daily for 5 days) followed by 80 mg orally with subsequent tapering for 7 weeks.

In September 2012, at a maintenance dose of 5 mg methylprednisolone per day and after improvement of symptoms, she experienced right-sided ON leading to a moderate decline in visual acuity (0.6). She received a further course of IVMP (1000 mg daily for 3 days) and oral therapy with decreasing doses of prednisolone (starting with 80 mg) for a total of 4 weeks with a permanent maintenance dose of 10 mg daily. This regimen prompted normalization of visual impairment. AQP4-IgG, tested in a CBA, was again negative. Long-term treatment with AZA and oral corticosteroids was started.

In October 2012 (6 weeks after onset of the last attack), just 4 days after termination of steroid therapy and 1 day following reduction of AZA from 150 mg to 100 mg daily because of elevated liver enzymes, the patient developed a third episode of painful ON with right-sided visual impairment. She received another course of 1000 mg IVMP daily for 5 days followed by oral tapering, which resulted in complete recovery. AZA was stopped in November 2012 and replaced by MTX in February 2013. The patient discontinued MTX in November 2014 because she wanted a second pregnancy. At that time, MRI showed mild residual signal hyperintensity in the left optic nerve, no brain lesions, and a residual short spinal T2 hyperintensity at the C4/5 level. At last follow-up in 09/2015, she had not experience new neurologic symptoms (EDSS 0). Three stored sera obtained in July 2012 were retrospectively tested positive for MOG-IgG at a titer of 1:5120 each.

### Case 13 – Recurrent ON and myelitis; hypesthesia of the tongue and cheek; lesions in the medulla oblongata and in the pons; ongoing disease activity during treatment with IFN-beta and GLAT

A 19-year old woman developed acute hypesthesia of both hands and Lhermitte's sign in 2007. Fifteen months before that event, she had suffered from an episode of extremely painful headache starting 4 weeks after influenza vaccination and lasting for 14 days (no previous

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history of headache). Brain MRI was normal at the time of attack onset, but spinal cord MRI revealed a single lesion at C2. CSF examination demonstrated lymphocytic pleocytosis (33 cells/µl) and intrathecal IgG and IgM synthesis. VEP, SSEP and MEP were normal. Two months later, she developed unilateral ON. Both attacks were treated with IVMP, which was followed by complete recovery in each case. Over the following 8 years, she developed at least seven more attacks of myelitis and two more attacks of ON despite immuno-modulatory or immunosuppressive treatment with IFN-beta 1a s.c., IFN-beta 1a i.m., GLAT, natalizumab or fin-golimod for suspected MS, with partial or full recovery following steroid treatment. While most myelitis attacks were characterized by sensory symptoms (including girdle-like or, later, generalized dysesthesia and pain), some resulted in spastic paresis of the lower extremities and impaired ambulation requiring unilateral assistance. Repeat MRI demonstrated both short spinal cord lesions and LETM lesions, partly with swelling of the spinal cord and contrast enhancement. ON attacks were mostly mild, but severe attacks occurred as well (VA 0.25, peripheral scotomas). At least twice, she experienced simultaneous ON and myelitis. Several repeat brain MRIs showed (partly contrast-enhancing) juxtacortical, deep white matter, callosal, and periventricular lesions. While the initial LP had revealed quantitative evidence for intrathecal synthesis of both IgG (33 % of total CSF IgG) and IgM (59 % of total CSF IgM), only intrathecal IgG synthesis was detected at repeat LP during another acute an acute attack 10 months later. Nineteen months after onset, she developed an attack of myelitis with hypesthesia in both legs, Lhermitte's sign, and lesions at C2, C6, and C7 which was accompanied by hypesthesia of the tongue and facial hypesthesia, dysesthesia and pain, suggesting brainstem encephalitis. However, no brainstem MRI was performed at that time. Two follow-up MRIs showed T2-hyperintense, non-contrast enhancing lesions in the medulla oblongata and in the pons, which remained detectable over a period of at least once year. Additional symptoms attributable to brainstem lesions included impaired coordination, impaired ambulation, and disturbed smooth pursuit. Microbiological examinations for *Borrelia burgdoferi*, HIV, HBV, HCV and VZV as well as serological examinations for rheumatic diseases were negative. At last follow-up in 2016, an EDSS score of 3.5 was documented.

### Case 14 – LETM with pontomedullary brainstem encephalitis and relapsing ON; almost full recovery

A female Caucasian patient developed a first attack of unilateral ON at the age of 17 but recovered fully after IVMP treatment. Brain MRI was normal at onset. CSF examination revealed mild pleocytosis (8 cells/µl) and evidence of BCSFB dysfunction but not of intrathecal

IgG synthesis. Seven months later, she developed paraparesis due to an attack of acute myelitis; IVMP treatment was followed by complete recovery. Over the next 56 months, five further attacks of ON occurred (three while on GLAT for suspected MS, one with transient unilateral blindness several weeks after GLAT had been discontinued due to an unplanned pregnancy, and one after 8 months of AZA treatment), all of which responded to IVMP therapy, as well as two further attacks of non-longitudinally extensive transverse myelitis, one of which was associated with internuclear ophthalmoplegia (INO). The latter attack occurred under treatment with IVIG. Previously, AZA had had to be discontinued due to an increase in liver enzymes and a planned pregnancy. During that attack, brain MRI showed a large, Gd-enhancing brainstem lesion affecting the pons bilaterally, both pedunculi cerebelli, and the paramedian pontomedullary junction. Spinal cord MRI showed two separate lesions at C2/3 and C5. In addition, Gd-enhancing supratentorial lesions located peritrigonally and in the corona radiata were noted. PEX was required in addition to IVMP to achieve remission of symptoms. Treatment with rituximab had to be stopped after the first infusion due to an allergic skin reaction. Under subsequent immunosuppression with ofatumumab (18 months so far, 4 cycles) one additional ON attack has occurred (14 months after commencement of therapy). Despite the high number of nine ON attacks and complete blindness during one of those attacks, the patient had almost normal VA (0.9) at last FU. VEP showed prolonged P100 latencies and reduced amplitudes.

### Case 15 – Hemihypesthesia including the face; bilateral lesion in the pons and the cerebellar peduncles; myelitis; partial recovery

This 25-year-old Caucasian woman first presented in February 2016 with tingling paresthesias of her left arm and leg, which had developed over the course of 2 days. She had a history of asthma bronchiale and neurodermitis, but except for a bipolar disorder and rare attacks of typical migraine no history of neurological symptoms. She had suffered from fever and fatigue 2 weeks before her neurological symptoms started. Neurological examination revealed paramedian moderate left-sided tactile hemihypesthesia including the face and a pathologically increased left knee-jerk without other pyramidal signs, as well as an unstable Romberg stance with eyes closed, but was otherwise unremarkable. In particular she had no limb ataxia and no further cerebellar signs. Auto-immune serology revealed a strongly increased ANA titer of 1:320 with a speckled/spindle apparatus staining pattern, while no ENA antibodies and no ANCA were detectable. Cranial MRI revealed bilateral lesions of the pons, more marked on the right side, and bilateral

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lesions in the cerebellar peduncles as well as a single small lesion directly adjacent to the left lateral ventricle, while MRI of the whole spinal cord showed no abnormalities. CSF analysis revealed pleocytosis (150 white cells/μl; 29 % lymphocytes, 45 % monocytes, 26 % granulocytes), total protein of 56.1 mg/dl (<50), and an increased albumin quotient of 11.3 (upper limit of age-corrected normal = 7.0); isoelectric focusing revealed identical OCB in serum and CSF; no intrathecal antibody synthesis against measles, rubella, varicella zoster, and herpes simplex virus was detectable. CSF PCR analyses of a panel of neurotropic viruses were negative. Serology of serum and CSF was negative for Lyme disease and syphilis. Visual, sensory, and motor evoked potentials were normal. Owing to the history of recent fever and CSF pleocytosis including neutrophils, treatment with aciclovir i. v. and ceftriaxone was started, based on a tentative diagnosis of infectious, primarily viral encephalitis on the day of admission. Aciclovir was discontinued after negative viral PCR results became available, while ceftriaxone was continued for 14 days. After an initial worsening of the patient's hemihypesthesia, occurrence of a mild left-sided hemiparesis, and newly occurring intermittent urinary hesitancy over a few days, the symptoms started to gradually improve. Another lumbar puncture 14 days after admission revealed a cell count of 98/μl. The patient was discharged with partial relief of symptoms. Positive anti-MOG IgG and negative aquaporin-4 antibody results arrived after discharge.

Four weeks later the patient was again admitted to our department with new sensory disturbances affecting her trunk and legs below T10 and stance and gait ataxia. MRI now revealed a lateral spinal cord lesion spanning from T8 to T9 without contrast enhancement. Another spinal tap showed pleocytosis (20 white cells/μl), a now normal albumin quotient of 5.6, and again identical OCB in serum and CSF. Flow cytometric CSF analysis revealed 10 % lymphocytes, all CD3-positive, and a marginally increased CD4/CD8 ratio of 4.4. Visual and sensory evoked potentials were again normal, while the patient rejected analysis of motor evoked potentials. Treatment with IVMP (5 × 1 g/day) induced partial remission of her new symptoms. Cranial MRI after steroid initiation revealed moderate regression of all known brain lesions and no new lesions. After a well-tolerated test dose, secondary prophylactic treatment with azathioprine 150 mg/day was started.

The patient next attended our neuroimmunological outpatient clinic for a routine follow-up appointment in June 2016. She reported that the residual left-sided sensorimotor symptoms from her first attack had intensified in the previous 4 weeks, indicating a potential mild relapse. Two weeks before the beginning of this intensification of symptoms she had had a feverish respiratory infection, for which she had received antibiotic treatment. Based on a peripheral blood lymphocyte count of 2470/μl, the azathioprine dose was increased. An EDSS of 3 was documented.

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### Author details
[1]Molecular Neuroimmunology Group, Department of Neurology, University Hospital Heidelberg, Otto Meyerhof Center, Im Neuenheimer Feld 350, 69120 Heidelberg, Germany. [2]Department of Neurology, Ruhr University Bochum, Bochum, Germany. [3]Department of Neurology, Charité – University Medicine Berlin, Berlin, Germany. [4]Department of Neurology and Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark. [5]Department of Neurology, Hannover Medical School, Hannover, Germany. [6]Department of Neurology, NeuroCure Clinical Research Center and Clinical and Experimental Multiple Sclerosis Research Center, Charité University Medicine, Berlin, Germany. [7]Experimental and Clinical Research Center, Max Delbrueck Center for Molecular Medicine, Berlin, Germany. [8]Department of Neurology, University of Rostock, Rostock, Germany. [9]Department of Neurology, Albert Ludwigs University, Freiburg, Germany. [10]Department of Neurology, Heinrich Heine University, Düsseldorf, Germany. [11]Department of Neuroradiology, Ruhr University Bochum, Bochum, Germany. [12]Institute of Experimental Immunology, affiliated to Euroimmun AG, Lübeck, Germany. [13]Department of Neurology, Julius Maximilians University, Würzburg, Germany. [14]IRCCS, C. Mondino National Neurological Institute, Pavia, Italy. [15]Department of Neurology, Medical University Innsbruck, Innsbruck, Austria.

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# 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

Florence Pache[1,2†], Hanna Zimmermann[1,2†], Janine Mikolajczak[1], Sophie Schumacher[1], Anna Lacheta[1], Frederike C. Oertel[1], Judith Bellmann-Strobl[1,12], Sven Jarius[3], Brigitte Wildemann[3], Markus Reindl[4], Amy Waldman[5], Kerstin Soelberg[6,7], Nasrin Asgari[6,7], Marius Ringelstein[8], Orhan Aktas[8], Nikolai Gross[9], Mathias Buttmann[10], Thomas Ach[11], Klemens Ruprecht[2], Friedemann Paul[1,2,12†], and Alexander U. Brandt[1*†]; in cooperation with the Neuromyelitis Optica Study Group (NEMOS)

## Abstract

**Background:** Antibodies against myelin oligodendrocyte glycoprotein (MOG-IgG) have been reported in patients with aquaporin-4 antibody (AQP4-IgG)-negative neuromyelitis optica spectrum disorders (NMOSD). The objective of this study was to describe optic neuritis (ON)-induced neuro-axonal damage in the retina of MOG-IgG-positive patients in comparison with AQP4-IgG-positive NMOSD patients.

**Methods:** Afferent visual system damage following ON was bilaterally assessed in 16 MOG-IgG-positive patients with a history of ON and compared with that in 16 AQP4-IgG-positive NMOSD patients. In addition, 16 healthy controls matched for age, sex, and disease duration were analyzed. Study data included ON history, retinal optical coherence tomography, visual acuity, and visual evoked potentials.

**Results:** Eight MOG-IgG-positive patients had a previous diagnosis of AQP4-IgG-negative NMOSD with ON and myelitis, and eight of (mainly recurrent) ON. Twenty-nine of the 32 eyes of the MOG-IgG-positive patients had been affected by at least one episode of ON. Peripapillary retinal nerve fiber layer thickness (pRNFL) and ganglion cell and inner plexiform layer volume (GCIP) were significantly reduced in ON eyes of MOG-IgG-positive patients (pRNFL = $59 \pm 23$ μm; GCIP = $1.50 \pm 0.34$ mm$^3$) compared with healthy controls (pRNFL = $99 \pm 6$ μm, $p < 0.001$; GCIP = $1.97 \pm 0.11$ mm$^3$, $p < 0.001$). Visual acuity was impaired in eyes after ON in MOG-IgG-positive patients ($0.35 \pm 0.88$ logMAR). There were no significant differences in any structural or functional visual parameters between MOG-IgG-positive and AQP4-IgG-positive patients (pRNFL: $59 \pm 21$ μm; GCIP: $1.41 \pm 0.27$ mm$^3$; Visual acuity = $0.72 \pm 1.09$ logMAR). Importantly, MOG-IgG-positive patients had a significantly higher annual ON relapse rate than AQP4-IgG-positive patients (median 0.69 vs. 0.29 attacks/year, $p = 0.004$), meaning that on average a single ON episode caused less damage in MOG-IgG-positive than in AQP4-IgG-positive patients. pRNFL and GCIP loss correlated with the number of ON episodes in MOG-IgG-positive patients ($p < 0.001$), but not in AQP4-IgG-positive patients.

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\* Correspondence: Alexander.Brandt@charite.de
†Equal contributors
Friedemann Paul and Alexander U Brandt are equally contributing senior authors.
Florence Pache and Hanna Zimmermann are equally contributing first authors.
[1]NeuroCure Clinical Research Center (NCRC), Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
Full list of author information is available at the end of the article

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**Conclusions:** Retinal neuro-axonal damage and visual impairment after ON in MOG-IgG-positive patients are as severe as in AQP4-IgG-positive NMOSD patients. In MOG-IgG-positive patients, damage accrual may be driven by higher relapse rates, whereas AQP4-IgG-positive patients showed fewer but more severe episodes of ON. Given the marked damage in some of our MOG-IgG-positive patients, early diagnosis and timely initiation and close monitoring of immunosuppressive therapy are important.

**Keywords:** Myelin oligodendrocyte glycoprotein antibodies (MOG-IgG), aquaporin-4 antibodies (AQP4-IgG), NMO-IgG, neuromyelitis optica, Devic syndrome, neuromyelitis optica spectrum disorders (NMOSD), optic neuritis, optical coherence tomography, visual evoked potentials, visual acuity, retinal neuro-axonal damage

## Background

Myelin oligodendrocyte glycoprotein (MOG) is expressed on the outer surface of oligodendrocytic myelin sheaths, representing approximately 0.05 % of all myelin-constituting proteins [1]. Antibodies against MOG (MOG-IgG) have been detected in a proportion of aquaporin-4 (AQP4)-IgG-seronegative patients with neuromyelitis optica spectrum disorder (NMOSD) phenotype [2–6]. MOG-IgG have further been reported in children with acute and relapsing-remitting inflammatory demyelinating encephalomyelitis as well as in a proportion of adults with inflammatory demyelinating diseases such as optic neuritis (ON) [7–9].

Currently it is debated whether MOG-IgG-associated encephalomyelitis should be classified as an NMOSD subtype or as a separate disease entity [10–12]. MOG-IgG-seropositive patients from NMOSD cohorts can show clinical features of recurrent transverse myelitis and ON, similar to AQP4-IgG-seropositive patients [4]. However, the cellular target of AQP4-IgG is an astrocytic water channel, suggesting a different mechanism of injury from MOG-IgG. This is supported by a recent case study of a MOG-IgG-seropositive patient who showed severe demyelination with no evidence of astrocytopathy [13] and by further brain biopsy case studies [14–16].

ON in NMOSD patients is often severe with marked retinal nerve fiber layer and ganglion cell layer loss, severe visual impairment including blindness, and a high frequency of bilateral events [17, 18]. In around 20 % of affected eyes, macular microcysts are found in the inner nuclear layer as a sign of severe ON-related retinal injury [19, 20]. In comparison, the extent of afferent visual system damage following ON in MOG-IgG-seropositive patients is less well understood.

Some previous studies, employing either structural or clinical assessment of visual function, suggested that MOG-IgG-positive patients have fewer attacks, better recovery from relapses, and less neuro-axonal retinal damage than AQP4-IgG-positive patients [4, 21, 22]. However, it is a potential drawback that observation periods were relatively short and sample sizes low in those studies. Moreover, some included mostly or exclusively Asian patients [4, 22]; this could be relevant in that genetic factors have been proposed to play a role in NMOSD pathogenesis [17]. By contrast, more recent studies by others [23, 24] and us [25] demonstrate that the disease follows a relapsing course in the long run in most MOG-IgG-positive patients.

The objective of this retrospective multicenter study was to investigate visual system damage after ON in a larger cohort of Caucasian patients with MOG-IgG-associated encephalomyelitis and long-term follow-up using a comprehensive assessment of the afferent visual system including structural, functional, and clinical parameters, and to compare it with that in AQP4-IgG-positive NMOSD patients.

## Methods

### Patients

MOG-IgG-seropositive patients with a history of ON and available optical coherence tomography (OCT) data were recruited from a large retrospective study [25, 26]. Sixteen patients (15 female; mean age 44.0 ± 15.2 years) were enrolled from six university hospitals in Europe (Germany: Berlin, Freiburg, Düsseldorf, Heidelberg, Würzburg; Denmark: Vejle). The inclusion criteria were age ≥18 years, a confirmed history of ON (more than 3 months prior to visual assessments), and seropositivity for MOG-IgG. A MOG-antibody serum titer of ≥1: 160 was classified as positive [26]. Clinical and paraclinical data on disease onset, relapse history, expanded disability status scale (EDSS) [27], visual acuity, OCT, magnetic resonance imaging (MRI), and immunotherapy were retrospectively collected.. Annualized relapse rate was calculated as the ratio of number of attacks and years since disease onset, excluding patients with disease duration of less than 1 year. All patients were of Caucasian descent; all MOG-IgG-positive patients tested seronegative for AQP4-IgG, and vice versa (Table 1). Eight (50 %) MOG-IgG-positive patients had a previous diagnosis of—mainly recurrent—ON, and eight (50 %) had been diagnosed with NMOSD based on the clinical symptoms of ON and myelitis before anti-MOG-IgG was tested.

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**Table 1** Demographic data

|  |  | MOG-IgG | AQP4-IgG | MOG-IgG vs. AQP4-IgG (MWU/Chi$^2$) |
|---|---|---|---|---|
|  |  |  |  | p |
| Patients | N | 16 | 16 |  |
| Age (years) | Mean ± SD | 44.0 ± 15.2 | 43.2 ± 13.9 | 0.838 |
| Sex (f/m) |  | 15/1 | 16/0 | >0.999 |
| Ophthalmologic comorbidities | N | 2[a)] (13 %) | 0 (0 %) |  |
| Age at onset (years) | Mean ± SD | 37.2 ± 15.1 | 34.7 ± 14.8 | 0.669 |
| Time since onset (years) | Mean ± SD | 6.9 ± 6.5 | 8.4 ± 6.8 | 0.287 |
| ON eyes | N (%) | 29 (91.6 %) | 25 (78.1 %) |  |
| Number of ON episodes | Median (range) | 4.5 (1–13) | 2 (1-4) | **0.012** |
| Myelitis prevalence | N (%) | 8 (50 %) | 15 (93.8 %) | **0.018** |
| ARR | Median (range) | 1.25 (0.38–7.14) | 0.64 (0.17–1.44) | **0.026** |
| ON ARR | Median (range) | 0.69 (0.17–7.14) | 0.29 (0.07–0.96) | **0.004** |
| EDSS | Median (range) | 3.0 (1.0–7.5) | 4.0 (1.0–6.5) | 0.064 |

*Abbreviations*: *AQP4-IgG* aquaporin-4 antibody-seropositive NMOSD patients, *ARR* annualized relapse rate, *EDSS* expanded disability status scale, *f* female, *m* male, *MOG-IgG* myelin oligodendrocyte glycoprotein antibody-seropositive patients, *MWU* Wilcoxon-Mann-Whitney *U* test, *ON* optic neuritis, *SD* standard deviation
[a)]Early stage dry macular degeneration in both eyes and suspect for early stage glaucoma, respectively
*p*-values in bold emphasis depict significant values (p < 0.05)

AQP4-IgG-positive NMOSD patients [28] (*n* = 16, all female, mean age 43.2 ± 13.9 years) and healthy controls (HC, *n* = 16, 15 female, mean age 43.9 ± 15.4 years) were randomly selected from the research database of the NeuroCure Clinical Research Center (Charité – Universitätsmedizin Berlin, Berlin, Germany), matched for sex and age on cohort basis. Two MOG-IgG-positive patients had co-occurring ophthalmologic conditions in both eyes: one had early-stage dry macular degeneration, and glaucoma was suspected in the other patient. These two patients and their matched AQP4-IgG-positive patients and HC were included in the case descriptions but excluded from statistical analyses of OCT and visual function parameters. Furthermore, only eyes with a previous ON were included in statistical analyses. The local ethics committees approved the study protocol in accordance with the Declaration of Helsinki (1964) in its currently applicable version. All participants provided informed written consent.

### MOG-IgG and AQP4-IgG assay
MOG-IgG antibodies were detected using a live cell-based assay and a fixed cell-based assay, both employing HEK293 cells transfected with human full-length MOG; mock-transfected cells were used as control substrates (see part 1 for details [26]). AQP4-IgG were detected using a commercially available cell-based assay (EUROIMMUN, Lübeck, Germany) [29, 30].

### Optical coherence tomography
OCT was performed using the Spectralis SD-OCT device (Heidelberg Engineering, Heidelberg, Germany) with the automatic real time function for image averaging.

Peripapillary retinal nerve fiber layer thickness (pRNFL) was derived from a standard ring scan around the optic nerve head (12°, 768 or 1536 A-scans, 16≤ART≤100). A macular volume scan (25° × 30°, 61 vertical or horizontal B-scans, 768 A-scans per B-scan, 9≤ART≤15) was acquired for retinal layer analysis. All scans underwent quality control [31] and post-processing by one experienced rater in a standardized manner. Layer segmentation was performed with the device's software (Eye Explorer 1.9.10.0 with viewing module 6.0.9.0). Automatic segmentation results were carefully checked for errors and corrected if necessary by an experienced rater masked for the diagnosis of the subjects. Combined ganglion cell and inner plexiform layer volume (GCIP), inner nuclear layer volume, and outer retinal layers volume including the outer plexiform and nuclear layer, inner and outer photoreceptor segments, and retinal pigment epithelium, were extracted from a 6-mm-diameter cylinder around the fovea [32]. Furthermore, all scans were examined for macular microcysts [19] and other retinal pathologies. The OCT parameters are visualized in Fig. 1.

### Visual function testing
Visual function testing was performed in MOG-IgG-positive and AQP4-IgG-positive patients at the same visit as OCT, except for one patient (see Additional file 1: Table S1). Visual evoked potentials (VEP) were recorded with checkerboard stimulation (1°) with the device routinely used at the sites. P100 peak latency was included in analysis and considered as abnormal when higher than 112 ms [33] or when no clear signal could be evoked. Habitually corrected visual acuity was tested with letter charts obtained as part of routine clinical care
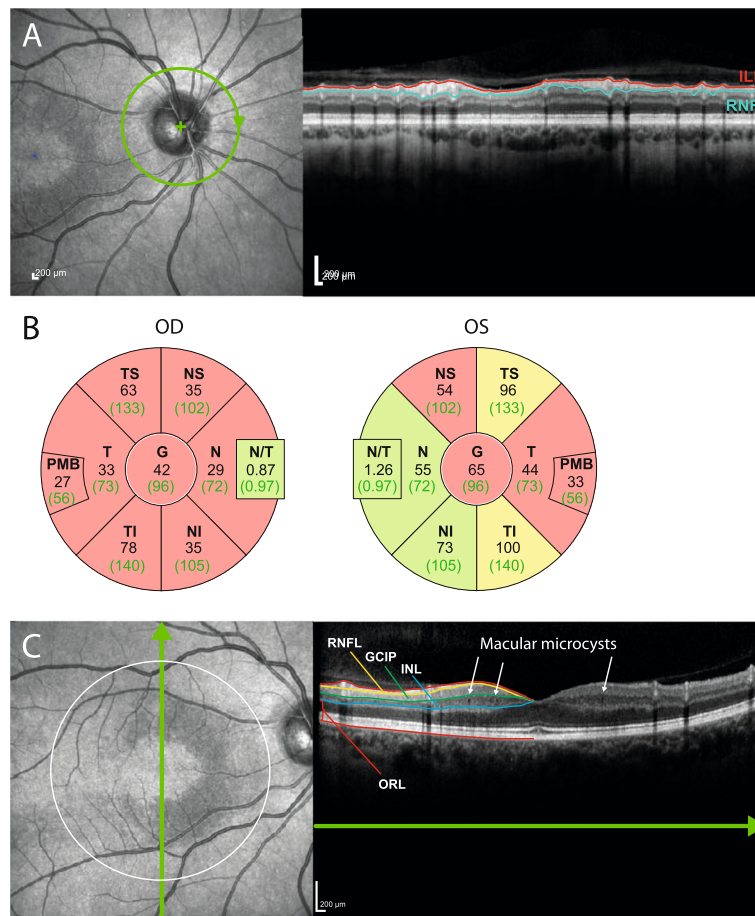
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**Fig. 1** Sample images from patient 1. **a** Sample images from a peripapillary ring scan. On the *left*, a scanning laser ophthalmoscopy image shows scan positioning (in *green*). On the *right*, an OCT scan shows severe peripapillary retinal nerve fiber layer (pRNFL) loss (between the inner limiting membrane [ILM], shown in *red*, and the lower border, in *turquoise*). **b** Ring-scan data in comparison with normative device data from both eyes of this patient. *Black numbers* display the thickness measurements (in μm) of the subject, *green numbers* the average thickness in the age-matched reference group. Sectors are classified in comparison with the reference group: *green*, thickness values within the 5th and 95th percentile range; *yellow*, 1st to 5th percentile range; *red*, below the 1st percentile. *Abbreviations*: G global, NS nasal-superior, N nasal, NI nasal-inferior, TI temporal-inferior, T temporal, TS temporal-superior. **c** Macular scan of the same patient. On the *left*, the dark, sickle-shaped area on and around the macula represents tissue with microcysts in the inner nuclear layer (INL). The *white circle* indicates the 6-mm-diameter cylinder in which intraretinal layers are analyzed. The *green line* with *arrow* shows the scanning position of the OCT scan on the right. Here, the defined layers are the RNFL, the ganglion cell and inner plexiform layer (GCIP), then INL and the outer retinal layers (ORL). Macular microcysts can be seen as small *black dots* in the INL

and converted into logMAR units. A visual acuity of 0.2 logMAR and worse was considered abnormal. When no letter could be recognized by the patient, visual acuity was registered with 2.0 logMAR for finger counting and 3.0 logMAR for hand motion recognition [34].

### Data analysis

Statistics were performed in R version 3.1.2 [35] using the packages psych, MASS, geepack and ggplot. Differences in demographics between the cohorts were tested with Pearson chi-square test and non-parametric tests (Mann-Whitney U for two cohorts and Kruskal-Wallis for three cohorts). Comparisons of visual system data between cohorts were performed using generalized estimating equation (GEE) models accounting for intra-subject inter-eye dependencies. GEE results are provided with regression coefficient (B) and standard error (SE). To investigate the extent of damage caused by subsequent ON episodes we employed a linear spline regression model as proposed by Ratchford et al. [36]. Due to the exploratory nature of this study, no correction for multiple comparisons was performed.

### Results

The demographic and clinical features of MOG-IgG-positive patients are presented in Table 1 and case-by-case clinical details are provided in Additional file 1: Table S1. One patient had pediatric onset of the disease,

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at 6 years of age; her case has been reported in an earlier publication [11]. All other patients had adult onset. All MOG-IgG-positive patients had experienced at least one episode of ON (median 4.5, range 1–13) and, except for one with a short follow-up period (8 months, patient 8), presented with an unequivocally relapsing disease course. Age at onset and disease duration at the time of examination did not differ between MOG-IgG-positive and AQP4-IgG-positive patients (Table 1). Detailed case studies, including therapy, are provided in parts 2 and 3 of this series of articles [25, 37].

### OCT and visual function in MOG-IgG-positive ON

Two eyes from two patients had to be excluded from the analysis owing to acute ON at the time of assessment. Thus, 23 eyes from 14 MOG-IgG-positive patients were analyzed at a median time of 16.4 months (range 3–125 months) since the most recent episode of ON. Detailed afferent visual system parameters of all patients are given in Table 2, and case-by-case descriptions are provided in Additional file 2: Table S2.

Reduced pRNFL thickness compared with the manufacturer's normative data was found in 18 of the 23 (78.2 %) ON-affected eyes of the MOG-IgG-positive group (mean $59 \pm 23$ μm). In addition, two fellow eyes without clinically evident previous ON and with normal VEPs showed reduced RNFL thickness (51 μm and 75 μm, respectively). Five ON eyes (21.7 %) but none of the non-ON eyes had macular microcysts in the inner

nuclear layer. Of 20 ON eyes with available VEP data, 12 (60 %) eyes had abnormal P100 latencies—two (10 %) of them despite normal pRNFL—while all four non-ON fellow eyes had normal VEPs. Visual acuity was on average reduced in ON eyes (mean $0.35 \pm 0.88$ logMAR), with three eyes being legally blind at a visual acuity of 1.0 logMAR and worse. On the other hand, 16 of 23 ON eyes (70 %) preserved visual acuity of 0.1 logMAR or better.

There were no significant differences in OCT and visual function measurements between MOG-IgG-positive patients with a history of both ON and myelitis ($n = 8$) and MOG-IgG-positive patients with a history only of recurrent ON ($n = 8$) (not shown).

### Comparison with HC and AQP4-IgG-positive NMOSD patients

We then compared the afferent visual system damage in ON eyes of MOG-IgG-positive patients with age- and sex-matched HC and with ON eyes of AQP4-IgG-positive NMOSD patients (Table 2, Fig. 2). As expected, pRNFL and GCIP were significantly lower than in HC both in the MOG-IgG-positive group (both $p < 0.001$) and in the AQP4-IgG-positive group (both $p < 0.001$). Furthermore, inner nuclear layer volume was significantly greater than HC in the MOG-IgG-positive subgroup ($p = 0.009$), but not in the AQP4-IgG-positive NMOSD subgroup. By contrast, no significant difference was noted between MOG-IgG-positive and AQP4-IgG-positive patients regarding retinal layer measures. Macular

**Table 2** Structural and functional data of MOG-IgG-positive patients' ON eyes in comparison to AQP4-IgG-positive patients and control data

| | MOG-IgG positive ON ($n = 23$ eyes from 14 subjects) | AQP4-IgG positive ON ($n = 21$ eyes from 14 subjects) | HC ($n = 28$ eyes from 14 subjects) | MOG-IgG positive vs. AQP4-IgG positive (GEE) | | | MOG-IgG positive vs. HC (GEE) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | B | SE | p | B | SE | p |
| Retinal OCT | | | | | | | | | |
| Average pRNFL (μm) | $59 \pm 23$ | $59 \pm 21$ | $99 \pm 6$ | −0.6 | 7.58 | 0.94 | 39.0 | 6.01 | **<0.001** |
| Nasal pRNFL (μm) | $44 \pm 21$ | $45 \pm 24$ | $74 \pm 12$ | 0.2 | 7.85 | 0.98 | 28.6 | 6.01 | **<0.001** |
| Temporal pRNFL (μm) | $44 \pm 16$ | $40 \pm 15$ | $71 \pm 10$ | −3.0 | 4.51 | 0.50 | 27.6 | 4.26 | **<0.001** |
| GCIP (mm³) | $1.50 \pm 0.34$ | $1.41 \pm 0.27$ | $1.97 \pm 0.11$ | −0.10 | 0.10 | 0.35 | 0.47 | 0.08 | **<0.001** |
| INL (mm³) | $1.03 \pm 0.10$ | $1.01 \pm 0.11$ | $0.95 \pm 0.04$ | −0.02 | 0.04 | 0.55 | −0.07 | 0.03 | **0.009** |
| ORL (mm³) | $4.86 \pm 0.26$ | $4.93 \pm 0.26$ | $4.73 \pm 0.21$ | 0.04 | 0.09 | 0.70 | −0.13 | 0.09 | 0.14 |
| Eyes with macular microcysts ($n$) | 5 (21.7 %) | 4 (19.0 %) | | Chi² | | >0.99 | | | |
| Visual function | | | | | | | | | |
| Visual acuity/logMAR | $0.35 \pm 0.88$ | $0.72 \pm 1.09$ | - | 0.33 | 0.32 | 0.30 | | | |
| Abnormal P100 latency* | 12 (57 %) | 10 (50 %) | - | Chi² | | 0.88 | | | |

OCT and visual function results are not including data from the two patients with early stage dry macular degeneration in both eyes and glaucoma, respectively, and their respective AQP4-IgG-positive controls and healthy controls. Furthermore, two eyes of two MOG-IgG positive patients were excluded due to acute ON at time of examination. Explanations: All data are given as mean ± standard deviation (minimum – maximum), if not declared different
*AQP4-IgG* aquaporin-4 antibody-seropositive NMOSD patients, *GCIP* ganglion cell and inner plexiform layer, *HC* healthy controls, *INL* inner nuclear layer, *ON* eyes with history of optic neuritis, *MOG-IgG* myelin oligodendrocyte glycoprotein antibody-seropositive patients, *ORL* outer retinal layers including layer from outer plexiform layer to Bruch's membrane, *pRNFL* peripapillary retinal nerve fiber layer
*p*-values in bold emphasis depict significant values ($p < 0.05$)
* VEP data were available for 20 out of 23 ON eyes of MOG-IgG positive patients and 20 out of 21 eyes of AQP4-IgG positive patients

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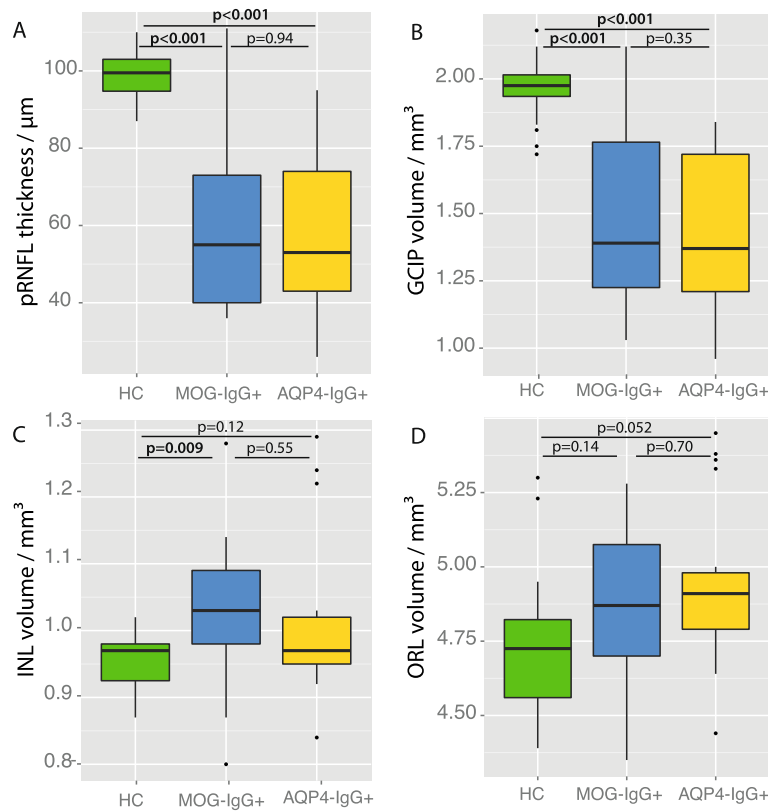
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**Fig. 2** Retinal layer measures of MOG-IgG-positive and AQP4-IgG-positive ON eyes. *Boxplots* for the comparison of retinal layer measures of the eyes in the healthy control group and the ON eyes of MOG-IgG-positive (MOG-IgG+) and AQP4-IgG-positive (AQP4-IgG+) NMOSD patients. (**a**) Peripapillary retinal nerve fiber layer thickness derived from a ring scan (pRNFL); (**b-d**) Intraretinal layer volumes quantified in a 6-mm-diameter cylinder around the fovea centralis: (**b**) ganglion cell and inner plexifom layer volume (GCIP); (**c**) inner nuclear layer volume (INL); (**d**) outer retinal layer volume comprising all layers from outer plexiform layer to Bruch's membrane

microcysts were found in both subgroups in similar prevalence, but differences in microcyst size or extend might have led to a high variability of inner nuclear layer volume values (Table 2). Visual acuity was less impaired in the MOG-IgG-positive subgroup (mean $0.35 \pm 0.88$ logMAR) than in the AQP4-IgG-positive subgroup ($0.72 \pm 1.09$); however, the difference was not significant ($p = 0.30$).

Of note, the MOG-IgG-positive patients showed a significantly higher annualized relapse rate both for all relapses and—even higher—for ON than the AQP4-IgG-positive patients ($p = 0.026$ and $p = 0.004$, respectively), despite similar disease duration (Table 1).

### Retinal damage and number of ON episodes

In MOG-IgG-positive patients, a higher number of ON episodes was associated with more severe pRNFL and GCIP loss (GEE: pRNFL B = −4.9, SE = 1.40, $p < 0.001$; GCIP B = −0.07, SE = 0.02, $p < 0.001$), but not with changes of the inner nuclear layer or outer retinal layers. By contrast, in AQP4-IgG-positive patients the extent of retinal layer changes did not correlate with the number of ON attacks.

In our cross-sectional data, the first ON episode caused a mean pRNFL loss of 12.8 μm ($p = 0.001$) in MOG-IgG-positive patients and 32.8 μm ($p < 0.001$) in AQP4-IgG-positive patients in comparison with HC eyes. In contrast, a second episode of ON caused additional pRNFL loss of 37.8 μm ($p < 0.001$) in MOG-IgG-positive patients and 20.8 μm in AQP4-IgG-positive patients, although that difference was not significant ($p = 0.07$) (Fig. 3). A similar association was found for GCIP volume (data not shown).

### Discussion

This study shows that ON in MOG-IgG-positive patients leads to severe pRNFL and GCIP thinning and visual function impairment, the extent of which is comparable to ON in patients with AQP4-IgG. Moreover, it suggests that the damage accrual may be driven by higher relapse rates in MOG-IgG-positive patients, in contrast to more severe ON-associated damage during a single ON episode in AQP4-IgG-positive patients.

Some earlier studies of MOG-IgG-positive patients, which were characterized by relatively short observation

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**Fig. 3** Retinal nerve fiber layer loss as a function of optic neuritis in MOG-IgG-positive and AQP4-IgG-positive patients. Peripapillary retinal nerve fiber layer (pRNFL) loss caused by sequential episodes of optic neuritis (ON), estimated from cross-sectional data, in comparison with eyes without optic neuritis from the healthy control (HC) cohort. (**a**) ON eyes from MOG-IgG-seropositive patients (MOG-IgG+); (**b**) ON eyes from AQP4-IgG-seropositive patients (AQP4-IgG+). P-values were computed with linear regressions

periods, suggested that MOG-IgG-seropositive patients present more often with monophasic disease and have a milder clinical phenotype and better recovery than patients with AQP4-IgG-seropositive NMOSD [4, 5, 38]. By contrast, all but one of our patients showed a relapsing disease course with a high frequency of attacks, protracted ON episodes, and, in some cases, severe visual impairment. In line with our findings, two more recent studies have also demonstrated that MOG-IgG seropositivity is frequently associated with a recurrent disease course in patients with ON [23, 24]. Concerning neuro-axonal damage of the retina, a recent study including 19 MOG-IgG-positive patients reported less retinal nerve fiber and ganglion cell layer damage than in AQP4-IgG-positive patients following ON [22]. As a limitation, however, that study included exclusively monophasic patients. By contrast, in our study we demonstrated that retinal neuro-axonal damage after ON in MOG-IgG-positive patients is at least as severe as in AQP4-IgG-positive NMOSD patients, compared with our own control cohort as well as with previously published AQP4-IgG-positive cohorts [39, 40] when patients with long-term follow-up (mean ~7 years) and, accordingly, relapsing disease course are included in the analysis.

Notably, although average visual function was impaired in relapsing ON of both MOG-IgG-positive and AQP4-IgG-positive patients, some MOG-IgG-positive patients performed comparably well on high-contrast visual acuity testing despite severe neuro-axonal retinal damage: 70 % of ON eyes retained a visual acuity of 0.1 logMAR or better after ON. However, visual acuity was obtained in non-standardized manner as high-contrast letter acuity in

clinical routine; thus the reliance on functional testing may underestimate the actual extent of damage to the afferent visual system. The impact of structural damage as demonstrated in the present study should be further investigated with low-contrast letter acuity, color vision testing, visual fields, and quality of life scales.

Our study features strengths and limitations. Among its strengths we count the relatively high number of patients included, given the low prevalence of the disease, the fact that reliable assays for detecting antibodies to full-length human MOG have become available only relatively recently, and the fact that OCT is not yet routinely and generally available. A further potential strength is that our cohort was genetically homogeneous with all patients and controls being of Caucasian origin. As a potential limitation, not all patients were systematically tested for other optic neuropathies, such as Leber's hereditary optic neuropathy (LHON). While a mitochondrial mutation may have contributed to the marked pRNFL thinning in the female patient with pediatric onset of disease (patient 4 in Additional file 1: Table S1), the time course (approximately 10 years before the contralateral eye demonstrated a mild decrease in visual acuity) is unusual for LHON, a condition which typically affects both eyes within months of each other without a relapsing and remitting course. Finally, data were collected retrospectively in a multicenter approach. As a result, additional data, e.g., the Multiple Sclerosis Function Composite or OCT scans obtained during acute optic neuritis, were not available. Moreover, we were not able to systematically correlate optic nerve MRI [23, 41] and OCT in this study,

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which would require highly standardized MRI protocols and a prospective study design. However, prospective studies as well as single-center studies in MOG-IgG-positive patients are difficult to perform due to the condition's rarity and the currently limited access to MOG-IgG testing. Moreover, all patients with available data seen at the various centers were included in the analysis, thereby reducing the risk of referral bias. Nonetheless, the preliminary evidence derived from this retrospective exploratory study needs to be confirmed in further prospective and independent studies.

## Conclusions

In summary, we demonstrate (a) that a substantial proportion of MOG-IgG-seropositive patients develop retinal neuro-axonal damage; (b) that visual impairment and structural damage increase with the number of attacks and thus with disease duration; and, importantly, (c) that the extent of neuro-axonal damage in MOG-IgG-positive patients with ON is not different from that in patients with AQP4-IgG-positive ON in the long-term course of the disease, i.e., when patients with relapsing rather than monophasic ON are taken into account. Given the marked structural and functional damage in some of our ON patients, early diagnosis, timely initiation of immunosuppressive therapy, and close monitoring of treatment efficacy seem paramount. Although no systematic investigations of drugs for relapse prevention in this condition have yet been conducted, retrospective data on treatment responses (see part 2 of this series [25]), as well as available evidence in favor of a pathogenic role of MOG-IgG [16, 30], suggest that—in accordance with treatment recommendations for AQP4-IgG-positive NMOSD [42]—patients with MOG-IgG-positive ON may benefit from high-dose intravenous methylprednisolone treatment and, possibly, plasma exchange for acute attacks as well as from immunosuppression for attack prevention.

## Additional files

**Additional file 1: Table S1.** Demographic, clinical and serological data. [a] Early stage dry macular degeneration in both eyes; [b] Suspected early stage glaucoma. [c] Visual assessments were performed during acute ON OS. Abbreviations: ON: optic neuritis. VEP P100: visually evoked potential P100 latency. n.e.: not evocable; pRNFL: peripapillary retinal nerve fiber layer thickness. GCIP: combined ganglion cell and inner plexiform layer volume. INL: inner nuclear layer volume. ORL: outer retinal layers volume including layers from outer plexiform layer to Bruch's membrane. (DOCX 21 kb)

**Additional file 2: Table S2.** Visual evoked potentials, visual acuity, and OCT results. [a] Protracted relapses were registered as one episode; [b] Early stage dry macular degeneration in both eyes; [c] Suspected early stage glaucoma; [d] Medication other than acute relapse therapy (immunotherapy). Abbreviations: AQP4-IgG = aquaporin-4 antibodies; CRION = chronic relapsing inflammatory optic neuropathy; EDSS = expanded disability status scale; F = female; MOG-IgG = myelin oligodendrocyte glycoprotein antibodies; NMOSD = neuromyelitis optica spectrum disorders; (r)ON = (recurrent) optic neuritis. (DOC 59 kb)

## Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

## Authors' contributions

FIP participated in the design of the study, acquired clinical information, and drafted the manuscript. HZ performed OCT post-processing and statistical analysis and drafted the manuscript. JM acquired OCT and visual function data. SS, AL, and FCO. acquired clinical and visual function data. JBS participated in the design of the study and acquired clinical data. MRe and SJ provided MOG-IgG results and contributed to interpretation of the data. SJ and BW contributed OCT and clinical data and contributed to interpretation of the data. AW contributed to interpretation of the data. KS acquired OCT and visual function data. NA acquired clinical data and contributed to interpretation of the data. MRi acquired OCT, clinical, and visual function data. OA acquired clinical data. NG acquired OCT, clinical, and visual function data. KR participated in the design of the study and contributed to interpretation of the data. MB acquired clinical data, contributed to interpretation of the data, and edited the manuscript. TA acquired OCT and visual function data. FrP contributed to the interpretation of the data and drafted the manuscript. AUB conceived the study and participated in its design and coordination contributed to analysis, and drafted the manuscript. All authors were involved in revising the manuscript for intellectual content and read and approved the final manuscript.

## Competing interests

FIP has received a research grant from Novartis Pharmaceuticals and travel grants from Genzyme, a Sanofi company. HZ has received speaking fees from Teva and Bayer. JM has received speaking fees from Teva and Biogen Idec. B.W. has received speaking/consultation honoraria and travel grants from Bayer Healthcare, Biogen Idec, Merck Serono, and Genzyme, a Sanofi company. ATW has received support from the National Institutes of Health and Biogen Idec. MRi has received speaker honoraria from Novartis and Bayer and travel reimbursements from Bayer Schering, Biogen Idec, and Genzyme. OA has received advisor fees or honoraria from Bayer HealthCare,

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**Consent for publication**
All participants provided informed written consent for publication.

**Ethics approval and consent to participate**
The study was approved by the local ethics committees of the participating centers. All participants provided informed written consent.

**Author details**
[1]NeuroCure Clinical Research Center (NCRC), Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany. [2]Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany. [3]Molecular Neuroimmunology Group, Department of Neurology, University of Heidelberg, Heidelberg, Germany. [4]Clinical Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria. [5]Division of Neurology, Children's Hospital of Philadelphia, Pennsylvania, USA. [6]Department of Neurology, Vejle Hospital, Vejle, Denmark. [7]Department of Neurobiology, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark. [8]Department of Neurology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany. [9]Department of Ophthalmology, Medical Faculty, University of Freiburg, Freiburg, Germany. [10]Department of Neurology, University of Würzburg, Würzburg, Germany. [11]Department of Ophthalmology, University of Würzburg, Würzburg, Germany. [12]Experimental and Clinical Research Center, Max Delbrück Center for Molecular Medicine and Charité – Universitätsmedizin Berlin, Berlin, Germany.

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# (NEI-VFQ & OCT)

Die Publikation

# Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

# Publikationsliste

## Artikel in Fachzeitschriften

Finke C, Zimmermann H, Pache F, Oertel FC, Chavarro VS, Kramarenko Y, Bellmann-Strobl J, Ruprecht K, Brandt AU, Paul F; Association of Visual Impairment in Neuromyelitis Optica Spectrum Disorder With Visual Network Reorganization, JAMA Neurol. 2018

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