Aus dem NeuroCure Clinical Research Center
der Medizinischen Fakultät Charité - Universitätsmedizin Berlin

DISSERTATION

Charakterisierung retinaler Veränderungen bei Patienten mit Multipler Sklerose mittels Optischer Kohärenztomographie

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Zusammenfassung

Abstrakt

Hintergrund: Die Netzhaut ist entwicklungsbiologisch Teil des zentralen Nervensystems und stellt daher ein vielversprechendes Zielgewebe für entzündliche und neurodegenerative Prozesse bei Patienten mit Multipler Sklerose (MS) dar. Mit der optischen Kohärenztomographie (OCT) ist es möglich, die retinale Anatomie schnell und nicht-invasiv zu untersuchen. Bisherige OCT Studien konnten einen axonalen und neuronalen Schaden in der Retina bei MS Patienten mit und ohne vorherige Sehnervenentzündung (ON) zeigen. Allerdings sind die Unterschiede zwischen den MS-Subtypen und der zeitliche Verlauf der retinalen Veränderungen bisher nur unzureichend erforscht und die Ergebnisse zum Teil widersprüchlich. Bei einem Teil der MS Patienten wurden zudem makuläre Mikrozysten in der inneren Körnerschicht (INL) festgestellt und eine Verdickung der INL konnte mit der Krankheitsprogression assoziiert werden. Für das Auftreten von Mikrozysten und der Korrelationen zwischen INL Dicke und Krankheitsprogression existieren unterschiedliche Erklärungsmodelle (z. B. Glaskörpertraktion), die bisher allerdings nur unzureichend auf Validität untersucht wurden.
Zielsetzung: Ziel dieser Arbeit war eine bessere Charakterisierung retinaler Veränderungen bei Patienten mit MS mittels hochauflösender *spectral-domain* OCT (SD-OCT).
Methodik: In den vier hier präsentierten Studien wurden Patienten mit MS, klinisch isoliertem Syndrom (CIS), Neuromyelitis optica (NMO) und *Chronic relapsing inflammatory optic neuropathy* (CRION) mit SD-OCT untersucht. Für den Vergleich der Kohorten untereinander und zu gesunden Kontrollen wurden durchschnittliche retinale Schichtdicken und räumliche Dickenkarten verwendet.
Ergebnisse: In einer großen Multicenterstudie konnten wir zeigen, dass retinale Schädigung bei Patienten mit progressiven Verlaufsformen stärker ausgeprägt ist als bei Patienten mit schubförmiger MS. Entgegen früheren Arbeiten zeigten die Ergebnisse der intra-retinalen Segmentierung bereits bei CIS Patienten ohne vorherige ON eine Reduzierung der Ganglienzellschichtdicke. Eine retrospektive Analyse ergab, dass Mikrozysten mit hoher Sensitivität und guter Spezifität auf Fundusbildern detektiert werden können. Mikrozysten sind allerdings nicht spezifisch für MS, sondern stehen im Zusammenhang mit vorherigen ON Ereignissen und finden sich vermehrt auch bei anderen Erkrankungen wie NMO und CRION. Auch ohne sichtbare Mikrozysten führt eine ON zu Verdickung der INL. In einem Fall kam es zu dynamischen Veränderungen der Mikrozysten, die aber nicht mit einer Zugwirkung der Glaskörpermembran an der Retina (Glaskörpertraktion) erklärt werden konnte.

Diskussion: Die OCT etabliert sich immer mehr als Marker von Neurodegeneration in der MS. Mit neuer hochauflösender SD-OCT Technik und retinaler Schichtsegmentierung wird die Sensitivität weiter gesteigert und klinische Studien mit OCT als primärer und sekundärer Endpunkt-Parameter laufen bereits oder stehen unmittelbar bevor. Zudem liefert die OCT in der MS Forschung neue Einblicke in den Pathomechanismus der Erkrankung.

Abstract

Background: During biological development, the retina originates from central nervous system tissue and therefore offers an interesting opportunity to monitor inflammatory and neurodegenerative processes in the context of multiple sclerosis (MS). Optical coherence tomography (OCT) is a fast and non-invasive technique to investigate the retinal architecture. While previous studies could show axonal and neuronal damage in the retina of patients with and without a history of optic neuritis (ON), only scarce and in part conflicting data exist on different MS subtypes and the temporal course of retinal alterations. Macular microcysts located in the inner nuclear layer (INL) were described in a subset of MS patients and an association between INL thickening and disease progression was found. Various theories exist explaining the appearance of macular microcysts and the correlation between INL thickening and disease progression (i. e. vitreous traction), but only insufficient tests for validity of these theories were performed.
Objective: To better characterize retinal alterations in patients with MS using high-resolution spectral-domain OCT (SD-OCT).
Methods: In the four studies presented here, patients with MS, clinically isolated syndrome (CIS), neuromyelitis optica (NMO) and chronic relapsing inflammatory optic neuropathy (CRION) were investigated with SD-OCT. Mean retinal layer thicknesses and spatial thickness maps were applied to compare the different cohorts with each other and to healthy controls.
Results: In a large multicentre study, we could show that patients with progressive MS subtypes exhibit more severe retinal neurodegeneration than relapsing-remitting MS patients. In contrast to previous works, intra-retinal layer segmentation was able to detect ganglion cell layer reduction in CIS patients even in the absence of previous ON events. A retrospective analysis showed that macular microcysts could be identified with high sensitivity and good specificity using fundus images. However, microcysts are not specific for MS but were strongly associated with a previous ON and also occurred in other diseases like NMO and CRION. Even without visible microcysts, the INL thickness increased after an ON. One case presented with dynamic changes of microcysts, which could not be explained by vitreous traction.

Discussion: OCT is gradually being established as a marker for neurodegeneration in MS. New high-resolution SD-OCT technique and retinal layer segmentation further increase the sensitivity, and clinical studies using OCT derived primary and secondary outcome parameters are on-going or about to commence. Furthermore, OCT continues to be an interesting tool in MS research and is providing new insights in the pathomechanisms of MS.

Einleitung

Multiple Sklerose (MS) ist eine chronisch-entzündliche und neurodegenerative Erkrankung des zentralen Nervensystems (ZNS) und eine der häufigsten neurologischen Erkrankungen im jungen Erwachsenenalter.[1] Charakteristisch für die MS sind lokale, demyelinisierende Entzündungsherde hauptsächlich in der weißen Substanz des ZNS, die sich klinisch in akuten Schüben mit unterschiedlichen neurologischen Symptomen, wie zum Beispiel motorischen Ausfällen, Sensibilitäts- oder Sehstörungen manifestieren. Der Pathomechanismus der MS ist bisher nicht vollständig aufgeklärt, es wird aber vermutet, dass die lokalen Entzündungsreaktionen durch autoreaktive T-Zellen vermittelt werden, die nach Durchwanderung der Blut-Hirn-Schranke zu einer Schädigung der Myelinscheide und der Myelin-bildenden Oligodendrozyten führen. Neben der Neuroinflammation beinhaltet die MS auch eine neurodegenerative Komponente, die bereits früh im Krankheitsverlauf auftritt und auch unabhängig von akuten Entzündungsreaktionen stattfinden kann.[2] Diese nicht-reversiblen axonalen und neuronalen Schädigungen haben einen entscheidenden Anteil an den bleibenden funktionellen Behinderungen, die im Krankheitsverlauf akkumulieren. Die Mehrheit der Patienten präsentiert sich zu Beginn der Erkrankung mit einem schubförmig remittierenden MS-Verlauf (*relapsing-remitting* MS, RRMS). Im späteren Krankheitsverlauf geht ein Teil der Patienten in den sogenannten sekundär chronisch-progredienten Verlauf (*secondary progressive* MS, SPMS) über, bei dem es zu einer weiteren progredienten Verschlechterung der klinischen Symptomatik mit oder ohne aufgesetzte Schübe kommt. Bei einem geringen Teil der Patienten besteht ein progredienter Verlauf von Beginn an (*primary progressive* MS, PPMS).[3] Die aktuellen Diagnosekriterien der MS erfordern den Nachweis von Krankheitsaktivität beziehungsweise fokaler Demyelinisierung in verschiedenen Bereichen des ZNS (örtliche Dissemination) und zu mehreren Zeitpunkten (zeitliche Dissemination).[4] Patienten, die einen ersten MS-typischen Schub bzw. Läsion haben, aber noch nicht alle MS-Diagnosekriterien vollständig erfüllen, werden als klinisch isoliertes Syndrom (*clinically isolated syndrom*, CIS) diagnostiziert.[5]

Ein häufig auftretendes Symptom der MS ist die Sehnervenentzündung (*optic neuritis*, ON) und frühe Studien mit optischer Kohärenztomographie (*optical coherence tomography*, OCT) konnten eine Verdünnung der retinalen Nervenfaserschicht (*retinal nerve fibre layer*, RNFL) in Augen mit vorangegangener ON nachweisen.[6] Interessanterweise zeigten auch viele OCT-Studien eine Reduktion der RNFL bei MS-Augen ohne vorherige ON; die OCT wurde daher als Marker für die Neurodegeneration postuliert. Allerdings basieren die meisten OCT-Studien entweder auf der älteren *time-domain* OCT (TD-OCT) Technologie oder die Studien verfügten nur über eine geringe Fallzahl. Auch der Einfluss der verschiedenen MS-Subtypen wurde in nur wenigen Studien untersucht, die zum Teil widersprüchliche Ergebnisse lieferten. In den meisten, aber nicht in allen

Studien waren SPMS und PPMS stärker von retinaler Atrophie betroffen als RRMS Patienten.[7, 8, 9, 10] Außerdem war in vielen Studien nicht ersichtlich, welchen Einfluss Krankheitsdauer und vorherige ON auf die Veränderung hatte. Bei Patienten mit CIS konnten zwei frühe Studien mit TD-OCT keine Veränderungen der Retina zeigen.[11, 12] Mit Einführung der *spectral-domain* OCT (SD-OCT), die gegenüber der älteren TD-OCT eine verbesserte Auflösung und eine deutlich erhöhte Aufnahmegeschwindigkeit hat, wurde es möglich, hochauflösende Volumenaufnahmen zu generieren, die eine Segmentation der inneren retinalen Schichten erlaubt.[13] Die OCT Ergebnisse wurden durch eine histologische post-mortem Studie bestärkt, die in 79% der untersuchten MS Augen einen Verlust an retinalen Axonen in der RNFL nachweisen konnte.[14] In dieser Studie war außerdem ein Untergang an Neuronen in der Ganglienzellschicht (*ganglion cell layer*, GCL) und in geringerem Ausmaß auch eine Atrophie der Horizontal- und Bipolarzellen in der inneren Körnerschicht (*inner nuclear layer*, INL) festzustellen.
In aktuellen OCT Studien gewinnt die INL stark an Interesse, nachdem in einer Studie bei ca. 5% der MS Patienten makuläre Mikrozysten nachgewiesen worden waren, die zu einer Verdickung der INL führten.[15] Makuläre Mikrozysten und die Dicke der INL konnten zudem mit der Krankheitsprogression sowie dem Auftreten von neuen Schüben und Läsionen assoziiert werden und wurden daher als Marker für die neuroinflammatorische Aktivität postuliert.[16] Die Ätiologie für das Auftreten von Mikrozysten ist allerdings weitgehend unbekannt. Zunächst wurde vermutet, dass eine lokale, eventuell entzündliche Reaktion auf die Degeneration der retinalen Ganglienzellen stattfindet. In einer lebhaften wissenschaftlichen Debatte, die noch nicht abgeschlossen ist, wurde als alternative Hypothese eine Zugwirkung der Glaskörpermembran an der Retina (Glaskörpertraktion, *vitreous traction*) vorgeschlagen.[17, 18]

Fragestellungen

Ziel der vorliegenden Arbeit ist die bessere Charakterisierung retinaler Veränderungen bei MS Patienten mittels neuester SD-OCT Technik und retinaler Schichtsegmentierung. Im Einzelnen untersuchen die hier vorgestellten Studien die folgenden Fragestellungen:

- Lassen sich mit SD-OCT unterschiedliche Muster in den retinalen Veränderungen bei verschiedenen MS-Subtypen feststellen? [19]
- Sind mit retinaler Schichtsegmentierung bereits frühzeitig neurodegenerative Prozesse bei CIS Patienten nachweisbar? [20]
- Inwieweit hängt das Auftreten von makulären Mikrozysten und eine Verdickung der INL mit einer vorangegangenen ON zusammen? [21]
- Kann das Auftreten von Mikrozysten durch Glaskörpertraktion erklärt werden? [22]

Methodik

Patienten und Kontrollen

Alle Patienten, die in den hier vorgestellten Studien teilnahmen, wurden in prospektiven Kohortenstudien eingeschlossen und an einem von drei deutschen MS Forschungszentren rekrutiert. Die Daten aus Studie [19] (nachfolgend Multicenter-Studie genannt) entstammen einer Zusammenarbeit des NeuroCure Clinical Research Center der Charité Universitätsmedizin (Berlin), der Neurologischen Klinik der Heinrich-Heine Universität (Düsseldorf) und dem Institut für Neuroimmunologie und klinische MS-Forschung (Hamburg). Die Teilnehmer der Studie [20] (nachfolgend CIS-Studie genannt) wurden in einer Kooperation der Standorte Berlin und Düsseldorf rekrutiert, während für Studie [21] (nachfolgend Retrospektive-Studie genannt) die komplette Berliner OCT Datenbank retrospektiv analysiert wurde. Davon wurden neun Patienten mit sichtbaren Mikrozysten in der Basisvisite in Studie [22] (nachfolgend Mikrozysten-Studie genannt) longitudinal nachverfolgt.
Je nach Studie wurden Patienten mit unterschiedlichen Diagnosen eingeschlossen. Für die Multicenter-Studie war eine eindeutige MS Diagnose nach den revidierten McDonald-Kriterien von 2005 und eine MS-Subtypen Klassifikation in RRMS, SPMS und PPMS nach Lublin-Kriterien Voraussetzung.[3, 23] Die untersuchten Patienten in der CIS-Studie wurden nach den revidierten McDonald-Kriterien von 2010 rekrutiert und in die Retrospektive-Studie wurden Patienten mit MS, CIS, *Neuromyelitis optica spectrum disorders* (NMOSD) und *Chronic relapsing inflammatory optic neuropathy* (CRION) eingeschlossen.[24, 25] Das Alter der Studienteilnehmer musste zwischen 18 und 60 Jahren (Multicenter-Studie) bzw. 18 und 65 Jahren (CIS-Studie) liegen. Für die Retrospektive-Studie war nur ein Mindestalter von 18 Jahren Voraussetzung. Ausschlusskriterien waren andere neurologische, ophthalmologische oder systemische Erkrankungen mit möglichem Einfluss auf die Retina (z.B. Glaukom, Diabetes mellitus, Altersabhängige Makuladegeneration, Epiretinale Membranen). Refraktionsfehler von 5 dpt oder mehr führten zum Ausschluss des betreffenden Auges.
Gesunde Kontrollprobanden rekrutierten sich aus Verwandten von Patienten, klinischen Mitarbeitern oder sonstigen Freiwilligen. In der CIS-Studie wurden Kontrollen so gewählt, dass sie nach Geschlecht und Alter (Abstand maximal ± 3 Jahre) zu den CIS Patienten passten. Nach gleichen Kriterien wurde für die Retrospektive-Studie eine Subgruppe aus allen Kontrollen gebildet, die optimal zu den Patienten mit unilateraler ON korrespondierten.

Ethik

Die Studienprotokolle wurden von den lokalen Ethikkommissionen genehmigt und in Übereinstimmung mit der Deklaration von Helsinki (1964), gemäß den Richtlinien zur guten klinischen Praxis (ICH-GCP) und dem anzuwendendem deutschen Recht durchgeführt. Alle Studienteilnehmer gaben zu Beginn der Studie ihre schriftliche Einwilligung.

Optische Kohärenztomographie

Die OCT ist ein nicht-invasives, bildgebendes Verfahren zur Untersuchung von optisch reflektierenden oder rückstreuenden Material, wie es auch biologisches Gewebe ist, und basiert auf dem Prinzip der Interferometrie.[26] Dabei wird kurzkohärentes Licht an einem Strahlteiler in einen Signal- und einen Referenzarm gespalten. Das Licht des Signalarms wird axial in das Untersuchungsobjekt geleitet und an rückstreuenden Mikrostrukturen zurückgeworfen. Der an einem Referenzspiegel reflektierte Referenzarm bildet mit dem Signalarm am Detektor ein Interferogramm. Die Tiefendarstellung des Untersuchungsobjektes erfolgt dabei entweder durch periodische Bewegung des Referenzspiegels (TD-OCT) oder der Aufspaltung des Lichtes in den Spektralbereich (SD-OCT). Die modernere SD-OCT Technik hat dabei gegenüber der TD-OCT den Vorteil einer höheren axialen und temporalen Auflösung, sowie eines besseren Signal-zu-Rauschverhältnisses.
In den letzten Jahren hat sich die OCT als wichtige Methode zur Untersuchung der Retina bewährt. Aufgrund der Eindringtiefe von 1 bis 3 mm und der hohen axialen Auflösung ($3 - 6$ μm) kann die Retina sehr genau vermessen werden. Die verschiedenen anatomischen Strukturen der Retina (Membranen bzw. Zellschichten) besitzen unterschiedliche Rückstreukoeffizienten was zu einer guten Abgrenzbarkeit dieser Strukturen im OCT führt. In Analogie zur Ultraschalldiagnostik wird eine Tiefenmessung an einer definierten Position auf der Retina als A-Scan bezeichnet und ein aus mehreren A-Scans zusammengesetztes 2-dimensionales Bild wird B-Scan genannt. Aufgrund der hohen Aufnahmegeschwindigkeit beim SD-OCT besteht die Möglichkeit ein Volumen aus mehreren B-Scans zu rekonstruieren. Auch wenn die OCT-Bilder nicht auf zellulärer Eben vollständig mit der Histologie übereinstimmen, bieten sie eine schnelle, günstige und relativ genaue *in vivo* Repräsentation der Retina.
Alle OCT Untersuchungen in den hier vorliegenden Studien wurden mit dem Heidelberg Spectralis® SD-OCT (Heidelberg Engineering, Heidelberg, Deutschland) von erfahrenen Untersuchern durchgeführt. Die Qualität der OCT Aufnahmen wurden gemäß aktuell empfohlenen Richtlinien (OSCAR-IB) kontrolliert.[27] In den Studien kamen verschiedene Aufnahmeprotokolle zum Einsatz; die Einstellungen sowie die aus den Aufnahmen abgeleiteten Ausgabeparameter sind der Tabelle 1 zu entnehmen. Das totale Makulavolumen (*total macular volume*, TMV) wurde definiert als das Volumen zwischen innerer Grenzmembran (*inner limiting membrane*, ILM) und Bruch'sche Membran in einem

	Peripapillärer Ringscan	**Makulärer Volumenscan (Berlin)**	**Makulärer Volumenscan (Hamburg und Düsseldorf)**
Zentrierung	Sehnervenkopf	Makula	Makula
Scanrichtung	Ringscan	vertikal	vertikal oder horizontal
Scanbereich	Ringdurchmesser: 12° ($\approx 3,4$mm)	$30° \times 25°$ ($\approx 9,0 \times 7,5$mm)	$20° \times 20°$ ($\approx 6,0 \times 6,0$mm)
Anzahl B-Scans	1	61	25
A-Scans pro B-Scan	1024	786	786
***Automatic realtime* (ART) Einstellung**	Max. 100	13	9
Ausgabe-parameter	Durchschnittliche RNFL Schichtdicke (pRNFL)	Totales Makulavolumen (TMV), durchschnittliche intra-retinale Schichtdicken bzw. Schichtdickenkarten	Totales Makulavolumen (TMV)

Tabelle 1: Einstellungen der verschiedenen OCT Parameter

Zylinder mit 6 mm Durchmesser um die *fovea centralis*. Für die Generierung von intra-retinalen Schichtdickenkarten wurden jeweils 13 B-Scans eines makulären Volumenscans automatisch vorsegmentiert und manuell nachkorrigiert. Dabei wurde immer der zentrale B-Scan durch die *fovea centralis* und jeweils sechs B-Scans zu jeder Seite (jeweils jeder 4. B-Scans) verwendet. Die folgenden intra-retinalen Schichten wurden aus den Segmentierungen bestimmt: makuläre RNFL (mRNFL), Ganglionzellschicht (GCL), innere plexiforme Schicht (*inner plexiform layer*, IPL) und die innere Körnerschicht (INL). Aufgrund der zum Teil schwierigen Abgrenzbarkeit der GCL und IPL wurde außerdem die kombinierte Schicht aus GCL und IPL analysiert (GCIPL).
Zur Detektion von Augen mit makulären Mikrozysten wurden alle OCT Aufnahmen von einem geschulten Anwender auf das Auftreten von klar umschlossenen zystenartigen Bereichen in mindestens zwei aufeinanderfolgenden B-Scans untersucht. Ein zweiter Anwender untersuchte Veränderungen auf den Fundusbildern, die parallel zu den OCT Aufnahmen von einem Scanning Laser Ophthalmoskop erstellt wurden. Die Flächen, die diese Veränderungen auf den Fundusbildern einnahmen, wurden von einem weiteren, unabhängigen Untersucher mit dem Programm ImageJ vermessen.

Statistische Auswertung

Gruppenunterschiede in demographischen Parametern wurden mit dem Mann-Whitney-U bzw. Chi-Quadrat Test untersucht. Für den Vergleich von OCT Ergebnissen zwischen den Studiengruppen wurden verallgemeinerte Schätzungsgleichungsmodelle (*generalized estimation equation models*, GEE) verwendet, welche die Korrelationseffekte zwischen

den beiden Augen eines Patienten berücksichtigen. Je nach Fragestellung wurden diese Modelle zusätzlich für Alter, Geschlecht und Krankheitsdauer korrigiert. Des Weiteren kamen folgende statistische Methoden zum Einsatz: 2-seitiger gepaarter Wilcoxon Rangsummentest, exakter Fischer-Test, Berechnung der *odds ratio*, lineare Regressionsmodelle und Berechnung der Sensitivität und Spezifität. Die statistischen Auswertungen wurden mit den Programmen R (Versionen zwischen 2.12.2 und 2.15.2) oder SPSS (Version 19 oder 20) durchgeführt. Statistische Signifikanz wurde bei einem P-Wert $< 0,05$ festgelegt.

Ergebnisse

Kohortenübersicht

Die Ergebnisse der hier vorgestellten Studien basieren zum Teil auf unterschiedlichen Kohorten. In Tabelle 2 findet sich eine Übersicht über die kompletten Patienten- und Kontrollpopulationen der verschiedenen Studien mit den demographischen Daten zu Alter und Geschlecht der Teilnehmer. Eine detaillierte Aufschlüsselung nach Subgruppen findet sich in den folgenden Ergebnissen zu den einzelnen Studien und in den jeweiligen Originalarbeiten.

		Name der Studie			
		Multicenter	**CIS**	**Retrospektive**	**Mikrozysten**
Anzahl Teilnehmer	Patienten	414	45	283	9
	Kontrollen	94	45	121	9
Anzahl Augen	Patienten	754	89	566	18
	Kontrollen	183	90	236	18
Geschlecht männlich / weiblich	Patienten	140 / 274	14 / 31	100 / 183	3 / 6
	Kontrollen	31/ 63	14 / 31	38/ 83	3 / 6
Alter in Jahren Mittelwert	Patienten	41,3 ± 9,6	31,9 ± 8,0	41,4 ± 11,1	38,7 ± 10,5
	Kontrollen	34,5 ± 10,3	31,7 ± 7,8	36,1 ± 12,4	36,9 ± 8,5

Tabelle 2: Kohortenübersicht mit demographischen Daten

Multicenter-Studie: Retinaler Schaden in verschiedenen MS-Subtypen

In dieser Studie wurde die Patientenpopulation in die MS-Subtypen RRMS (308 Patienten, 561 analysierte Augen), SPMS (65 Patienten, 116 analysierte Augen) und PPMS (41 Patienten, 77 analysierte Augen) stratifiziert und mit Augen von gesunden Kontrollen verglichen. Die Geschlechterverteilung der gesunden Kontrollen unterschied sich nicht von den RRMS und SPMS Kohorten, dem gegenüber hatte die PPMS Kohorte einen signifikant höheren Anteil an Männern. Die Kontrollen waren signifikant jünger als alle MS-Subgruppen und das Durchschnittsalter der RRMS Patienten war geringer als in den progredienten MS-Subgruppen. Aufgrund dieser Unterschiede in den Kohorten wurden Alter und Geschlecht als Kovariaten in die statistischen Modelle aufgenommen. Innerhalb der MS Kohorte war die Krankheitsdauer zwischen den Subgruppen unterschiedlich verteilt, mit einer signifikant längeren Zeit seit Diagnosestellung in der SPMS Kohorte. Gemessen am EDSS waren Patienten mit der Diagnose SPMS am schwersten betroffen (Median EDSS = 5,5), wohingegen PPMS (Median EDSS = 4,0) und RRMS (Median EDSS = 2,0) weniger stark betroffen waren. Bei Vergleichen innerhalb der MS-Subgruppen wurden die statistischen Modelle auf Krankheitsdauer und in einer weiteren Analyse zusätzlich auf EDSS korrigiert.
Die durchschnittliche pRNFL Schichtdicke bei gesunden Kontrollen lag bei 100,6 μm $\pm$ 9,4 μm und das TMV bei 8,75 mm^3 $\pm$ 0,34 mm^3. Unabhängig von der MS-Subgruppe waren pRNFL und TMV sowohl bei ON betroffenen Augen wie auch bei nicht ON betroffenen (NON) Augen signifikant reduziert im Vergleich zu der Kontrollpopulation (alle P-Werte $< 0,001$). Augen mit vorangegangener ON waren grundsätzlich stärker von der pRNFL und TMV Reduktion betroffen als NON Augen. Die niedrigsten Werte fanden sich bei SPMS-ON Augen (durchschnittliche pRNFL Dicke = 73,2 μm $\pm$ 11,9 μm; durchschnittliches TMV = 8,05 mm^3 $\pm$ 0,41 mm^3).
Der Vergleich zwischen den unterschiedlichen MS-Subtypen ergab eine signifikant stärkere Verdünnung der pRNFL bei SPMS-NON gegenüber RRMS-NON Augen. Das TMV war sowohl bei SPMS-NON wie auch bei PPMS-NON signifikant geringer als bei RRMS-NON. Die Krankheitsdauer hatte beim Vergleich von RRMS-NON zu den progressiven MS-Typen einen signifikanten Einfluss. Bei zusätzlicher Korrektur der Modelle mittels EDSS waren keine Unterschiede mehr in den OCT Parametern zwischen den Subgruppen festzustellen.
Für RRMS-NON Augen war eine signifikante Korrelation zwischen Krankheitsdauer und pRNFL und TMV nachzuweisen. Die aus den GEE Modellen ermittelten jährlichen Veränderungen beliefen sich auf -0,533 μm für die pRNFL und -0,016 mm^3 für das TMV.

CIS-Studie: Intra-retinale Schichtdickenanalyse bei CIS Patienten

In dieser Studie wurden insgesamt 45 Patienten (29 in Berlin und 16 in Düsseldorf) mit der Diagnose eines klinisch isolierten Syndroms eingeschlossen. In 17 Fällen hatten Patienten eine einseitige ON, 14 Patienten zeigten Symptome mit Beteiligung des Rückenmarks, 6 Patienten hatten Schübe vermutlich assoziiert mit infratentoriellen Hirnläsionen, 7 Patienten hatten Schübe mit MRT Auffälligkeiten in supratentoriellen Hirnarealen und ein Patient hatte klinische Anzeichen sowohl von supratentorieller als auch Rückenmarksbeteiligung. Durchschnittlich lagen zwischen dem Auftreten des Erstsymptoms und der OCT-Untersuchung 8,6 Monate (Standardabweichung: 12,2 Monate; Minimum: 1,4 Monate; Maximum: 59,7 Monate). Der Median des EDSS lag in der Patientenkohorte bei 1 (Minimum: 0; Maximum: 4). Die Augen der Patienten wurden in Abhängigkeit von der ON-Vorgeschichte in eine von drei Gruppen eingeteilt. Zusätzlich zu Augen mit einer dokumentierten ON Episode (abgekürzt: CIS-ON) und Augen ohne Anzeichen einer ON in der Vergangenheit (CIS-NON) wurde eine dritte Gruppe gebildet in der alle Augen zusammengefasst wurden, die zwar keine dokumentierte ON in der Anamnese aufwiesen, aber aufgrund von verlängerten P100-Latenzen von mehr als 115 ms im VEP verdächtig für eine subklinisch abgelaufene ON waren (CIS-SON). Die CIS-ON Gruppe umfasste 17 Augen, die CIS-NON Gruppe 66 Augen, und 7 Augen wurden als CIS-SON klassifiziert. Bei einem Patienten bestand der Verdacht auf beidseitige subklinische ON. Die weiteren CIS-SON Augen stellten jeweils das kontra-laterale Auge eines CIS-ON Auges dar. Ein Auge aus der CIS-ON Gruppe wurde aufgrund von Artefakten in den OCT Aufnahmen von der Analyse ausgeschlossen.

Lineare Korrelationen bestanden zwischen der P100-Latenz und pRNFL ($R^2 = 0{,}243$; $P < 0,001$) und zwischen der P100-Latenz und TMV ($R^2 = 0{,}124$; $P < 0,001$) in allen untersuchen Patientenaugen. Bei der Betrachtung der linearen Korrelation für die verschiedenen Subgruppen war nur eine schwache Korrelation zwischen P100 und pRNFL in der CIS-NON Gruppe festzustellen ($R^2 = 0{,}065$; $P = 0{,}039$).

Im Vergleich zu gesunden Kontrollen war die pRNFL in der CIS-ON Gruppe ($P < 0,001$) und CIS-SON Gruppe ($P = 0{,}014$) signifikant verdünnt, nicht allerdings in CIS-NON Augen ($P = 0{,}636$). Interessanterweise fand sich bei der Untersuchung der TMV auch bei CIS-NON Augen eine signifikante Reduktion im Vergleich zur Kontrollkohorte ($P = 0{,}031$).

Um die intra-retinalen Veränderungen in der CIS Kohorte im Vergleich zu den Kontrollen genauer zu untersuchen, wurden für jeden Studienteilnehmer Dickenkarten von den inneren retinalen Schichten erzeugt und aus diesen die durchschnittlichen Dicken der oben beschriebenen Schichten errechnet. Zusätzlich wurden aus allen Dickenkarten einer Gruppe Mittelwertkarten gebildet, so dass die Differenz zwischen den Mittelwertkarten

von Patienten- und gepaarter Kontrollkohorte topologische Differenzkarten ergab. Diese stellten die Unterschiede zwischen den Kohorten räumlich auf der Retina dar. Die durchschnittliche GCIPL Dicke war signifikant reduziert in allen Patientengruppen: CIS-ON: $P < 0,001$; CIS-SON: $P = 0.048$ und CIS-NON: $P = 0.027$. In den topologischen Differenzkarten zeigte sich eine ähnliche räumliche Verteilung der GCIPL Abnahme. Die mRNFL war nur bei CIS-ON signifikant reduziert, nicht aber in den anderen untersuchten Gruppen. Dieses Ergebnis könnte auf den verwendeten Makula-Scan zurückzuführen sein, da dieser mit der Makula einen Bereich der Retina untersucht, der physiologische nur eine geringe RNFL Dicke aufweist. Im Gegensatz zu RNFL und GCIPL zeigte die INL bei keiner Gruppe Veränderungen. Analog zu der Korrelationsanalyse von pRNFL und TMV mit VEP korrelierten auch die intra-retinalen Schichtdicken mRNFL und GCIPL aller Augen linear mit der P100 Latenz.

Retrospektive-Studie: Mikrozysten und INL Verdickung nach ON

Durch Analyse der OCT B-Scans konnten bei 15 Patienten (22 Augen) makuläre Mikrozysten nachgewiesen werden; 7 dieser Patienten hatten eine MS Diagnose, 5 waren als CRION klassifiziert und 3 Patienten als NMOSD. Bei Kontrollen und CIS Patienten waren keine Mikrozysten feststellbar. Alle Mikrozysten waren ausschließlich in der INL lokalisiert, lagen zwischen 925 μm und 2.200 μm distal von der *Fovea centralis* entfernt und hatten einen durchschnittlichen Durchmesser von 45 μm $\pm$ 14 μm (maximaler Durchmesser: 81 μm).
Aus den medizinischen Aufzeichnungen ging hervor, dass 21 der 22 betroffenen Augen (95,5%) mindestens ein akutes ON Ereignis in der Vergangenheit hatten. Für das einzige Auge mit Mikrozysten ohne eine ON in der Anamnese gab es ON typische Symptome (Sehstörungen und verlängerte VEP Latenz) in der klinischen Vorgeschichte, die auf eine mild, nicht eindeutig diagnostizierte ON hindeuten. Augen mit Mikrozysten zeigten gegenüber den nicht betroffenen Patientenaugen eine reduzierte Sehleistung ($P = 0,01$), eine reduzierte pRNFL Dicke ($P < 0,001$) und ein verringertes TMV ($P < 0,001$). Die Häufigkeit, mit der Mikrozysten nach einer ON auftraten, unterschied sich stark zwischen den verschiedenen Diagnosen: bei 6,3% der MS Augen kam es nach einer ON zum Auftreten von Mikrozysten, während es bei CRION Augen 53,8% und bei NMOSD Augen 21,0% waren. Bei MS Patienten mit einer vorherigen ON war das Auftreten von Mikrozysten mit einem höheren EDSS assoziiert. Patienten mit Mikrozysten wurden zu einem größeren Anteil als SPMS eingestuft als Patienten mit ON aber ohne Mikrozysten. Aufgrund der Tatsache, dass in dieser Studie das Vorhandensein von Mikrozysten stark mit einer vorangegangenen ON einherging, sollte zusätzlich untersucht werden, ob es nach einer ON bereits zu einer Verdickung der inneren Körnerschicht kommt, auch ohne dass

Mikrozysten im OCT sichtbar waren. Dazu wurden beide Augen aller Patienten mit einer einseitigen ON und ohne Anzeichen von Mikrozysten schichtweise segmentiert (n = 75) und mittels gepaarten Wilcoxon Rangsummentest dahingehend analysiert, ob zwischen ON betroffenen Augen und den jeweiligen nicht-betroffenen kontra-lateralen Augen ein systematischer Unterschied bestand. Die beiden inneren Schichten (mRNFL und GCIP) bei den von ON betroffenen Augen waren signifikant dünner (beide $P < 0,001$) als die nicht betroffenen Augen. Bezüglich der INL war hingegen eine signifikante Verdickung der ON Augen gegenüber den kontra-lateralen Augen festzustellen ($P < 0,001$). Die Differenzen der retinalen Schichtdicken zwischen beiden Augen eines Patienten wurden gegeneinander korreliert und sowohl mRNFL ($R^2 = 0{,}086$; $P < 0,001$) wie auch GCIPL ($R^2 = 0{,}043$; $P = 0{,}011$) waren invers zu der INL Differenz korreliert.
Die Augen mit Mikrozysten zeigten bei der intra-retinalen Segmentierung eine signifikant dünnere mRNFL und GCIPL (beide $P < 0,001$) im Vergleich zu den segmentierten ON Augen und auch zu einer nach Alter und Geschlecht gepaarten Kontrollkohorte. Demgegenüber war die INL bei Augen mit Mikrozysten erwartungsgemäß deutlich dicker (durchschnittliche Dicke = 39,0 μm $\pm$ 3,2 μm) als bei gesunden Kontrollen (33,0 μm $\pm$ 2,5 μm) und auch gegenüber ON Augen (34,5 μm $\pm$ 2,6 μm).
Mikrozysten konnten auf den Fundusbildern als dunklere, meist halbmondförmige Bereiche ausgemacht werden. Bei der Analyse der Fundusbilder wurden alle Augen mit Mikrozysten korrekt erkannt (Sensitivität = 100%), bei einer Spezifität von 95,2%. Das Zeitintervall seit der letzten ON und die Sehfähigkeit waren invers mit der Fläche korreliert, die auf dem Fundusbild als Mikrozysten markiert wurden.

Mikrozysten-Studie: Dynamische Mikrozysten unabhängig von Traktion

Zum aktuellen Zeitpunkt existieren unterschiedliche Erklärungsansätze für die Pathogenese von Mikrozysten im Bereich der Makula. Im Rahmen dieser Studie entwickelten wir ein Modell welches den Einfluss einer Zugwirkung des Glaskörpers auf die Retina bei Mikrozysten-Patienten longitudinal untersucht. Da eventuell auch nur sehr geringe Veränderungen für die Bildung von Mikrozysten verantwortlich sein könnten, wurde dieses Modell entwickelt, um den Zusammenhang von Glaskörper, Retina und den daraus resultierenden OCT Aufnahmen genauer untersuchen zu können. Im Fall von Glaskörpertraktion geht es von einer Vergrößerung der Distanz zwischen der hinteren Glaskörpermembran und der inneren Grenzmembran der Retina aus. Demgegenüber würde eine Schwellung der Retina durch einen primären retinalen Prozess zu einer Verringerung der Distanz der beiden Grenzflächen führen und dementsprechend auch zu einer Verschiebung der Kontaktstellen beider Membranen nach außen.
Eine Patientin (51 Jahre, Diagnose CRION, letzte ON 10 Monate vor Basisvisite) zeig-

te eine äußerst dynamische Veränderung der Mikrozysten über den Untersuchungszeitraum: nach 12 Monaten kam es zunächst zu einem leichten Anstieg der Anzahl und Größe der Mikrozysten, nach 19 Monaten wurde das Ausmaß der Mikrozysten drastisch größer und nach 25 Monaten war wieder eine deutliche Reduzierung festzustellen. Diese Veränderungen betrafen sowohl die OCT Aufnahmen, wie auch die Fundusbilder. Angewandt auf die Daten dieser Patientin mit dynamischem Mikrozystenverlauf, deuten die Ergebnisse des oben beschriebenen Modells auf das Vorhandensein eines primären retinalen Prozesses hin und nicht auf Glaskörpertraktion: Zwischen der zweiten und dritten Visite und dem massiven Anschwellen der Mikrozysten und INL, kam es zu keiner größeren Veränderung der Distanz zwischen den Grenzmembranen, dafür aber zu einer Verschiebung der Kontaktfläche der beiden Grenzmembranen nach außen. Dies deutet darauf hin, dass die Glaskörpermembran nicht unter Zugspannung stand, sondern auf der Retina auflag und sich passiv mit der Schwellung der Makula veränderte.
Insgesamt waren bei der longitudinalen Analyse der OCT Aufnahmen von 9 Patienten mit Mikrozysten keine Anzeichen von Glaskörpertraktion als Ursache für das Auftreten der Mikrozysten erkennbar. In einem Fall war sogar eine komplette Ablösung des Glaskörpers von der Netzhaut festzustellen.

Diskussion

In der vorliegenden Arbeit wurden verschiedene Aspekte von retinalen Veränderungen bei MS Patienten untersucht und in vier Publikationen veröffentlicht. Zunächst konnten in einer der bisher größten Querschnittsstudien mit neuester SD-OCT Technik frühere Ergebnisse zur retinalen Atrophie bei MS bestätigt werden.[19] Im Vergleich zu gesunden Kontrollen zeigten MS Patienten sowohl mit als auch ohne vorherige ON eine Reduktion der RNFL und TMV; grundsätzlich sind dabei ON Augen stärker betroffen als Augen ohne vorherige ON. Darüber hinaus erlaubte die große Kohorte auch eine Analyse der verschiedenen MS-Subtypen unter Einbeziehung möglicher Störvariablen wie Alter, Geschlecht und Krankheitsdauer in die statistischen Modelle. Dass Patienten mit progressiver Verlaufsform in dieser Studie einen stärkeren retinalen Schaden aufwiesen als RRMS Patienten, steht zum Teil im Widerspruch zur vorhandenen Literatur.[7, 8, 10] Mögliche Ursachen für die gefundenen Diskrepanzen zwischen den Studien sind die geringen Fallzahlen in vorangegangenen Studien, die Verwendung von unterschiedlichen statistischen Modellen und der Einsatz verschiedener OCT Generationen. Im Gegensatz zu der Subgruppen-Analyse werden die Ergebnisse zur Abschätzung der jährlichen Abnahme der Dicke von der aktuellen Literatur bestätigt. In Übereinstimmung mit den hier präsentierten Daten ermittelte eine kürzlich veröffentlichte longitudinale Studie bei MS Patienten mit SD-OCT einen Wert von ca. 0,5 μm/Jahr für die jährliche Abnahme der RNFL-Dicke.[28]

Allerdings ist der zeitliche Verlauf der retinalen Atrophie weiterhin noch nicht vollständig aufgeklärt. Insbesondere in der frühen Phase der Erkrankung konnten zwei frühere Studien mit TD-OCT keine Veränderungen der retinalen Architektur nachweisen.[11, 12] Im Gegensatz dazu konnten wir in einer Studie mit Hilfe von retinaler Schichtsegmentierung an hochauflösenden SD-OCT Aufnahmen bei Augen von CIS Patienten ohne Anzeichen einer vorherigen ON erstmalig eine Verdünnung der GCIPL nachweisen.[20] Dass es bereits früh im Krankheitsverlauf der MS zu neuronalen Schäden kommt, wird durch Studien mit Magnetresonanztomographie unterstützt.[2, 29] Heute wird allgemein angenommen, dass die nicht-reversiblen neuronalen Schädigungen zum großen Teil für die bleibenden Behinderungen und Beeinträchtigungen in der MS verantwortlich sind. Aus der Erkenntnis, dass Neurodegeneration bereits sehr früh im Krankheitsverlauf auftreten kann, ergeben sich wichtige Fragen zu Diagnosestellung und Therapiebeginn. Demnach ist es von enormer Bedeutung, frühzeitig Patienten zu erkennen, die mit hoher Wahrscheinlichkeit eine MS entwickeln und eine starke neurodegenerative Aktivität aufweisen, um möglichst schnell therapeutische Maßnahmen einleiten zu können. In Zukunft wird die OCT hier voraussichtlich einen wichtigen Beitrag leisten können.
Mit der Entdeckung von makulären Mikrozysten in der Retina von MS Patienten und der Assoziation von INL Dicke mit einer erhöhten Schubrate, neuen Läsionen in der MRT Bildgebung und verstärkter Krankheitsprogression bestimmt durch den EDSS, wurde die INL als interessanter möglicher Prädiktor für die immunologische Aktivität bei MS diskutiert.[15, 16] In einer großen retrospektiven Studie konnten wir zeigen, dass Mikrozysten nicht spezifisch für die MS sind, sondern auch bei anderen neuroimmunologischen Erkrankungen wie NMO und CRION zu finden sind.[21] Die Prävalenz für das Auftreten von Mikrozysten lag bei diesen Erkrankungen sogar deutlich höher als bei der MS und vergleichbare Zahlen wurden bei der NMO auch in anderen Studien berichtet.[30, 31] Aufgrund der geringen Inzidenzen von NMO und besonders CRION basieren die bisherigen Studien auf relativ niedrigen Fallzahlen und größere Kohorten sind hier wünschenswert, um die Ergebnisse zu bestätigen. In einer Analyse durch geblindete Untersucher konnte gezeigt werden, dass Fundusbilder, wie sie automatisch mit dem Scanning Laser Opthalmoscope bei einer OCT Untersuchung gemacht werden, sehr gut zur Identifikation von Mikrozysten geeignet sind (Sensitivität = 100% bei einer Spezifität von 95,2%). Dieses Verfahren stellt eine deutliche Vereinfachung für das Screening von Mikrozysten in der klinischen Routine dar. Während in vorherigen Studien bisher nur eine höhere ON Frequenz bei MS Patienten mit Mikrozysten gefunden werden konnte, waren in unserer Arbeit nahezu alle Patienten mit Mikrozysten von einer vorherigen ON betroffen. Der einzige Patient, der keine ON in der Anamnese aufwies, war zudem hochgradig verdächtig für eine mild abgelaufene ON (Sehstörungen in der Vergangenheit und eine verlängerte VEP Latenz). Diese Ergebnisse deuten darauf hin, dass eine pathophysiologische Korrelation zwischen dem Auftreten von Mikrozysten und

der Schädigung des Sehnervs durch eine ON besteht. Die Untersuchung von Patienten, die unilateral von einer ON betroffen waren, zeigte zudem eine INL Verdickung in ON Augen im Vergleich zu den nicht betroffenen kontra-lateralen Augen. Die INL Verdickung nach einer ON auch ohne sichtbare Mikrozysten ist somit ein möglicher Erklärungsansatz für die Assoziation von INL Schichtdicke und immunologischer Aktivität: Patienten mit höherer Schubrate und mehr Läsionen in der MRT Bildgebung haben hypothetisch auch mehr und / oder stärkere ONs. Daraufhin deutet auch die Korrelation von INL Zunahme und RNFL bzw. GCIPL Abnahme nach einer ON im Inter-Augen-Vergleich hin. Somit ist auch die INL potentiell als Marker für die frühe Risikostratifizierung bei MS Patienten anwendbar.

Aktuelle Arbeiten konnten bei verschiedenen anderen Erkrankungen mit unterschiedlicher Ätiologie Mikrozysten nachweisen, die denen bei MS ähneln; allerdings handelte es sich dabei meist um Einzelfallberichte.[17, 32, 33, 34] Als Ursache wurden entzündliche Prozesse nach Zusammenbruch der Blut-Retina-Schranke,[15] Glaskörpertraktion [17], Verlust von Müllerzellen [32] oder retrograde trans-synaptische Degeneration [34] vermutet. Die verschiedenen Hypothesen waren Anlass für eine lebhafte, wissenschaftliche Debatte und neue Methoden für die Überprüfung der Hypothesen haben eine hohe Relevanz.[35, 36] In einer retrospektiven Analyse aller Patienten, die longitudinal mit OCT nachverfolgt wurden, konnten wir zeigen, dass Mikrozysten sehr viel dynamischer sind als bisher angenommen.[22] Die Untersuchung der INL Schichtdicke und des Abstandes zwischen Retina und hinterer Glaskörpermembran deutet nicht auf Glaskörpertraktion als Ursache für die dynamischen Mikrozystenveränderungen hin. Im Gegenteil, die Daten sind eher so zu interpretieren, dass es zu einer Schwellung der Makula kommt und daraus resultierend zu einem Zusammendrücken der beiden Grenzmembranen. Glaskörpertraktion kann zwar nicht grundsätzlich als Ursache für Mikrozysten ausgeschlossen werden, aber es scheint nicht eine Grundvoraussetzung für deren Bildung zu sein. Für eine Validierung dieser Ergebnisse ist es nötig das hier entwickelte Modell in einer größeren Studie bei Patienten mit und ohne Mikrozysten anzuwenden.

Zusammenfassend bietet die OCT einen vielversprechenden Marker für neurodegenerative und neuroinflammatorische Prozesse in der MS. Mit dieser Arbeit konnte ein relevanter Beitrag zum besseren Verständnis der retinalen Veränderungen bei MS geleistet werden. Neue hochauflösende SD-OCT Technik und retinale Schichtsegmentierung führen zu einer weiteren Steigerung der Sensitivität der Methode. Klinische Studien mit OCT als primärer und sekundärer Endpunkt-Parameter laufen bereits oder stehen unmittelbar bevor.[37, 38] Die OCT wird darüber hinaus auch weiterhin für die MS Forschung interessant bleiben und voraussichtlich neue Einblicke in den Pathomechanismus der MS liefern.

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Eidesstattliche Versicherung

„Ich, Timm Oberwahrenbrock, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Charakterisierung retinaler Veränderungen bei Patienten mit Multipler Sklerose mittels Optischer Kohärenztomographie“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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30. Oktober 2014

Anteilserklärung an den erfolgten Publikationen

Timm Oberwahrenbrock hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: **Oberwahrenbrock T***, Schippling S*, Ringelstein M*, Kaufhold F, Zimmermann H, Keser N, Young KL, Harmel J, Hartung H-P, Martin R, Paul F, Aktas O, Brandt AU. Retinal Damage in Multiple Sclerosis Disease Subtypes Measured by High-Resolution Optical Coherence Tomography. Multiple Sclerosis International. 2012.
Beitrag im Einzelnen: Studienkoordination, Ausführung eines Teils der Experimente, Datenauswertung, Verfassung des Manuskriptes.

Publikation 2: **Oberwahrenbrock T***, Ringelstein M*, Jentschke S, Deuschle K, Klumbies K, Bellmann-Strobl J, Harmel J, Ruprecht K, Schippling S, Hartung H-P, Aktas O, Brandt AU, Paul F. Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome. Multiple Sclerosis Journal. 2013.
Beitrag im Einzelnen: Entwicklung der Analyse von retinalen Schichtdickenkarten und Differenzkarten, statistische Auswertung, Ausarbeitung des Manuskripts.

Publikation 3: Kaufhold F, Zimmermann H, Schneider E, Ruprecht K, Paul F, **Oberwahrenbrock T***, Brandt AU*. Optic Neuritis Is Associated with Inner Nuclear Layer Thickening and Microcystic Macular Edema Independently of Multiple Sclerosis. PLoS ONE. 2013.
Beitrag im Einzelnen: Mitwirken bei Konzeption und Design der Studie, teilweise Ausführung von Experimenten, Datenanalyse, Mitarbeit am Manuskript.

Publikation 4: Brandt AU, **Oberwahrenbrock T**, Kadas EM, Lagrèze WA, Paul F. Dynamic formation of macular microcysts independent of vitreous traction changes. Neurology. 2014.
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Oberwahrenbrock T*, Ringelstein M*, Jentschke S, Deuschle K, Klumbies K, Bellmann-Strobl J, Harmel J, Ruprecht K, Schippling S, Hartung H-P, Aktas O, Brandt AU, Paul F. Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome. Mult Scler. 2013. doi:10.1177/1352458513489757.
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Clinical Study

Retinal Damage in Multiple Sclerosis Disease Subtypes Measured by High-Resolution Optical Coherence Tomography

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Background. Optical coherence tomography (OCT) has facilitated characterisation of retinal alterations in MS patients. Only scarce and in part conflicting data exists on different MS subtypes. *Objective.* To analyse patterns of retinal changes in different subtypes of MS with latest spectral-domain technology. *Methods.* In a three-centre cross-sectional study 414 MS patients and 94 healthy controls underwent spectral-domain OCT examination. *Results.* Eyes of MS patients without a previous optic neuritis showed a significant reduction of both retinal nerve fibre layer (RNFL) thickness and total macular volume (TMV) compared to healthy controls independent of the MS subtype ($P < 0.001$ for all subtypes). RNFL thickness was lower in secondary progressive MS (SPMS) eyes compared to relapsing-remitting MS (RRMS) eyes ($P = 0.007$), and TMV was reduced in SPMS and primary progressive MS (PPMS) eyes compared to RRMS eyes (SPMS: $P = 0.039$, PPMS: $P = 0.005$). Independent of the subtype a more pronounced RNFL thinning and TMV reduction were found in eyes with a previous optic neuritis compared to unaffected eyes. *Conclusion.* Analysis of this large-scale cross-sectional dataset of MS patients studied with spectral-domain OCT confirmed and allows to generalize previous findings. Furthermore it carves out distinct patterns in different MS subtypes.

1. Introduction

Multiple sclerosis is an inflammatory and neurodegenerative disorder of the central nervous system that leads to a progressive axonal loss and degeneration of neurons. Whereas a vast majority of MS patients present with a relapsing-remitting disease course (RRMS) that may subsequently transforms into secondary progressive MS (SPMS), a smaller portion of patients shows a progressive course (PPMS) from the very beginning of the disease [1].

Optical coherence tomography (OCT) is an increasingly recognized, noninvasive tool in MS imaging that allows cost-effective investigation of the retina [2] in a disease in which pathology of the anterior visual system is common. Over the recent past, OCT has emerged as a potential marker of axonal retinal degeneration in MS patients [3]. Of note, an increasing number of studies have consistently shown an association between OCT measures of retinal atrophy and markers of degeneration derived from either structural magnetic resonance imaging or MR spectroscopy (MRS) studies

[4–11] and between OCT measures and functional visual parameters as well as physical disability and cognitive performance [12–17]. Retinal nerve fibre layer (RNFL) thickness and total macular volume (TMV) are the most frequently investigated OCT parameters. They provide a unique opportunity to quantify the integrity of nonmyelinated axonal tissue (RNFL) as well as all retinal layers (TMV), including cellular segments, by a noninvasive imaging technique. RNFL and TMV reduction can be detected in eyes with (MS-ON) or without a previous history of optic neuritis (MS-NON) applying different OCT devices [15, 18–29]. Data on distinct differences in OCT findings between MS subtypes are scarce, and the results are at least in part conflicting. In general, cross-sectional studies show a more profound thinning of RNFL in progressive forms of MS compared to RRMS patients. However, it remains unresolved to date whether these differences are a genuine effect of the disease subtype *per se* or rather a function of disease duration, the number of optic neuritis (ON) episodes in the patients' history, or disease severity [30–34].

Most OCT studies previously performed in MS applied time-domain technology. Only very recently spectral-domain technology became available which enables imaging at substantially higher resolution without an increase in scanning time [16, 22, 34–38]. Here we report results from the largest multicentre cohort of MS patients and healthy controls (HC) thus far, studied in three dedicated MS centres in Germany, applying latest high-resolution spectral-domain OCT (SD-OCT) technology. In this large cohort, we reliably identified patterns of RNFL thinning and TMV reduction among different MS subtypes both with and without a history of ON when controlling for disease duration and severity, age, and gender.

2. Materials and Methods

2.1. Patients and Controls. 414 patients and 94 healthy controls were recruited in three dedicated MS units in the respective outpatient clinics at the Charité University Medicine Berlin (NeuroCure Clinical Research Center (NCRC)), at the Department of Neurology of the Heinrich-Heine University Düsseldorf (UKD) and in Hamburg (Institute for Neuroimmunology and Clinical MS Research (inims)). Data from a subgroup has previously been reported in Brandt et al. [23]. Inclusion criteria were age between 18 and 60 years and a definite diagnosis of MS according to the revised 2005 McDonald criteria [39]. MS subtype classification in RRMS, SPMS, or PPMS was based on the clinical course as assessed by the treating physician using Lublin criteria [40]. A history of ON had to be clearly determinable either by existing medical records, a VEP suggestive of optic neuritis, or by patient self-reports. Only eyes in which a history of ON could either be confirmed by clinical records or ruled out were subsequently included in the analysis. Patients with a refractive error of ≥5.0 diopters or with a history of eye disease that may impact significantly on OCT measures (e.g., glaucoma, retinal disease, retinal surgery, and diabetes) were excluded. Other exclusion criteria were acute optic neuritis or any other acute relapse or steroid treatment less than six months prior to OCT assessment as well as any other neurological disease with possible ocular manifestations. Disease duration was calculated as time since diagnosis in months.

Participants were assessed in a clinical examination under supervision of board certified neurologists within 6 months of OCT. Extended disability status scale (EDSS) was calculated according to the current guidelines [41]. Time since diagnosis was determined by reviewing patients' medical records. Healthy control participants were recruited from the medical staff, patients' relatives, and other volunteers.

A total of 937 eyes (754 MS eyes and 183 HC eyes) were finally included, 79 eyes were excluded from the analysis either due to poor scan quality or incomplete clinical data, in detail missing data on history of ON.

2.2. Ethics. The study protocol was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki (1964) in its currently applicable version, the Guidelines of the International Conference on Harmonization of Good Clinical Practice (ICH-GCP) and applicable German laws. All participants gave written informed consent.

2.3. Optical Coherence Tomography. Participants underwent SD-OCT examination using the Heidelberg Engineering Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany). For both eyes of each participant, RNFL thickness around the optic disc was acquired using the 3.4 mm circle scan with the eye tracker system (TrueTrack) activated and the maximum number of averaging frames in ART-MEAN mode were tried to achieve. For assessing the macular volume two different scan protocols were used: the system built-in macula scan (25 B-scans, scanning angle = 15°× 15°, ART = 9) was performed at the Hamburg and Düsseldorf sites, while for the macular volume determination at the NCRC a custom protocol (61 B-scans, scanning angle = 30°× 25°, ART = 13) was used. Irrespective of the macular scan type, the TMV was calculated using the device's software. All scans were performed by trained operators and were reviewed for sufficient signal strength, correct centring, and beam placement as well as segmentation. Only eyes that passed the quality review were included in the subsequent analysis.

2.4. Statistical Analysis. Group comparisons of demographic factors were analysed using Mann-Whitney U test (for age and EDSS) and Pearson's Chi-square test (for gender, alpha = 0.05). Within the MS cohort Spearman's Rho analysis was used for correlation of EDSS and disease duration.

Generalized estimation equation (GEE) models accounting for within-subject intereye correlations were applied to test for differences of RNFL thickness and TMV between the study cohorts. GEE models were corrected for age and gender for the comparison of MS patients with HC and additionally for disease duration for MS subtype analysis. Associations of OCT with EDSS and regression analysis of disease duration with RNFL thickness and TMV were investigated with GEE models in a similar fashion. In all GEE

Table 1: Demographic and clinical data of multiple sclerosis patients (MS) and healthy controls (HC). MS patients are subdivided in the subtypes relapsing-remitting MS (RRMS), secondary-progressive MS (SPMS), and primary-progressive MS (PPMS).

		HC	MS			
			RRMS	SPMS	PPMS	All MS
No. of Subjects	Total	94	308	65	41	414
	Berlin	63	95	22	8	125
	Hamburg	0	158	23	22	203
	Düsseldorf	31	55	20	11	86
No. of Eyes (eyes with ON)	Total	183	561 (156)	116 (27)	77 (0)	754 (183)
	Berlin	122	187 (77)	44 (15)	16 (0)	247 (92)
	Hamburg	0	270 (54)	35 (2)	41 (0)	346 (56)
	Düsseldorf	61	104 (25)	37 (10)	20 (0)	161 (35)
Gender	Male (%)	31 (33)	87 (28)	29 (45)	24 (59)	140 (34)
	Female (%)	63 (67)	221 (72)	36 (55)	24 (41)	274 (66)
Age (in years)	Mean (SD)	34.47 (10.25)	39.10 (9.50)	48.23 (6.11)	46.90 (7.10)	41.31 (9.59)
	Range	19–56	19–58	33–59	32–59	19–59
Disease duration (in months)	Mean (SD)	NA	91.05 (80.26)	186.15 (87.94)	100.02 (93.33)	106.87 (89.51)
	Range	NA	0–384	39–403	4–426	0–426
EDSS	Median	NA	2.0	5.5	4.0	2.5
	Range	NA	0-7	3-8	2-8	0-8

models OCT measurements were included as the dependent variable. All statistical analyses were performed with R (R Version 2.12.2) including the *geepack* package for GEE models. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Cohort Description. An overview of the demographic and basic clinical data including MS subtypes is given in Table 1. Healthy controls showed no significant gender differences to RRMS (Chi-square: $P = 0.452$) and SPMS (Chi-square: $P = 0.186$) patients, while gender composition of PPMS patients differed compared to HC (Chi-square: $P = 0.010$). Mean age of HC was significantly lower compared to all MS patients and all subtypes (Mann-Whitney U tests, $P < 0.001$ for all subtypes). RRMS patients were significantly younger than the progressive subtypes (Mann-Whitney U tests, $P < 0.001$ for SPMS and PPMS). As a consequence, age and gender were included as covariates in all GEE models. Time since diagnosis of RRMS and PPMS was significantly shorter compared to SPMS (Mann-Whitney U tests, $P < 0.001$ for both). Therefore, GEE models for MS subtype comparison were additionally adjusted for disease duration. Disease severity estimated by the EDSS was heterogeneous between MS subtypes as evaluated by Mann-Whitney U tests (RRMS versus SPMS: $P < 0.001$; RRMS versus PPMS: $P < 0.001$; SPMS versus PPMS: $P = 0.03$).

3.2. RNFL and TMV in MS-NON Eyes of Different MS Subtypes Compared to HC. For MS-NON eyes, differences in RNFL thickness and TMV compared to HC are given in Table 2. In summary, average peripapillary RNFL thickness was reduced in the pooled cohort of all MS patients' eyes and in all MS subtypes compared to HC (Figure 1(a)). Likewise, TMV was reduced for the pooled cohort of all MS patients' eyes and in all MS subtypes when compared to HC (Figure 1(b)). All alterations of RNFL and TMV in MS-NON eyes compared to control eyes showed strong statistical significance based on GEE models (Table 2). EDSS was inversely correlated with RNFL in case of all MS subtypes without a history of prior ON (RRMS-NON: $P = 0.007$; SPMS-NON: $P = 0.034$; PPMS-NON: $P = 0.006$). In contrast, the TMV was only significantly correlated with the EDSS in RRMS-NON eyes ($P = 0.003$), but not in SPMS-NON ($P = 0.321$) or PPMS-NON ($P = 0.085$).

3.3. Comparison of MS-ON Eyes with MS-NON Eyes and HC. Irrespective of the MS subtype, MS-ON eyes were significantly different from HC eyes for RNFL thickness and TMV (Figure 2) and showed a more pronounced RNFL thinning and TMV reduction when compared to MS-NON eyes (Table 3). RNFL thickness of RRMS-ON eyes and SPMS-ON eyes was significantly thinner compared to RRMS-NON or SPMS-NON eyes, respectively. The same was true for the TMV of ON-affected MS eyes, which was significantly reduced when compared to HC and to unaffected eyes of the same MS subtype (Table 3, Figure 2). The extent of RNFL thinning as well as TMV reduction was comparable between RRMS-ON and SPMS-ON eyes (RNFL GEE: $P = 0.369$; TMV GEE: $P = 0.124$). RRMS-ON eyes showed a significant inverse correlation between EDSS and RNFL ($P = 0.012$) while TMV did not reach significance ($P = 0.085$). For SPMS-ON eyes no significant correlation of EDSS with OCT parameters was found (RNFL: $P = 0.169$; TMV: $P = 0.573$).

3.4. Differences between Subtypes in MS-NON Eyes. Among MS subtypes, RRMS patients showed less RNFL thinning when compared to progressive subtypes (Table 2). When

Table 2: OCT results for the subtypes of MS patients without a history of optic neuritis (NON). Total retinal nerve fiber layer (RNFL) thickness (in μm) and total macular volume (TMV in mm^3) are given as mean values with standard deviation (SD). Generalized estimation equation (GEE) models were used to compare the MS cohorts to healthy controls. GEE models estimate the effect size with standard error (SE) and the respective P value.

			GEE models comparing MS OCT parameters to the healthy control cohort						
				RNFL thickness			TMV		
	Total RNFL thickness mean (SD) [μm]	TMV mean (SD) [mm^3]	Effect	Effect size	SE	P value	Effect size	SE	P value
MS-NON ($n = 571$)	90.15 (12.27)	8.48 (0.43)	Group	−9.571	1.177	<0.001	−0.235	0.043	<0.001
			Age	−0.132	0.053	0.013	−0.006	0.002	0.002
			Gender	2.272	1.116	0.042	−0.075	0.041	0.064
RRMS-NON ($n = 405$)	92.03 (11.91)	8.54 (0.42)	Group	−8.470	1.188	<0.001	−0.197	0.044	<0.001
			Age	−0.080	0.061	0.189	−0.003	0.002	0.094
			Gender	2.933	1.224	0.017	−0.107	0.045	0.019
SPMS-NON ($n = 89$)	83.14 (12.07)	8.32 (0.41)	Group	−9.951	1.084	<0.001	−0.248	0.039	<0.001
			Age	0.139	0.098	0.158	0.003	0.003	0.267
			Gender	−1.807	1.788	0.312	−0.150	0.062	0.015
PPMS-NON ($n = 77$)	88.35 (11.31)	8.34 (0.42)	Group	−4.253	0.792	<0.001	−0.141	0.027	<0.001
			Age	0.037	0.093	0.691	−0.001	0.003	0.654
			Gender	0.802	1.796	0.655	−0.044	0.064	0.492
HC ($n = 183$)	100.60 (9.41)	8.75 (0.34)	Group	N/A	N/A	N/A	N/A	N/A	N/A
			Age	N/A	N/A	N/A	N/A	N/A	N/A
			Gender	N/A	N/A	N/A	N/A	N/A	N/A

adjusting GEE models for age, gender, and disease duration, the only significant difference based on the mean total RNFL thickness was found between RRMS and SPMS patients, while patients with PPMS did not differ from either RRMS or SPMS (Table 4, Figure 1). As opposed to RNFL thickness a different pattern was obtained for TMV measures, in which a significant reduction was found for SPMS and PPMS when compared to RRMS patients. GEE models in which we additionally corrected for EDSS to account for different stages of disease severity did not show differences in RNFL thickness or TMV between MS subtypes (data not shown).

3.5. Association with Disease Duration and Yearly Atrophy Estimate. GEE models were used to test for an association of disease duration and RNFL thickness or TMV in MS eyes. MS-NON eyes showed an association of RNFL thickness and TMV with disease duration in the pooled cohort of all MS subtypes (Table 5, Figure 3). This association was retained in RRMS and SPMS eyes only for RNFL thickness and only in RRMS eyes for TMV. In all MS subtypes the correlation between RNFL thickness and TMV and disease duration was lost in ON eyes (data not shown).

Based on the effect size of the association of disease duration and RNFL thickness or TMV we estimated RNFL thinning and TMV reduction per year of ongoing disease in MS-NON eyes only (Table 5). RRMS-NON eyes showed the strongest and highly significant yearly changes for RNFL thickness (−0.495 μm/year) and TMV (−0.0155 mm^3/year). Interestingly, the significant yearly RNFL thinning in SPMS-NON eyes (−0.464 μm/year) was not concomitantly associated with a significant correlation in TMV change (−0.0016 mm^3/year, $P = 0.838$). In contrast, PPMS-NON eyes showed a less pronounced yearly RNFL thinning (−0.105 μm/year) but in relation a distinct reduction of TMV (−0.0111 mm^3/year). However, correlations of RNFL and TMV with disease duration were not significant for PPMS-NON eyes.

4. Discussion

Here we present the largest ever performed cross-sectional study on retinal atrophy measures in MS subtypes applying latest SD-OCT technology. Groups of disease subtypes in our study were sufficiently large to contrast findings in ON versus ON-free eyes within subgroups. Hereby we show that both RNFL and TMV are reduced in MS-NON eyes versus HC when pooling all disease subtypes, but also when separately comparing disease subtypes (RRMS, SPMS,

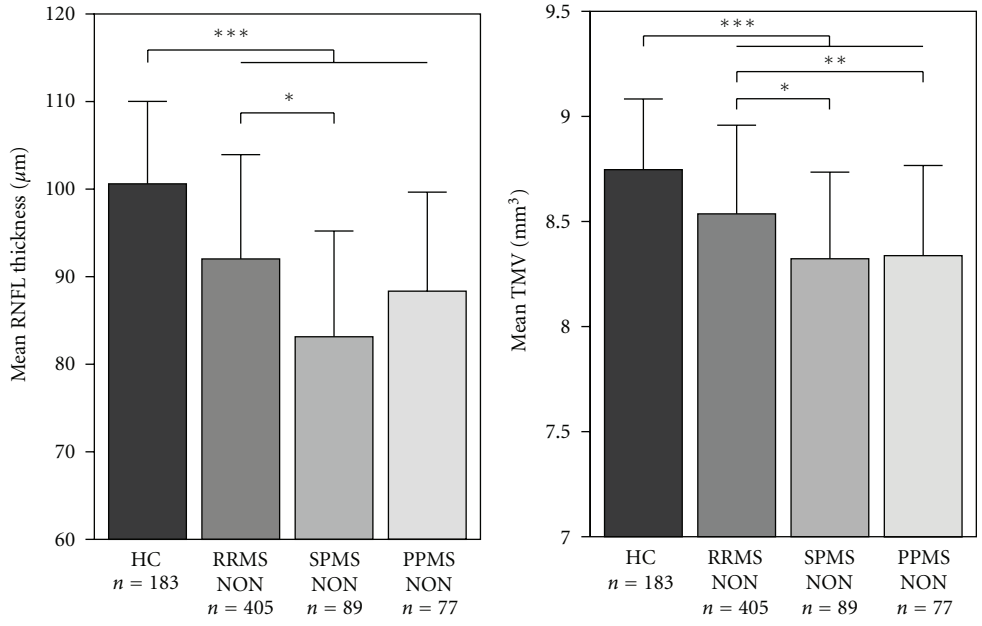

Figure 1: Mean retinal nerve fibre layer (RNFL) thickness (a) and mean total macular volume (TMV) (b) for the healthy controls (HC) and MS subtypes (RRMS, SPMS, and PPMS) without a history of optic neuritis (NON). Significant differences between the groups are indicated with $^{*}(P < 0.05)$, $^{**}(P < 0.01)$, and $^{***}(P < 0.001)$, respectively.

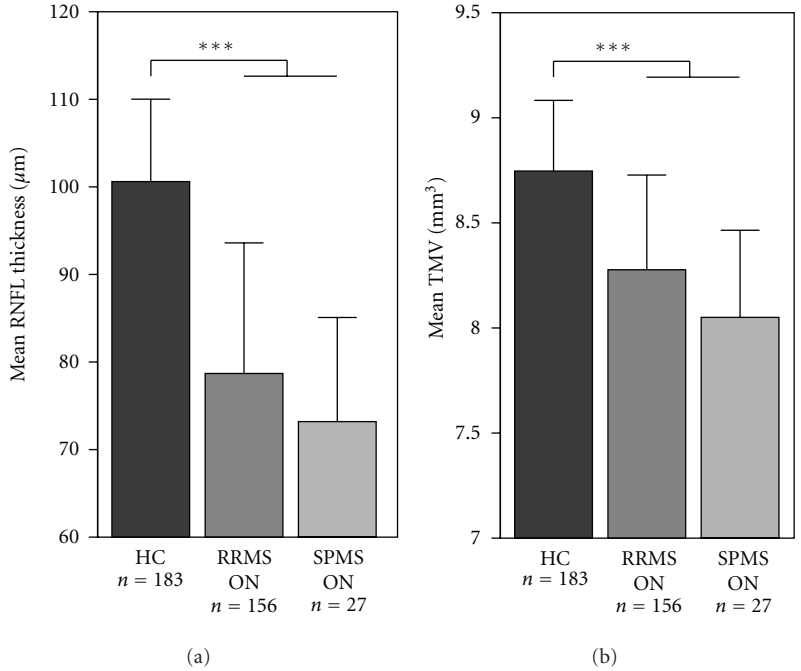

Figure 2: Mean retinal nerve fibre layer (RNFL) thickness (a) and mean total macular volume (TMV) (b) for the healthy controls (HC) and MS subtypes (RRMS, SPMS) with a history of optic neuritis (ON). Significant differences between the groups are indicated with $^{*}(P < 0.05)$, $^{**}(P < 0.01)$, and $^{***}(P < 0.001)$, respectively.

Table 3: OCT results for the subtypes of MS patients with a history of optic neuritis (ON). Total retinal nerve fiber layer (RNFL) thickness (in μm) and total macular volume (TMV in mm^3) are given as mean values with standard deviation (SD). ON eyes were compared to ON-non affected eyes of the same MS subtype using generalized estimation equation (GEE) models. GEE models estimate the effect size with standard error (SE) and the respective P value.

			GEE models comparing OCT parameters between ON-affected and unaffected eyes of the same subtype						
				RNFL thickness			TMV		
	Total RNFL thickness mean (SD) [μm]	TMV mean (SD) [mm^3]	Effect	Effect size	SE	P value	Effect size	SE	P value
MS-ON ($n = 183$)	77.88 (14.61)	8.24 (0.45)	Group	−12.199	1.336	<0.001	−0.237	0.043	<0.001
			Age	−0.147	0.056	0.008	−0.007	0.002	0.001
			Gender	2.989	1.161	0.010	−0.041	0.040	0.299
RRMS-ON ($n = 156$)	78.69 (14.91)	8.28 (0.45)	Group	−12.859	1.478	<0.001	−0.263	0.048	<0.001
			Age	−0.087	0.066	0.185	−0.004	0.002	0.071
			Gender	4.573	1.352	0.001	−0.048	0.046	0.295
SPMS-ON ($n = 27$)	73.19 (11.89)	8.05 (0.41)	Group	−9.297	2.802	0.001	−0.252	0.100	0.012
			Age	0.419	0.216	0.052	0.009	0.008	0.234
			Gender	−7.310	2.591	0.005	−0.250	0.091	0.006
PPMS-ON ($n = 0$)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
HC ($n = 183$)	100.60 (9.41)	8.75 (0.34)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 4: Differences between MS subtypes without a history of optic neuritis (NON) were analyzed with generalized estimation equations (GEE) models including age, disease duration, and gender as effects.

	GEE models comparing OCT parameters between NON eyes of different MS subtypes						
		RNFL thickness			TMV		
	Effect	Effect size	SE	P value	Effect size	SE	P value
RRMS-NON versus SPMS-NON	Group	−5.144	1.921	0.007	−0.137	0.066	0.039
	Age	0.062	0.077	0.419	−0.001	0.003	0.775
	Duration	−0.044	0.009	<0.001	−0.001	0.0003	<0.001
	Gender	1.864	1.377	0.176	−0.139	0.051	0.007
RRMS-NON versus PPMS-NON	Group	−1.204	1.022	0.239	−0.104	0.037	0.005
	Age	−0.016	0.073	0.823	−0.002	0.003	0.371
	Duration	−0.034	0.008	<0.001	−0.001	0.0003	<0.001
	Gender	2.785	1.393	0.045	−0.102	0.052	0.051
SPMS-NON versus PPMS-NON	Group	2.634	2.494	0.291	−0.053	0.090	0.553
	Age	0.339	0.175	0.053	0.002	0.006	0.729
	Duration	−0.031	0.011	0.007	−0.001	0.0004	0.224
	Gender	−3.932	2.287	0.086	−0.127	0.086	0.139

and PPMS) to HC. Not surprisingly and in accordance with previous studies, MS-ON eyes exhibited more severe RNFL and TMV damage than MS-NON eyes. Both findings have been previously described in a similar way by various groups so that the nature of our study appears to be largely confirmatory at first glance. However, our study has some methodological advances compared to previous works that have important implications for the interpretation of our and previous OCT findings. The large sample size of our study enabled a statistically robust comparison of various disease subgroups with the inclusion of possible confounding factors such as age, disease duration, and gender in the statistical

Table 5: Generalized estimation equation (GEE) models correlating disease duration with RNFL thickness and TMV, respectively. The yearly change based on the effect sizes of the respective GEE model.

	RNFL thickness				TMV			
	Effect size	SE	*P* value	Change per year (μm)	Effect size	SE	*P* value	Change per year (mm^3)
All MS-NON	−0.0444	0.0068	<0.001	**−0.533**	−0.0012	0.0002	<0.001	**−0.014**
RRMS-NON	−0.0413	0.0088	<0.001	**−0.495**	−0.0013	0.0003	<0.001	**−0.016**
SPMS-NON	−0.0387	0.0174	0.026	**−0.464**	−0.0001	0.0006	0.838	**−0.002**
PPMS-NON	−0.0088	0.0151	0.562	**−0.105**	−0.0009	0.0006	0.131	**−0.011**

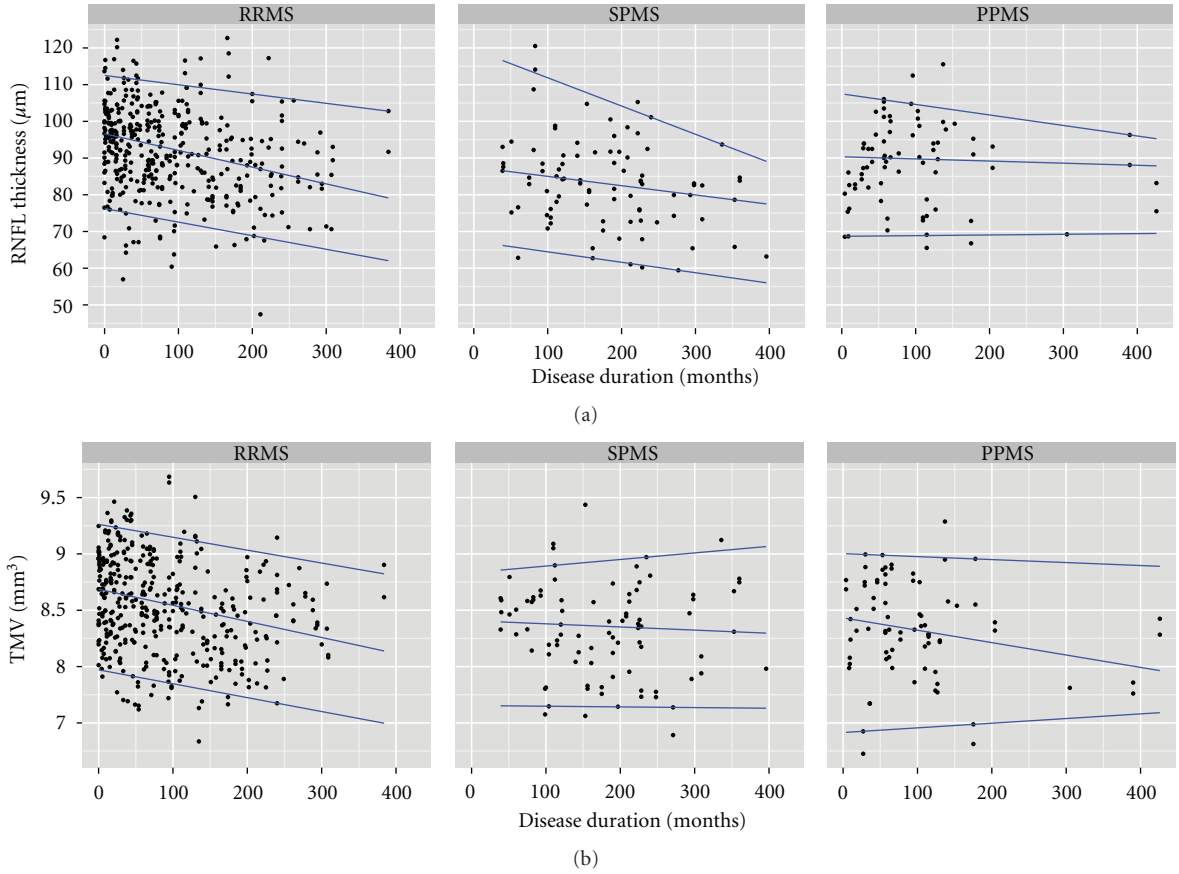

Figure 3: Association of RNFL thickness (a) and TMV (b) with disease duration for RRMS, SPMS and PPMS subtypes in eyes without previous optic neuritis. The blue lines indicate the 95%-, 50%- and 5%-quantiles.

models. In particular, we had larger numbers of patients in the progressive subgroups (65 SPMS, 41 PPMS) than any other previous study which allowed us to compare not only disease subtypes with HC but also with each other. This is of special interest against the background of the ongoing scientific debate on distinct pathogenetic mechanisms in, for example, PPMS versus RRMS. The subgroup comparisons revealed a significant reduction of RNFL thickness in SPMS patients versus RRMS after correction for age, gender, and disease duration and a significant reduction of TMV in both SPMS and PPMS patients versus RRMS.

In contrast, most previous works had only small sample sizes, especially for progressive subtypes which may—besides considerable differences in the statistical models—at least partly explain the inconsistent findings. Pulicken et al. (number of subjects: 135 RRMS, 16 SPMS, 12 PPMS, and 47 HC) found only trends towards lower RNFL thickness values in progressive disease versus RRMS and no difference in TMV in progressive MS versus RRMS [30]. Henderson et al. (number of subjects: 27 SPMS, 23 PPMS, and 20 HC) reported a significant reduction of RNFL and TMV versus HC only in NON eyes from SPMS patients but not PPMS patients and no difference between PPMS and SPMS [31]. Siepman et al. (number of subjects: 26 RRMS, 10 SPMS, and 29 PPMS) could not detect differences between PPMS-NON eyes and the pooled RRMS/SPMS-NON eyes [33].

Serbecic et al. (number of subjects: 42 RRMS, 17 SPMS, and 59 HC) did not specifically address differences in OCT measures between disease subtypes [34] as did numerous other studies with highly heterogeneous patient populations (Gordon-Lipkin et al. [6], number of subjects: 20 RRMS, 15 SPMS, 5 PPMS, and 15 HC; Toledo et al. [12], number of subjects: 7 CIS, 36 RRMS, 3 SPMS, 3 PPMS, 4 progressive-relapsing, and 18 HC; Fisher et al. [15], number of subjects: 90 MS, 76 of whom RRMS, and 36 HC; Sepulcre et al. [7], number of subjects: 22 CIS, 28 RRMS, 5 SPMS, 6 PPMS, and 29 HC), either because of insufficient subgroup sample sizes or owing to the fact that the study had goals other than comparing disease subtypes.

In line with several previous studies [16, 30, 31, 42, 43] we found higher RNFL measures in PPMS as compared to SPMS (88.4 μm versus 83.1 μm), which is in striking accordance with another study that also reported a difference of approximately 5 μm between PPMS and SPMS-NON eyes (93.9 μm versus 88.4 μm) [31]. Although these differences were not significant in both studies, these findings may point to a more severe RNFL damage in SPMS as compared to PPMS, which is in line with the clinical features of PPMS with a lower proportion of visual loss, less frequent ON attacks, a predominant clinical involvement of the spinal cord, and smaller brain lesions as compared to SPMS [31, 44, 45]. However, in contrast to Henderson et al. we found like in SPMS a significant reduction of TMV in NON eyes of PPMS patients versus RRMS and HC, which may display the neurodegenerative component of PPMS concomitantly reflected through brain atrophy measures [46].

Regarding the comparison of RNFL measures in RRMS and SPMS patients, we made another interesting observation: as described previously by Costello et al. [32], differences between SPMS-NON and RRMS-NON eyes were about twice that of differences between SPMS-ON and RRMS-ON eyes ($\sim$20 μm versus $\sim$10 μm in Costello's study, $\sim$10 μm versus $\sim$5 μm in our study). Costello et al. suggested that the impact of prior ON may outweigh the effects of disease subtype.

Further limitation of most of the previous studies is the utilization of time-domain OCT devices (TD-OCT) that only allow for 2-dimensional retinal imaging, limiting its use especially in the demanding macular investigations. The newer high-resolution spectral-domain OCT allows spatial imaging of the retina thus potentially greatly increasing the accuracy and value of OCT in MS [35, 36, 47]. First studies have already applied SD-OCT with intraretinal segmentation [16, 22, 26, 29]. Interestingly, the recent work by Saidha et al. supports the finding of a more severe neuroaxonal retinal damage in SPMS as compared to PPMS; a separate analysis of the combined ganglion cell layer and inner plexiform layer measured by Cirrus SD-OCT in different MS subtypes showed lowest values in SPMS [16]. In contrast, another study by Albrecht et al. [29] applying manual segmentation on Spectralis SD-OCT showed reduced measures in the deeper inner nuclear layer of PPMS but not SPMS patients versus HC. We presume that the ability of SD-OCT to measure spatial scans (earlier TD-OCT had to interpolate macular volume by using 6 radial linear scans) will in future greatly increase the value of macular scans over the currently preferred peripapillary ring scans. In addition, spatial scans allow for correction of positioning errors after scan acquisition by limiting the analysed area to a subset of the actual scan. For example, the Cirrus SD-OCT uses a spatial scan for the peripapillary ring scan, allowing for subsequent correction of alignment errors, whereas the Heidelberg Engineering Spectralis facilitates an eye tracker function to correct for eye movements. In TD-OCT, incorrect placement of peripapillary ring scans accounts to a significant extent for a weaker inter-measurement reliability and cannot be corrected after the scan has been acquired [48]. Next to the ability to analyse all intraretinal layers, improved test/retest-reliability distinguishes SD-OCT from TD-OCT and makes it an ideal instrument for the use in a longitudinal setting where inter-measurement reliability is crucial [49].

The time course of RNFL thinning and TMV reduction by atrophy of different retinal layers—be it in the context of ON or independent thereof—is an essential characteristic in rating the usefulness of OCT as a potential marker of axonal loss in longitudinal clinical trials. For MS-ON eyes it has previously been shown that RNFL thinning occurs within the first 6 months after the ON attack [21, 50]. Overall little is known about temporal dynamics of retinal thinning in MS-NON eyes. Based on published data from cross-sectional studies in MS patients with different disease duration a rough estimate of the yearly atrophy rate appears to be around 1 μm/year, which is ten times as much as what can be expected from normal ageing [3]. In previous cross-sectional studies significant inverse correlations of RNFL thickness and disease duration could be established by some authors [11, 15, 24], while others did not find a significant correlation [20, 31]. In PPMS, an MS subtype in which frequency of clinical attacks of ON is probably lowest, Henderson et al. [31] estimated an RNFL thinning of approximately 0.12 μm (TMV reduction: 0.01 mm^3) per year of disease, which is in good agreement with our results in PPMS eyes (RNFL thinning -0.105 μm/year; TMV reduction: -0.011 mm^3/year). Correlations of OCT measures of retinal atrophy and disease duration were not significant for PPMS patients in both studies. In case of RRMS and SPMS patients without ON we estimated higher yearly RNFL changes than for PPMS (nearly 0.5 μm/year). It is, however, important to note that yearly atrophy rates are considerably lower than the optimized axial resolution of SD-OCT devices, which is approximately 4–6 μm [51, 52]. This is of relevance in case OCT endpoints are taken into consideration for future clinical trials, for example, in proof-of-concept trials for neuroprotective agents. Depending on the disease subtypes, model timelines and sample sizes have to be planned accordingly.

In a first longitudinal OCT study by Talman et al. [53] a thorough examination of the time course of RNFL thinning in a mixed cohort of different MS subtypes was performed with TD-OCT (Stratus) revealing a yearly loss of approximately 2 μm in MS-NON eyes (GEE: $P < 0.001$). The study included a preliminary sample size calculation (supplementary data of [53]) for future clinical trials that aimed to detect a 30% reduction in the proportion of eyes with an RNFL

thinning greater than the test-retest variability of the Stratus OCT (6.6 μm) over a follow-up period of 2-3 years. With a power of 80–90% and a type 1 error of 0.05, the authors' sample size calculation estimated roughly a number of 400–600 patients per group. The yearly loss of 2 μm reported by Talman et al. from Stratus OCT is considerably higher than the yearly reduction of approximately 0.5 μm calculated from our dataset. Discrepancies may derive from the differences in the devices applied (TD-OCT versus SD-OCT) and the fact that our calculation is based on cross-sectional data only.

In sum, this study, based on a large SD-OCT data set, confirms previous data on neuroaxonal retinal damage in MS subtypes. At the same time, it extends previous findings by providing new insights into differences between MS-ON and MS-NON eyes in the various subgroups and—in addition—allowing for reliable correction for non-disease-related factors such as age, gender disease duration, and severity.

Authors' Contribution

T. Oberwahrenbrock, S. Schippling, and M. Ringelstein contributed equally to this work.

Acknowledgments

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Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome

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Abstract

Background: Axonal and neuronal damage are widely accepted as key events in the disease course of multiple sclerosis. However, it has been unclear to date at which stage in disease evolution neurodegeneration begins and whether neuronal damage can occur even in the absence of acute inflammatory attacks.

Objective: To characterize inner retinal layer changes in patients with clinically isolated syndrome (CIS).

Method: 45 patients with CIS and age- and sex-matched healthy controls were investigated using spectral domain optical coherence tomography. Patients' eyes were stratified into the following categories according to history of optic neuritis (ON): eyes with clinically-diagnosed ON (CIS-ON), eyes with suspected subclinical ON (CIS-SON) as indicated by a visual evoked potential latency of >115ms and eyes unaffected by ON (CIS-NON).

Results: CIS-NON eyes showed significant reduction of ganglion cell- and inner plexiform layer and a topography similar to that of CIS-ON eyes. Seven eyes were characterized as CIS-SON and likewise showed significant retinal layer thinning. The most pronounced thinning was present in CIS-ON eyes.

Conclusion: Our findings indicate that retinal pathology does occur already in CIS. Intraretinal layer segmentation may be an easily applicable, non-invasive method for early detection of retinal pathology in patients unaffected by ON.

Keywords

Clinically isolated syndrome, optical coherence tomography, retinal nerve fiber layer, retinal ganglion cell layer

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Introduction

Multiple sclerosis is an autoimmune disorder of the central nervous system that often manifests with optic neuritis (ON) as well as motor, sensory or cerebellar deficits in its earliest stage.[1] Current diagnostic criteria for MS require proof of dissemination of lesions or attacks in time and space.[2] In everyday clinical practice, patients presenting with a first clinical event that is highly indicative of MS are often instead diagnosed with a clinically isolated syndrome (CIS) or 'possible' MS.[3] A confirmed diagnosis of MS is possible once additional attacks or lesions present, as is the case for a significant proportion of such patients.[2]

In light of this, pinpointing the aspects of CIS that are most predictive for subsequent diagnosis with MS has high

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priority[3] so that patients at risk can be identified. Diagnosing MS as early as possible and thus allowing for the widest range of therapeutic options, is therefore highly in the patients' interest, in particular as irreversible axonal and neuronal injury is a key aspect and correlate of disability in MS in early disease stages.[3–5]

One easily-accessible means of assessing neuroaxonal damage in MS is the investigation of the retina. Optical coherence tomography (OCT) has shown specific retinal alterations in MS patients:[6] the retinal nerve fiber layer (RNFL) is reduced in MS,[7] not only in eyes with a history of ON[8] but also in eyes without any previous clinical event of ON.[9,10] Additionally, microcystic macular edema (MME) in the inner nuclear layer (INL) has been reported in a subset of MS patients.[11] Although MME might not be specific to MS, but instead ON-dependent,[12] the INL has become a key focus of clinical investigation of MS pathology after a postmortem histopathology study reported neuronal loss in the INL.[13,14]

Additionally, retinal changes in MS do not merely reflect the visual system, but potentially also overall disease pathology. RNFL thinning correlates closely with brain atrophy,[15–17] and with reduction of N-acetyl-aspartate as marker of neuroaxonal integrity in the visual cortex.[18]

These findings suggest that the retina and, in particular, intraretinal layers may be an effective means of detecting subtle neuronal and axonal damage already present in CIS. To investigate this theory, we performed a cross-sectional study analysing intraretinal changes in CIS patients. We were especially interested in retinal pathology in eyes that had not suffered from previous ON and therefore applied a rigorous classification of eyes not only on clinical assessments but also visual evoked potentials (VEP).

Methods

Study participants

Patients were prospectively recruited from outpatient clinics at two university medical centers (Berlin and Düsseldorf). Inclusion criteria were clinical and paraclinical (MRI, CSF, EP) diagnosis of CIS suggestive of MS after relevant differential diagnoses had been ruled out, and an age between 18 and 65 years.[2] Patients received MRI to exclude the possibility that the disease had developed into MS since first diagnosis of CIS. Neurological disability was assessed according to the Expanded Disability Status Scale (EDSS).[19] A history of ON was diagnosed by a treating physician and was cross-checked using medical records. Patients with a refractive error of more than ±5.0 dioptres or with any history of eye disease that could impact OCT measurements (i.e. glaucoma) were excluded. A second exclusion criterion was steroid therapy within 30 days prior to examination. A group of healthy controls matched by age (+/−3 years) and gender was recruited from patients' family members, medical staff or volunteers. Both centres assessed the matched controls to their patients. To exclude potential centre effects, we additionally performed centre-specific analysis or included centre as covariate. In these analyses, centre did not have a significant effect (data not shown). Local ethics committees approved the study and all participants gave written informed consent.

Visual evoked potentials

VEP were either performed during the clinical work-up or as part of the study protocol prior to or on the same day as the OCT assessment. We used the P100 latency values as a parameter to prove optic nerve conduction slowing potentially related to a history of ON. VEP amplitude was not analysed because the two centres involved in the study performed VEP using different devices in a non-standardized manner.

Optical coherence tomography

Experienced operators performed OCT on un-dilated eyes using Heidelberg Spectralis SD-OCT (Heidelberg Engineering, Germany). All scans were checked for appropriate image quality. All participants were examined using the peripapillary ring scan, which measures RNFL thickness (pRNFL) around the optic nerve head in a circle with an angle of 12°, resulting in a diameter of 3.4 mm (example shown in Figure 1(a)). Macular volume was assessed by a custom scan comprising 61 vertical B-scans (each with 768 A-Scans, Automatic Real-Time (ART) = 13 frames) with a scanning angle of 30° × 25° focusing on the fovea. Using this scan, TMV and intra-retinal layers thicknesses were determined within a cylinder of 6 mm diameter (Figure 1(b)).

Intraretinal layer segmentation

Heidelberg Engineering provided beta software that employed a multilayer segmentation algorithm for macular volume scans. To analyse the inner retinal layers, a subset of B-scans were segmented and manually corrected by an experienced assessor in a blinded fashion. The multilayer analysis was performed on the central B-scan through the fovea and on six B-scans each in nasal and temporal direction. Manual correction of automatically segmented B-scans is a time-consuming step. As a compromise, we manually corrected every fourth B-scan, thus analysing an area largely covering the 6 mm diameter ETDRS grid with a distance between adjacent B-scans of approximately 500 µm. For the combined analysis of both eyes, thickness maps of the left eye were mirrored vertically to match the topology of the right eye. The mean thickness maps within each of the study groups were calculated for the four innermost retinal layers: macular RNFL (mRNFL), ganglion cell layer (GCL), inner plexiform layer (IPL) and INL (Figure 1(c)). Because differentiating between GCL and IPL proved to be a hurdle, we used the combined thickness of GCL and IPL (GCIPL). Please see the supplementary data for individual analyses of GCL and IPL. By subtracting the group-specific mean

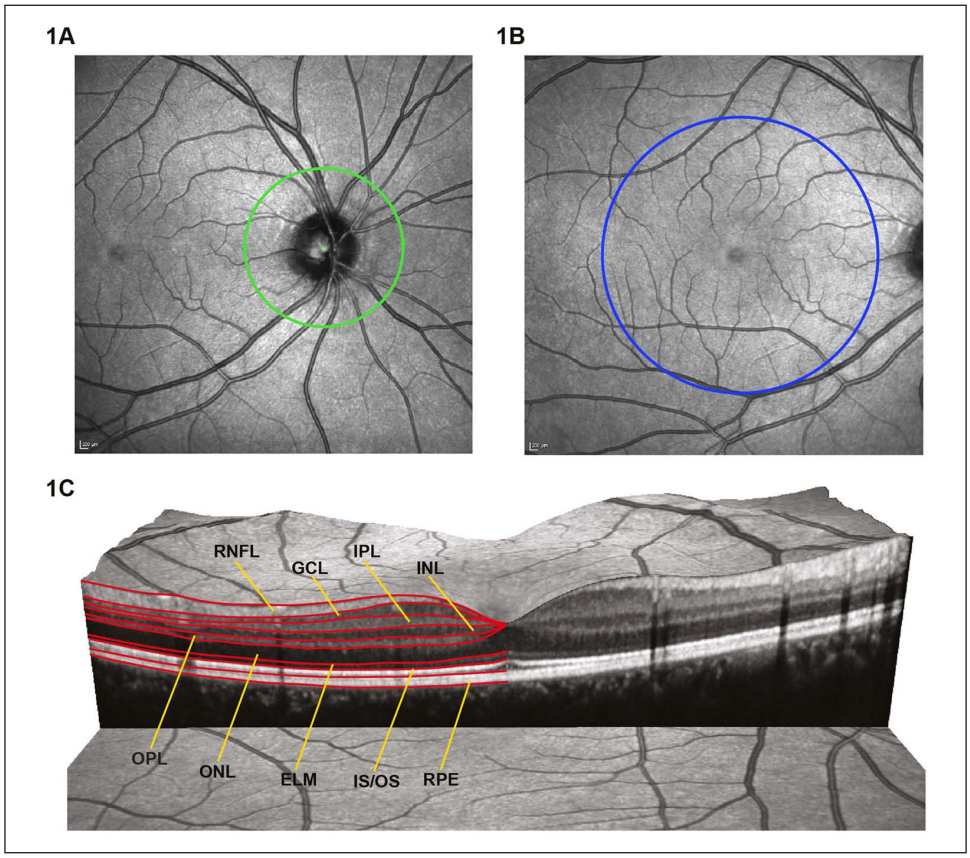

Figure 1. Examples of regions analysed in OCT.
A) Scanning laser ophthalmoscopy image showing the region of the peripapillary ring scan (green); B) Scanning laser ophthalmoscopy image of the macular scan with the blue circle indicating the area for total macular volume and intraretinal layer thickness determination; C) 3D reconstruction of a macular volume scan, depicting the identified intraretinal layers.
Abbreviations: RNFL = retinal nerve fibre layer; GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer; ELM = external limiting membrane; IS/OS = inner segments / outer segments; RPE = retinal pigment epithelium.

thickness maps we produced spatial difference maps (Figure 3), in which negative values indicate a thinning of the patients' group compared to matched healthy controls, whereas positive values indicated thickening.

Statistical analysis

Generalized estimation equation models (GEE) accounting for within-subject inter-eye effects were used to compare OCT results between the study cohorts. For the subgroup analysis, only controls that were matched to the respective CIS patients' eyes (NON, SON, ON) were used. Correlations between VEP and OCT results were performed by linear regression. All statistical analyses were performed and all figures were created using R version 2.15.0. Statistical significance was established at $p < 0.05$.

Results

Study participants

In total, 45 patients (Berlin 29, Düsseldorf 16) were enrolled and compared to matched healthy controls (Berlin 29, Düsseldorf 16). All patients were diagnosed with CIS at the time of OCT examination and diagnosis and non-progression towards MS was confirmed by means of MRI. Of the patients, 16 had unilateral optic neuritis (seven on the right, 10 on the left) and 14 patients presented with spinal cord symptoms. Six patients experienced relapses with findings suggestive of infratentorial brain lesions, in seven patients supratentorial signs were found, and one patient exhibited both supratentorial and spinal clinical signs. Examination of one patient's eye did not pass the quality criteria due to image artefacts and was excluded. Demographic and clinical data are summarized in Table 1.

ON classification according to VEP latency and correlation to standard OCT results

As a clinical diagnosis of ON may have been missed by patients or physicians, we created another category of subclinical (or suspected) ON in eyes without a clinical ON history, as assessed by VEP. In addition to the group of confirmed ON eyes (CIS-ON), we defined a group of suspected ON eyes (CIS-SON), defined as eyes with

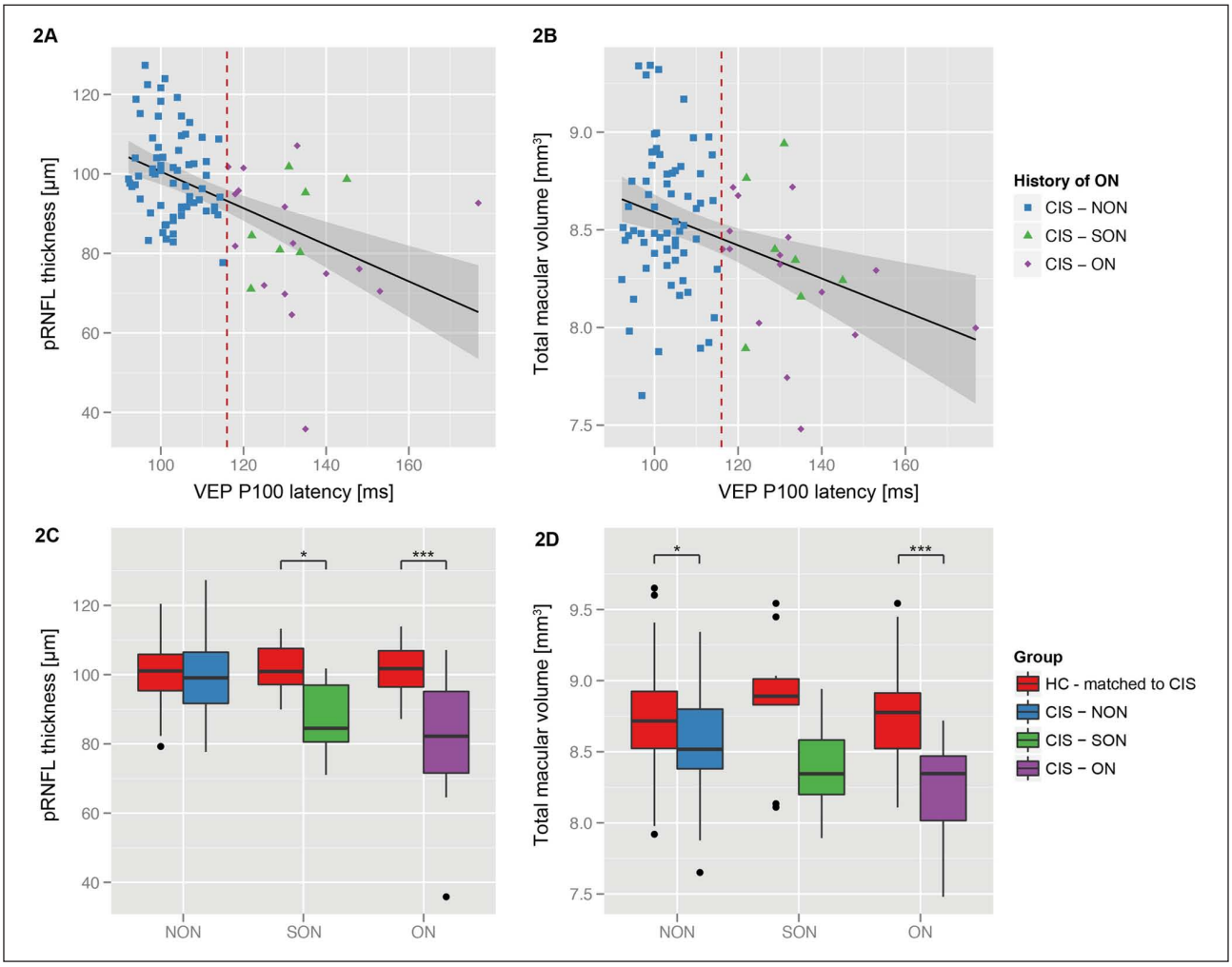

Figure 2. VEP and standard OCT results.
Scatterplots showing the relationship of the VEP P100 latencies with A) peripapillary RNFL (pRNFL) and B) total macular volume. The red dashed line at 115 ms indicates the threshold between CIS-NON and CIS-SON eyes. The black line is the result of the linear regression including all CIS eyes with the standard error given as gray shadow. Comparison of C) peripapillary RNFL thickness and D) total macular volume between the different CIS groups and the matching controls. Significant differences are marked with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).
Abbreviations: HC = healthy control eyes; CIS-NON = patient eyes without history of optic neuritis and VEP P100 ≤115 ms; CIS-SON = eyes with VEP P100 latency > 115 ms but no ON diagnosis; CIS-ON = patient eyes with clinical ON diagnosis.

prolonged P100 latency of over 115 ms but, as stated above, without a clinical history of ON. The latter value of a 115 ms limit for normal eyes is in accordance with literature[20] and proved an effective means of distinguishing between eyes diagnosed with ON and unaffected eyes (Figure 2(a) and 2(b)). In total, seven eyes were classified as CIS-SON. Both eyes of two patients were classified as suspected ON and all other CIS-SON eyes were contralateral to CIS-ON eyes. Figure 2(a) shows the correlation between P100 latencies and pRNFL thickness, while Figure 2(b) is a graph of the relationship between the TMV and the VEP results. Linear regression showed significant correlation between pRNFL and P100 VEP latencies in all CIS eyes ($R^2 = 0.243$, $p < 0.001$) and in CIS-NON eyes ($R^2 = 0.065$, $p = 0.039$) but not in CIS-SON and CIS-ON eyes. Similarly, TMV correlated significantly to P100 latencies for all CIS eyes ($R^2 = 0.124$, $p < 0.001$), but not for the other subgroups.

pRNFL and TMV comparison

When compared to the corresponding age- and sex-matched controls, pRNFL thickness was reduced in CIS-ON ($p < 0.001$) and CIS-SON ($p = 0.014$) but not in CIS-NON eyes ($p = 0.636$) (Figure 2(c)). Analysis of macular scans revealed significant TMV reduction in CIS-ON eyes ($p < 0.001$) and, importantly, also in CIS-NON eyes ($p = 0.031$) versus controls (Figure 2(d)). TMV reduction in the 7 CIS-SON eyes was not significant.

Table 1. Demographical and clinical parameters.

		CIS	HC
Subjects	*N*	45	45
Eyes	*N* total	89	90
	N with diagnosed ON	16	NA
	N with suspicion ON	7	NA
Age (years)	Mean (*SD*)	31.92 (7.95)	31.67 (7.80)
	Min–Max	19.13–56.25	18.64–54.20
Gender	*N* female (%)	31 (68.89)	31 (68.89)
First symptom (months)	Mean time between first symptom and OCT (*SD*)	8.60 (12.17)	NA
	Min–Max	1.37–59.70	
EDSS	Median	1	NA
	Min–Max	0–4	NA

HC: healthy control; CIS: clinically isolated syndrome; ON: optic neuritis; SD: standard deviation; OCT: optical coherence tomography; Min: minimum; Max: maximum, NA: not applicable.

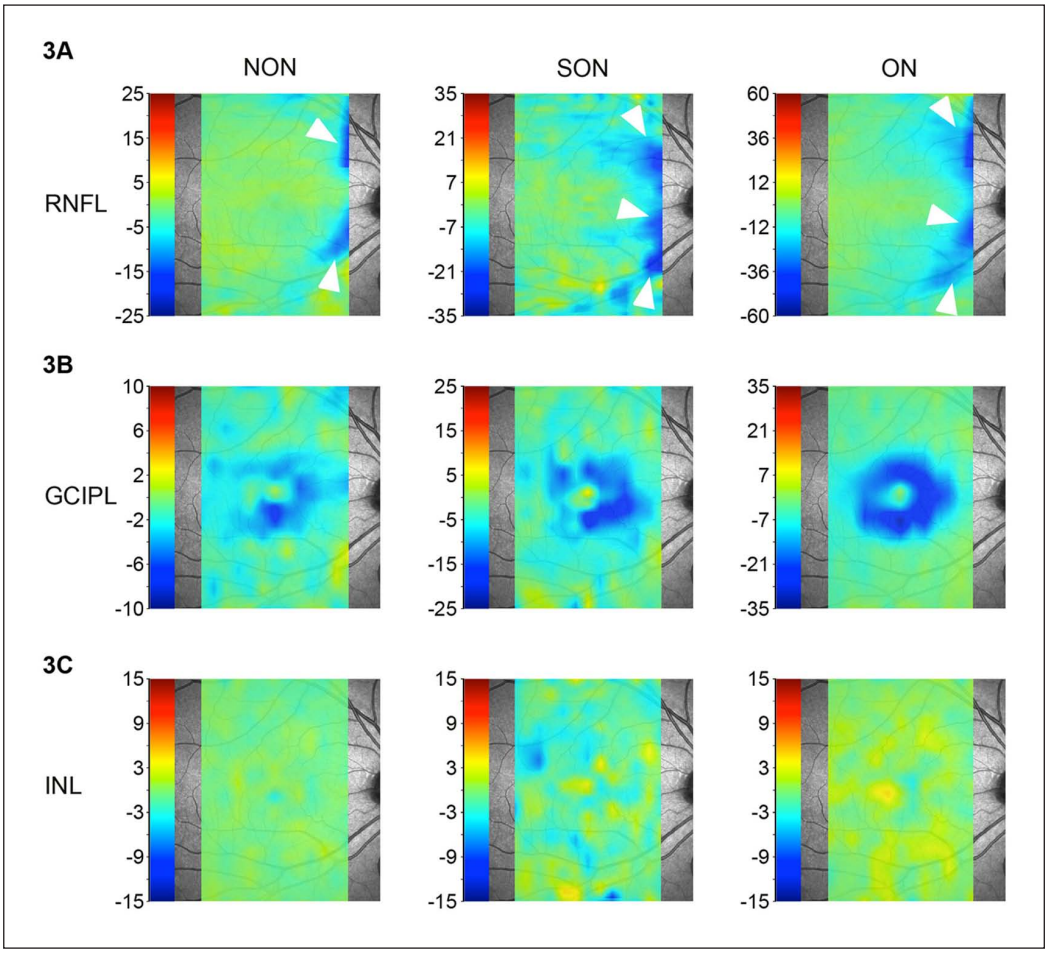

Figure 3. Spatial analysis of changes in CIS eyes versus healthy control eyes.
A) Changes in RNFL thickness between CIS patients and the corresponding group of age- and sex-matched healthy controls. Patients were stratified by history of ON: no history of ON (NON), suspected ON (SON) or clinically-diagnosed ON. Reduction in RNFL thickness was evident near the optic nerve head (white arrows) in all groups but was more pronounced in SON and ON eyes. B) Thickness changes in the GCIPL were identified in the perimacular region and were most evident in CIS-ON eyes. Significant thinning of the GCIPL in CIS-NON eyes compared to the matching controls were found in the perimacular area ($p = 0.027$). C) No group showed significant changes in the INL.
Abbreviations: CIS = clinically isolated syndrome; RNFL = retinal nerve fibre layer; GCIPL = combined ganglion cell and inner plexiform layer; INL = inner nuclear layer.

Table 2. Mean (*SD*) retinal layer thickness and total macular volume results.

Retinal layer	HC (matched to CIS-NON)	CIS-NON	Regression coefficient[a]	Standard error[a]	P value[a)]
pRNFL (µm)	100.69 (8.01)	99.94 (11.28)	−1.01	2.13	0.636
TMV (mm^3)	8.724 (0.321)	8.570 (0.362)	−0.16	0.07	**0.031**
mRNFL (µm)	39.73 (4.45)	38.76 (4.32)	−1.30	0.96	0.173
GCIPL (µm)	71.27 (4.52)	68.88 (5.52)	−2.48	1.12	**0.027**
INL (µm)	33.76 (2.19)	33.39 (2.01)	−0.22	0.46	0.626
Retinal layer	HC (matched to CIS-SON)	CIS-SON	Regression coefficient[a]	Standard error[a]	*P* value[a)]
pRNFL (µm)	101.75 (8.25)	87.49 (11.29)	−14.68	5.97	**0.014**
TMV (mm^3)	8.866 (0.465)	8.392 (0.358)	−0.44	0.26	0.091
mRNFL (µm)	42.01 (4.52)	37.31 (4.56)	−4.70	2.62	0.073
GCIPL (µm)	71.45 (4.87)	63.15 (7.43)	−7.62	3.85	**0.048**
INL (µm)	34.49 (2.42)	32.99 (0.96)	−1.31	1.05	0.212
Retinal layer	HC (matched to CIS-ON)	CIS-ON	Regression coefficient[a]	Standard error[a]	*P* value[a]
pRNFL (µm)	101.36 (7.44)	82.08 (18.02)	−20.15	4.62	**<0.001**
TMV (mm^3)	8.746 (0.335)	8.265 (0.350)	−0.48	0.11	**<0.001**
mRNFL (µm)	39.89 (4.57)	32.14 (5.64)	−8.05	1.68	**<0.001**
GCIPL (µm)	71.57 (4.62)	58.69 (9.77)	−3.68	2.64	**<0.001**
INL (µm)	34.34 (2.38)	34.86 (2.17)	0.64	0.75	0.397

HC: healthy control eyes; CIS-NON: patient eyes without history of optic neuritis and VEP P100 ≤ 115 ms; CIS-SON: eyes with VEP P100 latency > 115 ms but no ON diagnosis; CIS-ON: patient eyes with clinical ON diagnosis; SD: standard deviation; pRNFL: peripapillary retinal nerve fiber layer; TMV: total macular volume; mRNFL: macular retinal nerve fiber layer; GCIPL: combined ganglion cell and inner plexiform layer; INL: inner nuclear layer.
[a]Statistical parameters of the comparison of CIS patients to the corresponding matching controls using generalized estimation equation models.

Intraretinal multilayer segmentation

The mean macular thickness values for inner retinal layers (mRNFL, GCIPL, INL) of the different groups are summarized in Table 2. A graphical representation of the spatial changes of CIS patients compared to the matching controls is given in Figure 3.

Analysis of the central macular area (6 mm in diameter around the fovea) showed significant reduction in mRNFL thickness in CIS-ON eyes, but not for CIS-SON and CIS-NON in comparison to matched controls (Table 2). Spatial difference maps showed that mRNFL thinning was most prominent in close proximity to the optic nerve head (Figure 3(a), white arrows). Here, even for CIS-NON eyes mRNFL thinning was visible very close to the optic nerve head. It should be noted that macular volume scans are not designed to investigate the papillary region and that this area is highly penetrated by blood vessels, potentially causing segmentation errors; thus, the mRNFL results have to be evaluated with caution.

All patient groups showed reduced GCIPL thickness compared to the matched healthy controls. Spatial differences of the GCIPL were found in the perimacular region (Figure 3(b)) and statistical analysis of the GCIPL confirmed that the thickness in this area was significantly reduced for all patient groups compared to controls (Table 2). The thinning in CIS-ON and CIS-SON eyes was more pronounced than in the CIS-NON group, while the spatial distribution of changes was similar. Please refer to the supplementary material for detailed data on the analysis of the GCL and IPL individually.

Analogous to pRNFL and TMV, we analysed a potential correlation between VEP latencies and intraretinal layer thicknesses: mRNFL ($R^2 = 0.203$, $p < 0.001$) and GCIPL ($R^2 = 0.315$, $p < 0.001$) were significantly correlated to VEP latencies (supplementary Figure 2). There was no correlation of intraretinal layer thicknesses or VEP latencies with symptom onset in the CIS-NON group (supplementary Figure 3).

Discussion

We analysed intraretinal changes in a cohort of CIS patients, which included both eyes with confirmed previous ON, eyes with suspected ON, and eyes without evidence of ON compared to age- and sex-matched healthy controls. Notably, we identified significant thinning of GCIPL in the eyes of CIS patients without any clinical history of ON or suspected previous subclinical ON as determined by VEP changes. A supplementary analysis using distinct GCL and IPL thicknesses localized this GCIPL thinning to the GCL in CIS-NON patients. Additionally, and as expected, eyes with a confirmed history of ON showed an even more pronounced thinning of retinal layers. In contrast, INL appeared unaltered. Our data indicate that retinal neuronal damage can accompany CIS independently of a prior history of ON.

Three previous studies have investigated retinal changes in CIS patients: The first study failed to detect pRNFL or TMV reduction in the eyes of CIS patients without prior ON.[21] A second study reported no retinal damage in the eyes of patients with isolated unilateral ON.[22] However,

these studies were conducted before the introduction of spectral-domain OCT (SD-OCT), the superior spatial resolution of which over time-domain OCT (TD-OCT)[23] allows for the investigation of intraretinal layers.[24] Previously and in particular, in the above studies, retinal alterations may have simply not been detectable by TD-OCT and, more importantly, GCIPL changes that can only quantified using SD-OCT might be superior for detecting even subtle neurodegeneration in CIS over pRNFL. Peripapillary RNFL also failed to detect differences in our groups, suggesting that this parameter is in general less sensitive for detecting MS pathology than new intraretinal layer measurements like GCIPL. With this in mind, the failure to detect significant pRNFL alterations in our CIS-NON cohort may simply be a power issue. A third recent study comprising 45 CIS patients showed a reduction of pRNFL but not TMV using SD-OCT.[25]

The present study is the first to investigate intraretinal layer changes or detect retinal neurodegeneration independent from ON in a larger cohort of CIS patients. A recent study that reported reduction of the GCIPL in MS patients with and without a history of ON included seven CIS patients while the remaining patients had long-standing diagnoses of MS, which precluded reliable assessment of retinal damage in early disease stages.[26] Other studies have shown INL impairment (i.e. microcystic macular oedema) in MS patients with longer disease duration.[11,14] Such changes were not detected in our CIS patients, suggesting that INL impairment might be a symptom of later or more severe disease stages.

Our finding that damage to the GCIPL is detectable in CIS eyes without clinical history of ON and with normal VEP latency lends additional support to the increasingly widespread understanding of MS as both a demyelinating *and* neurodegenerative disease.[27] We show that neurodegeneration is not, in fact, limited to advanced disease stages, in which it is considered responsible for the continuous progression of neurological disability, even in the absence of relapses. Instead, neurodegeneration can begin very early in disease development. Our data corroborate MRI data showing neuroaxonal damage during the very earliest MS stages,[4,28] as well as histopathology data from brain [29] and eye,[13] and from experimental autoimmune encephalomyelitis.[30,31] In line with previous investigations, our study provides evidence that inflammatory attacks to the optic nerve to the extent of a clinical or subclinical ON may not be a pre-requisite for damage to the retinal GCIPL.[26]

Our finding that neuronal retinal damage begins during very early disease stages raises urgent questions, the answers to which may challenge our understanding of the underlying pathology and mechanisms of MS.[32] Is the damage we found in the retina a consequence of the retrograde degeneration of retinal nerve fibres that occurs as a consequence of autoimmune brain inflammation in MS? If the answer is yes, it follows that retrograde RNFL damage would subsequently initiate a degenerative process in the GCL via a *dying back* mechanism. Indeed, the hypothesis that retrograde retinal neuroaxonal damage takes place both after ON as well as brain inflammation without clinical ON is supported by experimental animal data from intracranial optic nerve sections.[33] Here, ocular pathology was shown to be limited to the inner retina. Evidence for inner retinal layer damage has been further provided by the first large scale pathological description of retinae from autopsied MS patients showing – apart from the anticipated extensive axonal damage – neuronal loss in both the GCL and the INL.[13] In contrast, a recent OCT study has suggested a primary retinal pathology as a novel distinct subtype of MS, which would implicate that a *dying back* pathomechanism does not apply to all patients:[24] the study identified MS patients exhibiting substantial reduction of TMV and significant thinning of the outer and inner nuclear layers despite normal RNFL values. The authors suggested that retinal pathology in this disease subtype (termed 'macular thinning predominant') occurs independently of optic nerve pathology and may be a harbinger of a more aggressive disease course. However, these findings have yet to be confirmed by other groups and with other OCT devices in larger cohorts.[34]

Some important caveats of our study should be noted. Firstly, undetected subclinical ON episodes in our patient cohort may have skewed our results. However, we dealt with this potential cohort bias swiftly by conducting a thorough clinical assessment and examination of the individual patients. Additionally, each patient had to undergo VEP: Eyes with P100 latency suspicious for ON were classified as subclinical ON and not as unaffected eyes. Furthermore, all patients received MRI as proof that a confirmed diagnosis of MS could not yet be established. Although this approach cannot be guaranteed to prevent all errors in ON identification, it does ensure that the risk of misclassification as CIS-NON or MS is negligible and that the conclusions drawn from our data are valid.

A further limitation of our study is that we could not correlate morphological data to functional visual measures such as low contrast letter acuity. However, we are currently addressing this aspect in an ongoing CIS study that includes Sloan charts as suggested by a previous study.[35] The high number of statistical analyses in comparison to the relatively low number of patients should also be noted. As we did not perform a previous power calculation and since OCT parameters are related and thus likely correlated, we did not correct for multiple comparisons, since doing so would have likely caused an overcorrection. We did carefully examine our cohorts for a possible influence of outliers and distribution effects, finding no such effect. However, it is important to reproduce our findings in an independent cohort.

Segmentation of intraretinal layers is a novel technique and no studies have been performed so far to better

understand how segmentation-derived results relate to in-vivo morphological changes that appear in MS (e.g. through histopathological studies). However, a number of recent studies have successfully applied intraretinal segmentation,[9,14,17,26,36] and comparison of different segmentation techniques showed excellent reproducibility and reliability.[37] We have investigated reliability of the novel algorithm applied in this study in a cross-centre inter-rater reliability study on a defined set of OCT macular B-scans. Results support the excellent reliability of intraretinal segmentation reported by others,[37] with the exception that no histopathological correlation has been performed so far (publication in preparation). However, GCL and IPL are still difficult to differentiate in OCT scans and therefore we based our study results mostly on the combined layer of both (GCIPL) and present individual layer analyses as supplementary data only.

Of note is the large amount of eyes that were classified as suspected ON ($n = 7$) in comparison to the number of eyes with definite clinical ON ($n = 16$). Retinal layer-thinning in these eyes was in-between NON and ON eyes, further supporting the notion that optic nerve inflammation is not a *yes* or *no* event. Instead, substantial damage might be caused by optic nerve inflammation before clinical visibility in form of an apparent clinical ON might be established. As our cohort comprised only patients with CIS, failure to detect subclinical ON potentially might compromise the discrimination between CIS patients and patients who already have definite MS. Clearly, detection of subclinical alterations in visual and other functional systems urgently needs improvement. Our study did not investigate the discriminatory properties of OCT and VEP between CIS and MS patients, and consequently, this question must be addressed by a future study.

In summary, our study shows that retinal neurodegeneration is already detectable in CIS patients and is dependent but importantly also independent of clinical relapses (i.e. ON). Accordingly, irreversible neuronal damage in MS might be much more prevalent than previously thought. Long-term follow-up of our study patients, who exhibited very early substantial and presumably irreversible neuroaxonal damage, is vital to ascertain diagnosis in patients likely to develop MS as early as possible.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Optic Neuritis Is Associated with Inner Nuclear Layer Thickening and Microcystic Macular Edema Independently of Multiple Sclerosis

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Abstract

Background: Microcystic macular edema (MME) and inner nuclear layer thickening (INL) were described in multiple sclerosis (MS) and neuromyelitis optica (NMO) patients using optical coherence tomography (OCT). The cause of these findings is currently unknown and a relation to inflammatory or degenerative processes in the optic nerve is discussed.

Objective: The aim of our study was to investigate whether INL thickening and MME are related to optic neuritis (ON) in various neuro-inflammatory disorders causingON: MS, NMO and chronic inflammatory optic neuropathy.

Methods: We retrospectively analyzed data from 216 MS patients, 39 patients with a clinically isolated syndrome, 20 NMO spectrum disorder patients, 9 patients with chronic inflammatory optic neuropathy and 121 healthy subjects. Intra-retinal layer segmentation was performed for the eyes of patients with unilateral ON. Scanning laser ophthalmoscopy (SLO) images were reviewed for characteristic ocular fundus changes.

Results: Intra-retinal layer segmentation showed that eyes with a history of ON displayed MME independent INL thickening compared to contralateral eyes without previous ON. MME was detected in 22 eyes from 15 patients (5.3% of all screened patients), including 7 patients with bilateral edema. Of these, 21 had a prior history of ON (95%). The SLO images of all 22 MME-affected eyes showed crescent-shaped texture changes which were visible in the perifoveal region. A second grader who was blinded to the results of the OCT classified all SLO images for the presence of these characteristic fundus changes. All MME eyes were correctly classified (sensitivity = 100%) with high specificity (95.2%).

Conclusion: This study shows that both MME and INL thickening occur in various neuro-inflammatory disorders associated with ON. We also demonstrate that detection and analysis of MME by OCT is not limited to B-scans, but also possible using SLO images.

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Introduction

Optic neuritis (ON) is a common symptom of inflammatory central nervous system disorders that often heralds a diagnosis of multiple sclerosis (MS) or neuromyelitis optica (NMO) [1–5]. In both diseases, ON can cause irreversible damage to axons in the retina and their neurons, the retinal ganglion cells, which can be quantified by optical coherence tomography (OCT) [6–10]. A number of OCT studies have demonstrated retinal neuro-axonal damage in MS patients with and without a history of ON, as well as in NMO patients [7,8,11–18]. Thanks to recent technical advances in OCT, including improved resolution and intra-retinal layer segmentation, pathologies can be detected in various retinal layers, such as the retinal ganglion cell layer (GCL) and the inner nuclear layer (INL) [19–24]. The new data this has yielded have substantially advanced our understanding of retinal pathology in MS, leading to intriguing, new hypotheses regarding MS pathophysiology [19].

Recently, a retinal finding termed microcystic macular edema (MME) was observed in MS [25,26]. While this paper was under review, two further studies reported MME in NMO [27,28]. In all studies, MME was predominantly located in the INL and was associated with reduced visual acuity and retinal nerve fiber layer (RNFL) thinning in both disorders. In MS, the INL was shown as a prominent site of retinal and microglial inflammation [29],

which led the authors to propose MME as a clinical correlate of pathological processes in the INL. Indeed, an earlier study had already reported an correlation between INL thickening at baseline with more severe disease progression, as measured by increased development of contrast-enhancing lesions, new T2 lesions, Extended Disability Status Scale (EDSS) [30] progression and relapses in patients with relapsing-remitting MS over the study period [26]. However, other reports have countered these findings, arguing that MME may not be MS- or NMO-specific [31] or even that it is entirely independent of inflammation and instead based on acute optic neuropathy e.g. mediated by mechanical stress [32,33].

Against this background, our first aim was determining whether INL thickening only occurs in MME and whether it is related to a previous episode of ON, which would suggest that ON is as a causative factor in INL pathology. We screened patients with three different neuro-inflammatory disorders including ON: MS, NMO and chronic relapsing inflammatory optic neuropathy (CRION) [34]. Secondly, we report that MME can be detected and quantified more easily in fundoscopic scanning laser ophthalmoscopy (SLO) images than on OCT B-scans, which has the potential to make screening and analysis of MME in research and clinical routine more robust.

Methods

Patients

Scans acquired between June 2010 and August 2012 archived in the NeuroCure Clinical Research Center's OCT database were screened for MME. The study included 216 MS patients diagnosed according to the revised 2005 McDonald criteria [35], 20 NMO-spectrum disorder (NMOSD) patients diagnosed according to current diagnostic criteria [36,37], 39 patients with clinically isolated syndrome (CIS), as identified by application of the revised McDonald criteria [38], 9 patients diagnosed with CRION and 121 healthy subjects. All NMOSD patients were tested for the presence of antibodies against aquaporin-4 (AQP4) in at least one of several assays, of which 16 tested positive (80%). Some ophthalmologic features of 17 patients from the NMOSD cohort have been previously reported by Schneider et al. [39].

Inclusion criteria were a history of ON or lack of ON clearly confirmed by medical records. In contrast, exclusion criteria were other neurological, ophthalmological and systemic diseases that damage the optic nerve or retina (i.e. glaucoma, diabetes, age-related macular degeneration, epiretinal membranes) or a history of fingolimod treatment, which is suspected to cause macular edema [40,41]. Clinical data, including disease duration and current medical treatment, were compiled as part of a comprehensive neurological examination under the supervision of a board-certified neurologist. MS patients were classified for MS subtypes according to the Lublin criteria [42]. Neurological disability was assessed using EDSS [30] and the Global Multiple Sclerosis Severity Score (MSSS) [43]. Finally, the high-contrast visual acuity of all subjects was quantified using Snellen charts or ETDRS charts [44].

Ethics Statement

The study was approved by the local ethics committee of the Charité – Universitätsmedizin Berlin and was conducted in accordance with the Declaration of Helsinki in its current version, the guidelines of the International Conference on Harmonisation of Good Clinical Practice (ICH-GCP) and applicable German laws. All participants gave informed, written consent.

Optical Coherence Tomography

Peripapillary RNFL thickness (pRNFL) and macular volume scans were performed using a spectral-domain OCT (Heidelberg Spectralis, Heidelberg Engineering, Germany, Heidelberg Eye Explorer viewing module versions 5.2.4.0–5.6.1.0) of each patient or healthy subject's eyes. pRNFL thickness was measured with a 12° circular scan (approx. 3.4 mm diameter) around the optic nerve head obtained using the device's standard protocol and segmentation algorithm with activated eye tracker. Whenever possible, the maximum number of averaging frames in the automatic-real-time mode (ART) was used. Macular volume was measured using a custom protocol that generated 61 vertical slices (B-scans) focusing the fovea, at scanning angle of 30°×25° and a resolution of 768 A-scans per B-scan. As this scan protocol limits averaging frames to 13, the sensitivity should be sufficient to detect MME in B-scans, which might be undetectable at higher ART settings [25]. Total macular volume (TMV) was calculated by estimating the distance between the inner limiting membrane and Bruch's membrane in a 6 mm-diameter cylinder using the OCT software's segmentation algorithm. All scans were acquired by experienced operators and were evaluated for sufficient signal strength, correct centering and segmentation based on the OSCAR-IB criteria by a second operator [45]. In total, 12 eyes of 12 different subjects (5 MS; 1 NMOSD; 6 healthy controls) had no usable OCT scan (neither RNFL circle nor macular volume scan) and were completely excluded from analysis for one of the following reasons: the RNFL scans of two eyes were truncated, one fundus image was weakly illuminated and another RNFL scan was not correctly centered and no eye-tracker had been used. The remaining RNFL and all macular volume scans had not been performed or archived because of lack of patient compliance or, in case of healthy controls, due to time constraints.

To identify eyes with MME, one operator applied the criteria published by Gelfand and colleagues [25] and reviewed all macular scans according to these criteria. MME was defined as clearly limited, insular and cystoid areas of hyporeflectivity in two or more consecutive B-scans. Shadowing in the retinal layers below the cystoid abnormalities was a second but optional criterion for MME inclusion. Blood vessels shadows were excluded as MME criterion.

Furthermore, we investigated the influence of MME and previous ON on the thickness of the inner retinal layers. Retinal layer segmentation was performed for patients with MME (n = 15) or with a previous unilateral ON event (excluding the patients with MME) (n = 75) and for 39 healthy controls. Controls were age- (±3 years) and gender-matched to patients with unilateral ON without MME and were chosen from all controls using the optmatch R package. Here, for each eye, the central B-scan through the fovea and every fourth B-scan in temporal and nasal direction were automatically segmented (Heidelberg Software 1.7.1.0), reviewed and manually revised by two graders to correct any overt segmentation errors. Both graders were blinded as to subject identity and clinical status (ON or eyes without a history of ON (NON)). In addition, graders were not aware which eyes were belonging together but in all cases, one and the same grader corrected both eyes of an individual subject The average layer thicknesses of the macular RNFL (mRNFL), the combined ganglion cell and inner plexiform layer (GCIPL) and the INL were assessed for the area calculated in the earlier TMV estimation. Grader consistency was estimated using 10 randomly selected patient eyes (all MS) each corrected by both graders. Intraclass correlation coefficients were 0.997 for the mRNFL and 0.999 for both the GCIPL and INL.

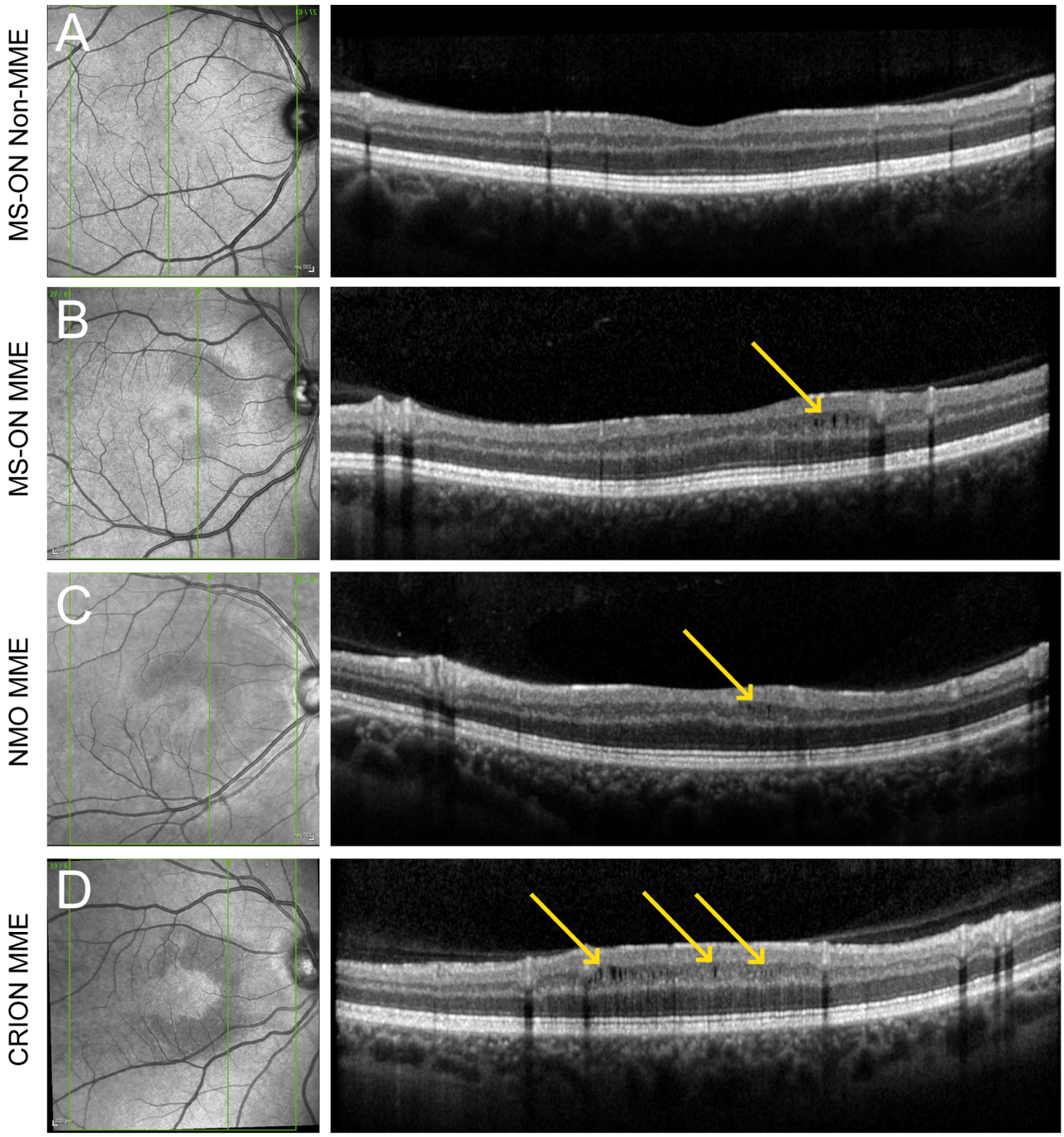

Figure 1. Sample scans showing MME in SLO and OCT images. Sample SLO images (on the left) and sample OCT B-scan images (on the right) from A) MS patient's eye with a history of ON but without MME, B) an MS patient's eye with history of ON and MME, C) an NMO patient's eye with history of ON and MME and D) an eye from a patient with CRION and MME. Whereas the eye from A does not show any signs of MME in either the SLO or OCT B-scan image, all eyes in B-D show similar findings. The SLO images were mirrored where necessary to standardize orientation.
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MME Detection in OCT SLO Images

While screening OCT B-scans for MME the operator (FK) detected distinct changes of the ocular fundus in the SLO-images from eyes diagnosed with MME using the Spectralis built-in confocal SLO (wavelength = 820 nm). In a second step, an independent reviewer (HZ) familiar with the SLO findings from a sample SLO image but blinded to the MME results from OCT scans screened all SLO images from the database. MME was considered present, if the SLO images showed: (i) a darker and dot-like pattern in the macular area compared to the surrounding tissue, and (ii) crescent-like configurations spanning parts of the macula around the fovea (see Results and Figure 1). A third operator (TO) quantified the affected SLO areas using ImageJ (version 1.44). A rigorous blinding procedure was followed to

Table 1. Demographic overview of the study cohort.

	Parameter	MME patients	Non-MME patients	HC
Subjects	Total (N)	15	268	121
	MS (N)	7	209	–
	CIS (N)	0	38	–
	NMO (N)	3	17	–
	CRION (N)	5	4	–
Age (years)	Mean ± SD	40±10	42±11	36±12
	Min – Max	23–54	17–72	20–68
Gender (female/male)	N/N	12/3	171/97	83/38
Disease Duration (months)	Mean ± SD	125±111	83±79	–
	Min – Max	10–317	0–403	–
MS-Subtypes	RRMS (N)	3	163	–
	SPMS (N)	4	31	–
	PPMS (N)	0	15	–

Abbreviations: MME = microcystic macular edema; MS = multiple sclerosis; NMO = neuromyelitis optica spectrum diseases; CRION = chronic relapsing inflammatory optic neuropathy; HC = healthy control; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis; PPMS = primary-progressive multiple sclerosis; CIS = Clinical isolated syndrome; SD = standard deviation; Min = minimum value; Max = maximum value.
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ensure classification by the three operators remained independent. All operators were blinded for age, gender, disease and ON history. All SLO-image evaluators were blinded to the corresponding OCT images and to the result of MME OCT screening.

Statistical Analysis

Comparison of age and EDSS between cohorts was performed using the Mann-Whitney-U test. Gender differences were assessed with Pearson's chi-squared test. Association between SLO area size and visual acuity, time since last ON, pRNFL thickness and TMV were analyzed using generalized estimating equation models (GEE) to account for inter-eye/intra-patient dependencies using

Table 2. Ocular key data of MME and non-MME eyes.

	Parameter	MME eyes	Non-MME eyes [a)]	HC eyes
Number of eyes	Total (N)	22	538	236
	MS (N)	11	416	–
	CIS (N)	0	76	–
	NMO (N)	4	35	–
	CRION (N)	7	11	–
ON prevalence	Total N (%)	21 (95.5)	186 (34.6)	–
	MS N (%)	10 (90.9)	150 (36.1)	–
	CIS N (%)	–	15 (19.7)	–
	NMO N (%)	4 (100)	15 (42.9)	–
	CRION N (%)	7 (100)	6 (54.5)	–
Time since last ON (months)	Mean ± SD	100±100	–	–
	Min – Max	1–304	–	–
Visual acuity	Mean ± SD	0.31±0.38	1.05±0.37	1.17±0.39
	Min – Max	0–1.25	0.01–1.6	0.13–1.6
pRNFL thickness (μm)	Mean ± SD	51.6±12.5	87.5±15.65	98.4±8.49
	Min – Max	31.8–78.2	29.1–129.9	79.9–120.0
TMV (mm^3)	Mean ± SD	8.00±0.41	8.40±0.44	8.67±0.33
	Min – Max	7.23–8.78	7.06–9.37	7.72–9.47

[a)]The contralateral eyes of unilateral MME eyes were excluded.
Abbreviations: MME = microcystic macular edema; MS = multiple sclerosis; NMO = neuromyelitis optica spectrum diseases; CRION = chronic relapsing inflammatory optic neuropathy; ON = optic neuritis; pRNFL = peripapillary retinal nerve fiber layer; TMV = total macular volume; SD = standard deviation; Min = minimum value; Max = maximum value.
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Table 3. Disease severity in MS patients.

		MME MS patients	Non-MME MS-ON patients	P-value
Subjects	N	7	100	
MS subtype	RRMS (N)/SPMS (N)	3/4	87/13	0.012
EDSS	Median	5	2.25	0.002
	Min - Max	2.5–6.5	0–6.5	
Disease duration (months)	Mean ± SD	172.14±109.44,	114.38±83.59,	0.174
	Min - Max	45–303	2–403	
MSSS (mean +- SD, min - max)	Mean ± SD	5.99±1.88	3.81±2.06	0.011
	Min - Max	3.69 8.83	0.22–8.3	

Abbreviations: MME = microcystic macular edema; MS = multiple sclerosis ON = optic neuritis; SD = standard deviation; Min = minimum value; Max = maximum value; EDSS = Expanded Disability Status Scale; MSSS = Multiple Sclerosis Severity Score.
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the working correlation matrix "exchangeable". In all GEE models, the SLO area was set as dependent variable. Likewise, group differences in visual acuity and OCT results were calculated using GEE models. Provided parameters from GEE models are effect size (B), standard error (SE) and significance (P). Frequency of MME occurrence was compared between the cohorts using Fisher's exact tests. Comparison of intra-patient/inter-eye differences was performed using the two-sided paired Wilcoxon signed rank test. Correlation of mRNFL and GCIPL differences to the INL differences was analyzed using linear regression models. Statistical tests were performed with IBM SPSS 20 (IBM, Armonk, NY, USA) or R (basic package ver. 2.15.2, including the packages: geepack (ver. 1.1.6), ggplot2 (ver. 0.9.3) and optmatch (ver. 0.8.1)). Statistical significance was established at $P<0.05$. As ours was an exploratory study, no power calculation had previously been performed, and the disorders of all included groups are related and their features therefore likely correlate, no correction for multiple comparisons was performed.

Results

Based on the criteria defined by Gelfand and colleagues [25], we identified MME in 22 eyes from 15 patients. Of these patients, 5 had been diagnosed with CRION, 3 with NMOSD and 7 with MS. No MME was observed in healthy controls and the screened CIS patients. A demographic overview of the study cohort is presented in Table 1. MME appeared bilaterally in 7 of the 15 patients and was located exclusively in the inner nuclear layer of the retina, between 925 µm and 2,200 µm (mean ± SD: 1,602±351 µm) distal to the foveal center. The maximum cyst diameter was 81 µm (mean ± SD: 45±14 µm).

MME is Associated with ON

A prior history of ON was found in all MME-affected patient eyes, with one exception (95.5%). In the latter case, the patient had been diagnosed with secondary progressive MS and reported experiencing visual disturbances in the eye during the 1980s. The most recent documented VEP from this eye had a latency of 116 ms. Eyes with MME showed reduced visual acuity ($P=0.010$), along with decreased pRNFL thickness ($P<0.001$) and TMV ($P<0.001$), compared to eyes unaffected by MME. A summary of the key ocular data of the MME and non-MME eyes, including ON prevalence, visual acuity, pRNFL thickness and TMV measures, is given in Table 2.

Frequency of MME in ON Eyes

In MS patients, 6.3% of all eyes with a history of ON showed MME, compared to 21.0% of all NMOSD patients' eyes with a history of ON. MME prevalence was highest in CRION patients at 53.8% of all eyes of patients with a history of ON. The prevalence of MME was significantly different between MS and CRION patients ($P<0.001$), but not between MS and NMOSD patients ($P=0.068$), or between NMOSD and CRION patients ($P=0.295$).

ON Eyes with MME are More Severely Affected

In eyes with a history of ON, MME-affected eyes showed significantly reduced pRNFL thickness (mean ± SD: 77.5±15.8 µm, $P<0.001$) and visual acuity (mean ± SD: 1.0±0.39, $P=0.049$) and non-significantly reduced TMV (mean ± SD: 8.15±0.43 mm^3, $P=0.155$), compared to eyes unaffected by MME.

Eyes with pRNFL below the first quartile of all ON eyes ($Q_1 = 64.6$ µm) were categorized as having a severe history of ON, which was a strong predictor for the development of MME (odds ratio = 13.6, 95% CI = 4.7–39.7).

Disease Severity in MS-ON Patients with and without MME

MS patients with MME were later in the disease course with a higher rate of secondary progressive patients ($P=0.01$) and had significantly higher EDSS and MSSS scores (Table 3), compared to MS patients with a history of ON but no MME.

Comparison of Retinal Layer Thickness between Patient Eyes with MME, Unilateral History of Optic Neuritis and Healthy Controls

Based on medical records, 75 patients (11 CIS, 44 RRMS, 9 SPMS, 4 CRION, 7 NMOSD) had previously experienced an unilateral ON event (excluding patients with present MME). The mean age of this group was 38.9 years (SD = 10.5 years), which was not significantly different from the matching controls (mean = 38.1 years; SD = 11.1 years; $P=0.639$). The gender composition in patients (47 female, 28 male) and controls (24 female, 15 male) did also not differ between both cohorts ($P=0.999$). The results of the intra-retinal layer segmentation of patients with unilateral ON, patients with MME and the matching controls are shown in Table 4. Unsurprisingly, the ON-affected eyes showed reduced mRNFL and GCIPL thickness compared to the unaffected contralateral eyes (both $P<0.001$). INL thickness

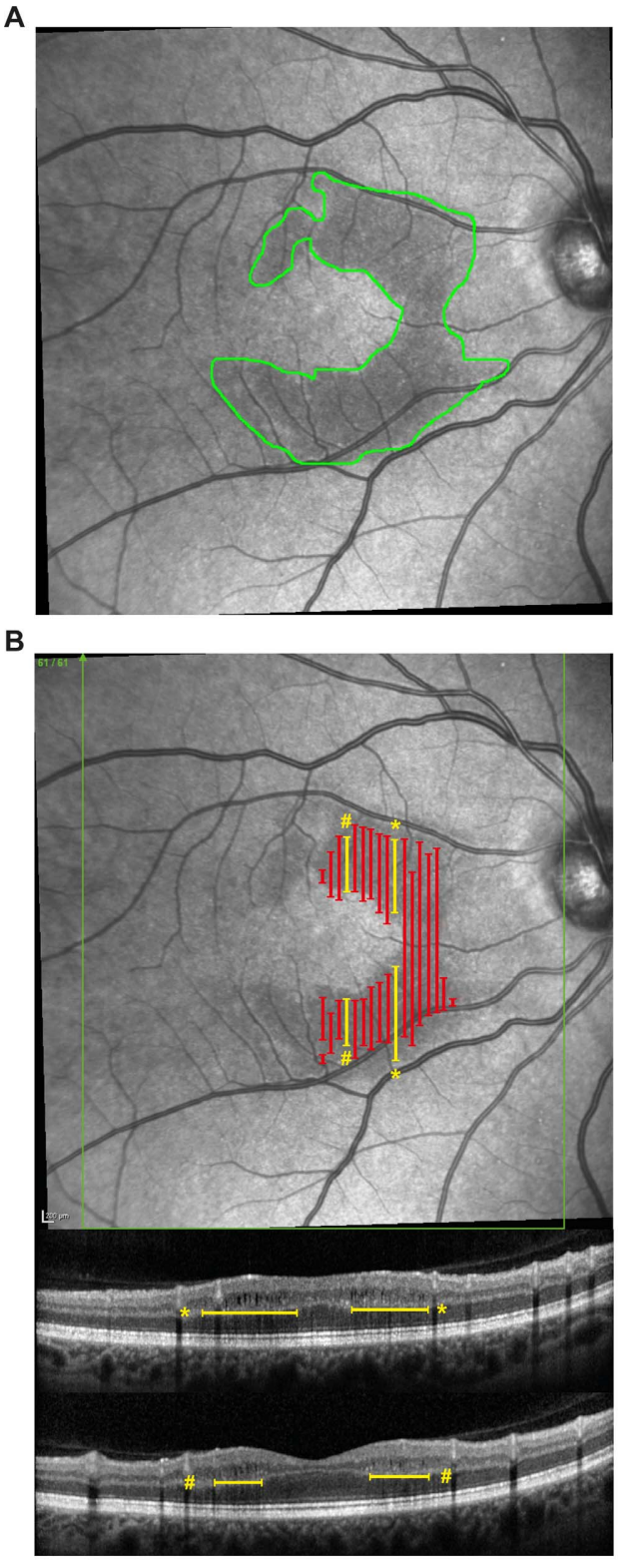

Figure 2. Inter-eye differences in patients with unilateral history of optic neuritis. A) Differences in inner nuclear layer (INL) thickness between affected and unaffected eyes of patients with a unilateral history of optic neuritis categorized by diagnosis. Eyes belonging to one patient are connected by lines. Lines in blue indicate eyes, which the INL of the optic neuritis eyes was thicker compared to the contralateral unaffected eyes, whereas red lines show the contrary. B) Correlation of inter-eye INL thickness differences with inter-eye macular retinal nerve fiber layer (mRNFL) thickness differences (LR: $P<0.001$). C) Correlation of inter-eye INL thickness differences with inter-eye ganglion cell and inner plexiform layer (GCIPL) thickness differences (LR: $P=0.011$).
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was increased in ON eyes without MME compared to contralateral NON eyes ($P<0.001$). We did not perform statistical testing of all disease cohorts separately, due to the relatively small sample size in some groups, but Figure 2A represents that – with some exceptions – ON eyes showed INL thickening in patients across all disease sub-groups. The intra-patient/inter-eye differences in INL were inversely correlated to mRNFL ($R^2=0.0863$; $P<0.001$, linear regression, Figure 2B) and GCIPL inter-eye differences ($R^2=0.0431$; $P=0.011$, Figure 2C).

Eyes with MME showed a more severe reduction in mRNFL and GCIPL thickness compared to controls and also to non-MME eyes with a previous unilateral ON, while the INL thickness was increased in patients with MME-affected eyes compared to controls and eyes with a history of unilateral ON (all $P<0.001$, Table 4).

Characteristics of MME in OCT SLO Images

The SLO images showed dark, dotted patterns in the ocular fundus in all 22 MME-affected eyes. These generally presented in a crescent shape around the fovea, which was itself recessed. Sample SLO images and their corresponding B-scans are shown in Figure 1.

To test the sensitivity and specificity of using these SLO changes to diagnose MME compared to using OCT B-scans, a second experienced operator screened all SLO images in a blinded fashion and was able to correctly classify all MME-affected eyes originally identified by OCT B-scans with no false negatives (sensitivity = 100%). An additional 27 eyes (from 17 subjects) not identified as potential MME eyes using OCT B-scans were flagged in the SLO analysis (specificity = 95.2%). Three of these eyes showed suspicious MME structures, but did not fulfill the criterion of visible microcysts on two adjacent B-scans.

MME Size Correlates with Visual Acuity

Alterations found in SLO images featured mostly clear contours that allowed us to quantify their area, which ranged from 1.7 to 11.1 mm^2 (mean ± SD: 5.9±2.3 mm^2; Figure 3A). Figure 3B maps the corresponding points of microcysts in OCT B-scans and SLO images. In GEE models, time since last ON in months ($B=-0.1$, $SE=0.04$, $P=0.025$) was a significant, inverse predictor for the size of MME-affected area shown in the SLO image. In other words, the shorter the time between the ON event and the OCT scan the larger the MME-affected area shown in the SLO image. Secondly, the size of the MME-affected area shown by SLO was in turn inversely correlated with visual acuity ($B=-2.2$, $SE=0.9$, $P=0.012$). In contrast, there was no correlation between SLO area and pRNFL thickness ($P=0.943$) or TMV ($P=0.233$).

Discussion

In this study we investigated the occurrence of INL changes including MME in neuro-inflammatory diseases associated with optic neuritis. Our main findings are: a) MME was almost exclusively limited to patients with a history of ON, regardless of the underlying nosologic entity, and – with the exception of one case – only occurred in eyes previously affected by ON; b) eyes without MME but with previous ON displayed INL thickening in

Table 4. Results of the retinal segmentation for patients with a history of unilateral ON, patients' eyes with MME and controls.

	HC eyes	MME eyes	Non-ON eyes	ON eyes	ON vs. NON eyes P value (paired Wilcoxon)	MME vs. HC eyes P value (GEE)	MME vs. ON eyes P value (GEE)
Number of eyes	78	22	75	75			
Mean mRNFL thickness (SD) [in μm]	38.4 (3.8)	22.6 (2.5)	35.4 (5.1)	29.4 (5.8)	<0.001	<0.001	<0.001
Mean GCIPL thickness (SD) [in μm]	70.4 (5.5)	48.1 (5.7)	66.0 (6.9)	57.1 (9.0)	<0.001	<0.001	<0.001
Mean INL thickness (SD) [in μm]	33.0 (2.5)	39.0 (3.2)	33.9 (2.4)	34.5 (2.6)	<0.001	<0.001	<0.001

Abbreviations: MME = microcystic macular edema; ON = optic neuritis; mRNFL = macular retinal nerve fiber layer; GCIPL = ganglion cell and inner plexiform layer; INL = inner nuclear layer; SD = standard deviation; GEE = generalized estimation equation.
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comparison to contralateral eyes without a history of ON; c) MME was easily detected and quantified using fundoscopic SLO images; d) the size of the affected MME-area in SLO images correlated inversely with the length of time since the last ON event and with visual acuity.

Previous studies investigating MME in MS patients have found higher ON frequencies in MME eyes compared to unaffected eyes [25,26]. For example, in one study, 50% of MME-affected eyes of MS patients had a history of ON, while two studies found past history of ON in 100% of the MME-affected NMO eyes [27,28]. In line with these findings, our data showed that almost all MME eyes had a history of ON, not only in NMO but also in MS and CRION patients. Overall, MME was more common in NMO and CRION than MS patients. This strongly points to a pathophysiological correlation between the development of MME and the extent of damage to the optic nerve, which would suggest that MME may result from ocular inflammation during or following ON, irrespective of the underlying nosologic entity. The finding that eyes with more severe optic neuritis were more likely to exhibit MME could indicate that the latter is an extreme manifestation of INL pathology, found in milder form as INL thickening in the majority of ON eyes. The fact that all previous studies have reported significantly reduced RNFL for MME-affected eyes, which is a common feature of ON-affected eyes, lends weight to our hypothesis [14]. Notably in this context, the only MME-affected eye in our study without a reported previous clinical ON event was that of an MS patient with long disease duration and a secondary progressive course. However, in this case, a previously recorded prolonged VEP latency and reports of visual disturbances in the past also suggest a subclinical ON event.

Importantly, previous studies did not detect general INL thickening in ON eyes from MS or NMO patients in comparison to healthy controls or non-ON eyes [26,27], probably due to the higher inter-individual variation compared to the effect size. One study found INL thinning in primary progressive MS patients compared to healthy controls, but not in other MS types [46]. Pairwise comparison of eyes from patients with only unilateral ON and no present MME consistently showed INL thickening in the eye with previous ON compared to its ON-unaffected counterpart, and was also true for MS, CRION and NMOSD patients alike. In MS, INL thickening has been previously reported to be correlated with higher inflammatory activity and more severe disease progression [26]. Whether the former was mainly a result on the more aggressive inflammatory activity in these patients, as suggested by Saidha et al. (i.e. MS patients with higher inflammatory activity could also have more clinical or subclinical ON episodes or other ocular inflammation, such as uveitis or periphlebitis that could be mistaken for optic neuritis) or represents an additional pathology requires further investigation. In our study, thinner INL in eyes with a history of ON compared to the contralateral eyes was only found in a few cases. Although a clear explanation for these exceptions is not evident from our data, but one reason might be that subclinical ON events not documented or reported by the patient had skewed our analysis.

Histopathological data on retinal pathology in MS have been provided in a comprehensive work by Green et al. [29]. Here, the INL was identified as a prominent site of reduced neuronal cell count, inflammation and microglial activation, presumably giving rise to development of microcystic edema. However, histopathological findings of reduced neuronal cell count may not necessarily translate into reduced thickness *in vivo*, since histopathology allows only quantification of cell counts, but not of tissue layer thicknesses. The latter parameter is usually lost or altered during the fixation process. It can be assumed that specific layer properties of the INL, including the presence of bipolar cells, promote loosening of intra-layer adhesion and in some cases even cause microcystic alterations or edematous changes, such as described by Gelfand and colleagues [25]. Whether the observed INL alterations are induced by bipolar cells, Müller cells, horizontal cells, amacrine cells and/or changes of the extracellular matrix, remains to be clarified. Of the latter, Müller cells indeed play a crucial role, as their dendrites are in contact with all other retinal layers and mediate the protective or detrimental processes of other retinal cells [47]. Overall, further studies are required to bridge the gap between post-mortem analyses of human retinal tissue and *in vivo* OCT.

The potential inflammatory etiology of INL changes posited by the paper of Gelfand et. al. [25] was challenged in two reply letters which reported retinal microcystic changes in non-inflammatory diseases affecting the optic nerve, leading the authors to propose alternative underlying pathomechanisms. Abegg et al. discussed MME as a possible consequence of optic nerve degeneration in optic nerve glioma [33], whereas Barboni et al. proposed vitreous traction, resulting in a schesis stretching the INL in Leber's hereditary optic neuropathy [32]. Both claimed that the resulting findings were identical to those reported for MS and NMO and would thus argue against disease specificity. Indeed, the included imaging data exhibited striking similarities to MME in MS, NMO and CRION. However, the small sample size and anecdotal nature of the two case reports presented in the letters hampers assessment of the proposed hypotheses [48]. Larger studies are needed to determine whether the described findings are indeed

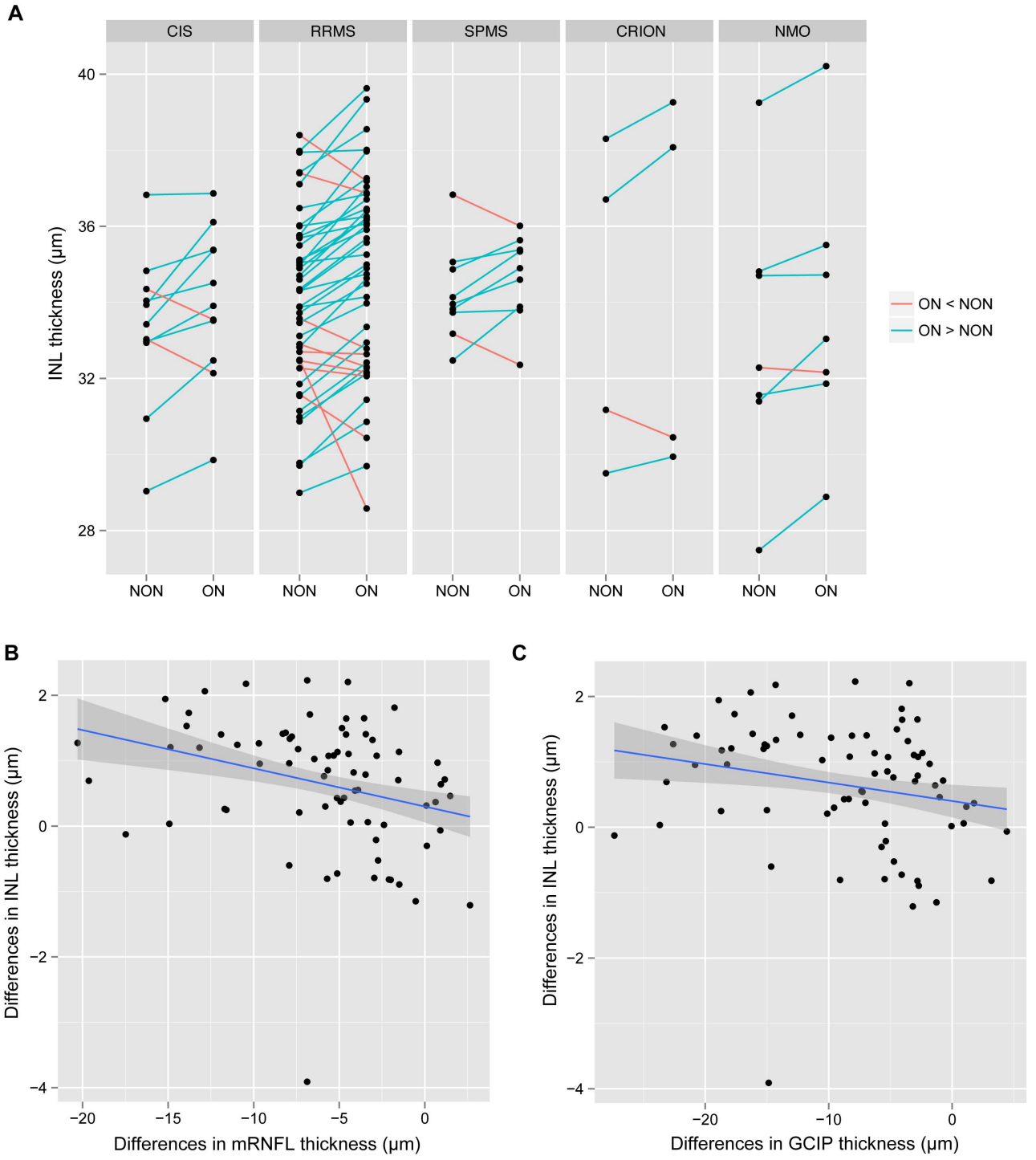

Figure 3. Sample quantification of SLO MME area. A) Quantification of the MME-affected area of a sample eye in a SLO image with ImageJ. B) The microcysts in OCT B-scans of the same sample eye mapped onto the SLO image. The yellow lines correspond to the spread of the macular edema in the two example B-scans at the bottom.
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based on same mechanisms as in MS and related inflammatory diseases or are rather merely similar symptoms with different etiologies. However, histopathological data on reduced neuronal cell counts in the INL in MS [29] and our findings of INL thickening in ON eyes strongly suggest that INL changes contribute significantly to retinal pathology in optic neuritis-associated diseases. Furthermore, INL changes were recently demonstrated in a rodent model of MS in the form of microcystic disruption or repair-associated loosening of the INL layer, which

supports the hypothesis of increased INL thickness in relation to optic nerve inflammation [49].

We could identify MME with high sensitivity and specificity using SLO images, a technique previously reported as promising in a case report [33]. In a blinded analysis, every MME detected on a B-scan had a clearly visible equivalent on the corresponding SLO image. Based on these findings, SLO image analysis may be superior to the more time-consuming inspection of a high number of B-scans for MME detection in daily routine. The fact that more eyes were classified as MME-affected in the blinded SLO analysis than in B-scan detection could be explained by the dynamics of microcystic changes, which might be different from the alterations found on OCT B-scans. Ultimately, with no gold standard means of confirmation (i.e. by histology), our study cannot determine whether the additional positive SLO scans were false positives or the corresponding OCT B-scans were false negatives.

The size of MME, as quantified by SLO images analysis, was inversely correlated with the length of time between prior ON and OCT examination: the shorter the interval between the clinical event of ON and OCT scan, the larger the MME-affected surface in the SLO image. Secondly, eyes with low visual acuity showed larger textural changes in SLO images than eyes with high visual acuity in patients with MME. Finally, MME-affected eyes showed significantly stronger RNFL thinning and poorer visual acuity than MME-unaffected eyes with a history of ON. These findings support the hypothesis that MME is a result of severe optic neuritis events and may contribute to a poor visual outcome following optic neuritis. However, given the cross-sectional design of our study and the in some cases long intervals between previous clinical ON, as documented or reported by patients, and MME diagnosis, these results should be interpreted with caution. Temporal dynamics of MME subsequent to ON will have to be addressed in longitudinal studies with frequent OCT scans following the clinical event.

Some important caveats deserve mention. Like the work by Gelfand et al. and Saidha et al., our study was a retrospective analysis, including assessment of previous ON events without the benefit of first-hand observation. While carries a theoretical risk of misclassification the high accordance of our findings with those in previous MME studies suggests methodological bias in our study is probably limited. Moreover, we examined a rather small cohort of CRION and NMOSD patients, which produced a very high rate of MME in these entities (56% and 15%, respectively) compared to MME prevalence in MS. The small number of these patients in our study reflect the relatively low prevalence of these diseases [34,50], but cohort size should be increased in further studies. Although the prevalence of MME varied between the three conditions, group comparisons should be interpreted with caution due to the relatively low sample size in the NMOSD and CRION groups and the overall low frequency of MME. Moreover, we cannot exclude the possibility that some of the CRION cases may evolve into an MS or NMOSD diagnosis with a longer duration of observation. Furthermore, the quantification of MME-affected areas in SLO images includes a potential grader bias. Further verification is required to establish the MME-affected area in SLO images as an accepted marker for the degree of retinal damage caused by MME.

In summary, we show that INL thickening and MME occur in various neuro-inflammatory disorders and are strongly linked to ON events and decreased visual acuity. We also provide data that identification and quantification of MME using SLO images generated by the OCT device is easier than using the established technique of B-scans, and may lend itself to clinical application in the future. In the final instance, the causative role of ON for MME and the dynamics of INL thickening and MME formation can only be elucidated in a longitudinal study on patients with acute optic neuritis.

Author Contributions

Conceived and designed the experiments: TO AUB FP. Performed the experiments: FK HZ TO ES. Analyzed the data: FK HZ TO ES. Contributed reagents/materials/analysis tools: KR FP. Wrote the paper: FK TO AUB FP.

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Die Publikation

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Publikationsliste

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* : geteilte Autorenschaft

Konferenzvorträge

1. **Oberwahrenbrock T**, Ringelstein M, Young KL, Kaufhold F, Zimmermann, H, Keser N, Hartung H-P, Martin R, Aktas O, Schippling S, Paul F, Brandt AU. Pattern of retinal alteration in different types of multiple sclerosis measured by high-resolution optical coherence tomography. In: Amsterdam, The Netherlands: Oral presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2011.

Konferenzposter

1. Zimmermann H, **Oberwahrenbrock T**, Pfueller CF, Gaede G, Bohn E, Paul F, Brandt AU. Daytime dependent retest-reliability of retinal nerve fibre layer thickness. In: Gothenburg, Sweden: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2010.

2. Brandt AU, Mohr C, Freing A, Weinges-Evers N, **Oberwahrenbrock T**, Pfueller C, Doerr JM, Wuerfel JT, Paul F. The Faces Symbol Test Shows Higher Correlation to Loss of Cortical Grey Matter Than Paced Auditory Serial Addition Test and Symbol Digit Modalities Test in Multiple Sclerosis. In: Honolulu, Hawaii: Poster presentation at the annual meeting of the American Academy of Neurology (AAN); 2011.

3. Brandt AU, Paul F, Freing A, **Oberwahrenbrock T**, Petzold A. Serum neurofilament in patients with relapsing-remitting multiple sclerosis. In: Amsterdam, The Netherlands: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2011.

4. Brandt AU, Pfueller C, Freing A, Doerr JM, **Oberwahrenbrock T**, Gaede G, Bellmann-Strobl J, Wuerfel JT, Paul F. Association of Retinal Nerve Fiber Layer Thickness, Macular Volume and Brain Atrophy in Secondary Progressive Multiple Sclerosis. In: Amsterdam, The Netherlands: Poster presentation at the annual meeting of the American Academy of Neurology (AAN); 2011.

5. Stricker S, **Oberwahrenbrock T**, Freing A, Rinnenthal J-L, Zimmermann H, Al-Sadoon B, Wuerfel J, Brandt AU, Friedemann P. Spinocerebellar Ataxia Type 1: Neurodegeneration beyond Cerebellum and Brainstem. In: Honolulu, Hawaii: Poster presentation at the annual meeting of the American Academy of Neurology (AAN); 2011.

6. Zimmermann H, Freing A, **Oberwahrenbrock T**, Gaede G, Kaufhold F, Schippling S, Paul F, Brandt AU. Association of retinal nerve fibre layer and optic nerve head in spectral-domain optical coherence tomography with brain atrophy in multiple sclerosis. In: Amsterdam, The Netherlands: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2011.

7. Kadas EM, Bilger F, Ludin L, **Oberwahrenbrock T**, Brandt AU, Paul F. Quantification of retinal vessels in multiple sclerosis. In: Lyon, France: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2012.

8. **Oberwahrenbrock T**, Gabilondo I, Nolan R, Songster C, Traber GL, Balk L, Pau F, Petzold A, Schippling S, Villoslada P, Brandt AU, Green A. Multicentre reliability of intra-retinal layer segmentation. In: Lyon, France: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2012.

9. **Oberwahrenbrock T**, Ringelstein M, Jentschke S, Schippling S, Deuschle K, Bellmann-Strobl J, Hartung H-P, Ruprecht K, Paul F, Aktas O, Brandt AU. Spatial Profile Analysis Detects Early Retinal Nerve Fiber and Ganglion Cell Layer Reduction in Patients with Clinically Isolated Syndrome. In: New Orleans, USA: Highlight Session and Poster presentation at the annual meeting of the American Academy of Neurology (AAN); 2012.

10. Schneider E, Zimmermann H, **Oberwahrenbrock T**, Brandt AU, Paul F. Differences in optic neuritis related retinal damage between patients with neuromyelitis optica and multiple sclerosis. In: Lyon, France: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2012.

11. Kaufhold F, Zimmermann H, Schneider E, Paul F, **Oberwahrenbrock T**, Brandt AU. Microcystic macular edema is associated with optic neuritis independently of multiple sclerosis. In: San Diego, USA: Poster presentation at the annual meeting of the American Academy of Neurology (AAN); 2013.

12. **Oberwahrenbrock T**, Kaufhold F, Zimmermann H, Schneider E, Ruprecht K, Paul F, Brandt AU. Inter-eye intraretinal layer thickness differences in patients with a history of unilateral optic neuritis. In: Copenhagen, Denmark: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2013.

13. **Oberwahrenbrock T**, Traber GL, Gabilondo I, Nolan R, Songster C, Balk L, Paul F, Petzold A, Villoslada P, Brandt AU, Green A, Schippling S. Multicentre

inter-rater reliability of retinal layer segmentation using spectral-domain optical coherence tomorgraphy (OCT). In: Copenhagen, Denmark: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2013.

14. Pfueller CF, Klumbies K, Freing A, **Oberwahrenbrock T**, Zimmermann H, Papazoglou S, Rinnenthal J-L, Wuerfel J, Paul F, Dörr J, Brandt AU. Retinal Inner Nuclear Layer Reflects Disease Severity and Grey Matter Atrophy in Secondary Progressive Multiple Sclerosis. In: San Diego, USA: Data Blitz session and poster presentation at the annual meeting of the American Academy of Neurology (AAN); 2013.

15. Sinnecker T, **Oberwahrenbrock T**, Metz I, Zimmermann H, Pfueller CF, Harms L, Ruprecht K, Hahn K, Brück W, Niendorf T, Paul F, Brandt AU, Dörr J, Wuerfel J. Axonal loss and trans-synaptic degeneration of the visual system in multiple sclerosis: a combined 7 Tesla MRI and optical coherence tomography (OCT) study. In: Copenhagen, Denmark: Poster presentation at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); 2013.

16. Traber GL, **Oberwahrenbrock T**, Gabilondo I, Nolan R, Songster C, Balk L, Paul F, Petzold A, Villoslada P, Brandt AU, Green A, Schippling S. Multicenter Inter-rater Reliability of Retinal Layer Segmentation Using Spectral-domain OCT. In: Utah, USA: Poster presentation at the annual meeting of the North American Neuro-Ophthalmology Society (NANOS); 2013.

17. **Oberwahrenbrock T**, Kadas EM, Lagrèze WA, Paul F, Brandt AU. Dynamic Formation of Macular Microcysts Independent of Vitreous Traction Changes. In: Philadelphia, PA, USA: Poster presentation at the annual meeting of the American Academy of Neurology (AAN); 2014.