Aus dem Experimental and Clinical Research Center
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DISSERTATION

Retinale Schichtdickenanalyse bei Multipler Sklerose *in vivo*: Anwendung im frühen Stadium und in einer klinischen Studie mit chronisch-progredientem Verlauf

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1. Zusammenfassung

1.1. Abstrakt

Die Multiple Sklerose (MS) ist eine Autoimmunerkrankung des zentralen Nervensystems (ZNS), welche sich durch entzündliche als auch neurodegenerative Prozesse auszeichnet. Typischerweise sind Frauen etwa dreimal so häufig betroffen wie Männer und erkranken im Schnitt zwischen dem 20. und 40. Lebensjahr. Die Ursache der Erkrankung ist weiterhin unbekannt. Diskutiert werden genetische und Umweltfaktoren. Klinisch kann sich die MS vielfältig präsentieren: jedes System des ZNS kann betroffen sein. Häufig kommt es zur Erstmanifestation im afferenten visuellen System mit Ausbildung einer einseitigen Entzündung des Sehnervs (sog. Optikusneuritis). In der Retina kann mit Hilfe der optischen Kohärenztomographie (OCT) Neurodegeneration des afferenten visuellen Systems quantifiziert werden.

Ziel der vorliegenden Arbeit war es, 1) retinale Veränderungen im frühen MS-Stadium zu untersuchen, 2) den Behandlungseffekt von grünem Tee, dessen Hauptbestandteil das Polyphenol Epigallocatechingallat (EGCG)/Sunphenon® ist, bei MS-Patient*innen mit progredienter Verlaufsform (PMS) mittels retinalem OCT als sekundäre Outcome-Parameter einer interventionellen Studie zu untersuchen. Zu 1) wurde in einer Kohorte von 45 Patient*innen mit klinisch-isoliertem Syndrom (CIS) gezeigt, dass es auch in Augen ohne abgelaufene Optikusneuritis zu einer signifikanten Abnahme der kombinierten Ganglienzell- und innerplexiformen Schicht (GCIP) kommt. Zu 2) ließ sich in der Analyse von 31 PMS-Patient*innen kein Therapieeffekt von EGCG nachweisen. Dies bestätigt zwar die Ergebnisse der primären Outcome-Parameter, allerdings war unsere Studie nicht ausreichend gepowert, um Behandlungseffekte zu zeigen. Die Arbeit zeigt zum einen, dass es schon sehr früh im Erkrankungsstadium zu Neurodegeneration des visuellen Systems kommt, und bekräftigt zum anderen den Einsatz von OCT in klinischen Studien mit höheren Fallzahlen, insbesondere bei PMS-Patient*innen.

1.2. Abstract

Multiple Sclerosis (MS) is an autoimmune inflammatory and degenerative central nervous system (CNS) disease, affecting around three times more women than men. The onset of the disease is usually in the twenties or thirties. The etiology of MS

remains unclear. Hereditary and environmental factors are assumed to play an important role in disease development. Clinically MS is presenting in various neurological manifestations, often starting in the visual system with an episode of optic neuritis (ON). Neurodegenerative visual system alterations can be examined via retinal optical coherence tomography (OCT).

The objective of this work was to examine 1) retinal alterations via OCT in a subset of early MS patients (clinically isolated syndrome, CIS cohort), 2) the impact of green tea, which is mainly comprising of epigallocatechin gallate (EGCG)/Sunphenon® on patients with progressive forms of MS (PMS) via OCT as secondary outcome analysis of an interventional study (SUPREMES cohort). 1) The OCT examination in our CIS cohort of 45 patients revealed a significant decrease of GCIP layer even in absence of previous optic neuritis. 2) The OCT analysis of 31 PMS patients showed no significant treatment effect of EGCG. While this is in line with the main outcome parameters, our study was probably underpowered to evaluate an effect. The presenting work shows that neurodegeneration of the visual system may be present from the beginning of the disease. This work also confirms OCT measurements as valuable outcome parameters in further clinical studies with higher sample size, especially in PMS cohorts.

1.3. Einführung

1.3.1. Das Krankheitsbild der Multiplen Sklerose

Der Begriff „Multiple Sklerose“ (MS) wurde im Jahre 1868 von Jean-Martin Charcot geprägt, der diese chronisch-entzündliche demyelinisierende ZNS-Erkrankung neben dem klinischen Erscheinungsbild auch pathologisch untersuchte [1]. Als „sclerose en plaques“ bezeichnete er das multiple Vorkommen sklerosierender Herde nahe den Hirnventrikeln und im Hirnstamm sowie den histologisch nachweisbaren Verlust der Markscheiden betroffener Areale. Es werden verschiedene Verlaufsformen der MS beschrieben: das klinisch isolierte Syndrom (clinically isolated syndrome, CIS), das radiologisch isolierte Syndrom (radiologically isolated syndrome, RIS), die schubförmig-remittierende Verlaufsform (relapsing-remitting MS, RRMS) sowie die sekundär (secondary progressive MS, SPMS) und primär progrediente Verlaufsform (primary progressive MS, PPMS). Das klinisch isolierte Syndrom ist die Erstmanifestation einer möglichen Multiplen Sklerose, die erstmalig mit einem neurologischen Defizit auffällig wird, jedoch die Diagnosekriterien der MS (noch) nicht erfüllt [2]. Das radiologisch isolierte Syndrom beschreibt Auffälligkeiten in der Magnetresonanztomographie (MRT) einer klinisch asymptomatischen Person, die mit einer Multiplen Sklerose vereinbar sind [3]. Die schubförmig-remittierende Verlaufsform (RRMS) zeichnet sich dadurch aus, dass sich ein Schub wieder vollständig oder teilweise zurückbildet und Phasen der Symptomfreiheit bestehen. Diese Verlaufsform besteht bei 85% der Patient*innen [4,5]. Die sekundär progrediente Form (SPMS) ist dadurch gekennzeichnet, dass zunächst eine RRMS besteht, wobei sich nach einiger Zeit (meist erst nach Jahren), die Schübe nicht mehr zurückbilden und Krankheitssymptome bleiben und durch weitere Schübe akkumulieren [6,7]. Bei der primär progredienten Verlaufsform ist seit Beginn der Symptome keine vollständige Rückbildung der Symptome zu verzeichnen. Im Vergleich zur RRMS sind hierbei vermehrt Männer betroffen in höherem Lebensalter (Mittelwert 40 Jahre vs. 30 Jahre bei RRMS). Diese Form betrifft 15% der Patient*innen [8,9].

Als Erstes sind insbesondere das visuelle und das sensible System betroffen. Jedoch können neurologische Defizite in allen Systemen auftreten [10]. Die Neuritis nervi optici (=Optikusneuritis, ON) beschreibt eine Entzündung des Sehnervs, wobei bei der MS meist der retrobulbäre Anteil betroffen ist, die sog. Retrobulbärneuritis. Diese ist meist einseitig und führt zu einer Visusminderung, zu Gesichtsfeldausfällen im Sinne eines Zentralskotoms, zu einer Farbsinnstörung und zu retrobulbären Schmerzen

insbesondere bei Augenbewegungen. Während des Krankheitsverlaufs kommt es bei 50-70% der Patient*innen zu einer akuten Optikusneuritis [11,12].

1.3.2. Optische Kohärenztomographie und intraretinale Segmentierung

Mit Hilfe der optischen Kohärenztomographie (optical coherence tomography, OCT) ist es möglich, die retinalen Schichten bei MS-Patient*innen *in vivo* darzustellen und für wissenschaftliche Zwecke auszuwerten [13]. Die ersten OCT-Messungen von MS-Patient*innen wurden im Jahre 1999 erhoben [14]. Damals wurden diese noch mit dem langsamen time domain (TD-) OCT durchgeführt. In den neueren und schnelleren spectral domain (SD-) OCT-Geräten ist der Hauptvorteil die halbautomatische intraretinale Segmentierung aufgrund höherer Auflösung. In der MS kommen vor allem peripapilläre Ringscans und Volumenscans der Makula zum Einsatz. Ersterer dient der Quantifizierung der peripapillären retinalen Nervenfaserschicht (pRNFL), bestehend aus den unmyelinisierten Axonen der Ganglienzellen, während die Ganglienzellschicht (GCL) die Zellkörper enthält. Die weiteren Schichten der Retina werden aus dem makulären Volumenscan segmentiert, da dort die Dichte der Ganglienzellen am höchsten ist. Die GCL wird als kombinierte Ganglienzell- und innerplexiforme Schicht (GCIP) zusammengefasst, da sich beide Schichten im OCT durch ähnliche Kontrastierung schlecht unterscheiden lassen. Eine Abnahme der pRNFL und GCIP wird mit einer neuroaxonalen Atrophie durch retrograde Neurodegeneration in Verbindung gebracht. Eine Verdünnung dieser beiden Schichten hat sich in diversen OCT-Studien als Marker für Krankheitsaktivität und neuroaxonalen Schaden erwiesen [15–17]. Die innere Körnerzellschicht (inner nuclear layer, INL), welche die Zellkörper des 2. Neurons (bipolare Zellen) sowie Gliazellen (Müller-Zellen), amakrine und Horizontalzellen enthält, hat sich als Marker für entzündliche Veränderungen an der Retina gezeigt. Dabei spielt der Nachweis von Ödemen in der Makula (microcystic macular edema, MME) eine große Rolle [18,19]. Es konnte gezeigt werden, dass die erhöhte Schichtdicke der INL mit dem Auftreten von Optikusneuritiden und der Schubrate korreliert [20]. Demgegenüber zeigt die verminderte bzw. normalisierte Schichtdicke der INL ein Ansprechen auf die verlaufsmodifizierende Therapie (disease-modifying therapy, DMT) bei RRMS-Patient*innen [21] an. Die anderen retinalen Schichten sind von untergeordneter Bedeutung.

1.3.3. Epigallocatechingallat (EGCG)/Sunphenon® als mögliche neuroprotektive Substanz

Der Therapieansatz der Multiplen Sklerose besteht aus: akuter Schubtherapie mit Methylprednisolon und/oder Plasmaaustauschverfahren, einer verlaufsmodifizierenden Therapie (DMT) und der symptomatischen Behandlung (mit z.B. Baclofen). Das Ziel der DMT ist die Reduktion der Schubrate, der Krankheitsaktivität und der Behinderungsprogression. Für die RRMS-Patient*innen stehen multiple immunmodulatorische und immunsuppressive Medikamente zur Auswahl [22]. Zunächst wird bei CIS- und RRMS-Patient*innen eine Basistherapie mit Interferon ß oder Glatirameracetat [23,24] angestrebt. Weiterhin kommen bei mildem und moderatem Verlauf Teriflunomid [25] und Dimethylfumarat [26] zum Einsatz. Bei mangelndem Ansprechen oder hoher Krankheitsaktivität kann auf eine Eskalationstherapie umgestellt werden. Hierbei werden als 1. Wahl Wirkstoffe wie Fingolimod [27] oder Natalizumab [28] verabreicht, falls keine Kontraindikationen bestehen. Ocrelizumab hat auch eine Wirksamkeit bei RRMS gezeigt [29]. Für die Therapie der SPMS mit aufgesetzten Schüben wurde für Interferon ß und Mitoxantron ein Effekt auf die Behinderungsprogression nachgewiesen [30]. SPMS-Patient*innen ohne aufgesetzte Schübe erhalten Mitoxantron [31]. Für die Therapie der PPMS sind nur Optionen in geringer Anzahl vorhanden [32]. Ocrelizumab ist ein humanisierter, monoklonaler Antikörper gegen CD20 auf B-Lymphozyten und hat bei PPMS eine Reduktion der Behinderungsprogression gezeigt [33]. Er ist das einzige zugelassene Medikament bei PPMS. Insgesamt sollte vor Beginn jeder Therapie eine individuelle Beratung und Anpassung in Bezug auf die Lebenssituation der Patient*innen erfolgen. Zum gegenwärtigen Zeitpunkt werden einige Medikamente zur Therapie der progredienten Verlaufsformen untersucht [34]. Es gibt aktuell keinen neuroprotektiven Wirkstoff, der eine ausreichende Wirkung gezeigt hätte.

Die Einnahme von grünem Tee und dessen Hauptbestandteil Epigallocatechingallat (EGCG) wird mit antiinflammatorischen, antioxidativen und antikanzerogenen Effekten verknüpft [35,36]. Verschiedene klinische und experimentelle Studien haben einen positiven Einfluss auf die Krankheitsaktivität von MS-Patient*innen bei Applikation von EGCG gezeigt [37–40]. In einer kürzlich veröffentlichten Studie [41] aus unserer Arbeitsgruppe hat sich nach Analyse von 122 RRMS-Patient*innen, die gleichzeitig mit Glatirameracetat behandelt wurden, kein Effekt von EGCG auf klinische und radiologische Parameter gezeigt. Jedoch ergab die Analyse der Subgruppen unter

anderem, dass die Patient*innen, die 12 Monate vor Studieneinschluss kein Schubereignis hatten, in der EGCG-Gruppe weniger neue T2-Läsionen im MRT aufwiesen (EGCG 12/21 vs. Placebo 5/19). Dieser Effekt von EGCG war jedoch statistisch nicht signifikant ($p = 0.062$).

1.3.4. Zielstellung

Ziel der vorliegenden Arbeit war es, retinale Veränderungen in verschiedenen Stadien der multiplen Sklerose zu beschreiben sowie als Outcome-Parameter in einer interventionellen Studie zu untersuchen.

Konkret untersucht die Arbeit folgende Fragestellungen, die in den jeweiligen Publikationen adressiert wurden:

1. Lassen sich in der CIS-Kohorte bereits Veränderungen der retinalen Schichten unabhängig vom Auftreten einer Optikusneuritis nachweisen? [42]
2. Gibt es einen Therapieeffekt von EGCG in PMS-Patient*innen
    a. auf klinische und radiologische Parameter? [43]
    b. auf OCT-Parameter? [44]

1.4. Material und Methodik

1.4.1. Patient*innen der CIS-Kohorte (Fragestellung 1)

Für unsere Auswertung der retinalen Veränderungen bei CIS-Patient*innen konnten 45 Patient*innen aus 2 Zentren (n=29 aus dem NeuroCure Clinical Research Center (NCRC) der Charité-Universitätsmedizin Berlin, n=16 aus der Klinik für Neurologie, Heinrich Heine Universität Düsseldorf) rekrutiert und mit entsprechender Anzahl von gesunden Kontrollen (healthy controls, HC) gematcht werden. Bei allen Patient*innen war zum Zeitpunkt des OCT ein CIS innerhalb der letzten 6 Monate diagnostiziert und die Diagnose einer MS gemäß der McDonald Kriterien von 2010 [45] ausgeschlossen worden. Eingeschlossen wurden Patient*innen über 18 Jahre mit Diagnosestellung einer MS in den letzten 2 Jahren. Ausschlusskriterien waren die Diagnose einer SPMS, Schwangerschaft oder Alkohol- und Drogenmissbrauch sowie Kontraindikationen gegen die MRT-Bildgebung. Zudem wurden Patient*innen mit ophthalmologischen Erkrankungen wie Glaukom, diabetischer Retinopathie oder starken Refraktionsfehlern (± 5dpt) nicht zugelassen. Weiterhin wurden in der CIS-Studie visuell evozierte Potentiale (VEP) durchgeführt. Dabei kommt es durch visuelle Reize (z.B.

Schachbrettmuster) auf der Retina zur Weiterleitung an die Sehrinde, wo mittels Elektroden am Hinterkopf elektrische Potentiale gemessen werden. Hierbei werden üblicherweise die Latenz (=Zeitdauer der Weiterleitung) und die Amplitude (=max. Auslenkung der Welle) ermittelt. Typischerweise weist das VEP 3 Spitzen (N75, P100, N145) auf: wir haben zur Auswertung die p100-Welle, also die positive Spitze bei 100ms herangezogen. Als pathologisch wurden Werte >115ms gewertet. Die Amplitude wurde bei unserer Analyse außen vor gelassen, da diese durch verschiedene Geräte zweier Zentren nicht vergleichbar war. Nun wurden die Patient*innen unterteilt in diagnostizierte Optikusneuritis (CIS-ON), vermutete Optikusneuritis mit Latenzverzögerung der p100-Welle von >115ms in den visuell evozierten Potentialen (VEP) (suspected ON, CIS-SON) und fehlende Optikusneuritis bei normaler p100 Latenz (CIS-NON). Weitere demographische Daten sind der Tab. 1 in der Publikation [42] zu entnehmen.

1.4.2. Patient*innen der PMS-Kohorte (Fragestellung 2)

Die SUPREMES-Studie (Sunphenon® in progressive forms of multiple sclerosis) war eine monozentrische, prospektive, doppelblind randomisierte Phase II – Studie, um den Effekt von EGCG (=Sunphenon®) auf Hirnatrophie im MRT über 36 Monate zu ermitteln (NCT00799890). Dafür konnten 61 Patient*innen mit progressiver Verlaufsform (davon n=38 SPMS und n=23 PPMS) aus der Studienambulanz des NeuroCure Clinical Research Center (NCRC) der Charité Berlin eingeschlossen und zur Einnahme von EGCG (n=30) oder Placebo (n=31) randomisiert werden. Eingeschlossen wurden Patient*innen zwischen 18 und 65 Jahren mit der Diagnose von PPMS oder SPMS entsprechend der McDonald Kriterien von 2005 [46]. Die Diagnose einer schubförmig-remittierenden MS war ein Ausschlusskriterium. Weiterhin war ein EDSS (expanded disability status scale) [47] von 3-8 zugelassen, das Auftreten des letzten Schubes (bei SPMS) musste mindestens 30 Tage zurück liegen. Ausgeschlossen wurden Patient*innen mit anderen neurologischen oder psychiatrischen Erkrankungen, ebenso wie diagnostizierte gastrointestinale, kardiovaskuläre oder immunologische Vorerkrankungen. Labortechnisch durfte es keine Anzeichen einer Leber-, Nieren- oder Knochenmarksdysfunktion geben. Für die MRT-Bildgebung durfte es keine Kontraindikationen geben. Weiterhin war den Patient*innen bis zu 3 Monaten davor keine Teilnahme an einer anderen klinischen Studie erlaubt. Ein weiteres Ausschlusskriterium war die Einnahme von Immunsuppressiva wie Mitoxantron,

Cyclophosphamid, Cyclosporin oder anderen immunomodulatorischen Medikamenten wie z.B. monoklonale Antikörper drei Monate vor Studienbeginn. Die Gabe von Methylprednisolon war erlaubt.

Für die Analyse des primären Endpunkts (Differenz der „Brain parenchymal fraction“ (BPF) vom Monat 36 zur Baseline) im MRT konnten 38 Patient*innen die Studie beenden. Die BPF wurde errechnet aus der Summe vom Volumen der grauen und weißen Substanz geteilt durch das gesamte intrakranielle Volumen. Dieses wurde dann als Differenz zwischen Behandlungsbeginn und den jeweiligen Visiten erstellt. Die MRT-Untersuchungen fanden zum Behandlungsbeginn, nach 12, 24 und 36 Monaten statt. Weitere Details zur Randomisierung, dem Visitenprotokoll und der Kohorte sind der Publikation [43] zu entnehmen. Als sekundäre Endpunkte galten folgende MRT-Parameter: die Hirnatrophie, gemessen durch die „percent brain volume change“ (PBVC), sowie Zahl und Volumen der T2-Läsionen (T2w) und der T1-Kontrastmittel-aufnehmenden Herde (contrast enhancing lesions, CELs). Weiterhin wurden klinische Parameter ausgewertet: die Behinderungsprogression in 36 Monaten, gemessen anhand des EDSS und die „confirmed disability progression“ (CDP), die als Zunahme des EDSS um 1 Punkt (Ausgangswert 3,0-5,5) oder um 0,5 Punkte (Ausgangswert ≥6) definiert war. Als weitere sekundäre Endpunkte wurden die Schubrate (annualized relapse rate, ARR), der „Multiple Sclerosis Functional Composite“ (MSFC) [48] und kognitive Tests (neuropsychologische Untersuchung, „Fatigue Severity Scale“ (FSS) [49], „Modified Fatigue Impact Scale“ (MFIS) [50], „Becks Depression Inventory I“ (BDI) [51]) ausgewertet. Zudem wurden Sicherheitsparameter wie „adverse events“ (AE) notiert sowie klinische und labortechnische Untersuchungen durchgeführt. Als sekundärer Outcome-Parameter wurde die Abnahme der RNFL-Dicke im OCT definiert. Wir untersuchten die einzelnen OCT-Parameter als experimentelle Outcome-Parameter wie folgt: die pRNFL und die segmentierten Schichten aus dem Volumenscan makuläre GCIP und INL.

Für die OCT-Bildgebung mussten alle Patient*innen mit starker Myopie (ab –5 dpt) oder ophthalmologischen Vorerkrankungen wie Glaukom oder rezidivierender Iritis ausgeschlossen werden. Für die Analyse der retinalen Schichten konnten wir letztendlich 31 Patient*innen einschließen, die restlichen waren wegen Augenvorerkrankungen, fehlender OCT-Messungen oder schlechter Qualität entsprechend der OSCAR-IB Kriterien [52] ausgeschlossen worden. Weitere Details zum Ein- und Ausschluss [44] sind der Publikation zu entnehmen.

1.4.3. Ethikvotum

Die CIS-Studie wurde von der Ethikkommission der Charité – Universitätsmedizin Berlin (EA1/182/10), die SUPREMES-Studie vom Landesamt für Gesundheit und Soziales (LaGeSo ZS EK 10 407/08, new: 08/0407-EK 15) genehmigt. Beide Studien wurden unter Einhaltung der Bestimmungen der Deklaration von Helsinki in ihrer jeweiligen aktuellen Version sowie den Richtlinien zur guten klinischen Praxis (International Conference on Harmonisation of Good Clinical Practice) und in Deutschland geltendem Recht durchgeführt. Alle Studienteilnehmer gaben ihre schriftliche Einwilligung nach erfolgter Aufklärung.

Beide Studien sind unter clinicaltrials.gov registriert: SUPREMES (NCT00799890) und CIS-COHORT (NCT01371071). Die SUPREMES-Studie ist zudem bei EudraCT (2008-005213-22) und beim Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) registriert.

1.4.4. Optische Kohärenztomographie (OCT)

Das Messprinzip der optischen Kohärenztomographie beruht auf der Weißlichtinterferometrie. Kurz zusammengefasst wird dabei Licht aus dem Infrarotbereich von den verschiedenen Schichten der Netzhaut reflektiert und vom Gerät ausgelesen und dreidimensional dargestellt [53,54]. Alle Untersuchungen wurden mit dem Spectralis-OCT (Heidelberg Spectralis SD-OCT, Heidelberg Engineering, Deutschland) an nicht dilatierten Augen durchgeführt. Bei allen Studienteilnehmer*innen wurde das Standardprotokoll für die Dicke der retinalen Nervenfaserschicht durchgeführt, welches einen peripapillären Ringscan mit einem Durchmesser von 3,4mm verwendet. Das Makulavolumen wurde mit einem selbst voreingestellten Scan gemessen, der mit 61 vertikalen B-Scans (jeder mit 768 A-Scans, ART = 13 frames) und einem Winkel von 30° x 25° um die Fovea centralis festgelegt war. Mit Hilfe dieses Scans konnte das Gesamtvolumen der Makula (total macular volume, TMV) zwischen der Membrana limitans interna und der Bruch-Membran mit einem Zylinder von 6mm im Durchmesser bestimmt werden. Für die Segmentierung und Untersuchung der Schichten im makulären Volumenscan stand für Fragestellung 1 eine Beta-Software-Version der Firma Heidelberg Engineering zur Verfügung (Spectralis software version 5.5.0.5, Eye Explorer Software 1.7.0.0). Um die Dicke der einzelnen retinalen Schichten korrekt zu bestimmen, mussten diese nachfolgend manuell segmentiert werden. Die manuelle Segmentierung erfolgte verblindet durch einen erfahrenen Auswerter nach

einem vorher festgelegten Schema: Von den 61 B-Scans wurden der zentral durch die Fovea centralis laufende B-Scan sowie 6 B-Scans in nasaler und 5 B-Scans in temporaler Richtung für die Auswertung ausgewählt. Demnach wurde jeder vierte B-Scan im Abstand von ca. 500µm ausgewertet. Für die Segmentierung der SUPREMES-Kohorte stand ein hauseigenes automatisiertes Programm SAMIRIX [55] zur Verfügung, welches von Hand nur noch minimal korrigiert werden musste. Alle OCT-Scans wurden auf ausreichende Bildqualität, retinale Veränderungen unabhängig von MS sowie Segmentierungsfehler kontrolliert. Folgende Schichten wurden in beiden Kohorten ausgewertet: die peripapilläre retinale Nervenfaserschicht (peripapillary retinal nerve fiber layer, pRNFL), die makuläre retinale Nervenfaserschicht (macular retinal nerve fiber layer, mRNFL), die kombinierte makuläre Ganglienzell- und innere plexiforme Schicht (macular ganglion cell and inner plexiform layer, GCIP) sowie die innere Körnerzellschicht (macular inner nuclear layer, INL). Die Ganglienzell- und die innere plexiforme Schicht wurden zu einer Schicht zusammengefasst (GCIP). Im Folgenden sind die makulären Schichten nur noch mit GCIP und INL gekennzeichnet. Die makuläre RNFL hat sich im Verlauf als unsicherer Parameter heraus gestellt, da diese Region von vielen Blutgefäßen umgeben ist und es somit zu Fehlern bei der Segmentierung kommen kann [42]. Im Folgenden wird die peripapilläre RNFL zur Auswertung herangezogen.

1.4.5. Magnetresonanztomographie (MRT)

In der SUPREMES-Studie wurden die MRT-Untersuchungen mit einem 1,5 Tesla MRT-Gerät (Siemens Sonata, Siemens Medical Systems, Erlangen, Deutschland) durchgeführt. Folgende MR-Sequenzen kamen zur Anwendung: T2-Wichtung (Echo times (= TE): 13ms, 81ms, 121ms; Repetition time (= TR) 5780ms, in plane-resolution 1x1mm, matrix 256x256; flip angle 150°); TIRM-Sequenz (= turbo-inversion recovery-magnitude; TR 10000ms, TE 108ms, Inversion time (= TI): 2500ms); MPRAGE-Sequenz (= 3D-T1-weighted magnetisation prepared rapid acquisition and multiple gradient echo; TE 4,38ms, TR 2110ms, TI 1100ms, flip angle 15°). Bei den T2- und TIRM-Sequenzen wurde das gesamte Hirnparenchym mit 44 axialen Schichten mit einer jeweiligen Schichtdicke von 3mm abgebildet. Das Volumen der grauen und weißen Substanz wurde mit dem Programm SIENAX (Structural Image Evaluation, using Normalization, of Atrophy; X = cross-sectional) [56] ausgewertet. Zunächst wurden die MRT-Aufnahmen des Gehirns und des Schädels des gesamten

Datensatzes extrahiert. Anschließend wurde Hirngewebe von Nichthirngewebe durch das Programm unterschieden. Danach erfolgte die Segmentierung der Schädel-Aufnahmen. Diese dienen als Referenz, um die individuelle Kopfgröße mit den Gehirnvolumina zu normalisieren. Danach erfolgte die automatische Differenzierung der weißen und grauen Substanz durch das Programm. Anschließend erfolgte die manuelle Segmentierung durch einen erfahrenen Spezialisten mit der Markierung von Volumes of interest (VOIs) der T2-gewichteten Bilder unter Verwendung der MIPAV 5.4.4 Software (Center for Information Technology, NIH, USA).

Somit konnten dann die MRT-Parameter „brain parenchymal fraction“ (BPF), prozentuale Hirnatrophie gemessen als „percent brain volume change“ (PBVC) sowie Zahl und Volumen der T2-Läsionen (T2w) und der T1-Kontrastmittel-aufnehmenden Herde (contrast enhancing lesions, CELs) ermittelt werden.

1.4.6. Statistik

Die Messwerte wurden entweder als Mittelwert und Standardabweichung, Median und Spannweite oder Interquartilsabstand angegeben. Als signifikant wurde ein p-Wert von $p < 0,05$ festgelegt. Die statistische Auswertung der CIS-Studie [42] wurde mit R Version 2.15.0 durchgeführt. Zur Analyse wurden verallgemeinerte Schätzungsgleichungen (Generalized estimation equations, GEE) verwendet, um die Werte *zweier Augen* in *einer Person* mit der Kontrollgruppe vergleichen und mit den klinischen und OCT-Parametern korrelieren zu können. Die GEE-Modelle wurden für Alter und Geschlecht korrigiert. In diesen Analysen war der jeweilige OCT-Messwert die abhängige Variable. Die statistische Auswertung der primären und sekundären Outcome-Parameter der SUPREMES-Studie [43] wurde mit SAS Version 9.4, SPSS Version 25 und mit R Version 3.0.2 durchgeführt. Die longitudinale OCT-Auswertung [44] der SUPREMES-Studie wurde mittels der „nichtparametrischen Analyse longitudinaler Daten“ [57] durchgeführt. Dabei wurden nur die Zeitpunkte nach 2 Jahren eingeschlossen, weil nach 3 Jahren zu viele OCT-Daten fehlten. Weiterhin wurden unsere Ergebnisse mit linear gemischten Modellen (linear mixed models, LMM) bestätigt, dabei wurden wieder alle Visiten auf volle Jahre aufgerundet. Die statistische Analyse wurde mit R Version 3.6.2 mit folgenden Paketen durchgeführt: nparLD [57], lme4, lmertest, tidyverse, tableone, ggplot2, beeswarm, ggplot, RMisc.

1.5. Ergebnisse

1.5.1. Fragestellung 1) Intraretinale Veränderungen bei CIS-Patient*innen mit und ohne Optikusneuritis [42]

Es handelt sich hierbei um eine querschnittliche Studie zur Analyse retinaler Veränderungen mittels OCT bei CIS-Patient*innen mit ON, mit vermuteter ON und ohne Nachweis einer ON. Diese wurden mit gesunden Kontrollen nach Alter und Geschlecht gematcht. Demographische und klinische Daten sind in der Tabelle 1 der Publikation [42] aufgeführt. Wir verwendeten das Heidelberg Spectralis SD-OCT und eine dazugehörige Software zur automatischen Segmentierung, die anschließend noch durch eine manuelle Korrektur kontrolliert werden musste. Als Messparameter ergaben sich die peripapilläre RNFL (pRNFL), das totale makuläre Volumen (TMV) und die makulären Schichten mRNFL, GCIP und INL. Beachtenswert war die signifikante Abnahme der Schichtdicke der GCIP auch bei Patient*innen ohne Optikusneuritis (NON) und vermuteter ON (SON). Wie zu erwarten zeigten die Patient*innen mit Optikusneuritis (ON) eine signifikante Abnahme aller retinalen Schichten außer der INL. Die INL zeigte in keiner Gruppe eine signifikante Veränderung im Vergleich zu HC. Einzelheiten sind in der Tabelle 2 der Publikation [42] zu finden. Weiterhin ergab sich eine signifikante Abnahme der pRNFL in CIS-ON und CIS-SON, aber nicht in der CIS-NON-Gruppe. Eine Reduktion des TMV zeigte sich in der CIS-ON und CIS-NON-Gruppe, aber nicht in CIS-SON. Eine signifikante mRNFL- Abnahme war in CIS-ON, aber nicht in den anderen beiden Gruppen nachweisbar.

1.5.2. Fragestellung 2a) Kein Effekt von EGCG auf MRT- und klinische Parameter in SUPREMES-Kohorte [43]

Für die SUPREMES-Kohorte wurden zunächst 61 Patient*innen mit progressiver MS (PMS) eingeschlossen und zur Verum (n=30)- oder Placebogruppe (n=31) randomisiert. Davon konnten jeweils 19 Patient*innen (n=38) die Studie nach 36 Monaten beenden. Die anderen mussten wegen Änderung der Medikation, unspezifischer Unverträglichkeit der Studienmedikation und ein Patient wegen erhöhten Leberenzymen auf Grund von antiepileptischer Medikation ausgeschlossen werden. Die meisten schieden aus persönlichen Gründen aus. Näheres hierzu in der Abb. 1 der Publikation [43]. Der primäre Endpunkt der Studie war die Hirnatrophie, die sich als Differenz vor Behandlungsbeginn und den verschiedenen Visiten ergab. Näheres hierzu im

Methodenteil der Publikation [43]. Hierbei konnte kein signifikanter Unterschied zwischen der Verum- und Placebogruppe nachgewiesen werden. Die weiteren sekundären Endpunkte zum Zeitpunkt des Monats 36 wie prozentuale Hirnvolumenänderung (PBVC), Anzahl und Volumen der T2-Läsionen (T2w lesions) bzw. T1-Kontrastmittel-aufnehmenden Läsionen zeigten ebenfalls keine Änderung zwischen EGCG- und Placebo-Gruppe. Details sind der Tabelle 1 der Publikation [43] zu entnehmen. Die Auswertung der klinischen sekundären Endpunkte wie EDSS, MSFC und des BDI und weiteren Fatigue-Fragebögen ergab auch keine signifikanten Unterschiede zwischen den Behandlungsgruppen. In der longitudinalen Auswertung zu den verschiedenen Zeitpunkten zu Monat 0, 12, 24 und 36 ergaben sich weder für die MRT- noch für die klinischen Untersuchungen signifikante Differenzen in den Behandlungsgruppen. Bezüglich der Sicherheit von EGCG wurden in der Verumgruppe 11 (36,7%) Patient*innen und in der Placebogruppe 10 (32,3 %) Patient*innen mit einem SAE (serious adverse event) beobachtet. Keines dieser SAEs konnte auf die Studienmedikation bezogen werden und war in den Behandlungsgruppen gleich verteilt. Am häufigsten waren grippale Infekte, Harnwegsinfektionen, Frakturen nach Sturz oder erhöhte Leberenzyme in der Labordiagnostik aufgetreten.

Weiterhin bestand die Möglichkeit für die Patient*innen, nach den 36 Monaten weiter an der Studie teilzunehmen (OE = open-label extension) bis zum Monat 48. Zu diesem Zeitpunkt waren noch 17 Patient*innen aus der EGCG- und 15 Patient*innen aus der Placebogruppe vorhanden. Auch jetzt zeigte sich kein signifikanter Unterschied im primären Endpunkt BPF und im PBVC sowie in den klinischen Parametern.

1.5.3. Fragestellung 2b) Longitudinale intraretinale Veränderungen bei PMS-Patient*innen ohne Effekt von EGCG [44]

Hierbei handelt es sich um eine longitudinale Auswertung von retinalen Veränderungen mittels OCT-Aufnahmen bei Patient*innen mit SPMS und PPMS. In der SUPREMES-Studie wurden zunächst 61 Patient*innen mit progressiver MS (PMS) eingeschlossen und zur Verum- oder Placebogruppe randomisiert [43,44]. Von diesen Patient*innen mussten 16 ausgeschlossen werden auf Grund fehlender OCT-Messungen, da das OCT-Gerät nicht ab Beginn der Studie vorhanden war. Weiterhin wurden 7 Patient*innen auf Grund fehlender Folgeuntersuchungen und 7 Patient*innen auf Grund von Augenvorerkrankungen ausgeschlossen, sodass letztendlich 31 Patient*innen in die Auswertung (62 Augen) einbezogen werden konnten. Weitere Details sind der

Abbildung 1 in der Publikation [44] zu entnehmen. Von den 31 Patient*innen war die Mehrheit (n=19) von SPMS und 12 Patient*innen von PPMS betroffen. Weitere demographische Einzelheiten sind der Tabelle 1 im Paper [44] zu entnehmen. Als OCT-Messparameter ergaben sich: die pRNFL und die makuläre GCIP und INL. Zunächst wurden Unterschiede querschnittlich zwischen den Gruppen Verum vs. Placebo zum Zeitpunkt der ersten OCT-Messung herausgearbeitet. Dabei hatte die EGCG-Gruppe eine signifikant dickere GCIP und INL im Vergleich zur Placebo-Gruppe.

Nachfolgend wurden die OCT-Parameter longitudinal ausgewertet. In der longitudinalen Betrachtung zeigte sich eine signifikante Abnahme der pRNFL in beiden Gruppen, aber nicht der GCIP und INL über die Zeit (s. hierzu Tabelle 4 in Publikation [44]). Für die Auswertung von Gruppenunterschieden im longitudinalen Verlauf bei kleiner Fallzahl und zunehmend fehlenden OCT-Daten zogen wir die „nichtparametrische Analyse longitudinaler Daten“ [57] heran. Hierbei ließ sich zwischen der EGCG- und der Placebo-Gruppe kein signifikanter Unterschied im Hinblick auf retinale Veränderungen und somit kein Behandlungseffekt von EGCG nachweisen (s. hierzu Tabelle 3 in Publikation [44]). Die Ergebnisse wurden zudem mittels linearer gemischter Modelle bestätigt, diese zeigten ebenfalls keinen Unterschied zwischen den Behandlungsgruppen (s. hierzu Tabelle 4 in Publikation [44]).

1.6. Diskussion

In der vorliegenden Arbeit haben wir einerseits retinale Veränderungen von Patient*innen im frühen Stadium der MS untersucht und andererseits OCT-Parameter als experimentelle Outcome-Analyse einer randomisierten Placebo-kontrollierten Studie zur Wirksamkeit von EGCG bei MS-Patient*innen mit progressiver Verlaufsform geprüft. Dabei kamen wir zu den Ergebnissen, dass es 1. bereits vor Diagnose einer MS und ohne Auftreten einer Optikusneuritis zu pathologischen Veränderungen insbesondere der GCIP kommt [42], und dass 2. die Gabe von EGCG keinen Effekt auf die klinischen, MRT- oder OCT-Parameter bei MS-Patient*innen mit progressiver Verlaufsform hat [43,44].

Wie wir in der Untersuchung der CIS-Kohorte zeigen konnten, scheint es bereits vor Auftreten einer Optikusneuritis zu einer Abnahme der GCIP und dementsprechend zu einer neuroaxonalen Schädigung, auch unabhängig von einem Schub, gekommen zu

sein. Bei Patient*innen mit nachgewiesener Optikusneuritis waren die pRNFL, die mRNFL und GCIP verringert im Vergleich zu gesunden Kontrollen.

Die Annahme, dass Neurodegeneration bereits vor Beginn der Erkrankung stattfindet, wird durch mehrere Arbeiten unterstützt: Bei einer Kohorte mit RIS konnte gezeigt werden, dass bereits vor Symptombeginn eine signifikante Atrophie im Thalamus im Vergleich zu HC nachweisbar war, hinweisend auf eine frühe Neurodegeneration [58]. Weiterhin konnte eine longitudinale Studie an 135 MS-Patient*innen über 2 Jahre zeigen, dass die pRNFL und GCIP signifikant abnimmt. Die Abnahme war vor allem im frühen Stadium der MS unabhängig von einer Optikusneuritis zu verzeichnen [59]. Eine weitere Studie, die auch 45 CIS-Patient*innen untersuchte, kam zu dem Ergebnis, dass es auch ohne vorheriges Auftreten einer Optikusneuritis zu einer signifikanten Abnahme der pRFNL komme [60]. Zusätzlich scheint eine Zunahme der INL mit der Schubrate und Neuroinflammation assoziiert zu sein [17,19,20]. Die INL zeigte sich in der CIS-Kohorte unverändert. Dies könnte damit zusammenhängen, dass das klinisch-isolierte Syndrom definitionsgemäß keine zeitliche und räumliche Dissemination aufweist und dementsprechend (noch) keine Inflammation oder nur in geringem Maße stattgefunden hat.

Für die SUPREMES-Kohorte konnten 61 Patient*innen eingeschlossen und jeweils zu Placebo oder EGCG randomisiert werden. Primärer Endpunkt der Hauptstudie [43] war die Hirnatrophie gemessen an der „brain parenchymal fraction“ wie oben beschrieben. Es konnte kein signifikanter Unterschied zwischen den Therapiegruppen gemessen werden. Auch für die weiteren MRT- und klinischen Parameter konnte kein Unterschied gefunden werden. Das Ausmaß der Hirnatrophie ist ein gut etablierter Surrogat-Parameter zur Anwendung in randomisierten klinischen Studien bei MS [61]. Unsere PMS-Kohorte zeigte eine stabil niedrigere jährliche PBVC Rate (0,2-0,3%/Jahr) im Vergleich zu anderen klinischen Interventionsstudien mit Ocrelizumab [33], Natalizumab [62] oder Fingolimod [63], die eine jährliche Atrophierate von 0,4-0,7% unabhängig von der Intervention beschreiben. Zudem waren die PMS-Patient*innen bereits zu Beginn der Studie neurologisch stark betroffen, was anhand des hohen EDSS von 6.0 als Mittelwert Ausdruck findet. Daher ist anzunehmen, dass unsere PMS-Kohorte zu stabil war, um einen positiven Effekt von EGCG anzuzeigen. Weiterhin kann es bei der Evaluation der Hirnatrophie auch zum Phänomen der „Pseudoatrophie“ kommen: dabei erscheint das Hirnvolumen auf Grund von abnehmender Entzündung und Ödemen nach Therapieansprechen kleiner und wird als Hirnatrophie missinterpretiert [64,65].

Zudem könnte das Alter eine Bedeutung spielen, da die PMS-Patient*innen in unserer Kohorte durchschnittlich 50 Jahre alt waren [66]. Die Schichten der Retina scheinen wiederum nicht anfällig für Alterungseffekte zu sein [55]. Daher haben wir als experimentellen Endpunkt die Analyse der OCT-Daten in der SUPREMES-Kohorte hinzugezogen [44]. Hierbei zeigten sich keine Unterschiede im longitudinalen Verlauf und somit kein Effekt von EGCG auf die retinalen Schichten. Einschränkend ist hierbei zu erwähnen, dass die Gruppen bezüglich der OCT-Parameter nicht gut gematcht waren, da dies bei der Randomisierung nicht berücksichtigt wurde. Konkret waren GCIP und INL in der EGCG-Gruppe signifikant dicker. Die Patient*innen wurden vorher zu den jeweiligen Gruppen randomisiert ohne Hinblick auf die OCT-Parameter.

Zusammengefasst zeigte die Untersuchung der SUPREMES-Kohorte keinen neuroprotektiven Behandlungseffekt von EGCG auf klinische, radiologische oder OCT-Outcome-Parameter. Die Ergebnisse stehen im Kontrast zu den vorherigen Arbeiten, die einen Effekt von EGCG auf inflammatorische Prozesse im Mausmodell und auch in klinischen Studien vermuten ließen [37,39,40]. Eine kürzlich veröffentlichte Studie [41], welche den Effekt von EGCG bei RRMS-Patient*innen untersuchte, fand ebenfalls keinen signifikanten Unterschied zu den Behandlungsgruppen in Bezug auf MRT- und klinische Parameter. Jedoch zeigte sich in der Subgruppenanalyse ein Trend, dass Patient*innen, die bereits 12 Monate vor Studieneinschluss kein Schubereignis hatten, weniger neue T2-hyperintense Läsionen in der EGCG-Gruppe aufwiesen im Vergleich zu Placebo. Eine weitere Studie untersuchte den Effekt von EGCG auf die Krankheitsprogression bei Multisystematrophie und konnte ebenfalls keinen Effekt nachweisen [67].

Mögliche Erklärungsversuche des fehlenden Effekts könnte die niedrige orale Bioverfügbarkeit von EGCG sein, die neuerdings beschrieben wurde [68]. Wir verabreichten eine tägliche Dosis EGCG von 800mg/d ab Monat 30 und 1200mg/d ab Monat 36 bis zur Erweiterung der Studie bis Monat 48. Frühere Studien hatten einen verbesserten Stoffwechsel im Muskel bei RRMS-Patient*innen mit einer Dosis von 600mg/d [40] und eine Plasma-Halbwertszeit von 5h nach Verabreichung von 800mg/d über 10 Tage bei gesunden Proband*innen [69] gezeigt. Daher wurde angenommen, dass eine maximale Dosis von 1200mg/d effektiv und sicher sei. Eine neuere Studie hatte gezeigt, dass die orale Bioverfügbarkeit von EGCG unter 1% [70] läge. Die Untersuchung der Plasma-Spiegel von EGCG in der SUNIMS-Kohorte [41] hatte eine große Bandbreite trotz gleicher Dosis ergeben. Obwohl die ZNS-Gängigkeit von EGCG

im Tiermodell [71] erwiesen schien, ist dies am Menschen bisher noch nicht bestätigt worden. Daher erscheint es möglich, dass EGCG in unserer Kohorte nicht die nötige Dosis und/oder die Eigenschaften zum Überwinden der Bluthirnschranke besaß, um einen Effekt zu generieren.

Eine weitere Limitation der SUPREMES-Studie ist die niedrige Fallzahl der Patient*innen, die zum Abschluss der Studie noch vorhanden waren. Eine kürzlich veröffentlichte Publikation [72] zu klinischen Studien mit progressiven MS-Verlaufsformen errechnete die Fallzahlen für eine dreijährige OCT-Studie mit n=173 (pRNFL) und n=125 (GCIP) Patient*innen. Dementsprechend beinhaltete unsere Studie möglicherweise zu wenig Patient*innen für eine aussagekräftige Auswertung. Die kürzlich publizierte MS-SMART-Studie [73], die 3 verschiedene neuroprotektive Medikamente im Vergleich zu Placebo bei SPMS über 96 Wochen testete, kam zu dem Ergebnis, dass selbst bei n>100 Patient*innen pro Therapiegruppe kein Effekt auf die PBVC zu eruieren war.

Zusammenfassend kann man sagen, dass neurodegenerative Prozesse schon im Frühstadium einer MS-Erkrankung vorhanden und mittels OCT erfassbar sind. Weiterhin haben wir bei PMS-Patient*innen keinen neuroprotektiven Effekt von EGCG auf klinische, radiologische oder OCT-Outcome-Parameter nachweisen können. Limitiert war die Studie durch die unzureichende Patient*innenanzahl sowie die niedrige Bioverfügbarkeit im ZNS von EGCG. Wir konnten zeigen, dass das OCT als Outcome-Parameter in klinischen Studien genutzt werden kann, jedoch insbesondere bei PMS-Kohorten mit ausreichender Anzahl an Patient*innen durchgeführt werden muss.

1.7. Literaturverzeichnis

[1] J.-M. Charcot, Histologie de la sclerose en plaques., Gaz. Des Hop. 41 (1868) 554–55.

[2] D.H. Miller, D.T. Chard, O. Ciccarelli, Clinically isolated syndromes, Lancet Neurol. 11 (2012) 157–169. https://doi.org/10.1016/S1474-4422(11)70274-5.

[3] A.G. Barboza, E. Carnero Contentti, M.C. Curbelo, M.J. Halfon, J.I. Rojas, B.A. Silva, V. Sinay, S. Tizio, M.C. Ysrraelit, R. Alonso, Radiologically isolated syndrome: from biological bases to practical management, Neurol. Sci. 42 (2021) 1335–1344. https://doi.org/10.1007/s10072-021-05069-6.

[4] R.M. Ransohoff, D.A. Hafler, C.F. Lucchinetti, Multiple sclerosis—a quiet revolution., Nat Rev Neurol. 11 (2016) 134–142. https://doi.org/10.1038/nrneurol.2015.14.

[5] A. Compston, A. Coles, Multiple sclerosis., Lancet. 372 (2008) 1502–17. https://doi.org/10.1016/S0140-6736(08)61620-7.

[6] F.D. Lublin, S.C. Reingold, J.A. Cohen, G.R. Cutter, A.J. Thompson, J.S. Wolinsky, B. Banwell, F. Barkhof, B. Bebo, P.A. Calabresi, M. Clanet, R.J. Fox, M.S. Freedman, A.D. Goodman, J.A. Lincoln, C. Lubetzki, A.E. Miller, X. Montalban, P.W.O. Connor, M.P. Sormani, Defining the clinical course of multiple sclerosis. The 2013 revisions, Neurology. 83 (2014) 278–286. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4117366/pdf/NEUROLOGY2013555623.pdf.

[7] M. Rovaris, C. Confavreux, R. Furlan, L. Kappos, G. Comi, M. Filippi, P. Wertheimer, C. Bernard, Secondary progressive multiple sclerosis: current knowledge and future challenges., Lancet Neurol. 5 (2006) 343–54.

[8] S. Faissner, J.R. Plemel, R. Gold, V.W. Yong, Progressive multiple sclerosis: from pathophysiology to therapeutic strategies, Nat. Rev. Drug Discov. 18 (2019) 905–922. https://doi.org/10.1038/s41573-019-0035-2.

[9] D.H. Miller, S.M. Leary, Primary-progressive multiple sclerosis., Lancet Neurol. 6 (2007) 903–12. https://doi.org/10.1016/S1474-4422(07)70243-0.

[10] W.J. Brownlee, T.A. Hardy, F. Fazekas, D.H. Miller, Diagnosis of multiple sclerosis: progress and challenges, Lancet. 389 (2017) 1336–1346. https://doi.org/10.1016/S0140-6736(16)30959-X.

[11] A. Petzold, M.P. Wattjes, F. Costello, J. Flores-Rivera, C.L. Fraser, K. Fujihara, J. Leavitt, R. Marignier, F. Paul, S. Schippling, C. Sindic, P. Villoslada, B. Weinshenker, G.T. Plant, The investigation of acute optic neuritis: a review and proposed protocol., Nat. Rev. Neurol. 10 (2014) 447–58. https://doi.org/10.1038/nrneurol.2014.108.

[12] L.J. Balcer, D.H. Miller, S.C. Reingold, J. a Cohen, Vision and vision-related outcome measures in multiple sclerosis., Brain. 138 (2015) 11–27. https://doi.org/10.1093/brain/awu335.

[13] F.C. Oertel, H.G. Zimmermann, A.U. Brandt, F. Paul, Novel uses of retinal imaging with optical coherence tomography in multiple sclerosis, Expert Rev. Neurother. 19 (2019) 31–43. https://doi.org/10.1080/14737175.2019.1559051.

[14] V. Parisi, G. Manni, M. Spadaro, G. Colacino, R. Restuccia, S. Marchi, M.G. Bucci, F. Pierelli, Correlation between morphological and functional retinal impairment in multiple sclerosis patients, Investig. Ophthalmol. Vis. Sci. 40 (1999) 2520–2527.

[15] A. Petzold, L.J. Balcer, P.A. Calabresi, F. Costello, T.C. Frohman, E.M. Frohman, E.H. Martinez-Lapiscina, A.J. Green, R. Kardon, O. Outteryck, F. Paul, S. Schippling, P. Vermersch, P. Villoslada, L.J. Balk, O. Aktas, P. Albrecht, J. Ashworth, N. Asgari, L. Balcer, L. Balk, G. Black, D. Boehringer, R. Behbehani, L. Benson, R. Bermel, J. Bernard, A. Brandt, J. Burton, P. Calabresi, J. Calkwood, C. Cordano, F. Costello, A. Courtney, A. Cruz-Herranz, R. Diem, A. Daly, H. Dollfus, C. Fasser, C. Finke, J. Frederiksen, E. Frohman, T. Frohman, E. Garcia-Martin, I.G. Suárez, G. Pihl-Jensen, J. Graves, A. Green, J. Havla, B. Hemmer, S.-C. Huang, J. Imitola, H. Jiang, D. Keegan, E. Kildebeck, A. Klistorner, B. Knier, S. Kolbe, T. Korn, B. LeRoy, L. Leocani, D. Leroux, N. Levin, P. Liskova, B. Lorenz, J.L. Preiningerova, E.H. Martínez-Lapiscina, J. Mikolajczak, X. Montalban, M. Morrow, R. Nolan, T. Oberwahrenbrock, F.C. Oertel, C. Oreja-Guevara, B. Osborne, O. Outteryck, A. Papadopoulou, F. Paul, A. Petzold, M.

Ringelstein, S. Saidha, B. Sanchez-Dalmau, J. Sastre-Garriga, S. Schippling, R. Shin, N. Shuey, K. Soelberg, A. Toosy, R. Torres, A. Vidal-Jordana, P. Villoslada, A. Waldman, O. White, A. Yeh, S. Wong, H. Zimmermann, Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis, Lancet Neurol. 16 (2017) 797–812. https://doi.org/10.1016/S1474-4422(17)30278-8.

[16] F. Costello, W. Hodge, Y.I. Pan, E. Eggenberger, M.S. Freedman, Using retinal architecture to help characterize multiple sclerosis patients., Can. J. Ophthalmol. J. Can. Dophtalmologie. 45 (2010) 520–526. https://doi.org/10.3129/i10-063.

[17] C.A. Wicki, J.V.M. Hanson, S. Schippling, Optical coherence tomography as a means to characterize visual pathway involvement in multiple sclerosis, Curr. Opin. Neurol. 31 (2018) 662–668. https://doi.org/10.1097/WCO.0000000000000604.

[18] J.M. Gelfand, R. Nolan, D.M. Schwartz, J. Graves, A.J. Green, Microcystic macular oedema in multiple sclerosis is associated with disease severity, Brain. 135 (2012) 1786–1793. https://doi.org/10.1093/brain/aws098.

[19] F. Kaufhold, H. Zimmermann, E. Schneider, K. Ruprecht, F. Paul, T. Oberwahrenbrock, A.U. Brandt, Optic Neuritis Is Associated with Inner Nuclear Layer Thickening and Microcystic Macular Edema Independently of Multiple Sclerosis, PLoS One. 8 (2013). https://doi.org/10.1371/journal.pone.0071145.

[20] L.J. Balk, D. Coric, B. Knier, H.G. Zimmermann, R. Behbehani, R. Alroughani, E.H. Martinez-lapiscina, A.U. Brandt, A. Vidal-jordana, P. Albrecht, V. Koska, J. Havla, M. Pisa, R.C. Nolan, L. Leocani, F. Paul, O. Aktas, X. Montalban, L.J. Balcer, P. Villoslada, Retinal inner nuclear layer volume reflects inflammatory disease activity in multiple sclerosis; a longitudinal OCT study, Mult. Scler. July-Septe (2019) 1–11. https://doi.org/10.1177/2055217319871582.

[21] B. Knier, P. Schmidt, L. Aly, D. Buck, A. Berthele, M. M??hlau, C. Zimmer, B. Hemmer, T. Korn, Retinal inner nuclear layer volume reflects response to immunotherapy in multiple sclerosis, Brain. 139 (2016) 2855–2863. https://doi.org/10.1093/brain/aww219.

[22] D.M. Wingerchuk, B.G. Weinshenker, Disease modifying therapies for relapsing

multiple sclerosis, BMJ. 354 (2016). https://doi.org/10.1136/bmj.i3518.

[23] L.D. Jacobs, R.W. Beck, J.H. Simon, R.P. Kinkel, C.M. Brownscheidle, T.J. Murray, N.A. Simonian, P.J. Slasor, A.W. Sandrock, and the CHAMPS study group, Intramuscular Interferon Beta-1a therapy initiated during a first demyelinating event in multiple sclerosis, N. Engl. J. Med. 343 (2000) 898–904.

[24] G. Comi, V. Martinelli, M. Rodegher, L. Moiola, O. Bajenaru, A. Carra, I. Elovaara, F. Fazekas, H.P. Hartung, J. Hillert, J. King, S. Komoly, C. Lubetzki, X. Montalban, K.M. Myhr, M. Ravnborg, P. Rieckmann, D. Wynn, C. Young, M. Filippi, Effect of glatiramer acetate on conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome (PreCISe study): a randomised, double-blind, placebo-controlled trial, Lancet. 374 (2009) 1503–1511. https://doi.org/10.1016/S0140-6736(09)61259-9.

[25] P. O'Connor, J.S. Wolinsky, C. Confavreux, G. Comi, L. Kappos, T.P. Olsson, H. Benzerdjeb, P. Truffinet, L. Wang, A. Miller, M.S. Freedman, Randomized Trial of Oral Teriflunomide for Relapsing Multiple Sclerosis, N. Engl. J. Med. 365 (2011) 1293–1303. https://doi.org/10.1056/nejmoa1014656.

[26] R. Gold, L. Kappos, D.L. Arnold, A. Bar-Or, G. Giovannoni, K. Selmaj, C. Tornatore, M.T. Sweetser, M. Yang, S.I. Sheikh, K.T. Dawson, Placebo-Controlled Phase 3 Study of Oral BG-12 for Relapsing Multiple Sclerosis, N. Engl. J. Med. 367 (2012) 1098–1107. https://doi.org/10.1056/nejmoa1114287.

[27] L. Kappos, E.-W. Radue, P. O´Connor, C. Polman, R. Hohlfeld, P. Calabresi, K. Selmaj, C. Agoropoulou, M. Leyk, L. Zhang-Auberson, P. Burtin, for the FREEDOMS study group, A Placebo-Controlled Trial of Oral Fingolimod in Relapsing Multiple Sclerosis, N. Engl. J. Med. 363 (2010) 2487–2498.

[28] C.H. Polman, P.W. O'Connor, E. Havrdova, M. Hutchinson, L. Kappos, D.H. Miller, J.T. Phillips, F.D. Lublin, G. Giovannoni, A. Wajgt, M. Toal, F. Lynn, M.A. Panzara, A.W. Sandrock, A Randomized, Placebo-Controlled Trial of Natalizumab for Relapsing Multiple Sclerosis, N. Engl. J. Med. 354 (2006) 899–910. https://doi.org/10.1056/nejmoa044397.

[29] S.L. Hauser, A. Bar-Or, G. Comi, G. Giovannoni, H.-P. Hartung, B. Hemmer, F.

Lublin, X. Montalban, K.W. Rammohan, K. Selmaj, A. Traboulsee, J.S. Wolinsky, D.L. Arnold, G. Klingelschmitt, D. Masterman, P. Fontoura, S. Belachew, P. Chin, N. Mairon, H. Garren, L. Kappos, Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis, N. Engl. J. Med. 376 (2017) 221–234. https://doi.org/10.1056/nejmoa1601277.

[30] X. Montalban, R. Gold, A.J. Thompson, S. Otero-Romero, M.P. Amato, D. Chandraratna, M. Clanet, G. Comi, T. Derfuss, F. Fazekas, H.P. Hartung, E. Havrdova, B. Hemmer, L. Kappos, R. Liblau, C. Lubetzki, E. Marcus, D.H. Miller, T. Olsson, S. Pilling, K. Selmaj, A. Siva, P.S. Sorensen, M.P. Sormani, C. Thalheim, H. Wiendl, F. Zipp, ECTRIMS/EAN guideline on the pharmacological treatment of people with multiple sclerosis, Eur. J. Neurol. 25 (2018) 215–237. https://doi.org/10.1111/ene.13536.

[31] H. Hartung, R. Gonsette, N. König, H. Kwiecinski, A. Guseo, S.P. Morrissey, H. Krapf, Mitoxantrone in progressive multiple sclerosis : a placebo- controlled , double-blind , randomised , multicentre trial, Lancet. 360 (2002) 2018–2025.

[32] G. Macaron, D. Ontaneda, Diagnosis and Management of Progressive Multiple Sclerosis, Biomedicines. 7 (2019) 56. https://doi.org/10.3390/biomedicines7030056.

[33] X. Montalban, S.L. Hauser, L. Kappos, D.L. Arnold, A. Bar-Or, G. Comi, J. De Seze, G. Giovannoni, H.P. Hartung, B. Hemmer, F. Lublin, K.W. Rammohan, K. Selmaj, A. Traboulsee, A. Sauter, D. Masterman, P. Fontoura, S. Belachew, H. Garren, N. Mairon, P. Chin, J.S. Wolinsky, Ocrelizumab versus placebo in primary progressive multiple sclerosis, N. Engl. J. Med. 376 (2017) 209–220. https://doi.org/10.1056/NEJMoa1606468.

[34] D. Ontaneda, A.J. Thompson, R.J. Fox, J.A. Cohen, Progressive multiple sclerosis: prospects for disease therapy, repair, and restoration of function, Lancet. 389 (2017) 1357–1366. https://doi.org/10.1016/S0140-6736(16)31320-4.

[35] T. Sato, G. Miyata, The Nutraceutical Benefit, Part I: Green Tea, Nutrition. 16 (2000) 315–317.

[36] A. Mähler, S. Mandel, M. Lorenz, U. Ruegg, E.E. Wanker, M. Boschmann, F.

Paul, Epigallocatechin-3-gallate: a useful, effective and safe clinical approach for targeted prevention and individualised treatment of neurological diseases?, EPMA J. 4 (2013) 1–17. https://doi.org/10.1186/1878-5085-4-5.

[37] O. Aktas, T. Prozorovski, A. Smorodchenko, N.E. Savaskan, R. Lauster, P.-M. Kloetzel, C. Infante-Duarte, S. Brocke, F. Zipp, Green Tea Epigallocatechin-3-Gallate Mediates T Cellular NF-κB Inhibition and Exerts Neuroprotection in Autoimmune Encephalomyelitis, J. Immunol. 173 (2004) 5794–5800. https://doi.org/10.4049/jimmunol.173.9.5794.

[38] J. Wang, Z. Ren, Y. Xu, S. Xiao, S.N. Meydani, D. Wu, Epigallocatechin-3-gallate ameliorates experimental autoimmune encephalomyelitis by altering balance among CD4 + T-cell subsets, Am. J. Pathol. 180 (2012) 221–234. https://doi.org/10.1016/j.ajpath.2011.09.007.

[39] K. Herges, J.M. Millward, N. Hentschel, C. Infante-Duarte, O. Aktas, F. Zipp, Neuroprotective effect of combination therapy of Glatiramer acetate and epigallocatechin-3-gallate in neuroinflammation, PLoS One. 6 (2011). https://doi.org/10.1371/journal.pone.0025456.

[40] A. Mähler, J. Steiniger, M. Bock, L. Klug, N. Parreidt, M. Lorenz, B.F. Zimmermann, A. Krannich, F. Paul, M. Boschmann, Metabolic response to epigallocatechin-3-gallate in relapsing-remitting multiple sclerosis: A randomized clinical trial, Am. J. Clin. Nutr. 101 (2015) 487–495. https://doi.org/10.3945/ajcn.113.075309.

[41] J. Bellmann-Strobl, F. Paul, J. Wuerfel, J. Dörr, C. Infante-Duarte, E. Heidrich, B. Körtgen, A.U. Brandt, C. Pfüller, H. Radbruch, R. Rust, V. Siffrin, O. Aktas, C. Heesen, J. Faiss, F. Hoffmann, M. Lorenz, B.F. Zimmermann, S. Groppa, K.-D. Wernecke, F. Zipp, Epigallocatechin Gallate in Relapsing-Remitting Multiple Sclerosis: A Randomized, Placebo-Controlled Trial, Neurol. Neuroimmunol. NeuroInflammation. 8 (2021) e981. https://doi.org/10.1212/NXI.0000000000000981.

[42] T. Oberwahrenbrock, M. Ringelstein, S. Jentschke, K. Deuschle, K. Klumbies, J. Bellmann-Strobl, J. Harmel, K. Ruprecht, S. Schippling, H.-P. Hartung, O. Aktas, A.U. Brandt, F. Paul, Retinal ganglion cell and inner plexiform layer thinning in

clinically isolated syndrome, Mult. Scler. J. 19 (2013) 1887–1895. https://doi.org/10.1177/1352458513489757.

[43] R. Rust, C. Chien, M. Scheel, A.U. Brandt, J. Dörr, J. Wuerfel, K. Klumbies, H. Zimmermann, M. Lorenz, K.D. Wernecke, J. Bellmann-Strobl, F. Paul, Epigallocatechin Gallate in Progressive MS: A Randomized, Placebo-Controlled Trial, Neurol. Neuroimmunol. Neuroinflammation. 8 (2021) e964. https://doi.org/10.1212/NXI.0000000000000964.

[44] K. Klumbies, R. Rust, J. Dörr, F. Konietschke, F. Paul, J. Bellmann-Strobl, A.U. Brandt, H. Zimmermann, Retinal thickness analysis in progressive multiple sclerosis patients treated with epigallocatechin gallate: optical coherence tomography results from the SUPREMES study., Front. Neurol. 12 (2021) in press. https://doi.org/10.3389/fneur.2021.615790.

[45] C.H. Polman, S.C. Reingold, B. Banwell, M. Clanet, J.A. Cohen, M. Filippi, K. Fujihara, E. Havrdova, M. Hutchinson, L. Kappos, F.D. Lublin, X. Montalban, P. O'Connor, M. Sandberg-Wollheim, A.J. Thompson, E. Waubant, B. Weinshenker, J.S. Wolinsky, Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria, Ann. Neurol. 69 (2011) 292–302. https://doi.org/10.1002/ana.22366.

[46] C.H. Polman, S.C. Reingold, G. Edan, M. Filippi, H.-P. Hartung, L. Kappos, F.D. Lublin, L.M. Metz, H.F. McFarland, P.W. O'Connor, M. Sandberg-Wollheim, A.J. Thompson, B.G. Weinshenker, J.S. Wolinsky, Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria," Ann. Neurol. 58 (2005) 840–846. https://doi.org/10.1002/ana.20703.

[47] J.F. Kurtzke, Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS)., Neurology. 33 (1983) 1444–1452. https://doi.org/10.1212/WNL.33.11.1444.

[48] J.S. Fischer, R. a Rudick, G.R. Cutter, S.C. Reingold, The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment., Mult. Scler. 5 (1999) 244–250. https://doi.org/10.1191/135245899678846168.

[49] L.B. Krupp, N.G. Larocca, J. Muir Nash, A.D. Steinberg, The fatigue severity scale: Application to patients with multiple sclerosis and systemic lupus erythematosus, Arch. Neurol. 46 (1989) 1121–1123. https://doi.org/10.1001/archneur.1989.00520460115022.

[50] J.D. Fisk, P.G. Ritvo, L. Ross, D.A. Haase, T.J. Marrie, W.F. Schlech, Measuring the functional impact of fatigue: Initial validation of the fatigue impact scale, Clin. Infect. Dis. 18 (1994) S79–S83. https://doi.org/10.1093/clinids/18.Supplement_1.S79.

[51] A.T. Beck, C.H. Ward, M. Mendelson, J. Mock, J. Erbaugh, An Inventory for Measuring Depression, Arch. Gen. Psychiatry. 4 (1961) 561–571. https://doi.org/10.1001/archpsyc.1961.01710120031004.

[52] P. Tewarie, L. Balk, F. Costello, A. Green, R. Martin, S. Schippling, A. Petzold, The OSCAR-IB consensus criteria for retinal OCT quality assessment., PLoS One. 7 (2012) e34823. https://doi.org/10.1371/journal.pone.0034823.

[53] D. Huang, E.A. Swanson, C.P. Lin, J.S. Schuman, W.G. Stinson, W. Chang, M.R. Hee, T. Flotte, K. Gregory, C.A. Puliafito, J.G. Fujimoto, Optical coherence tomography, Science (80-.). 254 (1991) 1178–1181. https://doi.org/10.1007/978-3-642-27676-7_21.

[54] M. Bock, F. Paul, J. Dörr, Diagnostik und Verlaufsbeurteilung der Multiplen Sklerose: Stellenwert der optischen Kohärenztomographie, Nervenarzt. 84 (2013) 483–492. https://doi.org/10.1007/s00115-012-3707-2.

[55] S. Motamedi, K. Gawlik, N. Ayadi, H.G. Zimmermann, S. Asseyer, C. Bereuter, J. Mikolajczak, F. Paul, E.-M. Kadas, A.U. Brandt, Normative data and minimally detectable change for inner retinal layer thicknesses using a semi-automated OCT image segmentation pipeline., Front. Neurol. 10 (2019) 1117. https://doi.org/10.3389/FNEUR.2019.01117.

[56] S.M. Smith, N. De Stefano, M. Jenkinson, P.M. Matthews, Normalized accurate measurement of longitudinal brain change., J. Comput. Assist. Tomogr. 25 (2001) 466–475. https://doi.org/10.1097/00004728-200105000-00022.

[57] K. Noguchi, Y.R. Gel, E. Brunner, F. Konietschke, nparLD : An R Software

Package for the Nonparametric Analysis of Longitudinal Data in Factorial Experiments , J. Stat. Softw. 50 (2012) 1–23. https://doi.org/10.18637/jss.v050.i12.

[58] C.J. Azevedo, E. Overton, S. Khadka, J. Buckley, S. Liu, M. Sampat, O. Kantarci, C.L. Frenay, A. Siva, D.T. Okuda, D. Pelletier, Early CNS neurodegeneration in radiologically isolated syndrome, Neurol. Neuroimmunol. NeuroInflammation. 2 (2015) e102. https://doi.org/10.1212/NXI.0000000000000102.

[59] L.J. Balk, A. Cruz-Herranz, P. Albrecht, S. Arnow, J.M. Gelfand, P. Tewarie, J. Killestein, B.M.J. Uitdehaag, A. Petzold, A.J. Green, Timing of retinal neuronal and axonal loss in MS: a longitudinal OCT study, J. Neurol. 263 (2016) 1323–1331. https://doi.org/10.1007/s00415-016-8127-y.

[60] J.M. Gelfand, D.S. Goodin, W.J. Boscardin, R. Nolan, A. Cuneo, A.J. Green, Retinal axonal loss begins early in the course of multiple sclerosis and is similar between progressive phenotypes, PLoS One. 7 (2012) 1–7. https://doi.org/10.1371/journal.pone.0036847.

[61] M. Moccia, N. de Stefano, F. Barkhof, Imaging outcome measures for progressive multiple sclerosis trials, Mult. Scler. 23 (2017) 1614–1626. https://doi.org/10.1177/1352458517729456.

[62] R. Kapoor, P.R. Ho, N. Campbell, I. Chang, A. Deykin, F. Forrestal, N. Lucas, B. Yu, D.L. Arnold, M.S. Freedman, M.D. Goldman, H.P. Hartung, E.K. Havrdová, D. Jeffery, A. Miller, F. Sellebjerg, D. Cadavid, D. Mikol, D. Steiner, E. Bartholomé, M. D'Hooghe, M. Pandolfo, B. Van Wijmeersch, V. Bhan, G. Blevins, D. Brunet, V. Devonshire, P. Duquette, M. Freedman, F. Grand'Maison, F. Jacques, Y. Lapierre, L. Lee, S. Morrow, M. Yeung, M. Dufek, E.K. Havrdová, P. Kanovsky, I. Stetkarova, M. Talabova, J. Frederiksen, M. Kant, T. Petersen, M. Ravnborg, F. Sellebjerg, L. Airas, I. Elovaara, J.P. Eralinna, T. Sarasoja, A. Al Khedr, D. Brassat, B. Brochet, W. Camu, M. Debouverie, D. Laplaud, C. Lebrun Frenay, J. Pelletier, P. Vermersch, S. Vukusi, K. Baum, A. Berthele, J. Faiss, P. Flachenecker, R. Hohlfeld, M. Krumbholz, C. Lassek, M. Maeurer, S. Meuth, T. Ziemssen, O. Hardiman, C. McGuigan, A. Achiron, D. Karussis, R. Bergamaschi, V.B. Morra, G. Comi, S. Cottone, L. Grimaldi, G.L. Mancardi, L. Massacesi, U.

Nocentini, M. Salvetti, E. Scarpini, P. Sola, G. Tedeschi, M. Trojano, M. Zaffaroni, S. Frequin, R. Hupperts, J. Killestein, H. Schrijver, R. Van Dijl, E. van Munster, M. Czarnecki, W. Drozdowski, W. Fryze, H. Hertmanowska, J. Ilkowski, A. Kaminska, G. Klodowska-Duda, M. Maciejowski, E. Motta, R. Podemski, A. Potemkowski, T. Rog, K. Selmaj, Z. Stelmasiak, A. Stepien, A. Tutaj, J. Zaborski, A. Boyko, Z. Chefranova, E. Evdoshenko, F. Khabirov, S. Sivertseva, E. Yakupov, J.C. Alvarez Cermeño, A. Escartin, O.F. Fernandez, A. Garcia-Merino, M.A. Hernandez Perez, G.I. Ayuso, J.M. Lallana, X.M. Gairin, C. Oreja-Guevara, A. Saiz Hinarejos, M. Gunnarsson, J. Lycke, C. Martin, F. Piehl, H. Roshanisefat, P. Sundstrom, M. Duddy, B. Gran, T. Harrower, J. Hobart, R. Kapoor, M. Lee, P. Mattison, R. Nicholas, O. Pearson, W. Rashid, D. Rog, B. Sharrack, E. Silber, B. Turner, A. Williams, J. Woolmore, C. Young, D. Bandari, J. Berger, A. Camac, S. Cohan, J. Conway, K. Edwards, M. Fabian, J. Florin, S. Freedman, D. Garwacki, M. Goldman, D. Harrison, C. Herrman, D. Huang, A. Javed, D. Jeffery, S. Kamin, G. Katsamakis, B. Khatri, A. Langer-Gould, S. Lynch, D. Mattson, T. Miller, A. Miravalle, H. Moses, S. Muley, J. Napier, A. Nielsen, A. Pachner, G. Pardo, M.A. Picone, D. Robertson, W. Royal, C. Sheppard, B. Thrower, C. Twyman, E. Waubant, J. Wendt, V. Yadav, R. Zabad, G. Zarelli, Effect of natalizumab on disease progression in secondary progressive multiple sclerosis (ASCEND): a phase 3, randomised, double-blind, placebo-controlled trial with an open-label extension, Lancet Neurol. 17 (2018) 405–415. https://doi.org/10.1016/S1474-4422(18)30069-3.

[63] F. Lublin, D.H. Miller, M.S. Freedman, B.A.C. Cree, J.S. Wolinsky, H. Weiner, C. Lubetzki, H.P. Hartung, X. Montalban, B.M.J. Uitdehaag, M. Merschhemke, B. Li, N. Putzki, F.C. Liu, D.A. Häring, L. Kappos, Oral fingolimod in primary progressive multiple sclerosis (INFORMS): A phase 3, randomised, double-blind, placebo-controlled trial, Lancet. 387 (2016) 1075–1084. https://doi.org/10.1016/S0140-6736(15)01314-8.

[64] N. De Stefano, D.L. Arnold, Towards a better understanding of pseudoatrophy in the brain of multiple sclerosis patients, Mult. Scler. 21 (2015) 675–676. https://doi.org/10.1177/1352458514564494.

[65] A. Vidal-Jordana, J. Sastre-Garriga, F. Pérez-Miralles, C. Tur, M. Tintoré, A.

Horga, C. Auger, J. Río, C. Nos, M.C. Edo, M.J. Arévalo, J. Castilló, A. Rovira, X. Montalban, Early brain pseudoatrophy while on natalizumab therapy is due to white matter volume changes, Mult. Scler. J. 19 (2013) 1175–1181. https://doi.org/10.1177/1352458512473190.

[66] C.J. Azevedo, S.Y. Cen, A. Jaberzadeh, L. Zheng, S.L. Hauser, D. Pelletier, Contribution of normal aging to brain atrophy in MS, Neurol. Neuroimmunol. Neuroinflammation. 6 (2019). https://doi.org/10.1212/NXI.0000000000000616.

[67] J. Levin, S. Maaß, M. Schuberth, A. Giese, W.H. Oertel, W. Poewe, C. Trenkwalder, G.K. Wenning, U. Mansmann, M. Südmeyer, K. Eggert, B. Mollenhauer, A. Lipp, M. Löhle, J. Classen, A. Münchau, J. Kassubek, F. Gandor, D. Berg, S. Egert-Schwender, C. Eberhardt, F. Paul, K. Bötzel, B. Ertl-Wagner, H.J. Huppertz, I. Ricard, G.U. Höglinger, E. André, C. Blankenstein, M. Canelo, M. Düring, J. Ebentheuer, C. Fricke, A. Gerbes, S. Groiss, D. Gruber, C. Hartmann, T. Kirchner, D. Kroneberg, M. Kunz, S. Lorenzl, A. Moldovan, A. Noda, H. Pape, G. Respondek, E. Schäffer, M. Schneider, A. Schnitzler, W. Schulz-Schaeffer, J. Schwarz, C. Skowronek, A. Storch, V. Tadic, D. Vadász, B. Zimmermann, Safety and efficacy of epigallocatechin gallate in multiple system atrophy (PROMESA): a randomised, double-blind, placebo-controlled trial, Lancet Neurol. 18 (2019) 724–735. https://doi.org/10.1016/S1474-4422(19)30141-3.

[68] L. Chakrawarti, R. Agrawal, S. Dang, S. Gupta, R. Gabrani, Therapeutic effects of EGCG: a patent review, Expert Opin. Ther. Pat. 26 (2016) 907–916. https://doi.org/10.1080/13543776.2016.1203419.

[69] U. Ullmann, J. Haller, J.D. Decourt, J. Girault, V. Spitzer, P. Weber, Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers, Int. J. Vitam. Nutr. Res. 74 (2004) 269–278. https://doi.org/10.1024/0300-9831.74.4.269.

[70] M. Pervin, K. Unno, A. Takagaki, M. Isemura, Y. Nakamura, Function of green tea catechins in the brain: Epigallocatechin gallate and its metabolites, Int. J. Mol. Sci. 20 (2019) 1–12. https://doi.org/10.3390/ijms20153630.

[71] L.C. Lin, M.N. Wang, T.Y. Tseng, J.S. Sung, T.H. Tsai, Pharmacokinetics of (-)-epigallocatechin-3-gallate in conscious and freely moving rats and its brain

regional distribution, J. Agric. Food Chem. 55 (2007) 1517–1524. https://doi.org/10.1021/jf062816a.

[72] E.S. Sotirchos, N. Gonzalez Caldito, A. Filippatou, K.C. Fitzgerald, O.C. Murphy, J. Lambe, J. Nguyen, J. Button, E. Ogbuokiri, C.M. Crainiceanu, J.L. Prince, P.A. Calabresi, S. Saidha, Progressive Multiple Sclerosis Is Associated with Faster and Specific Retinal Layer Atrophy, Ann. Neurol. 87 (2020) 885–896. https://doi.org/10.1002/ana.25738.

[73] J. Chataway, F. De Angelis, P. Connick, R.A. Parker, D. Plantone, A. Doshi, N. John, J. Stutters, D. MacManus, F. Prados Carrasco, F. Barkhof, S. Ourselin, M. Braisher, M. Ross, G. Cranswick, S.H. Pavitt, G. Giovannoni, C.A. Gandini Wheeler-Kingshott, C. Hawkins, B. Sharrack, R. Bastow, C.J. Weir, N. Stallard, S. Chandran, C.A.M. Gandini Wheeler-Kingshott, T. Williams, T. Beyene, V. Bassan, A. Zapata, D. Lyle, J. Cameron, D. Mollison, S. Colville, B. Dhillon, C.J. Weir, R.A. Parker, S. Gnanapavan, R. Nicholas, W. Rashid, J. Aram, H. Ford, J. Overell, C. Young, H. Arndt, M. Duddy, J. Guadagno, N. Evangelou, M. Craner, J. Palace, J. Hobart, D. Paling, S. Kalra, B. McLean, Efficacy of three neuroprotective drugs in secondary progressive multiple sclerosis (MS-SMART): a phase 2b, multiarm, double-blind, randomised placebo-controlled trial, Lancet Neurol. 19 (2020) 214–225. https://doi.org/10.1016/S1474-4422(19)30485-5.

2. Eidesstattliche Versicherung

„Ich, Katharina Klumbies, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: **„Retinale Schichtdickenanalyse bei Multipler Sklerose *in vivo*: Anwendung im frühen Stadium und in einer klinischen Studie mit chronisch-progredientem Verlauf“ (engl.: „Retinal thickness analysis in multiple sclerosis *in vivo*: Application in the early stages and in a clinical study with progressive disease course“)** selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

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

Datum Unterschrift

3. Anteilserklärung an den erfolgten Publikationen

Katharina Klumbies hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: CIS-Kohorte (Fragestellung 1) [42]
T. Oberwahrenbrock, M. Ringelstein, S. Jentschke, K. Deuschle, **K. Klumbies**, J. Bellmann-Strobl, J. Harmel, K. Ruprecht, S. Schippling, H.-P. Hartung, O. Aktas, A.U. Brandt, F. Paul; **Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome**, Multiple Sclerosis Journal, 2013
Journal Impact Factor: 5.412

Beitrag im Einzelnen:
Patient*innenakquisition (telefonische Anfrage und Information von in Frage kommenden Patient*innen über CIS-Studie), Erhebung einiger OCT-Daten und Qualitätskontrolle (Durchführung der OCT-Messungen am Spectralis-OCT sowie kritisches Überprüfen der OCT-Bilder und ggf. Neu-Messung oder Ausschluss von Patient*innen), aus der Erhebung der OCT-Daten sind die Werte für die Berechnungen entstanden, Rekrutierung von gesunden Kontrollen und teilweise Erstellung von OCT-Messungen, Erhebung der klinischen Daten (Durchführung von MSFC, neurokognitiver Testung, Sehtests, Kontrastsehen, Gewährleistung der Durchführung von MRT-, VEP- sowie Laboruntersuchungen), Bewertung und Qualitätskontrolle der klinischen Daten (Überprüfung auf Vollständigkeit und Plausibilität), Überarbeitung des Manuskripts (insbesondere Abstract und Methods).

Publikation 2: SUPREMES-Kohorte (Fragestellung 2a) [43]
R. Rust, C. Chien, M. Scheel, A.U. Brandt, J. Dörr, J. Wuerfel, **K. Klumbies**, H. Zimmermann, M. Lorenz, K.D. Wernecke, J. Bellmann-Strobl, F. Paul; **Epigallocatechin Gallate in Progressive MS: A Randomized, Placebo-Controlled Trial**, Neurology: Neuroimmunology & Neuroinflammation, 2021
Journal Impact Factor: 7.724

Beitrag im Einzelnen:
Erhebung von klinischen Daten (Durchführung von MSFC, Sehtests, Kontrastsehen, Fragebögen), aus der Erhebung der klinischen Daten sind die Werte für die Berechnungen entstanden, Bewertung der klinischen Daten (Überprüfung auf Vollständigkeit und Plausibilität), Überarbeitung des Manuskripts (insbesondere Abstract, Methods und Discussion).

Publikation 3: SUPREMES-Kohorte (Fragestellung 2b) [44]
K. Klumbies, R. Rust, J. Dörr, F. Konietschke, F. Paul, J. Bellmann-Strobl, A.U. Brandt, H. Zimmermann; **Retinal thickness analysis in progressive multiple sclerosis patients treated with epigallocatechin gallate: optical coherence tomography results from the SUPREMES study,** Frontiers in Neurology, 2021
Journal Impact Factor: 2.889

Beitrag im Einzelnen:
Durchführung der OCT-Segmentierung mit dem Samirix-Programm, daraus resultierend Erstellung der Werte der querschnittlichen und longitudinalen Berechnungen, Bewertung der klinischen Daten (Prüfung Ein- und Ausschlusskriterien für OCT), Teile der statistischen Analyse (querschnittliche Berechnungen zur Studienkohorte), aus meiner statistischen Auswertung ist Tabelle 1 entstanden, Erstellen der Abbildungen 1 und 2, Interpretation aller Ergebnisse, Erstellung (Schreiben von Abstract, Introduction, Material and Methods, Results, Discussion und References) sowie Überarbeitung des Manuskripts.

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Unterschrift, Datum und Stempel des/der erstbetreuenden Hochschullehrers/in

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Unterschrift des Doktoranden/der Doktorandin

4. Druckexemplare der ausgewählten Publikationen

T. Oberwahrenbrock, M. Ringelstein, S. Jentschke, K. Deuschle, K. Klumbies, J. Bellmann-Strobl, J. Harmel, K. Ruprecht, S. Schippling, H.-P. Hartung, O. Aktas, A.U. Brandt, F. Paul, Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome, Mult. Scler. J. 19 (2013) 1887–1895. https://doi.org/10.1177/1352458513489757.
Journal Impact Factor: 5.280

R. Rust, C. Chien, M. Scheel, A.U. Brandt, J. Dörr, J. Wuerfel, K. Klumbies, H. Zimmermann, M. Lorenz, K.D. Wernecke, J. Bellmann-Strobl, F. Paul, Epigallocatechin Gallate in Progressive MS: A Randomized, Placebo-Controlled Trial, Neurol. Neuroimmunol. Neuroinflammation. 8 (2021) e964. https://doi.org/10.1212/NXI.0000000000000964.
Journal Impact Factor: 7.724

K. Klumbies, R. Rust, J. Dörr, F. Konietschke, F. Paul, J. Bellmann-Strobl, A.U. Brandt, H. Zimmermann, Retinal thickness analysis in progressive multiple sclerosis patients treated with epigallocatechin gallate: optical coherence tomography results from the SUPREMES study., Front. Neurol. 12 (2021) in press.
https://doi.org/10.3389/fneur.2021.615790
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Research Paper

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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

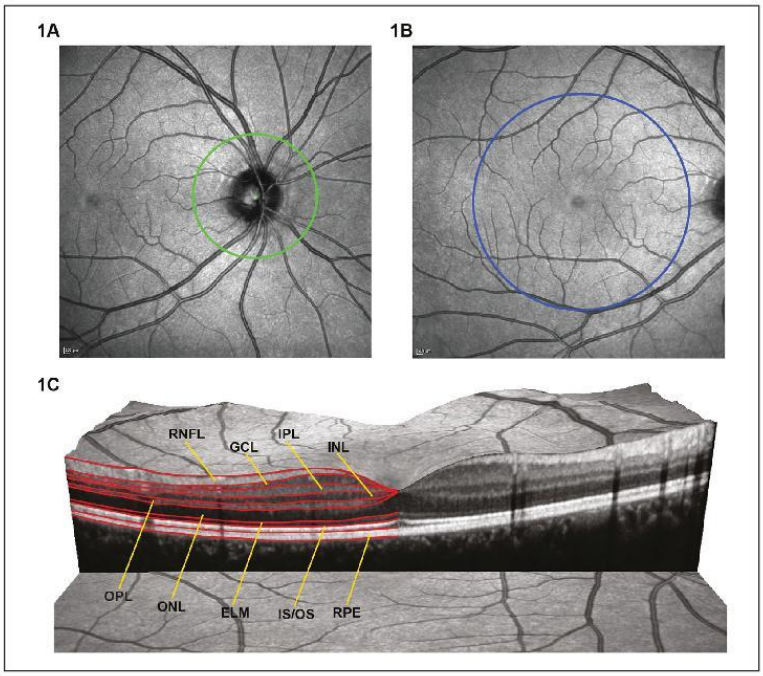

Figure 1. Examples of regions analysed in OCT.
A) Scanning laser ophthalmoscopy image showing the region of the peripapillary ring scan (green); B) Scanning laser ophthalmoscopy image of the macular scan with the blue circle indicating the area for total macular volume and intraretinal layer thickness 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

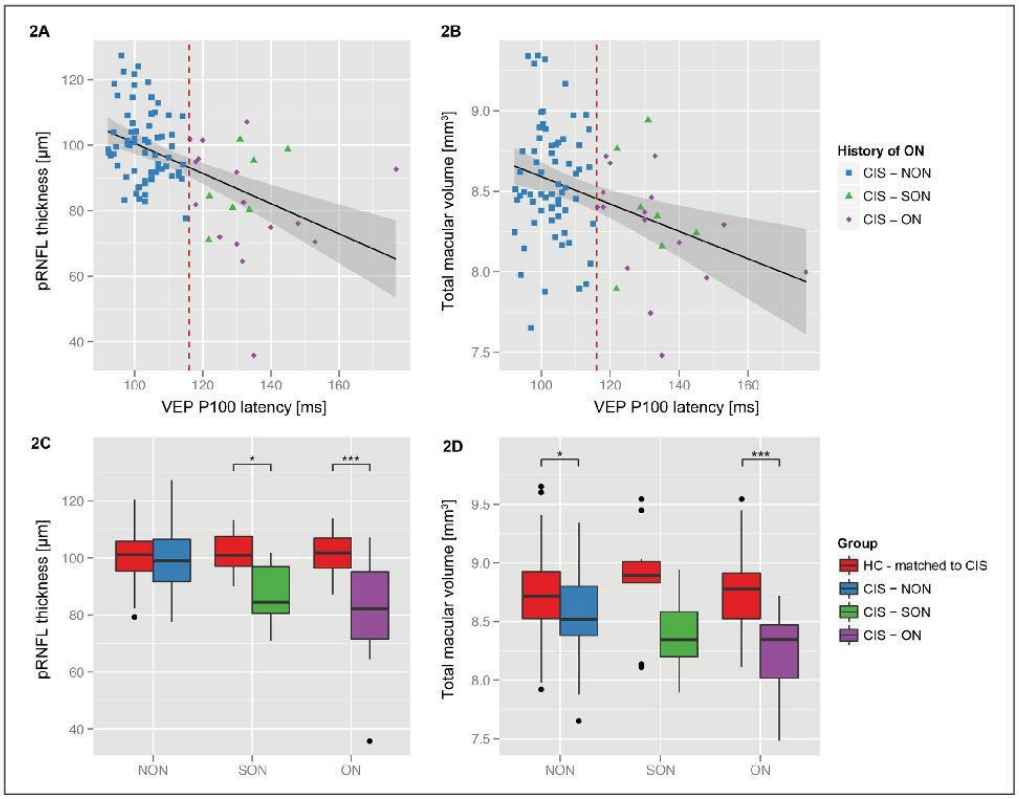

Figure 2. VEP and standard OCT results.
Scatterplots showing the relationship of the VEP P100 latencies with A) peripapillary RNFL (pRNFL) and B) total macular volume. The red dashed line at 115 ms indicates the threshold between CIS-NON and CIS-SON eyes. The black line is the result of the linear regression 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.

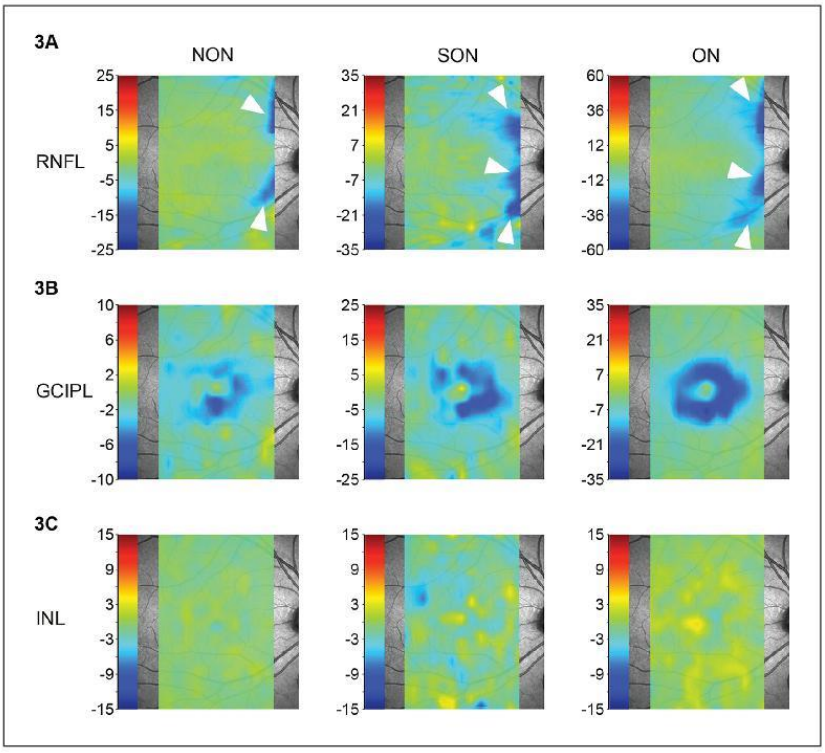

Figure 3. Spatial analysis of changes in CIS eyes versus healthy control eyes.
A) Changes in RNFL thickness between CIS patients and the corresponding group of age- and sex-matched healthy controls. Patients were stratified by history of ON: no history of ON (NON), suspected ON (SON) or clinically-diagnosed 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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References

1. Compston A and Coles A. Multiple sclerosis. *Lancet* 2008; 372: 1502–1517.
2. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69: 292–302.
3. Miller DH, Chard DT and Ciccarelli O. Clinically isolated syndromes. *Lancet Neurol* 2012; 11: 157–169.
4. Filippi M, Bozzali M, Rovaris M, et al. Evidence for widespread axonal damage at the earliest clinical stage of multiple sclerosis. *Brain*. 2003;126: 433–437.
5. Rovaris M, Gambini A, Gallo A, et al. Axonal injury in early multiple sclerosis is irreversible and independent of the short-term disease evolution. *Neurology* 2005; 65: 1626–1630.
6. Frohman E, Costello F, Zivadinov R, et al. Optical coherence tomography in multiple sclerosis. *Lancet Neurol* 2006; 5: 853–863.
7. Petzold A, De Boer JF, Schippling S, et al. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol* 2010; 9: 921–932.
8. Costello F, Coupland S, Hodge W, et al. Quantifying axonal loss after optic neuritis with optical coherence tomography. *Ann Neurol* 2006; 59: 963–969.
9. Albrecht P, Ringelstein M, Müller AK, et al. Degeneration of retinal layers in multiple sclerosis subtypes quantified by optical coherence tomography. *Mult Scler* 2012; 18: 1422–1429.
10. Oberwahrenbrock T, Schippling S, Ringelstein M, et al. Retinal Damage in Multiple Sclerosis Disease Subtypes Measured by High-Resolution Optical Coherence Tomography. *Multiple Sclerosis International* 2012; 2012: 1–10.
11. Gelfand JM, Nolan R, Schwartz DM, et al. Microcystic macular oedema in multiple sclerosis is associated with disease severity. *Brain* 2012; 135: 1786–1793.
12. Balk LJ, Killestein J, Polman CH, et al. Microcystic macular oedema confirmed, but not specific for multiple sclerosis. *Brain* 2012; 135: e226; author reply: e227. doi:10.1093/brain/aws216
13. Green AJ, McQuaid S, Hauser SL, et al. Ocular pathology in multiple sclerosis: retinal atrophy and inflammation irrespective of disease duration. *Brain* 2010; 133: 1591–1601.
14. Saidha S, Sotirchos ES, Ibrahim MA, et al. Microcystic macular oedema, thickness of the inner nuclear layer of the retina, and disease characteristics in multiple sclerosis: a retrospective study. *Lancet Neurol* 2012; 11: 963–972.
15. Gordon-Lipkin E, Chodkowski B, Reich DS, et al. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. *Neurology* 2007; 69: 1603–1609.
16. Dörr J, Wernecke KD, Bock M, et al. Association of retinal and macular damage with brain atrophy in multiple sclerosis. *PLoS ONE* 2011; 6: e18132.
17. Zimmermann H, Freing A, Kaufhold F, et al. Optic neuritis interferes with optical coherence tomography and magnetic resonance imaging correlations. *Mult Scler* 2013;19(4): 443–50.

18. Pfueller CF, Brandt AU, Schubert F, et al. Metabolic changes in the Visual cortex are linked to retinal nerve fiber layer thinning in multiple sclerosis. *PLoS ONE* 2011; 6: e18019.
19. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983; 33: 1444–1452.
20. Sisto D, Trojano M, Vetrugno M, et al. Subclinical visual involvement in multiple sclerosis: a study by MRI, VEPs, Frequency-Doubling Perimetry, Standard Perimetry, and Contrast Sensitivity. *IOVS* 2005; 46: 1264–1268.
21. Outteryck O, Zephir H, Defoort S, et al. Optical coherence tomography in clinically isolated syndrome: no evidence of subclinical retinal axonal loss. *Arch Neurol* 2009; 66: 1373–1377.
22. Kallenbach K, Sander B, Tsakiri A, et al. Neither retinal nor brain atrophy can be shown in patients with isolated unilateral optic neuritis at the time of presentation. *Mult Scler* 2011; 17: 89–95.
23. Bock M, Brandt AU, Dorr J, et al. Time domain and spectral domain optical coherence tomography in multiple sclerosis: a comparative cross-sectional study. *Mult Scler* 2010; 16: 893–896.
24. Saidha S, Syc SB, Ibrahim MA, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. *Brain* 2011; 134: 518–533.
25. Gelfand JM, Goodin DS, Boscardin WJ, et al. Retinal axonal loss begins early in the course of multiple sclerosis and is similar between progressive phenotypes. *PLoS ONE* 2012; 7: e36847.
26. Syc SB, Saidha S, Newsome SD, et al. Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis. *Brain* 2012; 135: 521–533.
27. Zipp F and Aktas O. The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. *Trends Neurosci* 2006; 29: 518–527.
28. Sbardella E, Tomassini V, Stromillo ML, et al. Pronounced focal and diffuse brain damage predicts short-term disease evolution in patients with clinically isolated syndrome suggestive of multiple sclerosis. *Mult Scler* 2011; 17: 1432–1440.
29. Lucchinetti CF, Popescu BFG, Bunyan RF, et al. Inflammatory cortical demyelination in early multiple sclerosis. *N Engl J Med* 2011; 365: 2188–2197.
30. Vogt J, Paul F, Aktas O, et al. Lower motor neuron loss in multiple sclerosis and experimental autoimmune encephalomyelitis. *Ann Neurol* 2009; 66: 310–322.
31. Fairless R, Williams SK, Hoffmann DB, et al. Preclinical retinal neurodegeneration in a model of multiple sclerosis. *J Neurosci* 2012; 32: 5585–5597.
32. Trapp BD, Peterson J, Ransohoff RM, et al. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; 338: 278–285.
33. Holländer H, Bisti S, Maffei L, et al. Electroretinographic responses and retrograde changes of retinal morphology after intracranial optic nerve section. A quantitative analysis in the cat. *Exp Brain Res* 1984; 55: 483–493.
34. Brandt AU, Oberwahrenbrock T, Ringelstein M, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. *Brain* 2011; 134: e193; author reply: e194.
35. Balcer LJ, Baier ML, Cohen JA, et al. Contrast letter acuity as a visual component for the Multiple Sclerosis Functional Composite. *Neurology* 2003; 61: 1367–1373.
36. Saidha S, Syc SB, Durbin MK, et al. Visual dysfunction in multiple sclerosis correlates better with optical coherence tomography derived estimates of macular ganglion cell layer thickness than peripapillary retinal nerve fiber layer thickness. *Mult Scler* 2011; 17: 1449–1463.
37. Seigo M, Sotirchos E, Newsome S, et al. In vivo assessment of retinal neuronal layers in multiple sclerosis with manual and automated optical coherence tomography segmentation techniques. *J Neurol* 2012; 259: 2119–2130.

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Epigallocatechin Gallate in Progressive MS

A Randomized, Placebo-Controlled Trial

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Abstract

Objective

To examine whether treatment with epigallocatechin gallate (EGCG) influences progression of brain atrophy, reduces clinical and further radiologic disease activity markers, and is safe in patients with progressive multiple sclerosis (PMS).

Methods

We enrolled 61 patients with primary or secondary PMS in a randomized double-blind, parallel-group, phase II trial on oral EGCG (up to 1,200 mg daily) or placebo for 36 months with an optional open-label EGCG treatment extension (OE) of 12-month duration. The primary end point was the rate of brain atrophy, quantified as brain parenchymal fraction (BPF). The secondary end points were radiologic and clinical disease parameters and safety assessments.

Results

In our cohort, 30 patients were randomized to EGCG treatment and 31 to placebo. Thirty-eight patients (19 from each group) completed the study. The primary endpoint was not met, as in 36 months the rate of decrease in BPF was 0.0092 ± 0.0152 in the treatment group and −0.0078 ± 0.0159 in placebo-treated patients. None of the secondary MRI and clinical end points revealed group differences. Adverse events of EGCG were mostly mild and occurred with a similar incidence in the placebo group. One patient in the EGCG group had to stop treatment due to elevated aminotransferases (>3.5 times above normal limit).

Conclusions

In a phase II trial including patients with multiple sclerosis (MS) with progressive disease course, we were unable to demonstrate a treatment effect of EGCG on the primary and secondary radiologic and clinical disease parameters while confirming on overall beneficial safety profile.

Clinicaltrial.gov Identifier

NCT00799890.

Classification of Evidence

This phase II trial provides Class II evidence that for patients with PMS, EGCG was safe, well tolerated, and did not significantly reduce the rate of brain atrophy.

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Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

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Glossary

AAR = annualized atrophy rate; **AE** = adverse event; **ARR** = annualized relapse rate; **BBB** = blood-brain barrier; **BDI** = Becks Depression Inventory I; **BPF** = brain parenchymal fraction; **CDP** = confirmed disability progression; **EAE** = experimental autoimmune encephalomyelitis; **EDSS** = Expanded Disability Status Scale; **EGCG** = epigallocatechin gallate; **FSS** = Fatigue Severity Scale; **GMV** = gray matter volume; **GTE** = green tea extract; **ITT** = intention to treat; **MFIS** = Modified Fatigue Impact Scale; **MS** = multiple sclerosis; **MSFC** = MS Functional Composite; **OCT** = optical coherence tomography; **OE** = open-label extension; **PAS** = primary analysis set; **PASAT** = Paced Auditory Serial Addition Test; **PBVC** = Percentage Brain Volume Change; **PMS** = progressive MS; **PP** = per protocol; **PPMS** = primary progressive MS; **RRMS** = relapsing-remitting MS; **SAE** = serious adverse event; **SPMS** = secondary progressive MS; **TIV** = total intracranial volume; **WMV** = white matter volume.

Safe and effective treatment options with neuroprotective properties for progressive MS (PMS) are an unmet clinical need.[1] In contrast to many approved therapies for the relapsing course,[2,3] there are only the monoclonal antibody ocrelizumab[4] for primary progressive MS (PPMS) and the chemotherapy agent mitoxantrone as well as newly the sphingosine 1-phosphate receptor modulator siponimod[5] for the treatment of secondary progressive MS (SPMS).

One of the main goals for PMS treatment is to slow progression of neurologic impairment arising from permanent tissue injury[1] often evaluated by the degree of brain atrophy.[6]

Epigallocatechin-3-gallate (EGCG) is a polyphenolic green tea catechin[7] with anti-inflammatory and neuroprotective properties demonstrated in animal and ex vivo studies.[8,9] In experimental autoimmune encephalomyelitis (EAE), an animal model of MS, it was shown to reduce brain inflammation and neuronal damage by influencing T-cell proliferation, inhibiting the activation of nuclear factor-κB (NF-κB), and exerting antioxidant effects.[10–12]

Its approval as a dietary supplement with a satisfactory long-term safety profile[13] could make EGCG an attractive treatment for patients with PMS with possible neuroprotective effects.

The present study investigated the effect of EGCG on brain atrophy, further radiologic parameters, and clinical disease activity and safety aspects in patients with PMS during a 36-month double-blind treatment period. This study was extended by an optional open-label period (OE) for another 12 months.

Methods

Primary Research Question

This monocentric, prospective, phase II, double-blinded, parallel-group, randomized controlled trial was designed to evaluate the question whether the oral intake of up to 1,200 mg EGCG reduces the rate of brain atrophy in patients with PMS and is safe and well tolerated. The study was conducted in Berlin, Germany, from May 2009 to February 2016. The study is rated Class II because less than 80% of enrolled patients completed the study.

Patients

Eligibility criteria comprised fulfillment of the 2005 revised McDonald criteria for MS[14] and the diagnosis of PPMS or SPMS, age between 18 and 65 years, Expanded Disability Status Scale (EDSS)[15] score of 3 to 8 at screening, and a relapse-free period of at least 30 days before randomization. No MS disease-modifying therapy was allowed.

Key exclusion criteria were a relapsing-remitting form of MS, a major systemic or CNS disease, especially such as Parkinson, Huntington, or Alzheimer disease as well as clinically relevant predefined laboratory abnormalities (aminotransferases >3.5 times above normal limit), and intake of any potentially hepatotoxic medication. Additional consumption of green tea or green tea extract (GTE) was prohibited.

Standard Protocol Approvals, Registrations, and Participant Consents

The study was approved by the local ethics committees (LaGeSo ZS EK 10 407/08, new: 08/0407-EK 15) and by the German Federal Institute for Drugs and Medical Devices (BfArM). This trial is registered with EudraCT (2008-005213-22) and clinicaltrials.gov (NCT00799890). It was conducted according to the Declaration of Helsinki in its applicable version, and every participant provided written informed consent before screening.

Data Availability

As far as permitted according to data protection requirements and consent provided by the participants, original data are available from the corresponding author on request from any qualified investigator within 5 years after publication.

Randomization and Blinding

To account for potential baseline data imbalances, patients were stratified before randomization for sex (female and male) and diagnosis (PPMS and SPMS). Patients were randomly (1:1) assigned to receive either Sunphenon/EGCG capsules (GTE containing >90% EGCG, product of Taiyo International, taiyointernational.com) or capsules of placebo with identical appearance.

A block randomization list was generated by the independent pharmacy to assign patients either to EGCG or to placebo for 36 months.

Only the pharmacist was aware of treatment allocation throughout the study; all staff and patients remained blinded to treatment allocation with the exception of 1 patient who was prematurely unblinded by having the study medication analyzed in an external laboratory at his own discretion. This led to the patient's exclusion from the study.

Following the blinded randomized part of the study (until month 36), the patients were offered the opportunity to participate in another 12-month OE, in which all patients received EGCG.

Procedures

Following randomization, patients started treatment with EGCG or placebo capsules 200 mg daily. Divided into 2 doses, they were escalated after 3 months to 400 mg daily, after 6 months to 600 mg daily, after 18 months to 800 mg, and after 30 months to 1,200 mg daily until the end of the study at month 36.

Patients initially treated with placebo and decided to participate in the 12-month OE started treatment with EGCG capsules 200 mg daily, then escalated every 2 weeks with 200 mg, reaching 1,200 mg after 10 weeks. For the patients treated with EGCG, the dosage was maintained during OE, until month 48 if they participated.

Patients received containers with EGCG capsules or placebo sufficient until the next study visit. At each of these visits, drug accountability was performed (number of taken capsules).

Standardized neurologic assessments including EDSS[15] and MS Functional Composite (MSFC)[16,17] consisting of 9-Hole Peg Test, timed 25-foot walk test, and Paced Auditory Serial Addition Test (PASAT) were performed by a blinded and especially trained examiner at the initial screening (which was at most 1 week before randomization), then every 6 months, and at every unscheduled visit when a relapse was suspected. To avoid any training effect in the PASAT, each participant underwent at least 3 test scorings before study scoring.

At baseline and at month 36, fatigue and depressive symptoms were assessed by the Fatigue Severity Scale (FSS)[18] and Modified Fatigue Impact Scale (MFIS)[19] as well as Becks Depression Inventory I (BDI).[20] An optical coherence tomography (OCT) was also performed every 12 months.

Safety assessments included reporting of adverse events (AEs), medical examinations, and laboratory examinations. Visits were scheduled every 2–3 months and with short-term follow-up in case of pathologic results.

MRI Data Acquisition and Analysis

MRI was performed on one 1.5 Tesla scanner (Sonata Siemens, Erlangen, Germany). The MRI protocol included a T2w fluid-attenuated inversion recovery sequence (TR/TE = 10,000/108 ms, 0.5 × 0.5 × 3 mm^3, no gap) and a high-resolution 3D T1-weighted sequence (magnetization prepared rapid acquisition gradient echo, MPRAGE: TR/TE = 2110/4.38 ms, 1 × 1 × 1 mm^3), before and after IV contrast agent administration. Brain parenchymal fraction (BPF), percent brain volume change (PBVC), and T2w hyperintense lesions were quantified at screening and months 12, 24, and 36, whereas contrast-enhancing T1-weighted lesions (CELs) were quantified at screening and month 36.

Brain atrophy was assessed from lesion infilled MPRAGE images using 2 approaches. BPF was assessed for each time point using the CAT12 software package (version 12.5—neuro.uni-jena.de/cat/). Here, gray matter volume (GMV) and white matter volume (WMV) and total intracranial volume (TIV) were segmented and visually checked for segmentation errors. BPF was calculated as follows: BPF = (GMV + WMV)/TIV. Atrophy was then calculated as the difference between baseline and subsequent time points. In an additional approach, the PBVC was quantified longitudinally using the SIENA pipeline (FMRIB software package, FSL Version 5.0.9).[21]

T2w lesion load and CELs were manually segmented using ITK-SNAP.[22] Lesions were infilled in MPRAGE images using the FSL lesion filling tool (FMRIB software package, FSL Version 5.0.9).[21]

Primary and Secondary Outcomes

The primary outcome was the change of BPF[23] from baseline to month 36. Secondary MRI outcome measures were PBVC[21] at month 36, increase (difference from month 36 to baseline) in number and volume of all T2-weighted (T2w) hyperintense lesions, and the number and volume of CELs at month 36. Secondary outcomes of OCT are reported in detail elsewhere.

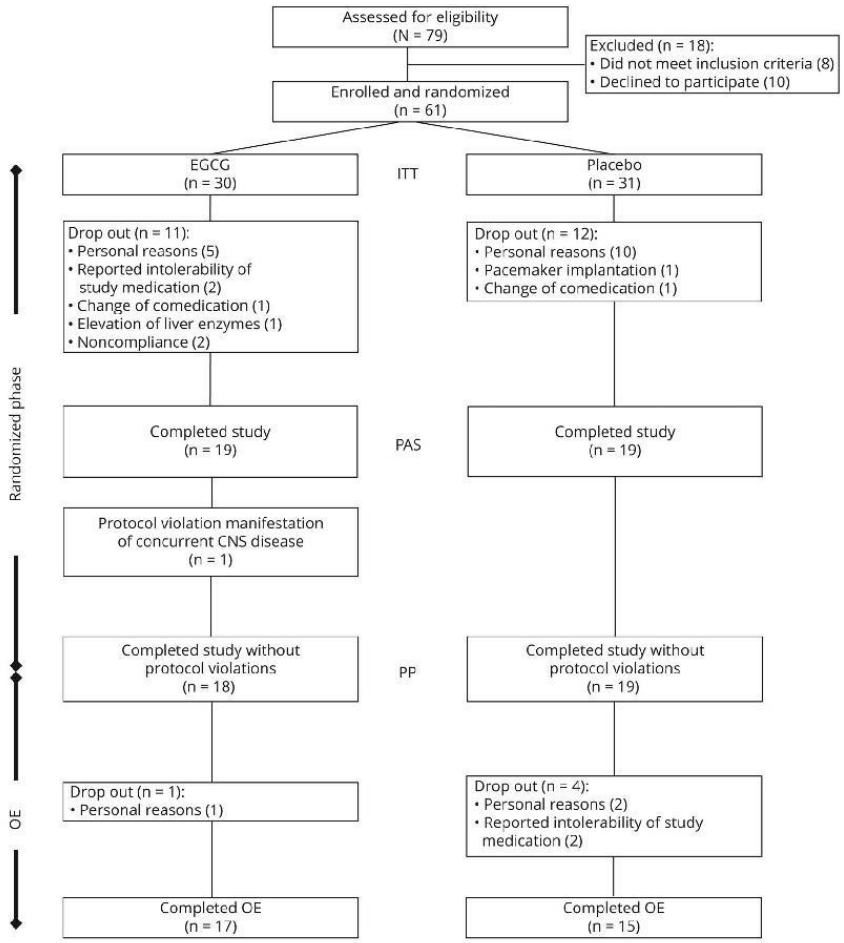
Secondary clinical outcome measurements were disability progression as measured by EDSS and confirmed disability progression (CDP) defined as a 1-point increase in the EDSS if the baseline score was 3.0–5.5, or a 0.5-point increase if the baseline score was 6.0 and above, confirmed at a scheduled visit 6 months later. Further secondary clinical outcome parameters were annualized relapse rate (ARR), MSFC, BDI, FSS, and MFIS.

Safety assessments were also part of the secondary outcomes. At the end of the OE, the primary and secondary outcome parameters were assessed again.

Statistical Analysis

The study was initially planned as a double-blind adaptive pilot study for the inclusion of an initial total of 60 patients with subsequent sample size recalculation.[24] The latter was not performed due to recruitment difficulties. At the end of the blinded phase (after 36 months), the study was unblinded, resulting in 61 patients altogether (30 randomized to verum

Figure 1 Consort Diagram



ITT = intention-to-treat population; OE = open-label extension; PAS = primary analysis set; PP = per-protocol population.

and 31 randomized to placebo) and an OE implemented (compare CONSORT diagram, figure 1).

Results are expressed as arithmetic mean ± SD, median (range), or frequencies (%). The primary end point BPF was assessed using the exact Mann-Whitney test.

Continuous secondary endpoints were tested for differences between groups by using the nonparametric (exact) Mann-Whitney test for independent groups. Differences in categorical variables were tested by the Fisher exact test.

Differences between the verum and placebo group with respect to the whole time course were analyzed using nonparametric analysis of longitudinal data in a 2-factorial design[25] (first factor (independent): treatment groups, second factor (dependent): study visits). This cumulates in 3 tests: differences in groups, significant changes in time, and interactions between groups and time. When appropriate, multivariate, nonparametric analysis of covariance[26] using baseline values as covariates was complemented.

Because of the large number of missings and lost to follow-up, we abstained from a full data set analysis according to the intention-to-treat (ITT) principle. Instead, we used a modified ITT approach, in which we excluded patients in both groups who dropped out of the study (primary analysis set [PAS], 38 patients). In addition, a per-protocol analysis (PP,

Table 1 MRI Outcome Parameters After 36 Months (Primary Analysis Set)

	EGCG (n = 19)	Placebo (n = 19)	*p* Value
BPF	0.6943 (0.0502)	0.6867 (0.0439)	0.608[a]
Change from baseline	0.0092 (0.0152)	0.0078 (0.0159)	0.670[a]
Median	0.7040 (0.6000 to 0.7710)	0.6840 (0.6020 to 0.7560)	
Percent brain volume change	−0.5659 (0.9818)	−0.8013 (1.1996)	0.603[a]
Median	−0.5869 (−2.3057 to 0.9561)	−0.9600 (−2.4856 to 0.9701)	
No. of T2w lesions	35.21 (16.84)	39.32 (19.28)	0.501[a]
Change from baseline	1.52 (4.23)	3.78 (4.88)	0.146[a]
Median	30 (8 to 63)	39 (5 to 76)	
Volume of T2w lesions (mL)	17.57 (16.47)	16.90 (17.30)	0.773[a]
Change from baseline (mL)	1.04 (1.48)	0.52 (2.36)	0.043[a]
Median	11.65 (1.64 to 64.63)	12.20 (0.91 to 67.98)	
No. of CELs[b]	0.00 (0.00)	0.13 (0.34)	0.964[a]
Median	0 (0 to 0)	0 (0 to 1)	
Volume of CELs (mL)[b]	0.00 (0.00)	0.00 (0.01)	0.984[a]
Median	0.00 (0.00 to 0.00)	0 (0.00 to 0.04)	

Abbreviations: BPF = brain parenchymal fraction; CEL = contrast-enhancing lesion; EGCG = epigallocatechin-3-gallate.
Data are mean (SD) or median (range).
[a] Exact Mann-Whitney test.
[b] Number and volume of CELs for 18 patients of EGCG and 16 patients of the placebo group.

37 patients) was performed, omitting patients who severely violated study protocol (see CONSORT diagram, figure 1).

A p value <0.05 was considered statistically significant. All tests of secondary end points were conducted as exploratory data analysis. Therefore, no adjustments for multiple testing were made.

Numerical calculations were performed using SAS version 9.4 [TS1M3] copyright 2002-2012 by SAS Institute Inc., Cary, NC, IBM SPSS Statistics, Version 25, Copyright 1989, 2010 SPSS Inc., an IBM Company, Chicago, IL. and The R Project for Statistical Computing, Version 3.0.2 (2017-04-21).

Results

Patients

Sixty-one participants were randomly assigned to receive either EGCG (n = 30) or placebo (n = 31) (figure 1). The EGCG and placebo group were similar for all baseline variables (table e-1, links.lww.com/NXI/A420). Thirty-seven percent of patients in the EGCG group and 39% of those in the placebo group had primary progressive disease; the others had secondary progressive disease. All included patients were of Caucasian ethnicity.

Thirty-eight patients (19 from each group) completed the study and were analyzed for the primary outcome. Twenty-three patients (11 EGCG [36.7%] and 12 placebo [38.7%]) withdrew from treatment (figure 1), mainly for personal reasons or change of comedication.

In the EGCG group, 2 patients reported partial intolerability to the study medication (not specified) and discontinued the study (dropout), and 1 patient dropped out due to elevated aminotransferases (>3.5 times above normal limit), which normalized after seizing medication. Reduction of study drug dosage was not required in any other patient.

All participants completing the full 36 months had a compliance of at least 80% when evaluating intake of study medication.

MRI Outcomes

The results of the ITT analyses for the MRI outcome parameters are summarized in table 1. Regarding the primary end point difference BPF (BPF [baseline–month 36]), we observed no difference between groups (EGCG = 0.0092 [SD 0.0152]; placebo = 0.0078 [SD 0.0159]; p = 0.670), giving annualized atrophy rates (AARs) of 0.31% for verum and 0.26% for the placebo group (difference 0.05%).

Regarding secondary end points at month 36, the EGCG and the placebo group did not differ in PBVC (p = 0.603, giving AAR of 0.19% for verum and 0.27% for placebo

Table 2 Clinical Outcome Parameters After 36 Months (Primary Analysis Set)

	EGCG	Placebo	*p* Value
EDSS	n = 19	n = 20	
Mean	6.08 (1.07)	5.73 (1.12)	0.098[a]
Change from baseline	0.26 (0.45)	0.57 (0.99)	0.421[a]
Median	6.5 (3.0–8.0)	6.0 (3.5–8.0)	
Annualized relapse rate	n = 19	n = 20	
Mean	0.24 (0.46)	0.19 (0.44)	0.513[a]
Progression by EDSS	n = 18	n = 19	
Number	6 (33.3%)	8 (42.1%)	0.737[b]
MS functional composite (*z*-score)	n = 12	n = 15	
Mean	0.56 (0.45)	0.07 (0.75)	0.931[a]
Change from baseline	0.16 (0.37)	−0.13 (0.38)	0.126[a]
Paced Auditory Serial Addition test	n = 17	n = 20	
Mean	51.35 (10.95)	42.05 (14.90)	0.051[a]
Change from baseline	3.82 (9.65)	1.00 (5.79)	0.292[a]
9-Hole Peg Test in s (average)	n = 16	n = 19	
Mean	27.64 (11.36)	31.27 (8.32)	0.117[a]
Change from baseline	1.48 (7.94)	3.00 (6.82)	0.172[a]
Timed 25-Foot Walk Test in s (average)	n = 14	n = 16	
Mean	14.19 (10.61)	10.98 (8.07)	0.275[a]
Change from baseline	1.99 (9.00)	0.23 (5.85)	0.880[a]
FSS	n = 10	n = 11	
Mean	4.41 (2.07)	4.54 (1.76)	0.931[a]
Change from baseline	−0.90 (1.86)	−0.38 (1.96)	0.813[a]
MFIS	n = 18	n = 19	
Mean	38.89 (21.65)	34.11 (13.59)	0.412[a]
Change from baseline	−3.76 (12.63)	2.06 (12.11)	0.178[a]
BDI	n = 18	n = 18	
Mean	9.78 (7.37)	9.00 (6.37)	0.820[a]
Change from baseline	0.13 (4.98)	0.41 (5.17)	0.610[a]

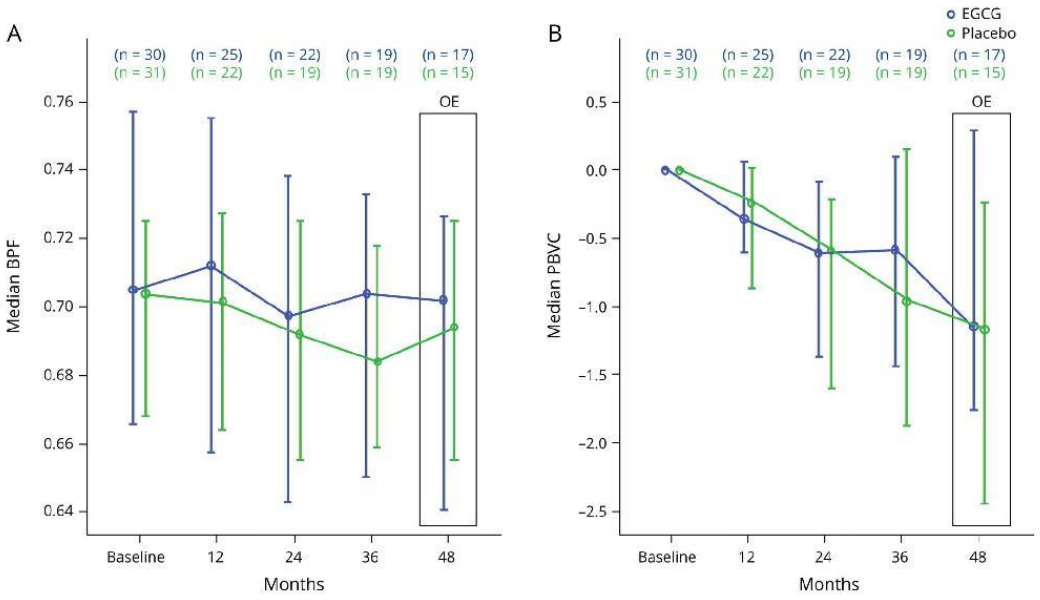
Abbreviations: BDI = Beck Depression Inventory; EDSS = Expanded Disability Status Scale; EGCG = epigallocatechin-3-gallate; FSS = Fatigue Severity Scale; MFIS = Modified Fatigue Impact Scale.
Data are mean (SD), number (%) or median (range).
[a] Exact Mann-Whitney test.
[b] Exact χ^2 test.

(difference 0.08%), T2w lesion count and volume, and in CELs (table 1).

Clinical Outcomes

When evaluating clinical end points (table 2), we found no difference between groups in EDSS, CDP, the mean change in EDSS between baseline and at month 36, MSFC and its subscores, and BDI as well as fatigue scores. Eighteen of 27 patients (66.67%) in the EGCG and 20/28 patients (71.43%) in the placebo group were relapse free during the study. The ARR until month 36 and CDP were similar in both groups. There was no difference between EGCG and placebo in the ARR between baseline and month 18 and between months 18 and 36 (data not shown).

Figure 2 Multivariate Longitudinal Analysis of Brain Atrophy Over 48 Months



(A) Primary outcome: brain parenchymal fraction; only a significant effect of time was observed ($p < 0.001$), no group difference ($p = 0.520$) and no interaction ($p = 0.647$). (B) Secondary outcome: percentage brain volume change; significant effect of time ($p < 0.001$), no group difference ($p = 0$ 0.476), and no interaction ($p = 0.807$). Bars represent 25%–75% quartiles. EGCG = epigallocatechin-3-gallate; OE = open-label extension.

The results of the PP analyses concerning primary and all secondary outcome parameters did not differ from those of the PAS analyses (data not shown).

Subgroup Analyses

In performed subgroup analyses for patients with lower and higher BPF (≤ median BPF vs >median BPF at baseline) and for patients with and without CEL during the study, the change in brain atrophy was not significantly different between groups. Also in subgroups with clinically milder disease (EDSS score <5) and in patients with lower Individual Progression Index (EDSS/years of symptoms), we could not detect a difference for the primary end point.

Furthermore, no sex effects were found relating to PBVC, BPF, and EDSS.

Longitudinal Analyses

Longitudinal analyses of the entire time course[25] including all available time points (0, 12, 24, and 36 months) also showed no difference in MRI and clinical parameters for the primary and secondary end points. These findings were confirmed by longitudinal covariance analyses[24] (see multivariate longitudinal analysis for brain atrophy in figure 2 and T2w lesions in figure 3).

Safety

Of the 30 participants in the EGCG group 29 (96.7%) and of the 31 participants in the placebo group, 28 (90.3%) experienced 1 or more AEs. Eleven (36.7%) in the EGCG and 10 (32.3%) in the placebo group had a serious adverse event (SAE). None of the SAEs were considered related to the study drug. All occurred due to hospitalization of study participants for various reasons (table e-2, links.lww.com/NXI/A420).

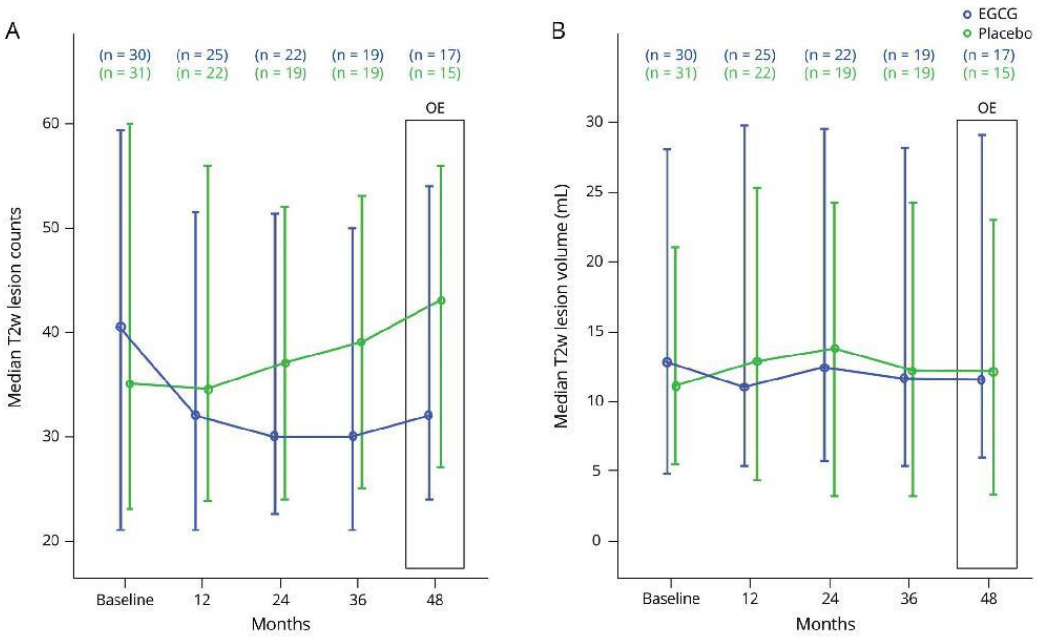
The incidence of SAEs and AEs was similar in both study groups. The most common AEs (>3%) were flu-like infections, urinary tract infections, fractures and contusions after falling, and elevated liver enzymes, without statistical difference between groups.

Open-Label Extension

Seventeen patients from the EGCG group and 15 patients from the former placebo group were available for follow-up assessments at the end of OE. At month 48, there were no significant differences in BPF (BPF former EGCG = 0.6911, BPF former placebo group = 0.6879; $p = 0.860$). PBVC and clinical progression parameters (EDSS, MSFC, and subscales) showed no significant difference between former groups and to the randomized phase of the study (data of the OE not shown).

During OE, AEs and SAEs were similar to the randomized phase, especially no elevation of liver enzymes or other hepatotoxic side effects occurred. However, 2 patients reported intolerability of study medication and decided to stop treatment.

Figure 3 Multivariate Longitudinal Analysis of T2w Lesions Over 48 Months



(A) Secondary outcome: median T2w lesion counts; a significant effect of time was observed ($p < 0.001$), no group difference ($p = 0.582$) and no interaction ($p = 0.417$). (B) Secondary outcome: median T2w lesion volume in mL; significant effect of time ($p < 0.001$), no group difference ($p = 0.821$), and no interaction ($p = 0.324$). Bars represent 25%–75% quartiles. EGCG = epigallocatechin gallate; OE = open-label extension.

Discussion

This randomized, placebo-controlled trial failed to show an effect of oral EGCG on radiographic (brain atrophy, T2w lesions) and clinical (EDSS, relapses, and MSFC) disease progression in patients with SPMS or PPMS. These results challenge preclinical data suggesting a neuroprotective and anti-inflammatory capacity of EGCG in an animal study with EAE[10] where it was shown that orally applied EGCG decreased T-cell proliferation and TNFα production of encephalitogenic T-cells via suppression of NF-κB activation and inhibited neuronal cell death by interference with reactive oxygen species formation. These findings provided the rationale for putative antioxidant and anti-inflammatory effects of EGCG also in human CNS. However, our results are in line with a study on EGCG in multiple system atrophy[27] and another study from our group that did not find an effect of oral EGCG on T2w lesion evolution, PBVC, and clinical disease measures in patients with relapsing-remitting MS (RRMS).[28]

A key issue of the negative outcome of our study seems to be the small sample size of the study. With only 61 patients included and a dropout rate of more than 30% (mostly due to personal reasons and less to side effects), our study was underpowered and the effect size was overestimated from the beginning as we have learned meanwhile.[29] A post hoc power calculation revealed a number of 1936 patients per group needed to detect the given effect size = 0.092 with a power of 80% and a type 1 error (α) of 5% (2 sided). With the 19 patients per group of our specific cohort, it would only be possible to detect a high effect size = 1.00.

Even in the recently published MS-SMART Study, investigating the effects of 3 different neuroprotective substances with about 100 patients per group, no difference in PBVC could be detected.[30]

Our cohort was a representative population of patients with PMS, including a large proportion of patients who were in a nonrelapsing stage of PMS and had a high level of established disability with a median EDSS score of 6.0 at study entry. Nevertheless, we unexpectedly detected a nonpathologic annual PBVC rate (0.2–0.3% per year) in our study population in comparison to various other PMS trials examining the effect of fingolimod,[31] siponimod,[5] lamotrigine,[32] ocrelizumab,[4] or natalizumab,[33] reporting an annual atrophy rate of 0.4–0.7%, disregarding the verum and placebo group. Only 2 studies with PMS reported a similarly low atrophy rate (ibudilast[6] and simvastatin/verum arm[34]). The possibility to prove a positive effect of an intervention depends on adequate

dynamics of the investigated variable. Therefore, we may speculate that our study population was too stable to detect a beneficial effect on radiographic disease progression markers.

Another possible reason for the negative outcome seems to be the insufficient bioavailability of oral EGCG in the doses used in these studies.[35] Previous studies had reported doses of up to 800 mg EGCG per day as safe and generally well tolerated, e.g., in healthy volunteers, where the plasma elimination half-life of EGCG was measured to be about 5 hours after repeated administration of 800 mg EGCG daily over 10 days.[36] Therefore, we chose a maximum daily dose of 800 mg EGCG until month 30, a maximum daily dose of 1,200 mg until month 36, and for the optional OE until month 48. Evidence was found that 600 mg EGCG beneficially influences muscle metabolism in patients with MS[11]; however, our dosages were not sufficient to achieve an effect in the CNS. Recently, a new study proposed the bioavailability of EGCG to be less than 1% in humans from ingestion, with a clearance from the systemic circulation within a few hours.[7] Although we did not measure plasma levels of EGCG in this study, our previous study in RRMS showed that plasma levels of EGCG are extremely variable across patients despite equal dosing.[28] Moreover, although passage of EGCG through the blood-brain barrier (BBB) was shown in animal studies,[7] proof of CNS entry of EGCG in humans is lacking.

In comparison to, e.g., the ocrelizumab ORATORIO trial (baseline: median EDSS score 4.5), the disease duration and the EDSS were higher in our study. Furthermore, active progression just before study entry was not mandatory for our trial. The nature of the EDSS as an ordinal scale results in scores that are unequally distributed, and the individuals remain at a step in the scale for different lengths of time, especially at higher EDSS scores despite progressive disability.[37] The considerations may explain why in a clinically stable cohort with high disability levels, subtle positive effects of EGCG at certain EDSS levels could not be demonstrated.

Although hepatotoxicity has been discussed as a potentially severe side effect of green tea dietary supplements[13] and Polyphenon,[38] we did not observe any related SAE with our EGCG dosing regimen. In our study, only 1 subject dropped out due to elevated liver enzymes. Also, in our study on EGCG in RRMS, no relevant liver toxicity occurred.[28] A possible explanation could be that pure EGCG is less harmful than GTE or Polyphenon regarding hepatotoxicity. GTE and Polyphenon contain several types of polyphenols. However, in the PROMESA study, 8 of 47 patients treated with EGCG up to a maximum dose of 1,200 mg for up to 40 weeks (48 weeks in total including the dosage phase) experienced hepatotoxicity. This was determined as increased aminotransferase concentrations of which 2 were regarded as SAEs (aminotransferase concentrations greater than 5 times the upper limit).[27] The concomitant medication with among others levodopa (which itself may cause elevated liver enzymes) and the mean age of the patients being 10 years older than in the MS studies (possibly leading to more concomitant diseases) may be an explanation for worse tolerability.

Recent studies reported beneficial effects of orally applied EGCG on cognitive functions in combination with cognitive training in patients with Down syndrome and fragile X syndrome.[39,40] Our study also found an improvement of the PASAT score in both study groups, favoring EGCG (change from baseline: EGCG 3.82 [SD 9.65], placebo 1.00 [SD 5.79]; p value = 0.051). The PASAT measures cognitive function such as calculation ability, auditory information processing speed, and flexibility. These findings may suggest that EGCG could have a positive effect on the cognitive functions of patients with PMS. Training effects of the PASAT due to 3 test scorings before the study are unlikely. However, this result should be interpreted carefully because it was observed as a statistical trend and our study was not designed to evaluate this outcome specifically.

EGCG at a dose of up to 1,200 mg daily was overall safe and well tolerated in patients with PMS over a period of 36 months and a 12-month open-label extension. However, we did not find an effect of treatment on MRI or clinical disease activity parameters. Possible explanations include the small sample size and the high dropout rate. First indications were found that EGCG treatment may beneficially affect cognitive functions also in MS. Thus, further investigation in larger MS cohorts may be warranted, especially for improvement of cognitive functions with adjuvant treatment. Such studies should consider using optimized formulations of EGCG for increased bioavailability and ideally with proven BBB passage.

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References

1. Fazekas F. Where to go next with neuroprotection in multiple sclerosis? Lancet Neurol 2010;9:647–648.
2. Reich DS, Lucchinetti CF, Calabresi PA. Multiple sclerosis N Engl J Med 2018;378:169–180.
3. Fiol MP, Ysrraelit MC, Gaitán MI, Correale J. Progressive multiple sclerosis: from pathogenic mechanisms to treatment. Brain 2016;140:527–546.
4. Montalban X, Hauser SL, Kappos L, et al. Ocrelizumab versus placebo in primary progressive multiple sclerosis. N Engl J Med 2017;376:209–220.
5. Kappos L, Bar-Or A, Cree BAC, et al. Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. Lancet 2018;391:1263–1273.
6. Chen JE, Glover GH. Phase 2 trial of ibudilast in progressive multiple sclerosis. N Engl J Med 2016;25:289–313.
7. Pervin M, Unno K, Takagaki A, Isemura M, Nakamura Y. Function of green tea catechins in the brain: epigallocatechin gallate and its metabolites. Int J Mol Sci 2019;20:1–12.
8. Wang J, Ren Z, Xu Y, Xiao S, Meydani SN, Wu D. Epigallocatechin-3-gallate ameliorates experimental autoimmune encephalomyelitis by altering balance among CD4+ T-cell subsets. Am J Pathol 2012;180:221–234.
9. Lorenz M, Paul F, Moobed M, et al. The activity of catechol-O-methyltransferase (COMT) is not impaired by high doses of epigallocatechin-3-gallate (EGCG) in vivo. Eur J Pharmacol 2014;740:645–651.
10. Aktas O, Prozorovski T, Smorodchenko A, et al. Green tea epigallocatechin-3-gallate mediates T cellular NF- B inhibition and exerts neuroprotection in autoimmune encephalomyelitis. J Immunol J Immunol 2004:173:5794-5800.
11. Mähler A, Steiniger J, Bock M, et al. Metabolic response to epigallocatechin-3-gallate in relapsing-remitting multiple sclerosis: a randomized clinical trial. Am J Clin Nutr 2015;101:487–495.
12. Mähler A, Mandel S, Lorenz M, et al. Epigallocatechin-3-gallate: a useful, effective and safe clinical approach for targeted prevention and individualised treatment of neurological diseases? EPMA J 2013;4:5.
13. Dekant W, Fujii K, Shibata E, Morita O, Shimotoyodome A. Safety assessment of green tea based beverages and dried green tea extracts as nutritional supplements. Toxicol Lett 2017;277:104–108.

14. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald criteria." Ann Neurol 2005;58:840–846.
15. Kurtzke JF. On the origin of EDSS. Mult Scler Relat Disord 2015;4:95–103.
16. Cohen JA, Reingold SC, Polman CH, Wolinsky JS. Disability outcome measures in multiple sclerosis clinical trials: current status and future prospects. Lancet Neurol 2012;11:467–476.
17. Fischer JS, Rudick RA, Cutter GR, Reingold SC. The multiple sclerosis functional composite measure (MSFC): an integrated approach to MS clinical outcome assessment. Mult Scler J 1999;5:244–250.
18. Krupp LB, Larocca NG, Muir Nash J, Steinberg AD. The fatigue severity scale: application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46:1121–1123.
19. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis 1994;18:S79–S83.
20. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561–571.
21. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage 2002;17:479–489.
22. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. Neuroimage 2006;31:1116–1128.
23. Kalkers NF, Ameziane N, Bot JCJ, Minneboo A, Polman CH, Barkhof F. Longitudinal brain volume measurement in multiple sclerosis: rate of brain atrophy is independent of the disease subtype. Arch Neurol 2002;59:1572–1576.
24. Friede T, Kieser M. Sample size recalculation in internal pilot study designs: a review. Biom J 2006;48:537–555.
25. Brunner E, Domhof S, Langer F. Nonparametric Analysis of Longitudinal Data in Factorial Experiments. J. Wiley; 2002. Available at: books.google.de/books?id=UxzvAAAAMAAJ.
26. Bathke A, Brunner E. A nonparametric alternative to analysis of covariance. In: Akritas MG, Politis DN, eds. Recent Advances and Trends in Nonparametric Statistics. Amsterdam, the Netherlands: Elsevier BV; 2003:109–120.
27. Levin J, Maaß S, Schuberth M, et al. Safety and efficacy of epigallocatechin gallate in multiple system atrophy (PROMESA): a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2019;18:724–735.
28. Bellmann-Strobl J, Paul F, Wuerfel J, et al. Epigallocatechin-gallate in relapsing-remitting MS: a randomized, double-blind, placebo-controlled trial. Neurol Neuroimmunol Neuroinflamm 2021;8:e981. doi: 10.1212/NXI.0000000000000981.
29. Altmann DR, Jasperse B, Barkhof F, et al. Sample sizes for brain atrophy outcomes in trials for secondary progressive multiple sclerosis. Neurology 2009;72:595601.
30. Chataway J, De Angelis F, Connick P, et al. Efficacy of three neuroprotective drugs in secondary progressive multiple sclerosis (MS-SMART): a phase 2b, multiarm, double-blind, randomised placebo-controlled trial. Lancet Neurol 2020;19:214–225.
31. Lublin F, Miller DH, Freedman MS, et al. Oral fingolimod in primary progressive multiple sclerosis (INFORMS): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet 2016;387:1075–1084.
32. Kapoor R, Furby J, Hayton T, et al. Lamotrigine for neuroprotection in secondary progressive multiple sclerosis: a randomised, double-blind, placebo-controlled, parallel-group trial. Lancet Neurol 2010;9:681–688.
33. Kapoor R, Ho PR, Campbell N, et al. Effect of natalizumab on disease progression in secondary progressive multiple sclerosis (ASCEND): a phase 3, randomised, double-blind, placebo-controlled trial with an open-label extension. Lancet Neurol 2018;17:405–415.
34. Chataway J, Schuerer N, Alsanousi A, et al. Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. Lancet 2014;383:2213–2221.
35. Chakrawarti L, Agrawal R, Dang S, Gupta S, Gabrani R. Therapeutic effects of EGCG: a patent review. Expert Opin Ther Pat 2016;26:907–916.
36. Ullmann U, Haller J, Decourt JD, Girault J, Spitzer V, Weber P. Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. Int J Vitam Nutr Res 2004;74:269–278.
37. Ontaneda D, Thompson AJ, Fox RJ, Cohen JA. Progressive multiple sclerosis: prospects for disease therapy, repair, and restoration of function. Lancet 2017;389:1357–1366.
38. Lovera J, Ramos A, Devier D, et al. Polyphenon E, non-futile at neuroprotection in multiple sclerosis but unpredictably hepatotoxic: phase I single group and phase II randomized placebo-controlled studies. J Neurol Sci 2015;358:46–52.
39. de la Torre R, de Sola S, Hernandez G, et al. Safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): a double-blind, randomised, placebo-controlled, phase 2 trial. Lancet Neurol 2016;15:801–810.
40. de la Torre R, de Sola S, Farré M, et al. A phase 1, randomized double-blind, placebo controlled trial to evaluate safety and efficacy of epigallocatechin-3-gallate and cognitive training in adults with fragile X syndrome. Clin Nutr 2020;39:378–387.

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Retinal Thickness Analysis in Progressive Multiple Sclerosis Patients Treated With Epigallocatechin Gallate: Optical Coherence Tomography Results From the SUPREMES Study

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Klumbies K, Rust R, Dörr J, Konietschke F, Paul F, Bellmann-Strobl J, Brandt AU and Zimmermann HG (2021) Retinal Thickness Analysis in Progressive Multiple Sclerosis Patients Treated With Epigallocatechin Gallate: Optical Coherence Tomography Results From the SUPREMES Study. Front. Neurol. 12:615790. doi: 10.3389/fneur.2021.615790

Background: Epigallocatechin gallate (EGCG) is an anti-inflammatory agent and has proven neuroprotective properties in animal models of multiple sclerosis (MS). Optical coherence tomography (OCT) assessed retinal thickness analysis can reflect treatment responses in MS.

Objective: To analyze the influence of EGCG treatment on retinal thickness analysis as secondary and exploratory outcomes of the randomized controlled *Sunphenon in Progressive Forms of MS* trial (SUPREMES, NCT00799890).

Methods: SUPREMES patients underwent OCT with the Heidelberg Spectralis device at a subset of visits. We determined peripapillary retinal nerve fiber layer (pRNFL) thickness from a 12° ring scan around the optic nerve head and thickness of the ganglion cell/inner plexiform layer (GCIP) and inner nuclear layer (INL) within a 6 mm diameter grid centered on the fovea from a macular volume scan. Longitudinal OCT data were available for exploratory analysis from 31 SUPREMES participants (12/19 primary/secondary progressive MS (PPMS/SPMS); mean age 51 ± 7 years; 12 female; mean time since disease onset 16 ± 11 years). We tested the null hypothesis of no treatment*time interaction using nonparametric analysis of longitudinal data in factorial experiments.

Results: After 2 years, there were no significant differences in longitudinal retinal thickness changes between EGCG treated and placebo arms in any OCT parameter

(Mean change [confidence interval] ECGC vs. Placebo: pRNFL: −0.83 [1.29] μm vs. −0.64 [1.56] μm, $p = 0.156$; GCIP: −0.67 [0.67] μm vs. −0.14 [0.47] μm, $p = 0.476$; INL: −0.06 [0.58] μm vs. 0.22 [0.41] μm, $p = 0.455$).

Conclusion: Retinal thickness analysis did not reveal a neuroprotective effect of EGCG. While this is in line with the results of the main SUPREMES trial, our study was probably underpowered to detect an effect.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier: NCT00799890.

Keywords: optical coherence tomography, retina, progressive multiple sclerosis, treatment response, epigallocatechin gallate

INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune inflammatory and degenerative central nervous system (CNS) disease, often resulting in sustained neurological deficits (1). The majority of patients manifest with a relapsing remitting (RRMS) disease course (2, 3), followed by a secondary progressive (SPMS) stage ∼20 years from onset (4). However, 15–20% show a primary progressive (PPMS) disease course from onset (3, 5, 6). Neurodegeneration may be present in any course from the onset of the disease (7–10).

The principle of disease modifying therapy (DMT) aims at decreasing relapse frequency and disability progression. Whereas various immunomodulatory drugs for the treatment of RRMS targeting the inflammatory aspect of the disease have been established in the last decades (11), treatment options for progressive MS are sparse (12, 13). Furthermore, due to the absence of clinical relapses, treatment response is difficult to measure in progressive MS and has to rely on measures not primarily associated with relapse activity (13).

Green tea anti-inflammatory, anti-oxidative, and anti-cancerogenic effects have been shown on various conditions such as energy metabolism, cell development, and neuroprotection (14–17). The most active agent is the polyphenol epigallocatechin-gallate (EGCG), comprising 50–80% of the total catechins in green tea (18). EGCG has shown immunomodulatory effects by inhibition of T cell proliferation and thus modulates the production of T cell-derived cytokines, e.g., Interferon-γ, Interleukin-2, and tumor necrosis factor (TFN) α (from T helper type 1 cell subset) (19–21). In an experimental animal model of MS (experimental autoimmune encephalomyelitis, EAE) the oral intake of EGCG suppressed inflammation via inhibition of TNFα and nuclear factor kappa-light-chain-enhancer of activated B cells in T cells, thus resulting in reduced clinical disease severity and fewer CNS lesions in mice (22–24). Furthermore, treatment with EGCG and glatiramer acetate in EAE mice delayed disease onset, reduced clinical disability and reduced inflammatory infiltrates (25). In clinical trials, oral intake of EGCG was associated with improved muscle metabolism during moderate exercise in RRMS (26) and improved cognitive rehabilitation in genetic disorders (27, 28).

Optical coherence tomography (OCT) allows quantification of anterior visual pathway damage in MS patients (29–33). While thinning of the peripapillary retinal nerve fiber layer (pRNFL), containing unmyelinated axons, and the ganglion cell layer, containing their cell bodies, reflect neuroaxonal atrophy as a consequence of retrograde neurodegeneration, the inner nuclear layer (INL) is associated with inflammation manifesting in thickening and edema (31, 34–40). The ganglion cell layer is usually—due to similar contrast on OCT images—analyzed in combination with the inner plexiform layer (GCIP). RNFL and GCIP changes are found even during early stages of MS and occur also in absence of a history of optic neuritis (ON) (8, 41–44). Response to DMT is reflected by decreased rates of GCIP thinning (45) and thinning of INL in RRMS patients (46). A recent study has shown faster retinal thinning—also compared to RRMS patients and no effect of DMT on thinning rates in progressive MS (47). The study has been discussed controversially (48).

The SUPREMES study (Sunphenon in progressive forms of multiple sclerosis) was a phase 2 monocentric, prospective, randomized double-blind placebo-controlled pilot study to evaluate the effect of EGCG/Sunphenon on brain atrophy in MRI over a period of 36 months in patients with primary and secondary progressive multiple sclerosis (NCT00799890). The primary results of the SUPREMES study have been published elsewhere (49). OCT parameters were assessed as secondary and exploratory outcomes. The aim of our study was to evaluate the impact of EGCG on longitudinal retinal component changes in patients with progressive MS.

MATERIALS AND METHODS

Patients and Study Design

In total, 61 patients were randomized to the SUPREMES trial (NCT00799890) at the NeuroCure Clinical Research Center (NCRC) at Charité—Universitätsmedizin Berlin, Germany. Inclusion and exclusion criteria, randomization, blinding process and primary and secondary endpoints are described in detail elsewhere (49). Primary outcome parameter of the main study was brain atrophy detected as the difference between brain parenchymal fraction after 36 months compared to baseline. Inclusion criteria were age between 18 and 65 years, diagnosis of primary progressive or secondary progressive multiple sclerosis according to the McDonald criteria version 2005 (50), expanded disability status scale (EDSS)

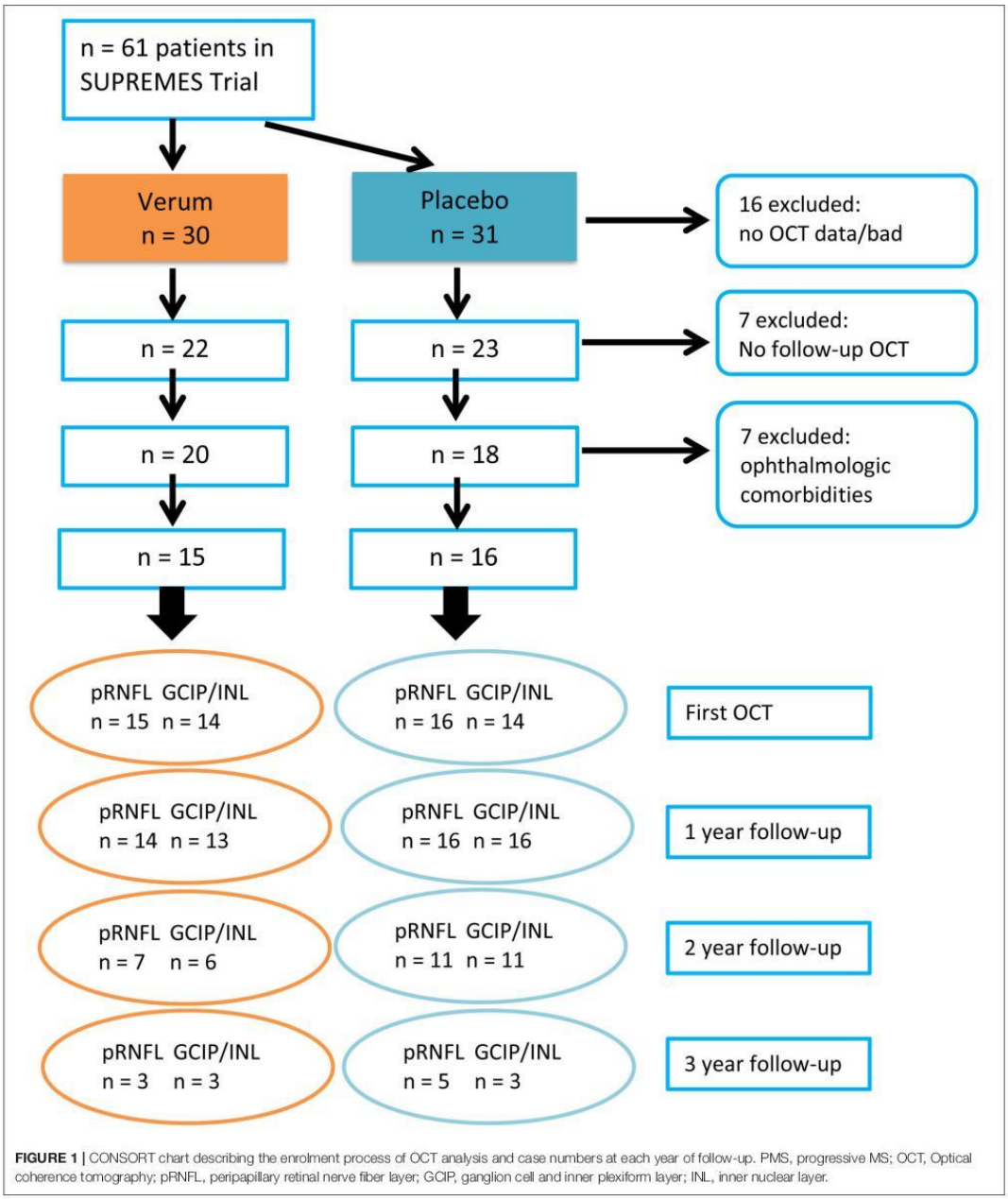

FIGURE 1 | CONSORT chart describing the enrolment process of OCT analysis and case numbers at each year of follow-up. PMS, progressive MS; OCT, Optical coherence tomography; pRNFL, peripapillary retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer.

TABLE 1 | Baseline cohort description.

		EGCG	Placebo	*p*
n		**15**	**16**	
Age [years]		50.8 ± 8.4	50.7 ± 6.9	0.968
Sex female [*n* (%)]		5 (33.3)	7 (43.8)	0.821
Diagnosis	**PPMS [*n* (%)]**	6 (40.0)	6 (37.5)	>0.999
	SPMS [*n* (%)]	9 (60.0)	10 (62.5)	
Disease duration [years] (median, [IQR])		13.69 [8.90, 29.41]	12.12 [7.47, 20.17]	0.406
EDSS (median, IQR)		6.00 [4.00, 6.50]	5.75 [4.00, 6.00]	0.138
Time on trial at OCT baseline (median, IQR) [years]		1.06 [0.00, 1.50]	1.04 [0.00, 1.53]	0.919
Follow-up duration (median, IQR) [years]		1.47 [1.27, 2.01]	1.95 [1.47, 2.90]	0.213

Abbreviations: EGCG: epigallocatechin-gallate, SPMS: secondary progressive multiple sclerosis, PPMS: primary progressive multiple sclerosis, EDSS: Expanded disability status scale, IQR: interquartile range, OCT: optical coherence tomography.

TABLE 2 | First OCT measurements.

	EGCG		Placebo		EGCG vs. placebo
	Mean ± SD	**RTE**	**Mean ± SD**	**RTE**	***p***
pRNFL/μm	87.3 ± 11.1	0.554	82.9 ± 11.4	0.450	0.297
GCIP/μm	65.4 ± 7.4	0.609	59.9 ± 6.1	0.381	0.024
INL/μm	37.8 ± 2.2	0.599	36.1 ± 2.3	0.392	0.049

Test statistics from "nonparametric analysis of longitudinal data" of first examination OCT data. EGCG, epigallocatechin-gallate; CI, confidence interval; RTE, Relative treatment effect; pRNFL, peripapillary retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer.

(51) between 3.0 and 8.0 and at least 30 days between the last exacerbation and study screening. Exclusion criteria were treatment with any immunomodulatory or immunosuppressive drugs, with exception of methylprednisolone up to 3 months before screening. Regarding OCT, pRNFL was a secondary outcome parameter; GCIP and INL were analyzed as exploratory endpoints. For inclusion in the analysis of OCT, ophthalmological diseases such as glaucoma, recurrent iritis, myopia <-5 dpt were considered as additional exclusion criteria. As for many patients OCT scanning was not available in the beginning, we only included patients to the OCT analysis who had at least one follow-up OCT at least 6 months from baseline OCT.

Study Medication

Patients in the treatment arm started treatment with one capsule containing Sunphenon 200 mg/day and placebo patients received identical capsules without active component. After 3 months, participants received two capsules per day of either EGCG or placebo medication. After 6 months, the medication increased to 600 mg/day, after 18 months to 800 mg/day and after 30 months they received the full amount of 1,200 mg/day.

Ethics

The SUPREMES trial was approved by the local ethics committee (LaGeSo ZS EK 10 407/08, new: 08/0407-EK 15) and by the German Federal Institute for Drugs and Medical Devices (BfArM). The trial is registered with EudraCT (2008-005213-22) and clinicaltrials.gov (NCT00799890) and was conducted in accordance with the current version of the Declaration of Helsinki and the applicable German law. All subjects provided written informed consent prior to enrolment.

Optical Coherence Tomography

Patients underwent spectral domain OCT (Spectralis SD-OCT; Heidelberg Engineering, Heidelberg, Germany) with the Eye Explorer 1.9.10.0 and automatic real-time (ART) image averaging. pRNFL was calculated from a standard ring scan around the optic nerve head (12°, 1536 A-scans, $16 \leq \text{ART} \leq 100$) using segmentation by the device's software with viewing module 6.0.14.0. A macular volume scan (25° × 30°, 61 B-scans, 768 A-scans per B-scan, $12 \leq \text{ART} \leq 15$) was acquired for intraretinal segmentation of GCIP and INL. Segmentation of macular scans was performed with SAMIRIX (52). All OCT scans were revised for retinal changes unrelated to MS, sufficient quality (53, 54), segmentation errors and were manually corrected by a blinded experienced grader if necessary. OCT methods are reported in line with the APOSTEL criteria (55).

Statistical Methods

Cohort baseline differences with subject reference in numerical variables were either given as mean ± standard deviation and analyzed with *t*-test, or as median and interquartile range (IQR) and analyzed with Wilcoxon rank-sum test, while Chi-Square test was applied for categorical variables. Due to overall low sample size and high number of missing data (**Figure 1**) we tested the OCT first examination and the longitudinal main hypothesis with "nonparametric analysis of longitudinal data in factorial experiments" as implemented in the R package nparLD (56). We modeled first OCT examination within an F1-LD-F1 design and used the ANOVA-Type test with treatment arm as whole-plot factor and eye as sub-plot factor for inference. We performed longitudinal analysis within the F1-LD-F2 experimental design with one whole-plot factor and two sub-plot factors, where the second sub-plot factor is the stratification of the first. Using this design, we used treatment group as whole-plot factor, time

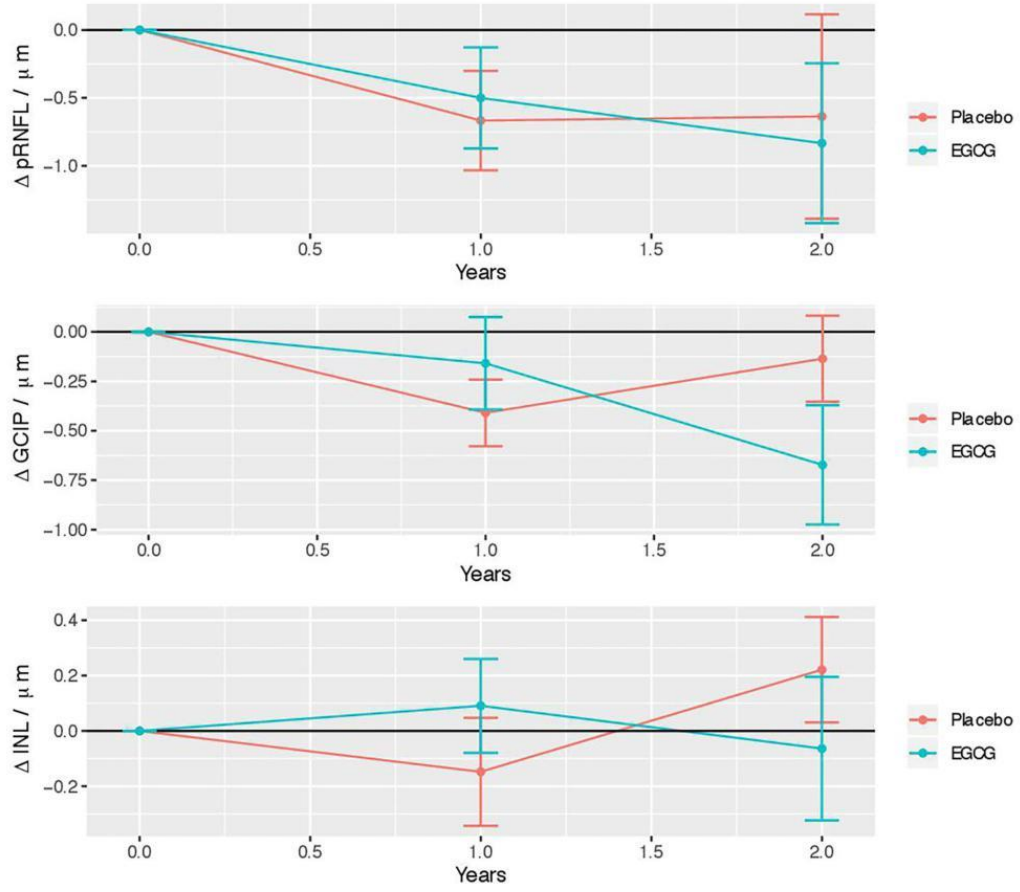

FIGURE 2 | Longitudinal retinal layer changes in the EGCG treated and placebo group. Error bars indicate the standard error to the mean. EGCG, epigallocatechin-gallate; pRNFL, peripapillary retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer.

as the first subplot factor, and eye as the second to account for two eye measurements per patient at each time point. We excluded three-year follow up because of potential bias resulting from missing data. The main question was whether the time profiles of the two groups were parallel or diverging, i.e., if there exists a statistical interaction between treatment group and time after 2 year follow up, which would indicate an effect of EGCG on OCT changes over time. The effect size is represented by the relative marginal treatment effect (RTE), indicating whether data tend to be smaller/larger under respective factor level combinations. The analysis set included missing values as described in the flow chart (**Figure 1**). In this data set we rounded follow-up time to full years in order to use time as a categorical variable. To confirm our findings, changes in OCT parameters were estimated with linear mixed models (LMM) using the formula: OCT value ~ group*time from baseline + (1 + time from baseline|patient/eye). In LMM, all sessions were considered including time since baseline as a continuous variable. No corrections for multiple comparisons were performed for this exploratory outcome analysis. Statistical analyses were performed with R (57) version 3.6.2 with packages nparLD (56), lme4, lmertest, tidyverse, tableone, ggplot2, beeswarm, ggplot, RMisc. Statistical significance was established at $p < 0.05$.

RESULTS

Cohort Description

Sixty-one patients with progressive MS were randomized in the SUPREMES trial to receive either EGCG treatment or placebo. From these patients, we had to exclude 16 patients because of

TABLE 3 | Longitudinal OCT changes in treatment arms—nonparametric analysis.

	EGCG		Placebo		EGCG vs. Placebo
	Mean change [CI]/μm	RTE treatment:time	Mean change [CI]/μm	RTE treatment:time	*p*
pRNFL/μm	−0.83 [1.29]	0.331	−0.64 [1.56]	0.492	0.156
GCIP/μm	−0.67 [0.67]	0.360	−0.14 [0.47]	0.429	0.476
INL/μm	−0.06 [0.58]	0.504	0.22 [0.41]	0.635	0.455

All results for the 2-year follow-up visit. Test statistics from "nonparametric analysis of longitudinal data." EGCG, epigallocatechin-gallate; RTE, Relative treatment effect; pRNFL, peripapillary retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer.

TABLE 4 | Longitudinal OCT changes in treatment arms—linear mixed models.

		B	SE	*p*	Lower CI	Upper CI	R^2m	R^2c
pRNFL	Treatment EGCG	3.194	4.014	0.433	−4.673	11.062	0.032	0.982
	Time	−0.788	0.306	0.018	−1.387	−0.189		
	Treatment EGCG:Time	0.766	0.463	0.111	−0.140	1.673		
GCIP	Treatment EGCG	4.389	2.432	0.082	−0.379	9.156	0.092	0.994
	Time	−0.221	0.111	0.068	−0.439	0.003		
	Treatment EGCG:Time	0.0138	0.160	0.933	−0.300	0.327		
INL	Treatment EGCG	1.866	0.838	0.034	0.223	3.509	0.136	0.956
	Time	−0.075	0.084	0.374	−0.240	0.089		
	Treatment EGCG:Time	0.064	0.119	0.589	−0.168	0.297		

All result for the maximum available follow-up time (continuous) under treatment. Test statistics from linear mixed models. B, non-standardized correlation coefficient; SE, standard error; CI, 95% confidence interval; R^2m, Marginal R^2; R^2c, Conditional R^2; pRNFL, peripapillary retinal nerve fiber layer; GCIP, ganglion cell and inner plexiform layer; INL, inner nuclear layer.

missing OCT data. From the 45 patients with OCT data, seven patients had no follow-up OCT data, and 7 patients had to be excluded due to ophthalmological diseases such as glaucoma, recurrent iritis, and myopia <-5 dpt. Thus, 31 patients were included in analysis. The inclusion process is detailed in **Figure 1**. Moreover, from 2 patients (1 EGCG, 1 placebo), one eye was excluded from all analyses because of unilateral retinopathy. Two pRNFL scans from 2 patients (both EGCG) and 34 macular scans from 28 sessions of 20 patients (8 EGCG, 12 placebo) failed the OSCAR-IB quality criteria and had to be excluded (53, 54).

Baseline OCT Findings

Baseline cohort details are described in **Table 1**. Patients had their first OCT examination median 1.05 (interquartile range 0.00–1.52) years after randomization. The OCT cohort comprised 15 patients from the treatment and 16 patients from the placebo group. There were no significant differences in age, sex, time since disease onset, EDSS, time in the trial, and follow-up duration between treatment and placebo groups (**Table 1**). Patients in the EGCG treated arm had thicker GCIP, INL, and—though not significant—pRNFL (**Table 2**).

Longitudinal OCT Results

Figure 2 illustrates changes over time in the EGCG treated and Placebo group. **Table 3** depicts changes over time separately for the treatment and the placebo arms and their statistical comparison from non-parametric longitudinal data analysis. There was no significant interaction of treatment and time for any parameter. **Table 4** includes results from LMMs, as well not detecting any significant differences in change over time between ECGC and placebo group.

DISCUSSION

In this study, we performed an analysis of OCT data as secondary (pRNFL) and exploratory (GCIP, INL) outcomes in the SUPREMES trial. Specifically, we investigated differences in retinal thickness changes over time between patients treated with EGCG vs. placebo. We found no difference between the treatment groups.

These results support the findings in the analysis of the primary and secondary outcome parameters of the SUPREMES trial: no evidence for treatment was found on brain atrophy, lesion load, and clinical scores (49). The primary outcome parameter of the SUPREMES trial was brain atrophy, a commonly used outcome for neuroprotective trials in MS (58). While brain atrophy measurement is widely established, retinal thickness analysis has been included as an additional outcome as the use of brain atrophy is not without challenge: a reduction of acute swelling by a potent anti-inflammatory intervention may lead to the phenomenon of "pseudoatrophy," which is referred to as decreased brain volume due to the resolution of edema and inflammation after treatment (59, 60). Furthermore, as our cohort had an average age of 50 years, treatment effects on brain atrophy may be confounded by non-linear aging effects (61).

In contrast, retinal thickness measurements are less prone to aging (52). Furthermore, GCIP is not prone to swelling (62), whereas a subtle swelling of pRNFL outside of acute ON has not been reported so far. While they may be inferior to brain atrophy at face value, GCIP and pRNFL may be superior for detecting neuroprotective effects due to a lack of pseudoatrophy. Nevertheless, we did not find a significantly reduced atrophy of pRNFL and GCIP in the EGCG group.

While pRNFL and GCIP thinning reflect neuroaxonal damage, the INL is considered a marker of inflammation. Treatment response is considered to be associated with INL thinning (46). However, the INL is also subject to atrophy as indicated by thinning in a large progressive MS study (47). In our study, the INL showed no overall thickness changes. This suggests that either no time-dependent change occur, or that both atrophy and inflammation occur in our cohort, masking a treatment-associated thinning.

Other clinical trials also failed to show a treatment effect of EGCG: The SUNIMS trial (63) reported no treatment effect of EGCG on clinical or MRI measures in RRMS patients. Moreover, a recently published study demonstrated no impact of EGCG after 48 weeks of treatment on disease progression in multiple system atrophy (64). A potential reason for the failure of EGCG in clinical trials could be the lower bioavailability of oral EGCG than previously assumed (65, 66).

Several limitations may impact our results. First, the low sample size of our cohort. A previous study estimated that the sample size for a progressive MS trial on neuroprotective agents should be at least $n = 173$ for pRNFL and $n = 125$ for GCIP per trial arm for a 3-year study (power 80%, effect size 50%), numbers way larger than achieved in this exploratory outcome analysis (47).

Another weakness is that treatment and placebo groups were not well-matched regarding baseline OCT, with a significantly thicker GCIP and INL in the treatment group. In our non-parametric analysis, we used the change of retinal parameters as outcome and the linear mixed models we computed additionally consider the individual intercept at baseline. Thus, we assume that the differences at OCT baseline had no influence on the longitudinal analysis.

To date, there are few studies applying OCT as an outcome parameter in clinical trials of MS. To the best of our knowledge, there is no published prospective interventional study that applied OCT as outcome parameter in trials in the progressive forms of the disease. While OCT detected differences in retinal thickness change between different treatment groups in RRMS (45), it is possible that the retina of SPMS and PPMS patients are less responsive to treatment. Another aspect is the high frequency of primary eye disorders in a usually elder progressive MS population. In our study, almost 20% of patients needed to be excluded due to eye comorbidities. Furthermore, due to increased disability, progressive MS patients are often less compliant with the OCT examination, leading to a high number of noise or cut-off scans failing the quality control. While this does not preclude OCT as endpoint from clinical trials in progressive MS, it suggests that careful ophthalmological examination for comorbidities and rigorous quality control of OCT scans are of paramount importance. A recent retrospective study showed a decreased macular RNFL thinning associated with 4-aminopyridine treatment in a mixed cohort of RRMS and progressive MS patients (67). These and our results encourage the further evaluation of OCT measurements as outcome parameters in clinical trials of progressive MS.

To conclude, our study shows no effect over time of EGCG on pRNFL, GCIP, or INL. As such, our study does not provide sufficient evidence for a neuroprotective effect of EGCG on retinal thickness in patients with SPMS and PPMS. While this is in line with the outcomes of the main SUPREMES trial, our study was probably underpowered to detect a treatment effect.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available upon request to the corresponding author to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The SUPREMES trial was approved by the local ethics committee (LaGeSo ZS EK 10 407/08, new: 08/0407-EK 15) and by the German Federal Institute for Drugs and Medical Devices (BfArM). The trial is registered with EudraCT (2008-005213-22) and clinicaltrials.gov (NCT00799890). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

KK drafting/revising the manuscript, analyzed and interpreted the data, and acquisition of data. RR acquisition of data, interpreted the data, and revised the manuscript for intellectual content. JD, FP, and JB-S study concept, acquisition of data, and revised the manuscript for intellectual content. FK analyzed and interpreted the data, statistical analysis, and revised the manuscript for intellectual content. AB study concept, analyzed and interpreted the data, statistical analysis, and drafting/revising the manuscript. HZ study concept, acquisition of data, analyzed and interpreted the data, statistical analysis, and drafting/revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Reich DS, Lucchinetti CF, Calabresi PA. Multiple sclerosis. *N Engl J Med.* (2018) 378:169–80. doi: 10.1056/NEJMra1401483
2. Ransohoff RM, Hafler DA, Lucchinetti CF. Multiple sclerosis-a quiet revolution. *Nat Rev Neurol.* (2016) 11:134–42. doi: 10.1038/nrneurol.2015.14
3. Weinshenker BG, Bass B, Rice GPA, Noseworthy J, Carriere W, Baskerville J, et al. The natural history of multiple sclerosis: a geographically based study: I. Clinical course and disability. *Brain.* (1989) 112:133–46. doi: 10.1093/brain/112.1.133
4. Krieger SC, Cook K, de Nino S, Fletcher M. The topographical model of multiple sclerosis: a dynamic visualization of disease course. *Neurol Neuroimmunol NeuroInflammation.* (2016) 3:e279. doi: 10.1212/NXI.0000000000000279
5. Faissner S, Plemel JR, Gold R, Yong VW. Progressive multiple sclerosis: from pathophysiology to therapeutic strategies. *Nat Rev Drug Discov.* (2019) 18:905–22. doi: 10.1038/s41573-019-0035-2
6. Miller DH, Leary SM. Primary-progressive multiple sclerosis. *Lancet Neurol.* (2007) 6:903–12. doi: 10.1016/S1474-4422(07)70243-0
7. Trapp BD, Nave K-A. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci.* (2008) 31:247–69. doi: 10.1146/annurev.neuro.30.051606.094313
8. Oberwahrenbrock T, Ringelstein M, Jentschke S, Deuschle K, Klumbies K, Bellmann-Strobl J, et al. Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome. *Mult Scler J.* (2013) 19:1887–95. doi: 10.1177/1352458513489757
9. Azevedo CJ, Overton E, Khadka S, Buckley J, Liu S, Sampat M, et al. Early CNS neurodegeneration in radiologically isolated syndrome. *Neurol Neuroimmunol NeuroInflammation.* (2015) 2:e102. doi: 10.1212/NXI.0000000000000102
10. Kuchling J, Paul F. Visualizing the central nervous system: imaging tools for multiple sclerosis and neuromyelitis optica spectrum disorders. *Front Neurol.* (2020) 11:450. doi: 10.3389/fneur.2020.00450
11. Wingerchuk DM, Weinshenker BG. Disease modifying therapies for relapsing multiple sclerosis. *BMJ.* (2016) 354:i3518. doi: 10.1136/bmj.i3518
12. Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, et al. Ocrelizumab versus placebo in primary progressive multiple sclerosis. *N Engl J Med.* (2017) 376:209–20. doi: 10.1056/NEJMoa1606468
13. Ontaneda D, Fox RJ, Chataway J. Clinical trials in progressive multiple sclerosis: lessons learned and future perspectives. *Lancet Neurol.* (2015) 14:208–23. doi: 10.1016/S1474-4422(14)70264-9
14. Sato T, Miyata G. The nutraceutical benefit, part I: green tea. *Nutrition.* (2000) 16:315–7. doi: 10.1016/S0899-9007(99)00301-9
15. Bogdanski P, Suliburska J, Szulinska M, Stepien M, Pupek-Musialik D, Jablecka A. Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutr Res.* (2012) 32:421–7. doi: 10.1016/j.nutres.2012.05.007
16. Syarifah-Noratiqah S-B, Naina-Mohamed I, Zulfarina MS, Qodriyah HM. Natural polyphenols in the treatment of Alzheimer's disease. *Curr Drug Targets.* (2017) 19:927–37. doi: 10.2174/1389450118666170328122527
17. Mähler A, Mandel S, Lorenz M, Ruegg U, Wanker EE, Boschmann M, et al. Epigallocatechin-3-gallate: a useful, effective and safe clinical approach for targeted prevention and individualised treatment of neurological diseases? *EPMA J.* (2013) 4:1–17. doi: 10.1186/1878-5085-4-5
18. Ashihara H, Deng WW, Mullen W, Crozier A. Distribution and biosynthesis of flavan-3-ols in *Camellia sinensis* seedlings and expression of genes encoding biosynthetic enzymes. *Phytochemistry.* (2010) 71:559–66. doi: 10.1016/j.phytochem.2010.01.010
19. Wu D, Wang J, Pae M, Meydani SN. Green tea EGCG, T cells, and T cell-mediated autoimmune diseases. *Mol Aspects Med.* (2012) 33:107–18. doi: 10.1016/j.mam.2011.10.001
20. Pae M, Wu D. Immunomodulating effects of epigallocatechin-3-gallate from green tea: Mechanisms and applications. *Food Funct.* (2013) 4:1287–303. doi: 10.1039/c3fo60076a
21. Wu D. Green tea EGCG, T-cell function, and T-cell-mediated autoimmune encephalomyelitis. *J Investig Med.* (2016) 64:1213–9. doi: 10.1136/jim-2016-000158
22. Aktas O, Prozorovski T, Smorodchenko A, Savaskan NE, Lauster R, Kloetzel P-M, et al. Green tea epigallocatechin-3-gallate mediates T cellular NF-κB inhibition and exerts neuroprotection in autoimmune encephalomyelitis. *J Immunol.* (2004) 173:5794–800. doi: 10.4049/jimmunol.173.9.5794
23. Wang J, Ren Z, Xu Y, Xiao S, Meydani SN, Wu D. Epigallocatechin-3-gallate ameliorates experimental autoimmune encephalomyelitis by altering balance among CD4 + T-cell subsets. *Am J Pathol.* (2012) 180:221–34. doi: 10.1016/j.ajpath.2011.09.007
24. Sun Q, Zheng Y, Zhang X. Novel immunoregulatory properties of EGCG on reducing inflammation in EAE. *Front Biosci.* (2013) 18:332–342. doi: 10.2741/4104
25. Herges K, Millward JM, Hentschel N, Infante-Duarte C, Aktas O, Zipp F. Neuroprotective effect of combination therapy of Glatiramer acetate and epigallocatechin-3-gallate in neuroinflammation. *PLoS One.* (2011) 6:e25456. doi: 10.1371/journal.pone.0025456
26. Mähler A, Steiniger J, Bock M, Klug L, Parreidt N, Lorenz M, et al. Metabolic response to epigallocatechin-3-gallate in relapsing-remitting multiple sclerosis: a randomized clinical trial. *Am J Clin Nutr.* (2015) 101:487–95. doi: 10.3945/ajcn.113.075309
27. de la Torre R, de Sola S, Hernandez G, Farré M, Pujol J, Rodriguez J, et al. Safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Neurol.* (2016) 15:801–10. doi: 10.1016/S1474-4422(16)30034-5
28. de la Torre R, de Sola S, Farré M, Xicota L, Cuenca-Royo A, Rodriguez J, et al. A phase 1, randomized double-blind, placebo controlled trial to evaluate safety and efficacy of epigallocatechin-3-gallate and cognitive training in adults with Fragile X syndrome. *Clin Nutr.* (2020) 39:378–87. doi: 10.1016/j.clnu.2019.02.028
29. Oberwahrenbrock T, Traber GL, Lukas S, Gabilondo I, Nolan R, Songster C, et al. Multicenter reliability of semiautomatic retinal layer segmentation using OCT. *Neurol Neuroimmunol Neuroinflammation.* (2018) 5:e449. doi: 10.1212/NXI.0000000000000449
30. Nolan-Kenney RC, Liu M, Akhand O, Calabresi PA, Paul F, Petzold A, et al. Optimal intereye difference thresholds by optical coherence tomography in multiple sclerosis: an international study. *Ann Neurol.* (2019) 85:618–29. doi: 10.1002/ana.25462
31. Petzold A, Balcer LJ, Calabresi PA, Costello F, Frohman TC, Frohman EM, et al. Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol.* (2017) 16:797–812. doi: 10.1016/S1474-4422(17)30278-8
32. Oertel FC, Zimmermann HG, Brandt AU, Paul F. Novel uses of retinal imaging with optical coherence tomography in multiple sclerosis. *Expert Rev Neurother.* (2019) 19:31–43. doi: 10.1080/14737175.2019.1559051
33. Zimmermann HG, Knier B, Oberwahrenbrock T, Behrens J, Pfuhl C, Aly L, et al. Association of retinal ganglion cell layer thickness with future disease activity in patients with clinically isolated syndrome. *JAMA Neurol.* (2018) 75:1071–9. doi: 10.1001/jamaneurol.2018.1011
34. Costello F, Hodge W, Pan YI, Eggenberger E, Freedman MS. Using retinal architecture to help characterize multiple sclerosis patients. *Can J Ophthalmol J Can dophtalmologie.* (2010) 45:520–6. doi: 10.3129/i10-063
35. Wicki CA, Hanson JVM, Schippling S. Optical coherence tomography as a means to characterize visual pathway involvement in multiple sclerosis. *Curr Opin Neurol.* (2018) 31:662–8. doi: 10.1097/WCO.0000000000000604
36. Kaufhold F, Zimmermann H, Schneider E, Ruprecht K, Paul F, Oberwahrenbrock T, et al. Optic neuritis is associated with inner nuclear layer thickening and microcystic macular edema independently of multiple sclerosis. *PLoS One.* (2013) 8:e71145. doi: 10.1371/journal.pone.0071145
37. Gelfand JM, Nolan R, Schwartz DM, Graves J, Green AJ. Microcystic macular oedema in multiple sclerosis is associated with disease severity. *Brain.* (2012) 135:1786–93. doi: 10.1093/brain/aws098
38. Saidha S, Sotirchos ES, Ibrahim M a., Crainiceanu CM, Gelfand JM, Sepah YJ, et al., Newsome SD, et al. Microcystic macular oedema, thickness of the inner nuclear layer of the retina, and disease characteristics in multiple sclerosis: a retrospective study. *Lancet Neurol.* (2012) 11:963–72. doi: 10.1016/S1474-4422(12)70213-2
39. Balk LJ, Coric D, Knier B, Zimmermann HG, Behbehani R, Alroughani R, et al. Retinal inner nuclear layer volume reflects inflammatory disease activity in multiple sclerosis; a longitudinal OCT study. *Mult Scler.* (2019) 5:1–11. doi: 10.1177/2055217319871582
40. Brandt AU, Oberwahrenbrock T, Kadas EM, Lagrèze WA, Paul F. Dynamic formation of macular microcysts independent of vitreous

traction changes. *Neurology*. (2014) 83:73–7. doi: 10.1212/WNL.0000000000000545
41. Green AJ, McQuaid S, Hauser SL, Allen I V., Lyness R. Ocular pathology in multiple sclerosis: Retinal atrophy and inflammation irrespective of disease duration. *Brain*. (2010) 133:1591–601. doi: 10.1093/brain/awq080
42. Balk LJ, Cruz-Herranz A, Albrecht P, Arnow S, Gelfand JM, Tewarie P, et al. Timing of retinal neuronal and axonal loss in MS: a longitudinal OCT study. *J Neurol*. (2016) 263:1323–31. doi: 10.1007/s00415-016-8127-y
43. Oberwahrenbrock T, Schippling S, Ringelstein M, Kaufhold F, Zimmermann H, Keser N, et al. Retinal damage in multiple sclerosis disease subtypes measured by high-resolution optical coherence tomography. *Mult Scler Int*. (2012) 2012:530305. doi: 10.1155/2012/530305
44. Gelfand JM, Goodin DS, Boscardin WJ, Nolan R, Cuneo A, Green AJ. Retinal axonal loss begins early in the course of multiple sclerosis and is similar between progressive phenotypes. *PLoS One*. (2012) 7:e36847. doi: 10.1371/journal.pone.0036847
45. Button J, Al-Louzi O, Lang A, Bhargava P, Newsome SD, Frohman T, et al. Disease-modifying therapies modulate retinal atrophy in multiple sclerosis: a retrospective study. *Neurology*. (2017) 88:525–2. doi: 10.1212/WNL.0000000000003582
46. Knier B, Schmidt P, Aly L, Buck D, Berthele A, Mühlau M, et al. Retinal inner nuclear layer volume reflects response to immunotherapy in multiple sclerosis. *Brain*. (2016) 139:2855–63. doi: 10.1093/brain/aww219
47. Sotirchos ES, Gonzalez Caldito N, Filippatou A, Fitzgerald KC, Murphy OC, Lambe J, et al. Progressive multiple sclerosis is associated with faster and specific retinal layer atrophy. *Ann Neurol*. (2020) 87:885–96. doi: 10.1002/ana.25738
48. Cordano C, Yiu HH, Oertel FC, Gelfand JM, Hauser SL, Cree BAC, et al. Retinal INL Thickness in multiple sclerosis: a mere marker of neurodegeneration? *Ann Neurol*. (2021) 89:192–3. doi: 10.1002/ana.25933
49. Rust R, Chien C, Scheel M, Brandt AU, Dörr J, Wuerfel J, et al. Epigallocatechin gallate in progressive MS: a randomized, placebo-controlled trial. *Neurol Neuroimmunol NeuroInflammation*. (2020) 8:e964. doi: 10.1212/NXI.0000000000000964
50. Polman CH, Reingold SC, Edan G, Filippi M, Hartung H-P, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria." *Ann Neurol*. (2005) 58:840–6. doi: 10.1002/ana.20703
51. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. (1983) 33:1444–52. doi: 10.1212/WNL.33.11.1444
52. Motamedi S, Gawlik K, Ayadi N, Zimmermann HG, Asseyer S, Bereuter C, et al. Normative data and minimally detectable change for inner retinal layer thicknesses using a semi-automated OCT image segmentation pipeline. *Front Neurol*. (2019) 10:1117. doi: 10.3389/fneur.2019.01117
53. Tewarie P, Balk L, Costello F, Green A, Martin R, Schippling S, et al. The OSCAR-IB consensus criteria for retinal OCT quality assessment. *PLoS One*. (2012) 7:e34823. doi: 10.1371/journal.pone.0034823
54. Schippling S, Balk LJ, Costello F, Albrecht P, Balcer L, Calabresi PA, et al. Quality control for retinal OCT in multiple sclerosis: validation of the OSCAR-IB criteria. *Mult Scler J*. (2015) 21:163–70. doi: 10.1177/1352458514538110
55. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, Saidha S, Martinez-Lapiscina EH, Lagreze WA, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology*. (2016) 86:2303–9. doi: 10.1212/WNL.0000000000002774
56. Noguchi K, Gel YR, Brunner E, Konietschke F. nparLD : an R Software Package for the nonparametric analysis of longitudinal data in factorial experiments. *J Stat Softw*. (2012) 50:1–23. doi: 10.18637/jss.v050.i12
57. R Core Team. *R: A Language and Environment for Statistical Computing* (2014).
58. Moccia M, de Stefano N, Barkhof F. Imaging outcome measures for progressive multiple sclerosis trials. *Mult Scler*. (2017) 23:1614–26. doi: 10.1177/1352458517729456
59. De Stefano N, Arnold DL. Towards a better understanding of pseudoatrophy in the brain of multiple sclerosis patients. *Mult Scler*. (2015) 21:675–6. doi: 10.1177/1352458514564494
60. Vidal-Jordana A, Sastre-Garriga J, Pérez-Miralles F, Tur C, Tintoré M, Horga A, et al. Early brain pseudoatrophy while on natalizumab therapy is due to white matter volume changes. *Mult Scler J*. (2013) 19:1175–81. doi: 10.1177/1352458512473190
61. Azevedo CJ, Cen SY, Jaberzadeh A, Zheng L, Hauser SL, Pelletier D. Contribution of normal aging to brain atrophy in MS. *Neurol Neuroimmunol neuroinflammation*. (2019) 6:e616. doi: 10.1212/NXI.0000000000000616
62. Syc SB, Saidha S, Newsome SD, Ratchford JN, Levy M, Ford E, et al. Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis. *Brain*. (2012) 135:521–33. doi: 10.1093/brain/awr264
63. Bellmann-Strobl J, Paul F, Wuerfel J, Dörr J, Infante-Duarte C, Heidrich E, et al. Epigallocatechin gallate in relapsing-remitting multiple sclerosis: a randomized, placebo-controlled trial. *Neurol Neuroimmunol NeuroInflammation*. (2021) 8:e981. doi: 10.1212/NXI.0000000000000981
64. Levin J, Maaß S, Schuberth M, Giese A, Oertel WH, Poewe W, et al. Safety and efficacy of epigallocatechin gallate in multiple system atrophy (PROMESA): a randomised, double-blind, placebo-controlled trial. *Lancet Neurol*. (2019) 18:724–35. doi: 10.1016/S1474-4422(19)30141-3
65. Ullmann U, Haller J, Decourt JD, Girault J, Spitzer V, Weber P. Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. *Int J Vitam Nutr Res*. (2004) 74:269–78. doi: 10.1024/0300-9831.74.4.269
66. Chakrawarti L, Agrawal R, Dang S, Gupta S, Gabrani R. Therapeutic effects of EGCG: a patent review. *Expert Opin Ther Pat*. (2016) 26:907–16. doi: 10.1080/13543776.2016.1203419
67. Dietrich M, Koska V, Hecker C, Göttle P, Hilla AM, Heskamp A, et al. Protective effects of 4-aminopyridine in experimental optic neuritis and multiple sclerosis. *Brain*. (2020) 143:1127–42. doi: 10.1093/brain/awaa062

Conflict of Interest: RR received speaking honoraria from Roche. JD reports research support by Bayer and Novartis, honoraria for lectures and advisory by Bayer, Novartis, Sanofi-Aventis, Merck-Serono, Biogen and Roche and travel support by Bayer, Novartis, Biogen, and Merck-Serono. FP reports non-financial support from Taiyo International, grants from TEVA GmbH, other from German Research Council (DFG), during the conduct of the study; He serves on scientific advisory boards of Novartis's OCTIMS study steering committee and MedImmune/Viela Bio steering committee. He received funding for travel or speaker honoraria from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, and Merck Serono, Alexion, Chugai, MedImmune, Shire, Roche, Actelion, Celgene and serves on editorial Boards at PLos ONE (academic editor) and Neurology Neuroimmunology and Neuroinflammation (Associate Editor). He provided consultancies for SanofiGenzyme, BiogenIdec, MedImmune, Shire, Alexion; He received research support from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, Alexion and Merck Serono, German Research Council (DFG Exc 257), Werth Stiftung of the City of Cologne, German Ministry of Education and Research (BMBF Competence Network Multiple Sclerosis), Arthur Arnstein Stiftung Berlin, EU FP7 Framework Program (combims.eu) Guthy Jackson Charitable Foundation, and National Multiple Sclerosis Society of the USA. JB-S has received travel grants and speaking honoraria from Bayer Healthcare, Biogen Idec, Merck Serono, Sanofi Genzyme, Teva Pharmaceuticals, Roche, and Novartis all unrelated to this work. AB is cofounder and shareholder of technology startups Motognosis GmbH and Nocturne GmbH. He is named as inventor on several patent applications describing serum biomarkers for multiple sclerosis, perceptive computing for motor symptoms and retinal image analysis using optical coherence tomography. HZ received research grants from Novartis and speaking fees from Bayer, unrelated to this study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5. Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

6. Publikationsliste

K. Deuschle, J. Hofmann, C. Otto, J. Bellmann-Strobl, O. Scherner, **K. Klumbies**, E. Schneider, J. Broddack, F. Paul, K. Ruprecht, **Are there Epstein-Barr virus seronegative patients with multiple sclerosis?**, Mult. Scler. (2013). https://doi.org/10.1177/1352458512472751.
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T. Oberwahrenbrock, M. Ringelstein, S. Jentschke, K. Deuschle, **K. Klumbies**, J. Bellmann-Strobl, J. Harmel, K. Ruprecht, S. Schippling, H.-P. Hartung, O. Aktas, A.U. Brandt, F. Paul, **Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome**, Mult. Scler. J. 19 (2013) 1887–1895. https://doi.org/10.1177/1352458513489757.
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R. Rust, C. Chien, M. Scheel, A.U. Brandt, J. Dörr, J. Wuerfel, **K. Klumbies**, H. Zimmermann, M. Lorenz, K.D. Wernecke, J. Bellmann-Strobl, F. Paul, **Epigallocatechin Gallate in Progressive MS: A Randomized, Placebo-Controlled Trial**, Neurol. Neuroimmunol. Neuroinflammation. 8 (2021) e964. https://doi.org/10.1212/NXI.0000000000000964.
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K. Klumbies, R. Rust, J. Dörr, F. Konietschke, F. Paul, J. Bellmann-Strobl, A.U. Brandt, H. Zimmermann, **Retinal thickness analysis in progressive multiple sclerosis patients treated with epigallocatechin gallate: optical coherence tomography results from the SUPREMES study.**, Front. Neurol. 12 (2021) in press. https://doi.org/10.3389/fneur.2021.615790
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K. Klumbies, K. Kiening, A.W. Unterberg, B. Ishak; **High rate of pulmonary cement embolism (PCE) in patients with cement augmented pedicle screw placement with intraoperative perivertebral cement leakage - 10-year single-centre experience**; ePoster auf der 71. Jahrestagung der Deutschen Gesellschaft für Neurochirurgie (DGNC), Juni 2020.

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