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

DISSERTATION

Optische Kohärenztomographie im Vergleich zu Magnetresonanztomographie und Magnetresonanzspektroskopie als Parameter der Neurodegeneration bei Multipler Sklerose

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Alexander Ulrich Brandt

aus Hannover

Datum der Promotion: 05.12.2014

Inhaltsverzeichnis der Zusammenfassung

Optische Kohärenztomographie im Vergleich zu Magnetresonanztomographie und Magnetresonanzspektroskopie als Parameter der Neurodegeneration bei Multipler Sklerose

Alexander Ulrich Brandt

Abstract (Deutsch)

Hintergrund: Die Untersuchung der Netzhaut des Auges mit Hilfe der Optischen Kohärenztomographie (OCT) erlaubt die Darstellung von retinalen Schichten. Die retinale Nervenfaserschicht (RNFL) ist bei Patienten mit Multipler Sklerose (MS) im Vergleich zu gesunden erniedrigt. Erste Studien haben zudem einen Zusammenhang zwischen RNFL Dicke und Hirnatrophie bei Patienten mit MS gezeigt. Zurzeit wird OCT als möglicher Verlaufsparameter für Neurodegeneration bei MS Patienten diskutiert.

Zielsetzung: Den Zusammenhang zwischen Veränderungen der retinalen Nervenfaserschichtdicke und Hirnatrophie-Markern in einer geplanten Studie zu belegen und weiter zu untersuchen.

Methodik: In vier Studien wurden Patienten mit schubförmig-remittierender MS oder klinisch-isoliertem Syndrom mittels OCT, Magnetresonanztomographie und Magnetresonanzspektroskopie untersucht.

Ergebnisse: Ein Zusammenhang zwischen RNFL und Hirnparenchymvolumen konnte bestätigt werden. Zudem besteht ein Zusammenhang zu neurochemischen Parametern der Neurodegeneration im visuellen Cortex. Eine Sehnervenentzündung beeinflusst den Zusammenhang zwischen RNFL und Hirnatrophie. Bei Patienten mit sehr geringer Erkrankungsdauer steht ein Zusammenhang zwischen RNFL und weißer Substanz im Vordergrund.

Zusammenfassung: OCT ist ein vielversprechender Parameter zur Verlaufsbeobachtung von MS Patienten. Mit Einschränkungen bei Erkrankungsdauer und Sehnervenentzündungen lassen sich potentiell Aussagen zum Krankheitsverlauf stellen.

Abstract (English)

Background: Imaging the eye's retina with optical coherence tomography (OCT) allows investigating retinal layers. It is now known that the retinal nerve fiber layer (RNFL) is reduced in patients with multiple sclerosis (MS) in comparison to healthy subjects. Furthermore, a correlation between RNFL thickness and brain atrophy assessed by magnetic resonance imaging (MRI) was shown. Currently, OCT is a potential candidate for monitoring disease progression in MS.

Objective: To further investigate the association between retinal markers from OCT with brain atrophy markers from MRI.

Methods: In four studies, patients with relapsing-remitting MS or clinically isolated syndrome were investigated with OCT, MRI and magnetic resonance spectroscopy.

Results: An association between RNFL and brain atrophy was confirmed. Furthermore, RNFL was associated with neurochemical parameters for neurodegeneration in the visual cortex. A previous optic neuritis interferes with the link between brain atrophy and RNFL. Furthermore, in early MS patients a correlation between RNFL and brain atrophy could only be seen for white matter.

Summary: OCT is a promising candidate for monitoring disease progression in MS patients. Potential conclusions about general or brain disease progression are limited by disease duration and optic neuritis.

Einleitung

Multiple Sklerose (MS) ist die häufigste autoimmune Erkrankung des zentralen Nervensystems (ZNS) im jungen Erwachsenenalter und ist gekennzeichnet durch entzündliche Schübe mit begleitenden neurologischen Symptomen wie Sehnervenentzündungen, sensiblen Ausfälle oder motorischen Störungen (schubförmig remittierende MS).(1) Bei adäquater Schubtherapie - und teilweise auch ohne diese - können sich viele Symptome wieder zurückbilden oder zumindest deutlich verbessern. Im Laufe der Erkrankung kann zusätzlich eine kontinuierlich progrediente Verschlechterung der klinischen Symptome in den Vordergrund treten (sekundär progrediente MS), bei einer Minderzahl der Patienten besteht diese von Anfang an (primär progrediente MS).(2)

Lokal begrenzte Entzündungsreaktionen gegen von Oligodendrozyten gebildete Myelinscheiden im ZNS und vermittelt durch autoimmune T-Zellen werden ursächlich als wichtigstes pathologisches Korrelat für MS assoziierte Schübe gesehen.(3) Durch diese Entzündungsreaktionen kommt es fokal zu Demyelinisierungen im ZNS, die ursächlich für Schub-assoziierte funktionelle Einschränkungen sind. Darüber hinaus führen neurodegenerative Prozesse zu progredienten und andauernden funktionellen Einschränkungen. Neurodegeneration tritt dabei schon früh im Krankheitsverlauf auf und wird heutzutage neben der Neuroinflammation als wichtigstes Krankheitskorrelat verstanden, ohne dass die genauen Anteile an Ursache und Wirkung abschließend geklärt sind.(4) Neurodegenerative Prozesse in Form von neuro-axonaler Schädigung treten bei MS sowohl fokal im Rahmen entzündlicher Läsionen auf,(4) werden aber auch an entfernten Stellen ohne unmittelbare Entzündungsreaktion gesehen.(5)

Magnetische Resonanztomographie (MRT) ist neben der klinischen Diagnostik die wichtigste Säule der Primär- und Sekundärdiagnostik und dominiert so auch die aktuellen Diagnoserichtlinien zur MS.(6) Kommt es während einer Entzündungsreaktion zu einem Zusammenbrechen der Bluthirnschranke, stellen sich aktive MS Läsionen durch Kontrastmittelanreicherung in T1 gewichteten Aufnahmen dar („Contrast Enhancing Lesion“, CEL). Auch nicht aktive MS spezifische Läsionen können in T2 gewichteten Sequenzen dargestellt werden („T2 Läsionen“), allerdings korrelieren Ausmaß und Progression der T2 Läsionen nur schlecht mit dem klinischen Verlauf der Erkrankung.(7) Wesentlich bessere Korrelate der klinischen

Progression sind MRT-Marker, die Neurodegeneration und Hirnatrophie abbilden: Bei MS etabliert ist hier vor allem die Hirnparenchymfraktion („Brain Parenchymal Fraction“, BPF), die das Parenchymvolumen in Relation zum Gesamthirnvolumen beschreibt.(8) Als Weiterentwicklung der BPF sind die Beschreibung des normalisierten Gesamtvolumens („Normalized Brain Volume“, NBV), der Grauen Substanz („Normalized Grey Matter Volume“, NGMV) und der Weißen Substanz („Normalized White Matter Volume“, NWMV) heute üblich.(9) Eine inverse Korrelation zwischen Hirnatrophie, beschrieben z.B. durch die BPF, und Erkrankungsschwere sowie Progression wurde mehrfach bestätigt.(10)

Eine weitere Methode zur Bestimmung des neuronalen und axonalen Schadens bei MS ist Bestimmung der N-acetyl-aspartat(NAA)-Konzentration mittels Magnetresonanzspektroskopie („Proton magnetic resonance spectroscopy“, ^{1}H-MRS). Ein Zusammenhang zwischen dem metabolischen Parameter NAA und dem Ausmaß der Neurodegeneration ist belegt, dennoch hat die Methode bisher keinen Einzug in die klinische Routine gefunden, da Messgenauigkeit und damit Aussagekraft nur eingeschränkt sind. Die MRS ist daher primär wissenschaftlichen Fragestellungen vorbehalten.(11)

In den letzten Jahren ist die Untersuchung der Netzhaut (Retina) mit Hilfe der optischen Kohärenztomographie („Optical Coherence Tomography“, OCT) in den Fokus der MS-Forschung gerückt. Die Retina ist entwicklungsphysiologisch Teil des Gehirns und weist eine ähnliche zelluläre Komposition aus Neuronen, Gliazellen sowie Gefäßstruktur wie das Gehirn auf.(12) Zudem betrifft die Sehnervenentzündung, eine der häufigsten klinischen Schubmanifestationen der MS, direkt die Retina bzw. den retinalen Teil des Sehnerven, die retinale Nervenfaserschicht („Retinal Nerve Fiber Layer“, RNFL): Obwohl die als auch Retrobulbärneuritis bezeichnete Sehnervenentzündung im myelinisierten Teil des Sehnervens außerhalb bzw. des Auges stattfindet, betrifft sie Axone, deren Nervenzellkörper in der retinalen Ganglienzellschicht liegen. Eine Vielzahl von Studien konnte inzwischen belegen, dass die RNFL bei MS Patienten erniedrigt ist: Vor allem nach einer Sehnervenentzündung kommt es zu einer starken Verdünnung als Ausdruck der Neurodegeneration.(13) Aber auch Augen von MS-Patienten, die nie klinisch eine Sehnervenentzündung hatten, weisen eine dünnere RNFL auf.(13,14) Die Retina scheint daher ausgezeichnet geeignet, sowohl entzündliche als auch degenerative Prozesse bei MS zu untersuchen. Folglich wird die Messung

von Veränderungen der RNFL inzwischen auch als Marker für die neuro-axonalen Degeneration bei MS empfohlen.(15)

Zielsetzung

Ausgangspunkt dieser Arbeit war eine explorative Studie von Gordon-Lipkin et al., die einen Zusammenhang zwischen der RNFL und BPF bei MS berichtete.(16) Dabei korrelierte die Dicke der RNFL gut mit der Hirnatrophie bei MS. Verglichen mit OCT Untersuchungen sind MRT Messungen vergleichsweise teuer und zeitaufwendig, so dass die Hoffnung besteht, mit der OCT-Untersuchung der Retina einen zusätzlichen, möglicherweise überlegenen Surrogat-Marker für Neurodegeneration bei MS etablieren zu können. Das Ziel dieser Arbeit war daher, den Zusammenhang zwischen OCT-Messparametern und etablierten MRT-Atrophie-Markern weitergehend zu untersuchen. Die Fragestellungen waren im Einzelnen:

1) Können die Ergebnisse der explorativen Studie von Gordon-Lipkin et al.(16) in einer Studie mit primärem Endpunkt und geplanter Fallzahl bestätigt werden?
2) Lassen sich neben den morphologischen auch metabolische/neurochemische Zusammenhänge der Neurodegeneration zwischen Retina und Gehirn finden?
3) Wie wirkt sich eine stattgehabte Sehnervenentzündung auf den Zusammenhang zwischen retinalem Schaden im OCT und Hirnatrophie im MRT aus?
4) Wie unterscheidet sich der OCT- und MRT-Zusammenhang bei früher MS von dem publizierten Zusammenhang bereits langjährig Erkrankter?

Die Arbeiten wurden am NCRC der Charité – Universitätsmedizin Berlin teilweise in Zusammenarbeit mit dem Institut für Neuroimmunologie und klinischer MS-Forschung (inims) des Universitätsklinikums Hamburg-Eppendorf durchgeführt und sollen im Folgenden kurz zusammengefasst dargestellt werden.

Methodik

Patienten und Studiendesign

Patienten mit schubförmig-remittierender Multipler Sklerose (RRMS), die die 2005 revidierte Fassung der McDonald-Richtlinien zur MS-Diagnose erfüllten (17) wurden aus Screening-Untersuchungen laufender Therapiestudien am NeuroCure Clinical Research Center der Charité – Universitätsmedizin Berlin und der MS

Studiensprechstunde des Universitätsklinikum Hamburg-Eppendorf rekrutiert. Einschlusskriterien waren: Alter zwischen 18 und 55 Jahren, stabile immunmodulatorische Behandlung oder keine kausale Therapie seit mindestens sechs Monaten, Expanded Disability Status Scale (EDSS) (18) zwischen 0 und 6,5. Spezifische Ausschlusskriterien waren: akuter Schub oder systemische Kortikosteroidgabe innerhalb von 30 Tagen vor Untersuchung, akute Sehnervenentzündung innerhalb der letzten drei Monate, ophthalmologische Erkrankungen mit Netzhautveränderungen (insbesondere Glaukomerkrankungen und Diabetes mellitus).

Ethik

Die lokalen Ethik-Kommissionen der Charité - Universitätsmedizin Berlin und des Universitätsklinikum Hamburg-Eppendorf haben die Studien genehmigt. Alle Teilnehmer haben ihr schriftliches Einverständnis in Anlehnung an die Deklaration von Helsinki 1964 erklärt.

Klinische Untersuchung und visuelle Funktionstestung

Bei allen Studienteilnehmern wurde eine detaillierte medizinische Anamnese erhoben, gefolgt von einer standardisierten klinisch-neurologischen Untersuchung unter Anleitung eines neurologischen Facharztes zur Beurteilung des neurologischen Behinderungsgrades auf Basis der EDSS. In Berlin erhielten Teilnehmer zudem eine Refraktionsmessung und Bestimmung der Sehschärfe anhand Snellen Tafeln und der Kontrastsensitivität mittels Functional Acuity Contrast Testing (FACT).(19)

Optische Kohärenztomographie (OCT)

Alle Teilnehmer wurden mittels OCT untersucht. In den Publikationen Dörr et al.(20) und Pfüller & Brandt et al.(21) erfolgte die Untersuchung mit einem Time Domain OCT (TD-OCT), dem Stratus 3000 OCT (Carl Zeiss Meditec, Dublin, Kalifornien, USA). Die peripapilläre RNFL-Dicke wurde mit dem “Fast RNFL 3.4” Protokoll (Software Version 4.0) bestimmt, das die Dicke der RNFL mit einem 3,4 mm durchmessenden Ringscan um den Sehnervenkopf misst. Das Totale Makulavolumen („Total Macular Volume“, TMV) wurde mit dem “Fast Macular Thickness Map” Protokoll bestimmt. Dieser Scan interpoliert sechs Linienscans durch die Fovea, um einen 6 mm durchmessenden Kreis zu beschreiben, der die Makula umfasst. Das TMV ist dann definiert als Volumen zwischen der Inneren

Begrenzungsmembran („Inner Limiting Membrane“, ILM) und dem retinalen Pigmentepithel („Retinal Pigment Epithelium“, RPE) in diesem Kreisareal. In die Auswertung wurden nur Scans eingeschlossen, die eine gute Signalqualität (>=7) aufwiesen und in denen die begrenzenden Schichten eindeutig bestimmt werden konnten.

In Zimmermann et al. (22) und Young & Brandt et al. (23) wurden die Untersuchungen mit einem schnelleren und auflösungsstärkeren Spectral Domain OCT (SD-OCT), dem Cirrus HD-OCT Version 5.1 (Carl-Zeiss Meditec, Dublin, Kalifornien, USA) durchgeführt, das 3D Aufnahmen zulässt.(24) Die peripapilläre RNFL-Dicke wurde mit dem „Optic Disc Cube 200 × 200“ Protokoll bestimmt, das einen 6 mm × 6 mm großen Volumenscan um den Sehnervenkopf misst. Die RNFL Dicke wurde dann in einem simulierten 3,4 mm durchmessenden Ringscan um den Sehnervenkopf bestimmt. TMV wurde entweder mit dem „Macular Cube 200 × 200“ oder dem „Macular Cube 512 × 128“ Protokoll bestimmt, die einen 6 mm × 6 mm großen Volumenscan um die Fovea messen. Das TMV ist dann als das Volumen zwischen der ILM und dem RPE in einem 6 mm Kreisareal um die Fovea definiert, das die Makula umfasst. In Zimmermann et al. (22) wurde zudem die Dicke der retinalen Ganglienzellschicht und der inneren plexiformen Schicht („ganglion cell and inner plexiform layer“, GCIPL) mit einer Beta-Software von Carl Zeiss Meditec bestimmt (HD-OCT software Version 6.0). Die beiden Schichten wurden kombiniert gemessen, da aufgrund der sehr ähnlichen Kontrastniveaus im OCT eine Einzelmessung zu fehleranfällig ist.

Magnetische Resonanztomographie (MRT)

MRT Messungen wurden mit 1.5 Tesla Scannern Avanto (Berlin) und Sonata (Hamburg) (Siemens Medical Systems, Erlangen, Deutschland) durchgeführt. 3D T1 gewichtete Aufnahmen („magnetization-prepared rapid acquisition and multiple gradient echo technique“, MPRAGE) wurden mit folgenden Parametern aufgenommen: Avanto: TE 3,09 ms, TR 1.900 ms, Auflösung 1 mm^3; Sonata: TE 3,82 ms, TR 1.900 ms, Auflösung 1 mm^3).

Auf Basis der MPRAGE-Aufnahmen wurden in Dörr et al.(20), Pfüller & Brandt et al.(21) und Young & Brandt et al.(23) die BPF, das Volumen der Grauen Substanz (GMV) und der Weißen Substanz (WMV) mit FSL SIENAX Version 4.1.4 berechnet. In Zimmermann et al.(22) wurden NBV, NGMV und NWMV mit FSL SIENAX Version

4.1.6 berechnet. FSL SIENAX extrahiert zunächst das eigentliche Hirnareal von den umgebenden Schädelaufnahmen. Anschließend wird das extrahierte Hirn auf einen MNI152 Standardraum registriert, um die Normalisierungsfaktoren zu generieren. Abschließend werden die einzelnen Gewebstypen (ventrikulärer Liquor, Graue und Weiße Substanz) segmentiert und die partiellen Volumen berechnet.(9)

Magnetische Resonanzspektroskopie (MRS)

In Pfüller et al.(21) wurde eine Untergruppe an Teilnehmern von Dörr et al.(20) beschrieben, die mit MRS untersucht wurden. Die MRS wurde durchgeführt an einem experimentellen 3 Tesla Scanner (MEDSPEC 30/100, Bruker Biospin, Ettlingen, Germany) in der Physikalisch Technischen Bundesanstalt (PTB) in Berlin. Es wurden zwei Voxel in periventrikulärer normal erscheinender Weißer Substanz („normal appearing periventricular white matter“, NAWM) mit der Größe 2 x 2 x 2 cm sowie ein gemischter Graue-/Weiße-Substanz Voxel im visuellen Cortex mit der Größe 3 x 2 x 2 cm aufgenommen.(25) Hieraus wurden NAA und Creatinin(Cr)-Spektren zur Bestimmung von NAA und normalisiertem NAA/Cr isoliert.

Statistische Analyse

Alle Messwerte wurden auf Normalverteilung unter Zuhilfenahme von Histogrammen, Analyse der Schiefe und Steilheit der Verteilung und mit Shapiro-Wilk-Tests überprüft. Die statistischen Analysen wurden im Wesentlichen mit verallgemeinerten Schätzungsgleichungen („Generalized Estimating Equation Models“, GEE) durchgeführt, die die intraindividuellen Abhängigkeiten zwischen den zwei Augen der Teilnehmer berücksichtigen. Da GEE keine (oder eine nur sehr eingeschränkte) Abschätzung der Effektstärke erlauben, wurden zwei weitere Verfahren implementiert: Zum einen wurden alle Eingangsvariablen zu z-Werten normalisiert und anschließend die GEE durchgeführt. Auf diese Weise wurde näherungsweise ein standardisiertes Beta bestimmt. Zum anderen wurden lineare Regressionen mit nur einer Messung (Bsp.: minimale RNFL -> die niedrigere RNFL Messungen der beiden Augen) durchgeführt. Beide Verfahren erlauben näherungsweise die Berechnung der Effektstärke in Form von R^2 und damit einen Vergleich der Ergebnisse untereinander und zu anderen Arbeiten.

Zusätzliche Analysen, primär zur Untersuchung von Einflussgrößen außerhalb der OCT-Messungen wurden mittels verschiedener parametrischer und nicht-parametrischer Verfahren durchgeführt (Varianzanalyse, partielle Korrelation,

Spearman und Pearson Korrelation, Pearson's Chi^2 Test, Kruskal-Wallis-Test, Mann-Whitney U Test).

Alle Analysen wurden mit SPSS Version 18 bis 20 (Chicago, IL, USA) durchgeführt. Signifikanz wurde in allen Tests bei $p < 0,05$ erreicht. Mit Ausnahme des primären Endpunktes waren alle Analysen explorativ, das heißt, es wurden keine vorherige Fallzahlplanung oder anschließende Korrektur für multiples Testen durchgeführt.

Fallzahlplanung

Für Dörr et al.(20) und Young & Brandt et al.(23) wurde eine Fallzahlplanung mit 95% Power auf Basis der Daten von Gordon-Lipkin durchgeführt.(16) Der primäre Endpunkt wurde mit einem Regressionsmodell auf Basis von GEE mit BPF als Ziel- und RNFL als unabhängige Variable sowie Alter und stattgehabte Sehnervenentzündung als Korrekturfaktoren definiert. Da es keine verfügbaren Verfahren zur Fallzahlschätzung bei GEE gibt, wurde ein lineares multiples Regressionsmodell zur Berechnung genutzt. Die Fallzahlkalkulation wurde mit G*Power 3.1.2 durchgeführt (Universität Düsseldorf, Deutschland).(26)

Ergebnisse

OCT Zusammenhang mit Hirnatrophie (Dörr et al.)

Die Fallzahlplanung ergab eine notwendige Teilnehmerzahl von n = 86 (mit 20% kalkulierten Ausfällen n = 103). Es wurden insgesamt 104 Patienten (208 Augen) eingeschlossen und untersucht (Alter 39,7 ± 8,8 Jahre, 69 Frauen/35 Männer, Erkrankungsdauer 68 ± 59 Monate, Median EDSS 2,0). 62 Patienten hatten anamnestisch keine, 29 eine einseitige und 13 Patienten eine beidseitige Sehnervenentzündung. Die Ergebnisse von Gordon-Lipkin et al. konnten in dieser Studie bestätigt werden. Die RNFL-Dicke war assoziiert mit BPF (GEE $p < 0.0001$, partielle Korrelation kontrolliert für Alter $R = 0,269$, $p = 0,006$). Zusätzliche korrelierte auch TMV mit BPF (GEE $p < 0,0001$, partielle Korrelation kontrolliert für Alter $R = 0,369$, $p < 0,001$). In einer explorativen Analyse über die Zusammenhänge zwischen Alter, Erkrankungsdauer (als Zeit seit Diagnosestellung), EDSS und Anamnese einer Sehnervenentzündung mit BPF, RNFL Dicke oder TMV korrelierte BPF mit Alter (stand. Beta = - 0,334, $p < 0,001$) und EDSS (stand. Beta -0,223, $p < 0,001$). RNFL war assoziiert mit Erkrankungsdauer (stand. Beta = -0,299, $p < 0,001$) und Anamnese einer Sehnervenentzündung (stand. Beta = -0,675, $p < 0,001$). TMV war

nur assoziiert mit Anamnese einer Sehnervenentzündung (stand. Beta -0,614, p < 0,001).

OCT Zusammenhang mit NAA im visuellen Cortex (Pfüller & Brandt et al.)

In einer Untergruppe der Studienkohorte von Dörr et al. von 86 Patienten wurde zusätzlich eine MRS durchgeführt. In GEE Analysen war NAA im visuellen Cortex Voxel mit der RNFL-Dicke assoziiert (stand. Beta = 0,191, p = 0,047). Ähnliches galt auch, wie in der Studie von Dörr et al., für BPF (stand. Beta = 0,269, p = 0,001). Um sicherzustellen, dass die NAA Assoziation ein vom Hirnvolumen unabhängiger Effekt ist, wurde zusätzlich überprüft, ob BPF mit NAA im visuellen Cortex Voxel korreliert. Dieses war nicht der Fall. In einer multivariaten GEE Analyse waren zudem NAA im visuellen Cortex (Stand. Beta = 0,188, p = 0,042) und BPF (Stand. Beta = 0,244, p = 0,002) unabhängig voneinander assoziiert mit der RNFL-Dicke. Für NAA in den NAWM Voxeln gab es keinen Zusammenhang mit der RNFL-Dicke.

Einfluss von Sehnervenentzündungen (Zimmermann et al.)

In bisherigen Studien wurde der Einfluss von früheren Sehnervenentzündungen auf den Zusammenhang zwischen OCT und MRT vernachlässigt. Zwar wurde vereinzelt die Angabe einer abgelaufenen Sehnervenentzündung als Korrekturfaktor in die statistischen Modelle aufgenommen (so auch in den obigen, eigenen Arbeiten), aber isolierte Analysen wurden nicht durchgeführt. Des Weiteren wurde inzwischen mit der GCIPL eine weitere Messgröße mit neuen 3D SD-OCT Messungen möglich, die sich als überlegen in der Darstellung einiger MS-spezifischer Veränderungen herausgestellt hat.(27,28)

In einer Zusammenarbeit mit dem Universitätsklinikum Hamburg-Eppendorf wurden 63 Patienten mit schubförmig-remittierender MS (Alter 41 ± 9 Jahre, 46 Frauen/17 Männer, Monate seit Diagnosestellung 79 ± 58, Median EDSS 2,0) mit SD-OCT und MRT untersucht. Es zeigte sich, wie erwartet, eine dünnere RNFL in Augen, die zuvor eine Sehnervenentzündung erlitten hatten (82 ± 12 µm vs. 90 ± 10 µm, p < 0,001). Gleiches galt für die GCIPL (im Manuskript „GCLT“ abgekürzt; 70 ± 10 µm vs. 78 ± 7 µm, p < 0,001). RNFL war bei Augen ohne vorherige Sehnervenentzündung mit NWMV (GEE B = 0,083, SE = 0,027, p = 0,002) und NGMV (GEE B = 0,085, SE = 0,026, p = 0,001) assoziiert. Bei Augen mit vorheriger

Sehnervenentzündung war nur noch ein Zusammenhang mit NWMV gegeben (GEE B = 0,116, SE = 0,045, p = 0,010). GICPL verhielt sich analog zu RNFL.

OCT Zusammenhang mit Hirnatrophie bei früher MS (Young & Brandt et al.)

Ebenfalls in einer Zusammenarbeit mit dem Universitätsklinikum Hamburg-Eppendorf wurde die Assoziation zwischen OCT und MRT in einer Kohorte von 44 schubförmig-remittierenden MS Patienten und Patienten mit klinisch isoliertem Syndrom („clinically isolated syndrome“, CIS) mit kurzer Erkrankungsdauer und ohne bestehende verlaufsmodifizierende Therapie untersucht (Alter 41 ± 9 Jahre, 46 Frauen/17 Männer, Jahre seit Diagnosestellung 3,2 ± 2,7, Median EDSS 1,5). Die Patienten wurden sowohl im OCT untersucht als auch im MRT ausführlich beschrieben.

Der primäre Endpunkt dieser mit 95% Power durchgeführten Studie war signifikant (RNFL Zusammenhang mit BPF mit den Kovariaten Alter und Angabe einer früheren Sehnervenentzündung, p = 0,005). In einer explorativen Analyse dieser frühen MS Patienten gab es lediglich einen Zusammenhang zwischen Weißer Substanz und RNFL (R^2 = 0,319, p < 0,001; GEE p = 0,003), während das Volumen der Grauen Substanz primär altersabhängig war (R^2 = 0,374, p < 0,001; GEE p = 0,003), jedoch kein Zusammenhang zur RNFL zeigte (p = 0,717). Es gab keinerlei Zusammenhänge mit dem T2 Läsions-Volumen oder dem CEL-Volumen.

Diskussion

In dieser Arbeit wurde der Zusammenhang zwischen retinalen OCT Parametern und MRT Markern der Hirnatrophie untersucht. Es konnte in vier Publikationen gezeigt werden, dass

1) sich ein Zusammenhang zwischen RNFL und BPF in einer geplanten Studie bestätigt.(20)
2) RNFL nicht nur mit globalen MRT-Parametern der Hirnatrophie korreliert sondern es auch einen Zusammenhang zu metabolischen/neurochemischen neurodegenerativen Veränderungen im visuellen Cortex gibt (NAA Reduktion).(21)
3) Es einen Zusammenhang sowohl zwischen dem Volumen der grauen als auch der Weißen Substanz mit OCT-Parametern (RNFL und GCIPL) gibt. Eine stattgehabte Sehnervenentzündung den Zusammenhang jedoch beeinflusst

und vor allem den Zusammenhang zwischen Grauer Substanz und OCT beeinträchtigt.(22)

4) In frühen MS Patienten ein Zusammenhang zwischen Weißer Substanz und OCT Parametern (RNFL) existiert, während das Volumen der Grauen Substanz stark vom Alter der Teilnehmer abhängt.(23)

Die Arbeiten haben einen relevanten Beitrag geleistet, das Verhalten von retinalen Veränderungen bei MS zu verstehen und im Vergleich zu MRT Parametern der Neurodegeneration zu interpretieren.

Ein gemeinsames Modell der Ergebnisse der einzelnen Arbeiten lässt sich wie folgt hypothetisieren: In früher MS besteht ein Zusammenhang zwischen RNFL und dem Volumen der Weißen Substanz, welcher das Übergewicht von Krankheitsprozessen in der Weißen Substanz in dieser Erkrankungsphase reflektiert. Dieser Zusammenhang bleibt auch nach einer Sehnervenentzündung bestehen, was darauf hindeutet, dass sich sowohl im Sehnerven als auch in der Weißen Substanz ein vergleichbares Entzündungsniveau als Ausmaß einer neuroimmunologischen Komponente der MS widerspiegeln könnte. Mit zunehmender Erkrankungsdauer treten sowohl im Hirn als auch in der Retina neurodegenerative Prozesse hinzu, die darüber hinaus eine Assoziation zwischen Grauer Substanz und RNFL und GCIPL bilden. Die retinale Neurodegeneration in Augen ohne vorherige Sehnervenentzündung reflektiert daher potentiell die allgemeine Erkrankungsschwere bzw. die globale Neurodegeneration auch im Gehirn. Kommt es zu einem zusätzlichen, fokalen immunologischen Schaden im Gebiet der Retina, insbesondere durch eine Sehnervenentzündung, wird die retinale Neurodegeneration darüber hinausgehend verstärkt und der Zusammenhang mit der globalen Neurodegeneration durchbrochen. Dieses Modell wird durch Arbeiten unterstützt, die unterschiedliche neuroimmunologische und neurodegenerative Phänotypen der MS beschreiben.(29)

Die aktuelle Studienlage ist dabei noch einigen Einschränkungen unterworfen. Zum einen sind Daten zum Zusammenhang zwischen OCT und MRT bei gesunden Personen nur sehr eingeschränkt verfügbar, deuten aber ebenfalls auf existierende Assoziationen hin.(30) In der Konsequenz müssen krankheitsspezifische Erklärungsmodelle ggf. angepasst werden. Zudem entwickeln sich sowohl OCT als MRT-Technologie kontinuierlich weiter. Nur wenige Informationen gibt es zu den

Zusammenhängen zwischen fokalen Veränderungen im Hirn und/oder Netzhautveränderungen in einzelnen Schichten, die nicht nur Hinweise auf Netzwerk-bedingte Zusammenhänge bei MS erlauben, sondern z.B. auch zellulär-pathologische Ähnlichkeiten aufweisen können. Bisher nicht zufriedenstellend waren auch Studien, die longitudinale OCT Veränderung bei MS außerhalb von Sehnervenentzündungen untersucht haben, da die jährlichen Veränderungen von ca. 0,5 bis 1 µm pro Jahr im Bereich der Messungenauigkeit heutiger Geräte liegen.(31)

Zusammengefasst bietet die Optische Kohärenztomographie die einmalige Chance, mit fast zellulärer Auflösung sowohl neuroimmunologische als auch neurodegenerative Prozesse an der Retina bei MS Patienten zu beobachten. Sie sollte und wird sich als zusätzlicher Parameter in der Neurologie etablieren und das MRT sinnvoll ergänzen, wenn es um Krankheitsprozesse am Sehnerven oder der Retina geht. Direkte OCT-basierte Surrogatmarker für MRT-Untersuchungen sind jedoch insbesondere im Rahmen von Sehnervenentzündungen nur eingeschränkt nutzbar. Ein besonderer Vorteil von OCT gegenüber MRT könnte hier sein, dass sich die Retina im Gegensatz zum Hirnvolumen bis zum 50. Lebensjahr kaum altersbedingt verändert.(32)

Literaturverzeichnis

1. Compston A, Coles A. Multiple sclerosis. The Lancet. 25;372(9648):1502–17.

2. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology. 1996 Apr;46(4):907–11.

3. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat. Immunol. 2007 Sep;8(9):913–9.

4. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. N. Engl. J. Med. 1998 Jan 29;338(5):278–85.

5. De Stefano N, Matthews PM, Filippi M, Agosta F, De Luca M, Bartolozzi ML, et al. Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. Neurology. 2003 Apr 8;60(7):1157–62.

6. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol. 2011 Feb;69(2):292–302.

7. Fisniku LK, Brex PA, Altmann DR, Miszkiel KA, Benton CE, Lanyon R, et al. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. Brain. 2008 Mar;131(Pt 3):808–17.

8. Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. Neurology. 1999 Nov 10;53(8):1698–704.

9. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 2004;23 Suppl 1:S208–219.

10. Fisher E, Rudick RA, Simon JH, Cutter G, Baier M, Lee J-C, et al. Eight-year follow-up study of brain atrophy in patients with MS. Neurology. 2002 Nov 12;59(9):1412–20.

11. Filippi M, Rocca MA, De Stefano N, Enzinger C, Fisher E, Horsfield MA, et al.

Magnetic resonance techniques in multiple sclerosis: the present and the future. Arch. Neurol. 2011 Dec;68(12):1514–20.

12. Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J. Anat. 2005 Apr;206(4):319–48.

13. 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. Multiple Sclerosis International. 2012;2012:1–10.

14. Petzold A, de Boer JF, Schippling S, Vermersch P, Kardon R, Green A, et al. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. Lancet Neurol. 2010 Sep;9(9):921–32.

15. Barkhof F, Calabresi PA, Miller DH, Reingold SC. Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. Nat Rev Neurol. 2009 May;5(5):256–66.

16. Gordon-Lipkin E, Chodkowski B, Reich DS, Smith SA, Pulicken M, Balcer LJ, et al. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology. 2007 Oct 16;69(16):1603–9.

17. 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 Dec;58(6):840–6.

18. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983 Nov;33(11):1444–52.

19. Bock M, Brandt AU, Kuchenbecker J, Dörr J, Pfueller CF, Weinges-Evers N, et al. Impairment of contrast visual acuity as a functional correlate of retinal nerve fibre layer thinning and total macular volume reduction in multiple sclerosis. Br J Ophthalmol [Internet]. 2011 Mar 3 [cited 2011 Apr 15]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/21378002

20. Dörr J, Wernecke KD, Bock M, Gaede G, Wuerfel JT, Pfueller CF, et al. Association of Retinal and Macular Damage with Brain Atrophy in Multiple Sclerosis.

PLoS ONE. 2011 Apr 8;6(4):e18132.

21. Pfueller CF, Brandt AU, Schubert F, Bock M, Walaszek B, Waiczies H, et al. Metabolic changes in the visual cortex are linked to retinal nerve fiber layer thinning in multiple sclerosis. PLoS ONE. 2011;6(4):e18019.

22. Zimmermann H, Freing A, Kaufhold F, Gaede G, Bohn E, Bock M, et al. Optic neuritis interferes with optical coherence tomography and magnetic resonance imaging correlations. Mult. Scler. [Internet]. 2012 Aug 30 [cited 2012 Sep 6]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/22936335

23. Young KL, Brandt AU, Petzold A, Reitz LY, Lintze F, Paul F, et al. Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis. Eur. J. Neurol. 2013 May;20(5):803–11.

24. Bock M, Brandt AU, Dörr J, Pfueller CF, Ohlraun S, Zipp F, et al. Time domain and spectral domain optical coherence tomography in multiple sclerosis: a comparative cross-sectional study. Mult. Scler. 2010 Jul;16(7):893–6.

25. Schubert F, Gallinat J, Seifert F, Rinneberg H. Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla. Neuroimage. 2004 Apr;21(4):1762–71.

26. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007 May;39(2):175–91.

27. Saidha S, Syc SB, Ibrahim MA, Eckstein C, Warner CV, Farrell SK, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. Brain. 2011 Feb;134(Pt 2):518–33.

28. 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: A Journal of Neurology [Internet]. 2011 Oct 17 [cited 2011 Dec 6]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/22006982

29. Bielekova B, Kadom N, Fisher E, Jeffries N, Ohayon J, Richert N, et al. MRI as a marker for disease heterogeneity in multiple sclerosis. Neurology. 2005 Oct 11;65(7):1071–6.

30. Saidha S, Sotirchos ES, Oh J, Syc SB, Seigo MA, Shiee N, et al.

Relationships Between Retinal Axonal and Neuronal Measures and Global Central Nervous System Pathology in Multiple Sclerosis. Arch. Neurol. 2012 Oct 1;1–10.

31. Talman LS, Bisker ER, Sackel DJ, Long DA, Galetta KM, Ratchford JN, et al. Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. Ann. Neurol. 2010 Jun;67(6):749–60.

32. Ooto S, Hangai M, Tomidokoro A, Saito H, Araie M, Otani T, et al. Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. Invest. Ophthalmol. Vis. Sci. 2011;52(12):8769–79.

Eidesstattliche Versicherung

„Ich, Alexander Ulrich Brandt, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Optische Kohärenztomographie im Vergleich zu Magnetresonanztomographie und Magnetresonanzspektroskopie als Parameter der Neurodegeneration bei Multipler Sklerose“ 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 beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -*www.**icmje**.org*) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.
Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.
Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum _____ Unterschrift

Anteilserklärung an den erfolgten Publikationen

Alexander Ulrich Brandt (AUB) hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: Dörr J, Wernecke KD, Bock M, Gaede G, Wuerfel JT, Pfueller CF, Bellmann-Strobl J, Freing A, **Brandt AU***, Paul F*. *Association of Retinal and Macular Damage with Brain Atrophy in Multiple Sclerosis.* PLoS ONE. 2011 Apr 8;6(4):e18132

Beitrag im Einzelnen:
AUB hat die Studie in weiten Teilen mit JD und FP konzipiert, die klinischen Untersuchungen und Messungen anteilig durchgeführt und ausgewertet, die Fallzahlkalkulation und die statistische Auswertung mit KDW zusammen geplant und durchgeführt und mit JD das Manuskript geschrieben.

Publikation 2: Pfueller CF*, **Brandt AU***, Schubert F, Bock M, Walaszek B, Waiczies H, Schwenteck T, Dörr J, Bellmann-Strobl J, Mohr C, Weinges-Evers N, Ittermann B, Wuerfel J, Paul F. *Metabolic changes in the visual cortex are linked to retinal nerve fiber layer thinning in multiple sclerosis.* PLoS ONE. 2011;6(4):e18019

Beitrag im Einzelnen:
AUB hat das Konzept der Studie mit erstellt, die klinischen Untersuchungen und Messungen anteilig durchgeführt und ausgewertet, die statistische Auswertung durchgeführt und das Manuskript mit CP geschrieben.

Publikation 3: Zimmermann H, Freing A, Kaufhold F, Gaede G, Bohn E, Bock M, Oberwahrenbrock T, Young KL, Dörr J, Wuerfel J, Schippling S, Paul F, **Brandt AU**. *Optic neuritis interferes with optical coherence tomography and magnetic resonance imaging correlations*. Mult Scler. 2013 Apr;19(4):443-50

Beitrag im Einzelnen:
AUB hat die Studie konzipiert, die statistische Auswertung geplant und koordiniert und das Manuskript finalisiert mit maßgeblicher Beteiligung and Einleitung und Diskussion der Ergebnisse im aktuellen wissenschaftlichen Kontext.

Publikation 4: Young KL*, **Brandt AU***, Petzold A, Reitz LY, Lintze F, Paul F, Martin R, Schippling S. *Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis.* Eur J Neurol. 2013 May;20(5):803-11

Beitrag im Einzelnen:
AUB hat die Studienfragestellung hergeleitet, die statistische Fallzahlplanung und Auswertung durchgeführt und das Manuskript in weiten Teilen mit FP und SS geschrieben.

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers/der betreuenden Hochschullehrerin

_____

Unterschrift des Doktoranden/der Doktorandin

_____

OPEN ACCESS Freely available online

PLoS one

Association of Retinal and Macular Damage with Brain Atrophy in Multiple Sclerosis

Jan Dörr[1]*, Klaus D. Wernecke[2], Markus Bock[1], Gunnar Gaede[1], Jens T. Wuerfel[3], Caspar F. Pfueller[1], Judith Bellmann-Strobl[1,4], Alina Freing[1], Alexander U. Brandt[1,5☯], Paul Friedemann[1,4☯]

1 NeuroCure Clinical Research Center, Charité - Universitaetsmedizin Berlin, Berlin, Germany, **2** Sophisticated Statistical Analysis GmbH and Charité - Universitaetsmedizin Berlin, Berlin, Germany, **3** Institute of Neuroradiology, University Luebeck, Luebeck, Germany, **4** Experimental and Clinical Research Center, Charité - Universitaetsmedizin Berlin and Max-Delbrück Center for Molecular Medicine Berlin, Berlin, Germany, **5** gfnmediber GmbH, Berlin, Germany

Abstract

Neuroaxonal degeneration in the central nervous system contributes substantially to the long term disability in multiple sclerosis (MS) patients. However, in vivo determination and monitoring of neurodegeneration remain difficult. As the widely used MRI-based approaches, including the brain parenchymal fraction (BPF) have some limitations, complementary in vivo measures for neurodegeneration are necessary. Optical coherence tomography (OCT) is a potent tool for the detection of MS-related retinal neurodegeneration. However, crucial aspects including the association between OCT- and MRI-based atrophy measures or the impact of MS-related parameters on OCT parameters are still unclear. In this large prospective cross-sectional study on 104 relapsing remitting multiple sclerosis (RRMS) patients we evaluated the associations of retinal nerve fiber layer thickness (RNFLT) and total macular volume (TMV) with BPF and addressed the impact of disease-determining parameters on RNFLT, TMV or BPF. BPF, normalized for subject head size, was estimated with SIENAX. Relations were analyzed primarily by Generalized Estimating Equation (GEE) models considering within-patient inter-eye relations. We found that both RNFLT ($p = 0.019$, GEE) and TMV ($p = 0.004$, GEE) associate with BPF. RNFLT was furthermore linked to the disease duration ($p < 0.001$, GEE) but neither to disease severity nor patients' age. Contrarily, BPF was rather associated with severity ($p < 0.001$, GEE) than disease duration and was confounded by age ($p < 0.001$, GEE). TMV was not associated with any of these parameters. Thus, we conclude that in RRMS patients with relatively short disease duration and rather mild disability RNFLT and TMV reflect brain atrophy and are thus promising parameters to evaluate neurodegeneration in MS. Furthermore, our data suggest that RNFLT and BPF reflect different aspects of MS. Whereas BPF best reflects disease severity, RNFLT might be the better parameter for monitoring axonal damage longitudinally. Longitudinal studies are necessary for validation of data and to further clarify the relevance of TMV.

Citation: Dörr J, Wernecke KD, Bock M, Gaede G, Wuerfel JT, et al. (2011) Association of Retinal and Macular Damage with Brain Atrophy in Multiple Sclerosis. PLoS ONE 6(4): e18132. doi:10.1371/journal.pone.0018132

Editor: Rafael Linden, Universidade Federal do Rio de Janeiro, Brazil

Received November 3, 2010; **Accepted** February 24, 2011; **Published** April 8, 2011

Funding: This work was supported by grants from the Excellence Cluster 257 of the German Research Foundation to NeuroCure Clinical Research Center, grant KF2286101FO9 from the German Ministry of Economics to NeuroCure Clinical Research Center and gfnmediber GmbH (www.gfnmediber.de) and a limited research grant by TEVA Pharma GmbH, Germany (www.teva-deutschland.de). These funders played no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Besides their scientific affiliation at the Charité, Alexander U. Brandt and Klaus-Dieter Wernecke are employed by gfnmediber (AU Brandt) and SoStAna (KD Wernecke, www.sostana.com). Alexander U. Brandt contributed to this project (study design, statistical analysis and preparation of the manuscript) in his role as scientist and not as employee of gfnmediber GmbH. Apart from partial funding (grant KF2286101FO9 from the German Ministry of Economics) the company gfnmediber GmbH had no further role in this project. Klaus-Dieter Wernecke contributed to this project (study design, statistical analysis) in his role as scientist at the Charité and not as CEO of SoStAna. The company SoStAna itself had no role in this project.

Competing Interests: Alexander U. Brandt is deputy CEO of gfnmediber GmbH and guest scientist at the NeuroCure Clinical Research Center (NCRC). Alexander U Brandt contributed to the study merely in his role of a guest scientist at the NCRC. Klaus-Dieter Wernecke is CEO of Sophisticated Statistical Analysis (SoStAna) and scientist at the Charité, Berlin. Klaus-Dieter Wernecke contributed to the study merely in his role as a scientist at the Charité. There are no patents, products in development or marketed products to declare with the current study and these affiliations did not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials. All other authors have declared that no competing interests exist.

* E-mail: jan-markus.doerr@charite.de

☯ These authors contributed equally to this work.

Introduction

Increasing evidence documents that neuronal and axonal damage within the central nervous system (CNS) contributes substantially to the development of permanent disability in multiple sclerosis (MS) [1,2,3]. However, in vivo quantification and longitudinal monitoring of neurodegenerative processes remain a challenging task. Whole brain atrophy expressed by brain parenchymal fraction (BPF) is a frequently used MRI-based surrogate parameter for neurodegeneration within the CNS [4,5,6] and an inverse relation of BPF and disability progression has been consistently demonstrated [7,8]. However, all MRI-based measures of brain atrophy have some important disadvantages. Besides limited availability, time consumption and costs, crucial confounders such as hydration status, inflammation, demyelination and age have to be accounted for [5]. Thus, a need for reliable, inexpensive and easily assessable complementary surrogate markers for neuroaxonal degeneration still remains. During the past two decades, optical coherence tomography (OCT) has emerged into a fascinating tool for the non invasive and reproducible in vivo studying of retinal neuroaxonal damage [9]. In MS patients, OCT has been consistently shown to detect

thinning of the peripapillary retinal nerve fiber layer (RNFL) which is most probably due to a diffuse damage of retinal axons and at least in part independent of a previous optic neuritis (ON) [10,11,12]. Moreover, the determination of total macular volume (TMV) has been suggested as a marker for neuronal loss in MS patients [13]. Therefore, OCT might be a valuable tool for quantification and monitoring of both axonal and neuronal damage in MS [14,15]. However, data on association between retinal nerve fiber layer thickness (RNFLT) and MS-determining parameters such as disease severity and disease duration are still inconsistent [15]. Whereas some studies found an association between RNFLT and disease duration [11,16] both parameters were not related in other studies [17,18]. Furthermore, data regarding the relation between OCT parameters and MRI measures for neurodegeneration are not yet consistent and a consensus on the most relevant parameter has yet to be reached [5,15]. To date, the association between OCT parameters and BPF has been addressed in only two studies. Gordon-Lipkin et al. reported an association between RNFLT and BPF but not between TMV and BPF in a small cohort of 40 MS patients [19]. In another small study, Siger et al. found a correlation between RNFLT and BPF only in the subgroup without a history of ON [20]. Thus, the relation between OCT-based measures and BPF as an established MRI-based measure for neurodegeneration is not yet clear.

The aims of our prospective cross-sectional study were (i) to investigate the association between retinal neuroaxonal damage, measured by RNFLT and TMV, and cerebral neurodegeneration, measured by BPF, in a homogenous and sufficiently large cohort of patients with relapsing remitting (RR)MS and (ii) to evaluate the influence of important aspects such as age, disease duration, disease severity and ON history on RNFLT, TMV and BPF, respectively.

Methods

Ethics Statement

The study was approved by the local ethics committee of Charité Universitätsmedizin Berlin, Germany, and all participants gave informed written consent according to the 1964 Declaration of Helsinki.

Patients

RRMS patients fulfilling the current panel criteria [21] were prospectively recruited from baseline visit of an ongoing clinical trial. All patients met the following criteria: age between 18 and 60 years, definite RRMS [21], expanded disability status scale (EDSS) between 0 and 6.5 [22], stable immunomodulatory treatment with glatiramer acetate for at least six months (this was an inclusion criterion of the ongoing clinical trial, patients were recruited from), no acute relapse (including optic neuritis) and no systemic steroid treatment within 30 days prior to enrolment. Medical history, particularly with respect to visual symptoms was taken from all study participants. All participants underwent a complete ophthalmologic examination including visual acuity testing, spheric and cylindric refractive error testing and non-contact tonometry. Patients with ophthalmologic disorders or medical conditions with impact on OCT parameters (e.g. diabetes, glaucoma) were not included.

Optical Coherence Tomography

All OCT examinations were carried out on a Stratus 3000 OCT (OCT3, Carl Zeiss Meditec, Dublin, USA). RNFLT was measured using the "fast RNFL 3.4" protocol (software version 4.0). Three 3.4 mm diameter circular scans were acquired over 1.92 seconds. The OCT A-scan data were digitally exported in a blinded fashion and average RNFLT was calculated. As no specific real time volume scan protocol is available we used the "Fast Macular Thickness Map" protocol for determination of TMV which interpolates the area between the real time line scans to construct a circular model of the fovea and macula. Six radial line scans with 128 A-scans per line and a scan area of 6-mm diameter circle were acquired over 1.92 seconds. The maximum of 786.432 data points for fast protocols was obtained. For controlled manual export of the TMV data in mm^3 we used the analysis protocol "Retinal Thickness/Volume Tabular". A good quality image was defined as an image with generalized signal distribution, a reflectance signal from RNFL or retinal pigment epithelium strong enough to identify either layer, no missing parts caused by eye movements and a signal strength of $\geq$8 of 10 [23]. The segmentation line that defines the upper border of the retina was required to be on the internal limiting membrane and the lower border was required to be on the lower border of the RNFL (for RNFLT) or between the inner and outer photoreceptor layer of the RNFL (for TMV). Images not meeting these criteria were excluded.

Magnetic Resonance Imaging

All MRI measurements were performed on a 1.5 Tesla scanner (Avanto, Siemens Medical Systems, Erlangen, Germany). A three-dimensional T1-weighted sequence (MPRAGE) was acquired from all participants according to the following protocol: TR 1.900 ms, TE 3.09 ms, TI 1,100 ms, flip angle 15°, resolution 1 mm^3). Brain tissue volume, normalized for subject head size, was estimated with SIENAX [24,25], part of FSL [26]. SIENAX starts by extracting brain and skull images from the single whole-head input data [27]. The brain image is then affine-registered to MNI152 space (using the skull image to determine the registration scaling) [28,29]. This is primarily in order to obtain the volumetric scaling factor, to be used as normalization for head size. Next, tissue-type segmentation with partial volume estimation is carried out in order to calculate total volume of brain tissue [30].

Statistical Analysis

The study was a prospective observational study with a primary endpoint defined by a regression approach with BPF as target and RNFLT and age as independent variables, allowing for the history of ON. The necessary sample size was calculated using assumptions from the study of Gordon-Lipkin [19]. For this calculation the reported, less pronounced R^2 values from the whole MS group were used, since the RRMS subgroup's sample size was small and therefore of limited validity. Since no sample size calculation is available for that approach in Generalized Estimating Equations (GEE's) so far, the sample size calculation was based on linear multiple regression using the random model that supposes both target and predictor variables as random and should thus deliver a feasible estimation of the sample size required for GEE. A squared multiple correlation R^2 of 0.21 for RNFLT plus age on BPF (Gordon-Lipkin *et al.*, 2007) would be detected with 95% power (alpha = 0.05, two-sided, k = 3 predictors) for n = 86 patients using the random model with an exact distribution. Considering a drop-out rate of 20% the final required sample size was estimated to n = 103 patients. Sample size was calculated using G*Power 3.1.2 (University of Duesseldorf, Germany) [31].

Normal distribution of outcome parameters BPF, RNFLT and TMV (the latter two considering an influence of history of ON) was tested using Shapiro-Wilk's test. For BPF, RNFLT and TMV the assumption of normality was not rejected.

The evaluation of the primary endpoint was accomplished by a GEE analysis with BPF as target variable and RNFLT and age as independent variables, taking into account the history of ON. Data of both eyes were included as repeated measures in order to account for inter-eye correlations. The working correlation matrix was defined as exchangeable (compound symmetry), i.e. the two eye-measurements were supposed equally correlated and independent from the sequence.

To address the diagnostic comparability of RNFLT or TMV with BPF in a second step, GEE analyses were performed with average RNFLT or TMV as independent variables and BPF as dependent variable while allowing for the history of ON, including again the data of both eyes as repeated measures and with an exchangeable corresponding working correlation matrix.

The influence of age, disease duration and disease severity (as expressed by EDSS) on BPF, RNFLT or TMV was analyzed in a third step: GEE analyses were performed with age, disease duration and EDSS (while allowing for the history of ON) as independent variables and with either BPF, RNFLT or TMV as dependent variable, using the same GEE-model specifications as before.

Since GEE in PASW 18 does not provide an output for standardized effect sizes or regression coefficients, we actualized this issue in the following way: age, disease duration, EDSS, RNFLT, TMV and BPF were transformed to standardized values and each GEE was performed with these z-values instead of the original values, thus achieving a better comparability with other data and understanding the effect sizes and associations.

To be able to compare our data to results reported by Gordon-Lipkin [19] the thinner RNFLT and TMV from each patient's eyes were selected (''minimum RNFLT'' and ''minimum TMV'') and used in partial correlations between BPF, minimum RNFLT and minimum TMV controlling for age. Since partial correlations do not account for inter-eye correlations, results from these tests should be interpreted carefully and used only in the context of comparability to the mentioned paper.

Significance in all tests was achieved with $p<0.05$. Beside the primary endpoint, all statistical evaluations should be understood as constituting exploratory data analysis, such that no adjustments for multiple testing have been made.

Statistical analysis was performed using PASW 18 (SPSS/IBM, Chicago, IL, USA).

Results

Cohort demographics

Our cohort included 104 patients (208 eyes) with RRMS. All patients underwent clinical evaluation, OCT examination and brain MRI. All patients with a complete data set were included in the subsequent analysis. 62 (60%) patients never had ON on either eye, whereas 29 patients (28%) had a history of unilateral and 13 patients (12%) of bilateral ON. Mean BPF, normalized for subject head size was 0.852 (SD 0.033), mean RNFLT was 95.2 µm (SD 14.2 µm) and average TMV was 6.769 mm^3 (SD 0.489 mm^3). Disease duration in this study was defined as time from establishment of MS diagnosis to enrolment in the trial. Patients' demographics and statistics are summarized in Table 1.

Association of OCT parameters with brain atrophy

The primary endpoint of our study was defined as the association between BPF and RNFLT, using BPF as target and RNFLT and age as independent variables and taking into account the history of ON, which corresponds to the study by Gordon-Lipkin [19]. In our study, the association was evaluated by GEE analysis accounting for within-patient inter-eye dependencies. In our cohort, BPF was predicted by patients' age and RNFLT, however the standardized coefficient for the association between RNFLT and BPF was extremely low (table 2).

Table 1. Description of study cohort with demographic and disease parameters.

RRMS-Patients	n	104
Eyes	n	208
Gender	Male	35 (34%)
	Female	69 (66%)
Age	Mean (SD)	39.7 (8.8)
	Range	20–59
Disease Duration [Months]	Mean (SD)	68.2 (58.6)
	Range	3–269
EDSS	Median	2.0
	Range	0.0–6.0
History of ON	NON/NON	62 (60%)
	NON/ON	29 (28%)
	ON/ON	13 (12%)
BPF	Mean (SD)	0.852 (0.032)
	Range	0.77–0.922
Average RNFLT [µm]	Mean (SD)	95.2 (14.2)
	Range	46–133
TMV [mm^3]	Mean (SD)	6.769 (0.489)
	Range	5.455–7.674

RRMS = relapsing remitting Multiple sclerosis; (N)ON = (non) optic neuritis; SD = standard deviation, BPF = brain parenchymal fraction, RNFLT = retinal nerve fiber layer thickness, TMV = total macular volume.
doi:10.1371/journal.pone.0018132.t001

Next, we analyzed the associations between RNFLT or TMV and BPF by a GEE model, which only factors the history of ON and thus reflects better the diagnostic situation. GEE with BPF as target and RNFLT as independent variable and accounting for the history of ON showed a stronger though still weak association for RNFLT (table 3 and figure 1a). Interestingly, TMV was also associated with BPF when using the same model with TMV as independent variable (table 3 and figure 1b).

With respect to the comparability of our data to results reported by Gordon-Lipkin [19], we additionally performed partial correlation analyses controlling for age and using minimum RNFLT or minimum TMV and BPF as variables. In line with the

Table 2. Generalized Estimating Equations for the association of RNFLT with BPF as primary endpoint.

		Beta	stand. Beta	CI95% Low	CI95% High	p
BPF	**ON**					0.617
	age	−0.002	−0.457	−0.611	−0.303	<0.001
	RNFLT	<0.0001	<0.0001	<0.0001	<0.001	0.021

Results from GEEs with RNFLT and age as independent variables and controlling for history of optic neuritis and BPF as dependent variable. The standardized Beta was calculated as described in the methods section. RNFLT = retinal nerve fiber layer thickness, ON = history of optic neuritis, CI = confidence interval.
doi:10.1371/journal.pone.0018132.t002

Table 3. Generalized Estimating Equations for the association of RNFLT or TMV with BPF.

	GEE controlled for ON			Partial Correlation controlled for age	
	Beta	**stand. Beta**	**p**	**R**	**p**
RNFLT	<0.0001	0.0001	0.019	0.269	0.006
TMV	<0.0001	0.0002	0.004	0.369	<0.001

Results from GEEs with RNFLT or TMV as independent values and controlling for history of optic neuritis and BPF as dependent variable are given in columns 2–4. The standardized Beta was calculated as described in the methods section. Additionally, partial correlation coefficients controlling for age are displayed in the last two columns to allow comparability to previous results [19]. P-values are given in parentheses. RNFLT = retinal nerve fiber layer thickness, TMV = total macular volume, ON = history of optic neuritis, GEE = Generalized Estimating Equations.

doi:10.1371/journal.pone.0018132.t003

GEE analyses, we found a significant but moderate correlation between RNFLT and BPF and between TMV and BPF (table 3).

Influence of age, disease duration and severity

Having demonstrated that on the one hand both RNFLT and TMV associate with brain atrophy and on the other hand, in the same cohort age is strongly predictive for BPF, we asked whether the three parameters RNFLT, TMV and BPF are linked to distinct aspects of the disease such as age, disease duration and disease severity as determined by EDSS. Therefore, we performed GEE modeling with BPF, RNFLT or TMV as dependent variables and age, disease duration and EDSS as independent variables and correcting for history of ON. Data are presented in table 4. In summary, the analyses confirmed that BPF is substantially determined by both the patients' age ($p<0.001$) and EDSS ($p<0.001$). Notably, in our cohort BPF was not linked to disease duration. For RNFLT our data confirmed the impact of a previous ON as the history of ON was associated with a lower RNFLT ($p<0.001$). Importantly, the only other parameter that showed a significant impact on RNFLT was disease duration ($p<0.001$). Neither age nor EDSS were linked to RNFLT. For TMV the only determining parameter was ON history ($p<0.001$). TMV was neither linked to the duration or severity of the disease nor to the patients' age.

Discussion

Investigating the associations between OCT parameters and BPF as an established MRI measure for neurodegeneration in a cross-sectional prospective study on 104 RRMS patients we here report an association between global brain atrophy and both thinning of the RNFL and reduction of macular volume. In line with previous studies, reduction of both average RNFLT and TMV was linked to the history of ON. Importantly, apart from the impact of ON, RNFL thinning correlated closely with disease duration whereas BPF was determined by age and disease severity. TMV associated neither with disease duration, disease severity nor age.

The strengths of our study are the large sample size, the prospective design, the homogeneity of our study cohort including exclusively RRMS patients on a stable immunomodulatory treatment, the evaluation of the influence of disease duration, disease severity and age on RNFLT, TMV or BPF in the same population, and a statistical approach taking within-patient inter-eye relations into account. On the other hand, with respect to our cohort characteristics which reflect patients with relatively short disease duration and mild to moderate disability, our results should not be uncritically transferred to MS populations with different characteristics.

Although OCT receives increasing attention as future tool for the detection and monitoring of neurodegenerative processes in MS the evaluation of the actual value of this technique remains difficult.

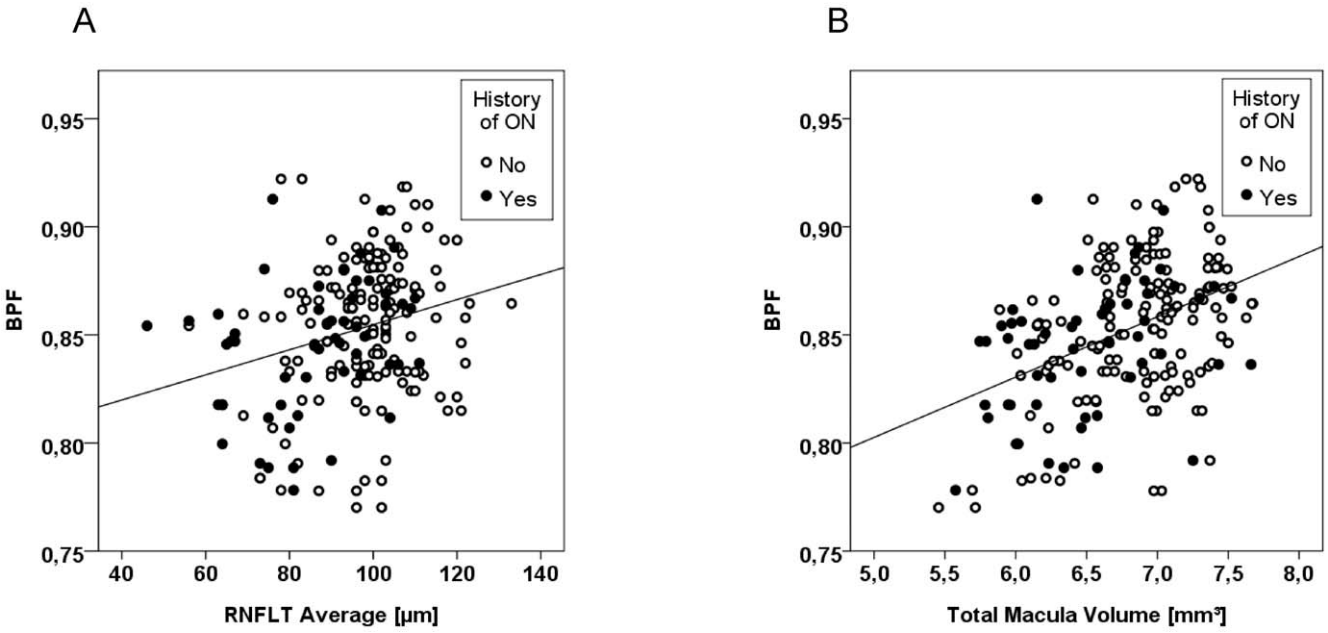

Figure 1. Association between BPF and OCT parameters in patients with RRMS. Patients (individual eyes) are labeled according to the history of optic neuritis (ON). Lines are derived from linear regression analyses with R^2 given in parentheses. Statistical significance level was calculated by Generalized Estimating Equation models controlling for the history of ON. A) Retinal nerve fiber layer thickness (RNFLT) vs. BPF (0.073, p = 0.019). B) Total macular volume (TMV) vs. BPF (0.113, p = 0.001).

doi:10.1371/journal.pone.0018132.g001

Table 4. Correlation between age, disease duration and severity with BPF, RNFLT and TMV.

		Beta	stand. Beta	CI95% Low	CI95% High	p
BPF	**age**	−0.001	−0.334	−0.499	−0.168	<0.001
	duration					0.098
	EDSS	−0.005	−0.223	−0.390	−0.057	<0.001
	ON					0.067
RNFLT	**age**					0.585
	duration	−0.073	−0.299	−0.471	−0.128	<0.001
	EDSS					0.201
	ON	−9.597	−0.675	−0.906	−0.444	<0.001
TMV	**age**					0.230
	duration					0.088
	EDSS					0.240
	ON	−0.300	−0.614	−0.818	−0.410	<0.001

Beta coefficients, standardized Beta coefficients, confidence interval for standardized beta coefficients and p values are provided as calculated by Generalized Estimating Equations accounting for inter-eye dependencies with age, duration, EDSS and history of optic neuritis as independent variables and RNFLT, TMV or BPF as dependent variables. For better clarity, coefficients are only given for factors that reached statistical significance. Standardized Beta was calculated as described in the Methods section. BPF = brain parenchymal fraction, RNFLT = retinal nerve fiber layer thickness, TMV = total macular volume, ON = history of optic neuritis, CI = confidence interval.
doi:10.1371/journal.pone.0018132.t004

This is mainly, because on the one hand, the interrelations between neurodegenerative processes in the retina and the brain are still under investigation and on the other hand, the impact of disease-related aspects such as duration and severity and disease-unrelated parameters such as age on OCT parameters is not yet clear [5,15]. The only published two previous studies addressing the correlation of OCT parameters with BPF included only a limited number of patients (between 18 and 40) or inhomogeneous disease courses and did not account for within-patient inter-eye relations [19,20]. In the present cohort we used a statistical model which allows adjusting for within-patient inter-eye relations and corrected for the history of ON, which we consider an appropriate approach in a population with different ON status. Our primary endpoint reflects the statistical model used by Gordon-Lipkin and was primarily defined for the estimation of the sample size. However, for the evaluation of associations between BPF and RNFLT or TMV we favor our second GEE model which does not account for disease duration, disease severity or age and thus represents a rather diagnostic than pathophysiological point of view. Since GEE does not provide standardized output for correlation coefficients we additionally calculated a "standardized Beta" in order to provide a better conception of the effect sizes. Using this model, the association between BPF and RNFLT was significant but weak (table 3, figure 1a). Interestingly, when applying the same statistical approach as Gordon-Lipkin et al., which however does not account for inter-eye dependencies, we also found an association between minimum RNFLT and BPF but with a moderate partial correlation coefficient (table 3), which is in line with Gordon-Lipkin [19]. In contrast to Siger et al. who found an association of RNFLT and BPF only in a cohort subgroup without ON [20], in our study the correlation was evident in the total cohort.

With respect to TMV our data (according to both GEE and partial correlation analyses; table 3) contrast Gordon-Lipkin et al., who did not find a correlation between TMV and BPF and suggested that "in a cohort of patients with MS with a mean duration of disease of approximately 10 years, TMV may be less informative than in a cohort with a longer history of MS" [19]. Our data rather indicate, that TMV in fact reflects global brain atrophy already after a mean disease duration of approximately five years and even in patients with a predominantly mild clinical disability (table 1) and suggest that damage to retinal ganglion cells occurs already in earlier phases of the disease. That in turn would be in line with grey matter damage detected early in the disease course by MRI or histopathology [32,33,34]. The differences in our data compared to previous data [19,20] may be at least partially explained by the larger sample size in our study and the different cohort characteristics. The different effect sizes estimated by GEE and partial correlations might indicate, that at least in a cohort with a comparably short disease duration and mild disability, partial correlation analysis with minimum RNFLT/TMV not factoring inter-eye dependencies, might overestimate the strength of associations. It remains to be seen whether in a currently running longitudinal study factoring the changes within the parameters over time the standardized beta will conform to the partial r.

Numerous studies have addressed the relations between RNFLT, TMV or BPF with disease duration, disease severity, ON history and age in individual cohorts with different characteristics (summarized and meta-analyzed in two recent major review articles [5,15]). However, data are still inconsistent. For example, a correlation between disease duration and thinning of RNFL has been reported in some studies [11,16,20,35] whereas others did not find a correlation [17,18]. Consequently, the ability of each marker RNFLT, TMV and BPF, respectively, to capture distinct aspects of the disease remains unclear. Evaluating the impact of disease duration, severity and age on each of the MRI and OCT parameters within the same cohort by GEE and correcting for the ON history, we here demonstrate that BPF might be a good parameter for the evaluation of the disease severity as, in line with previous reports [8], it associated best with the EDSS. On the other hand, our observations that in the same experimental setting RNFLT but not BPF were linked to the disease duration and that furthermore BPF but not RNFLT was substantially confounded by the patients' age, which is in line with previous reports [19], suggest that RNFLT is the better parameter for duration-related issues such as longitudinal monitoring in clinical trials. The relevance of TMV remains elusive, as TMV was not associated with any of the parameters. TMV captures not only the retinal nerve fiber layer, but also deeper layers of the retina, which in particular in combination with the use of time-domain OCT might render TMV a less specific parameter for neuroaxonal degeneration. Furthermore, as the sample size calculation was based on RNFLT this study was not powered to primarily investigate the role of TMV. Not surprisingly, our data moreover confirmed the impact of ON on RNFLT and TMV demonstrated in previous studies [12,15].

In summary, our cross-sectional data on the association between both RNFLT and TMV with BPF point to a significant but weak association which was at least in our cohort independent of a previous ON. RNFLT and BPF but not TMV are linked to certain aspects of MS. Whereas BPF reflects in the first place the severity of the disease, RNFLT might be the better parameter for monitoring axonal damage longitudinally. Thus we conclude that in addition to BPF at least RNFLT is a promising complementary parameter to evaluate early neurodegenerative processes in RRMS patients. The eligibility of TMV as surrogate marker requires further evaluation. Longitudinal studies and studies on patients with a longer disease duration and higher disability are necessary to corroborate the relevance of these parameters and to clarify the remaining questions. We therefore suggest that both

RNFLT and TMV should be included as standard secondary endpoints in clinical trials addressing neurodegeneration in MS.

Acknowledgments

We thank our study nurses Cordula Rudolph, Franziska Lipske, Katharina Stoesslein, and Antje Els for excellent support and Susan Pikol for excellent technical assistance.

Author Contributions

Conceived and designed the experiments: FP AUB JTW. Performed the experiments: JD MB GG CFP JBS. Analyzed the data: KDW AUB JD AF. Contributed reagents/materials/analysis tools: KDW JTW AF. Wrote the paper: JD AUB. Revised article for important intellectual content: JD KDW MB GG JTW CFP JBS AF AUB FP.

References

1. De Stefano N, Matthews PM, Fu L, Narayanan S, Stanley J, et al. (1998) Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. Brain 121(Pt 8): 1469–1477.
2. De Stefano N, Narayanan S, Francis GS, Arnaoutelis R, Tartaglia MC, et al. (2001) Evidence of axonal damage in the early stages of multiple sclerosis and its relevance to disability. Arch Neurol 58: 65–70.
3. Trapp BD, Ransohoff R, Rudick R (1999) Axonal pathology in multiple sclerosis: relationship to neurologic disability. Curr Opin Neurol 12: 295–302.
4. Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L (1999) Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. Neurology 53: 1698–1704.
5. Barkhof F, Calabresi PA, Miller DH, Reingold SC (2009) Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. Nat Rev Neurol 5: 256–266.
6. Chard DT, Griffin CM, Parker GJ, Kapoor R, Thompson AJ, et al. (2002) Brain atrophy in clinically early relapsing-remitting multiple sclerosis. Brain 125: 327–337.
7. Kalkers NF, Bergers E, Castelijns JA, van Walderveen MA, Bot JC, et al. (2001) Optimizing the association between disability and biological markers in MS. Neurology 57: 1253–1258.
8. Fisher E, Rudick RA, Simon JH, Cutter G, Baier M, et al. (2002) Eight-year follow-up study of brain atrophy in patients with MS. Neurology 59: 1412–1420.
9. Hee MR, Izatt JA, Swanson EA, Huang D, Schuman JS, et al. (1995) Optical coherence tomography of the human retina. Arch Ophthalmol 113: 325–332.
10. Frohman E, Costello F, Zivadinov R, Stuve O, Conger A, et al. (2006) Optical coherence tomography in multiple sclerosis. Lancet Neurol 5: 853–863.
11. Fisher JB, Jacobs DA, Markowitz CE, Galetta SL, Volpe NJ, et al. (2006) Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. Ophthalmology 113: 324–332.
12. Bock M, Brandt AU, Dorr J, Kraft H, Weinges-Evers N, et al. (2010) Patterns of retinal nerve fiber layer loss in multiple sclerosis patients with or without optic neuritis and glaucoma patients. Clin Neurol Neurosurg.
13. Burkholder BM, Osborne B, Loguidice MJ, Bisker E, Frohman TC, et al. (2009) Macular volume determined by optical coherence tomography as a measure of neuronal loss in multiple sclerosis. Arch Neurol 66: 1366–1372.
14. Sergott RC, Frohman E, Glanzman R, Al Sabbagh A (2007) The role of optical coherence tomography in multiple sclerosis: expert panel consensus. J Neurol Sci 263: 3–14.
15. Petzold A, de Boer JF, Schippling S, Vermersch P, Kardon R, et al. (2010) Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. Lancet Neurol 9: 921–932.
16. Pueyo V, Martin J, Fernandez J, Almarcegui C, Ara J, et al. (2008) Axonal loss in the retinal nerve fiber layer in patients with multiple sclerosis. Mult Scler 14: 609–614.
17. Henderson AP, Trip SA, Schlottmann PG, Altmann DR, Garway-Heath DF, et al. (2008) An investigation of the retinal nerve fibre layer in progressive multiple sclerosis using optical coherence tomography. Brain 131: 277–287.
18. Klistorner A, Arvind H, Nguyen T, Garrick R, Paine M, et al. (2008) Axonal loss and myelin in early ON loss in postacute optic neuritis. Ann Neurol 64: 325–331.
19. Gordon-Lipkin E, Chodkowski B, Reich DS, Smith SA, Pulicken M, et al. (2007) Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology 69: 1603–1609.
20. Siger M, Dziegielewski K, Jasek L, Bieniek M, Nicpan A, et al. (2008) Optical coherence tomography in multiple sclerosis: thickness of the retinal nerve fiber layer as a potential measure of axonal loss and brain atrophy. J Neurol 255: 1555–1560.
21. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, et al. (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 58: 840–846.
22. Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33: 1444–1452.
23. Cheung CY, Leung CK, Lin D, Pang CP, Lam DS (2008) Relationship between retinal nerve fiber layer measurement and signal strength in optical coherence tomography. Ophthalmology 115: 1347–1351, 1351 e1341–1342.
24. Smith SM (2002) Fast robust automated brain extraction. Hum Brain Mapp 17: 143–155.
25. Smith SM, De Stefano N, Jenkinson M, Matthews PM (2001) Normalized accurate measurement of longitudinal brain change. J Comput Assist Tomogr 25: 466–475.
26. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, et al. (2004) Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23 Suppl 1: S208–219.
27. Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, et al. (2002) Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage 17: 479–489.
28. Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17: 825–841.
29. Jenkinson M, Smith S (2001) A global optimisation method for robust affine registration of brain images. Med Image Anal 5: 143–156.
30. Zhang Y, Brady M, Smith S (2001) Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 20: 45–57.
31. Faul F, Erdfelder E, Buchner A, Lang AG (2009) Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods 41: 1149–1160.
32. De Stefano N, Matthews PM, Filippi M, Agosta F, De Luca M, et al. (2003) Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. Neurology 60: 1157–1162.
33. Audoin B, Davies G, Rashid W, Fisniku L, Thompson AJ, et al. (2007) Voxel-based analysis of grey matter magnetization transfer ratio maps in early relapsing remitting multiple sclerosis. Mult Scler 13: 483–489.
34. Schirmer L, Albert M, Buss A, Schulz-Schaeffer WJ, Antel JP, et al. (2009) Substantial early, but nonprogressive neuronal loss in multiple sclerosis (MS) spinal cord. Ann Neurol 66: 698–704.
35. Talman LS, Bisker ER, Sackel DJ, Long DA, Jr., Galetta KM, et al. (2010) Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. Ann Neurol 67: 749–760.

OPEN ACCESS Freely available online

PLoS one

Metabolic Changes in the Visual Cortex Are Linked to Retinal Nerve Fiber Layer Thinning in Multiple Sclerosis

Caspar F. Pfueller[1*9], Alexander U. Brandt[1,2 9], Florian Schubert[4], Markus Bock[1], Bernadeta Walaszek[4], Helmar Waiczies[4,5], Thomas Schwenteck[4], Jan Dörr[1], Judith Bellmann-Strobl[5], Christian Mohr[3], Nicholetta Weinges-Evers[1], Bernd Ittermann[4], Jens T. Wuerfel[1,3¶], Friedemann Paul[1,5¶]

1 NeuroCure Clinical Research Center, Charité Universitaetsmedizin Berlin, Berlin, Germany, **2** gfnmediber GmbH, Berlin, Germany, **3** Institute of Neuroradiology, University Luebeck, Luebeck, Germany, **4** Physikalisch-Technische Bundesanstalt (PTB), Braunschweig und Berlin, Germany, **5** Experimental and Clinical Research Center, Charité Universitaetsmedizin Berlin and Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany

Abstract

Objective: To investigate the damage to the retinal nerve fiber layer as part of the anterior visual pathway as well as an impairment of the neuronal and axonal integrity in the visual cortex as part of the posterior visual pathway with complementary neuroimaging techniques, and to correlate our results to patients' clinical symptoms concerning the visual pathway.

Design, Subjects and Methods: Survey of 86 patients with relapsing-remitting multiple sclerosis that were subjected to retinal nerve fiber layer thickness (RNFLT) measurement by optical coherence tomography, to a routine MRI scan including the calculation of the brain parenchymal fraction (BPF), and to magnetic resonance spectroscopy at 3 tesla, quantifying N-acetyl aspartate (NAA) concentrations in the visual cortex and normal-appearing white matter.

Results: RNFLT correlated significantly with BPF and visual cortex NAA, but not with normal-appearing white matter NAA. This was connected with the patients' history of a previous optic neuritis. In a combined model, both BPF and visual cortex NAA were independently associated with RNFLT.

Conclusions: Our data suggest the existence of functional pathway-specific damage patterns exceeding global neurodegeneration. They suggest a strong interrelationship between damage to the anterior and the posterior visual pathway.

Citation: Pfueller CF, Brandt AU, Schubert F, Bock M, Walaszek B, et al. (2011) Metabolic Changes in the Visual Cortex Are Linked to Retinal Nerve Fiber Layer Thinning in Multiple Sclerosis. PLoS ONE 6(4): e18019. doi:10.1371/journal.pone.0018019

Editor: Christoph Kleinschnitz, Julius-Maximilians-Universität Würzburg, Germany

Received September 24, 2010; **Accepted** February 22, 2011; **Published** April 6, 2011

Funding: This work was supported by grants from the Excellence Cluster 257 of the German Research Foundation to NeuroCure Clinical Research Center and the grant KF2286101FO9 from the German Ministry of Economics awarded both to NeuroCure Clinical Research Center and gfnmediber, a company co-managed by Alexander U Brandt, who is also a guest scientist in the NeuroCure Clinical Research Center. Therefore the authors mention gfnmediber as a funder of this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Alexander U Brandt is the deputy CEO of gfnmediber and a guest scientist in the NeuroCure Clinical Research Center (NCRC). There is no conflict of gfnmediber's company interests with the current study; Alexander U Brandt contributed to the study merely in his role of a guest scientist in the NCRC. As the grant KF2286101FO9 from the German Ministry of Economics was awarded both to NeuroCure Clinical Research Center and gfnmediber and gfnmediber is employing Alexander U Brandt, the authors include gfnmediber GmbH, Berlin, Germany as a funding source. This does not alter their adherence to all the PLoS ONE policies on sharing data and materials.

* E-mail: caspar.pfueller@charite.de

9 These authors contributed equally to this work.

¶ These authors also contributed equally to this work.

Introduction

Already in the 19th century, Charcot reported a regular occurrence of neuronal and axonal degeneration beyond demyelination in multiple sclerosis (MS) [1]. Unfortunately, these findings were neglected by the research community for a long time, and consequently MS was seen as a primarily demyelinating condition, with relative preservation of axons and neurons [2]. However, within the past two decades, Charcot's initial descriptions enjoyed a revival, mainly by the advent of advanced microscopic imaging techniques, such as the combination of fluorescent immunocytochemistry with confocal microscopy. Several groups could show that independently of the demyelination process neuronal and axonal breakdown contribute to central nervous system (CNS) tissue damage and the resulting functional deficits in different stages in the course of MS [3]. It is now well accepted that MS is not only a demyelinating CNS disease but has also a considerable neurodegenerative component [4]. In the light of these findings, therapeutic strategies that specifically address the neurodegenerative component of MS are in the focus of the research. Also in neuroimaging, there is a shift of research interest from a mere depiction of the inflammatory aspects of the disease such as T2- and contrast enhancing lesion load which only correlate modestly with the clinical disease course

and neurological disability (the so-called clinico-radiological paradox[5]) to improved techniques to quantify and monitor neurodegeneration. Brain atrophy is considered to represent at least partially axonal and neuronal loss in MS[6] and shows a strong association with some clinical disease-related measures. It can be quantified by various techniques, e.g. the calculation of the so-called brain parenchymal fraction (BPF) [7–8] but its appropriateness as primary endpoint in clinical trials on neuroprotective therapies still remains to be proven.

In recent years, optical coherence tomography (OCT) evolved as a valuable non-invasive diagnostic tool to image unmyelinated retinal CNS axons and thus to depict MS-related neurodegeneration (reviewed in [9],[10]). Based on the concept that ongoing diffuse neurodegeneration in the brain will also affect the retinal CNS axons, different groups reported reduced retinal nerve fiber layer thickness (RNFLT) in MS patients versus healthy controls[11–12] and could show that RNFLT correlates well with brain atrophy and physical and cognitive disability[13–16].

Proton magnetic resonance spectroscopy (^{1}H-MRS) emerged as technique to quantify MS-related neuronal and axonal damage by measuring the brain N-acetyl-aspartate (NAA) concentration, a presumed marker of axonal and neuronal integrity (reviewed in [17]). In line with the change of paradigm on MS pathology, ^{1}H-MRS provides evidence for metabolic alterations in normal appearing white matter in MS [18–19].

Against the background of these findings, we were interested whether changes in RNFLT indicating alterations of the anterior visual pathway are linked to impaired neuronal and axonal integrity in the visual cortex as part of the posterior visual pathway. We performed a cross-sectional study to investigate the association of RNFLT with NAA of the normal appearing white matter and the visual cortex as measured by ^{1}H-MRS, and with BPF as a parameter of global brain tissue loss.

Methods

Participants

Using an exploratory cross-sectional study design, relapsing-remitting multiple sclerosis (RRMS) patients fulfilling the current panel criteria[20] were prospectively recruited between September 2007 and February 2009. The study was approved by the ethics committee of Charité Universitätsmedizin Berlin, Germany and all participants gave informed written consent according to the 1964 Declaration of Helsinki. Patients with MS met the following criteria: age 18-55 years, stable immunomodulatory therapy with glatiramer acetate for at least six months prior to inclusion, EDSS between 0 and 6.5, no acute relapse and no systemic steroid treatment within 30 days prior to enrolment. Patients with ophthalmologic disorders or medical conditions with impact on retinal nerve fiber layer (e.g. diabetes, glaucoma) were not included. The patients included in this study are the sub-group of patients recruited for an ongoing clinical drug trial with glatiramer acetate as required co-medication, from whom baseline data of both 1.5T MR imaging and 3T magnetic resonance spectroscopy were available. Demographic data are summarized in Table 1.

Clinical and visual assessment

Medical history, particularly with respect to visual symptoms, was taken from all study participants. Based on the documented previous history of optic neuritis (ON), we defined three subgroups of patients – patients with no, unilateral and bilateral optic neuritis. None of the study subjects had suffered from acute optic neuritis within the last 6 months before recruitment to the study. All participants underwent a complete ophthalmologic examination, including non-contact tonometry, visual acuity testing by using Snellen charts, Nieden charts and functional acuity contrast testing, spheric refractive error testing and cylindric refractive error testing. Patients who showed a non-MS related eye pathology were excluded from OCT measurements. Neurological disability in MS patients was assessed by the expanded disability status scale (EDSS) [21].

Optical coherence tomography

RNFLT was measured with a Stratus 3000 OCT (Carl Zeiss Meditec, Dublin, California) using the "fast RNFL 3.4" protocol (software version 4.0). Three 3.4 mm diameter circular scans were acquired over 1.92 seconds. A good quality image was defined as an image with generalised signal distribution, a reflectance signal from either RNFL or retinal pigment epithelium strong enough to identify either layer, no missing parts caused by eye movements, and a signal strength of $\geq$8 of 10 [22]. The segmentation line defining the upper and lower border of the RNFL was required to be on the internal limiting membrane and lower border of the

Table 1. Summary of demographic data, mean RNFLT, mean BPF, mean normal-appearing white matter (NAWM) NAA concentrations, mean visual cortex (VC) NAA concentrations.

	All Patients	NON/NON Patients	NON/ON Patients	ON/ON Patients
Patients (%)	86 (100)	53 (61.6)	20 (23.3)	13 (15.1)
Age, mean (range), y	41 (21–60)	40 (21–60)	41 (24–60)	41 (32–56)
Disease Duration, mean (range), m	71 (4–271)	65 (4–271)	92 (11–193)	68 (7–147)
EDSS, median (range)	2.0 (0.0–6.0)	2.0 (0.0–6.0)	2.0 (1.0–4.5)	2.5 (0–4.5)
Min. RNFLT Average, mean (SD; range), μm	91.3 (15; 46–123)	97.3 (10; 74–123)	84.6 (17.3;46–111)	75 (14.2; 56–104)
BPF, mean (SD; range)	0.851 (0.031; 0.77–0.918)	0.855 (0.032; 0.77–0.918)	0.849 (0.03; 0.791–0.913)	0.838 (0.026; 0.789–0.872)
NAWM NAA, mean (SD; range), mmol/l	13.079 (1.354; 7.652–15.807)	13.192 (1.25; 10.909–15.807)	12.994 (1.714; 7.652–15.65)	12.746 (1.212; 11.026–15.344)
VC NAA, mean (SD; range), mmol/l	13.471 (1.017; 11.176–16.086)	13.601 (1.023; 11.176–16.086)	13.43 (0.996; 11.57–14.783)	13.002 (0.948; 11.401–14.651)

(NON/NON – no previous optic neuritis, NON/ON – previous unilateral optic neuritis, ON/ON - previous bilateral optic neuritis).
doi:10.1371/journal.pone.0018019.t001

RNFL. Images which did not meet these criteria were excluded. The OCT A-scan data were digitally exported in a blinded fashion.

Magnetic resonance imaging and brain parenchymal fraction calculation

MRI measurements were performed on a 1.5 tesla scanner (Avanto, Siemens Medical Systems, Erlangen, Germany). A three-dimensional T1-weighted image (MPRAGE) was acquired according to the following protocol: T_R 1.9 ms, T_E 3.09 ms, T_I 1.1 ms, flip angle 15°, matrix size 1 mm^3. Brain tissue volume, normalized for subject head size, was estimated applying SIENAX[23–24],part of FSL [25]. SIENAX starts by extracting brain and skull images from the single whole-head input data [26]. The brain image is then affine-registered to MNI152 space (using the skull image to determine the registration scaling) [27–28] in order to obtain the volumetric scaling factor to be used as normalization for head size. Next, tissue-type segmentation with partial volume estimation is carried out in order to calculate total volume of brain tissue [29].

MR spectroscopy

MR measurements were carried out on a 3 tesla scanner (MEDSPEC 30/100, Bruker Biospin, Ettlingen, Germany). T_1-weighted images were acquired using MDEFT (modified driven equilibrium Fourier transform, with $T_E = 3.8$ ms, $T_R = 20.53$ ms; 128 contiguous slices, 1.5 mm thick; 1-mm in-plane (x–y) resolution). After localized shimming, magnetic resonance spectra were recorded from two voxels located in left and right normal appearing periventricular white matter ($2\times2\times2$ cm^3), and a voxel centered on the visual cortex ($3\times2\times2$ cm^3) (Fig. 1). The PRESS (point resolved spectroscopy) sequence preceded by water suppression (3 Gauss CHESS pulses of 25.6 ms duration) was used throughout. Details of the procedure for metabolite quantification were previously published [30]. For one metabolite spectrum eight subspectra of 16 phase cycled scans each were recorded with $T_R = 3$ s and $T_E = 80$ ms. Before further processing, the 8 metabolite subspectra were corrected for eddy currents using water-unsuppressed spectra (T_R and T_E as above), automatically corrected for frequency and phase shifts, and added together to give 128 averages. Spectral quantification was carried out using a time domain-frequency domain fitting procedure that involves background estimation by regularization [31]. Any residual contributions by macromolecules are accommodated in the baseline by the fitting procedure. Mean uncertainties corresponding to Cramér-Rao lower bounds with added uncertainties from the background modelling[31] for the fitting of NAA were as small as 2.1% for the visual cortex voxel and 2.4% for the normal-appearing white matter voxels. The fitted NAA amplitudes were corrected for different coil loading by an aqueous metabolite phantom used for spectrum analysis and the individual subject's head (principle of reciprocity), and for transverse relaxation effects using mean T_2 values measured earlier at 3 T for normal-appearing white matter [32] and cortical regions [30]. Longitudinal relaxation effects were neglected because T_1 was assumed to be similar in the aqueous phantom and in brain tissue. Metabolite concentrations were corrected for cerebrospinal fluid (CSF) in the voxels studied by using the CSF fractions obtained by segmenting the T_1-weighted images with SPM2 (www.fil.ion.ucl.ac.uk/spm/spm2.html).

Statistical analysis

RNFLT, ^{1}H-MRS and BPF data were analyzed for normal distribution using skewness and kurtosis of data histograms. All data was within +/-1.5 skewness and kurtosis. Additionally, Shapiro-Wilk tests were performed to check for normal distribution. According to these tests, RNFLT, BPF, visual cortex NAA were normally distributed whereas NAA in normal-appearing white matter was not (Shapiro-Wilk test, $p = 0.037$).

Correlation between normal-appearing white matter NAA and visual cortex NAA and BPF was assessed using Pearson's correlation coefficient, counterchecked with Spearman's correlation due to the distribution of the normal-appearing white matter NAA. Association of normal-appearing white matter and visual cortex NAA and BPF with RNFLT was tested with Generalized Estimating Equation Models (GEE) to adjust for inter-eye dependencies within patients using RNFLT as the dependent variable and BPF, visual cortex voxel NAA or normal-appearing white matter NAA as single independent variables. Finally, a GEE with BPF and visual cortex NAA as independents and RNFLT as dependent variable was used to calculate the independent association of BPF and visual cortex NAA with RNFLT (combined model). Since GEE function in PASW 18 does not provide standardized output for coefficients, we approached this issue in the following way: RNFLT, BPF and visual cortex NAA were transformed to standardized z-values and each GEE was performed again with these z-values instead of the original values (standardized Beta, see Table 2).

Analyses of variance were performed with BPF, visual cortex NAA or normal-appearing white matter NAA as dependent variable and history of ON as nominal independent factor to identify group differences regarding BPF, visual cortex NAA and normal-appearing white matter NAA between patients with history of bilateral optic neuritis, patients with history of unilateral optic neuritis and without previous optic neuritis. Differences in age, EDSS and disease duration between these groups defined by history of optic neuritis were assessed with Kruskal-Wallis tests, differences in gender with Pearson's Chi Square analysis.

All statistical tests were performed using PASW 18 (SPSS, Chicago, IL, USA). For all calculations, statistical significance was established at $p<0.05$. Data sets with partly missing data as indicated under results were not excluded from sub-analyses. All tests should be understood as constituting exploratory data analysis, such that no adjustments for multiple testing were made.

Results

86 RRMS patients were recruited. Three patients were excluded from OCT analysis due to non-MS related retinal pathologies. All other eyes were included and were analyzable with an RNFLT signal strength ≥8. Data from ^{1}H-MRS measurements were available for all patients. In five patients BPF analysis was not performed due to insufficient image quality (e.g. motion artifacts). Patients in the three subgroups defined by history of optic neuritis did not differ significantly regarding age (Kruskal-Wallis, $p = 0.947$), disease duration (Kruskal-Wallis, $p = 0.172$), EDSS (Kruskal-Wallis, $p = 0.829$) or gender (Chi-Square, $p = 0.768$). Clinical and demographical data including history of optic neuritis, RNFLT, BPF and ^{1}H-MRS are given in Table 1.

BPF correlates with RNFLT but not with NAA concentration in visual cortex and normal-appearing white matter

BPF correlated with RNFLT (GEE, CI95% low = 0.55 µm/%, high = 2.11 µm/%, $p<0.001$). There was no correlation between visual cortex NAA concentration and BPF (Pearson, $p = 0.161$) or normal-appearing white matter NAA concentration and BPF (Pearson, $p = 0.540$). Comparing the BPF in the three subgroups

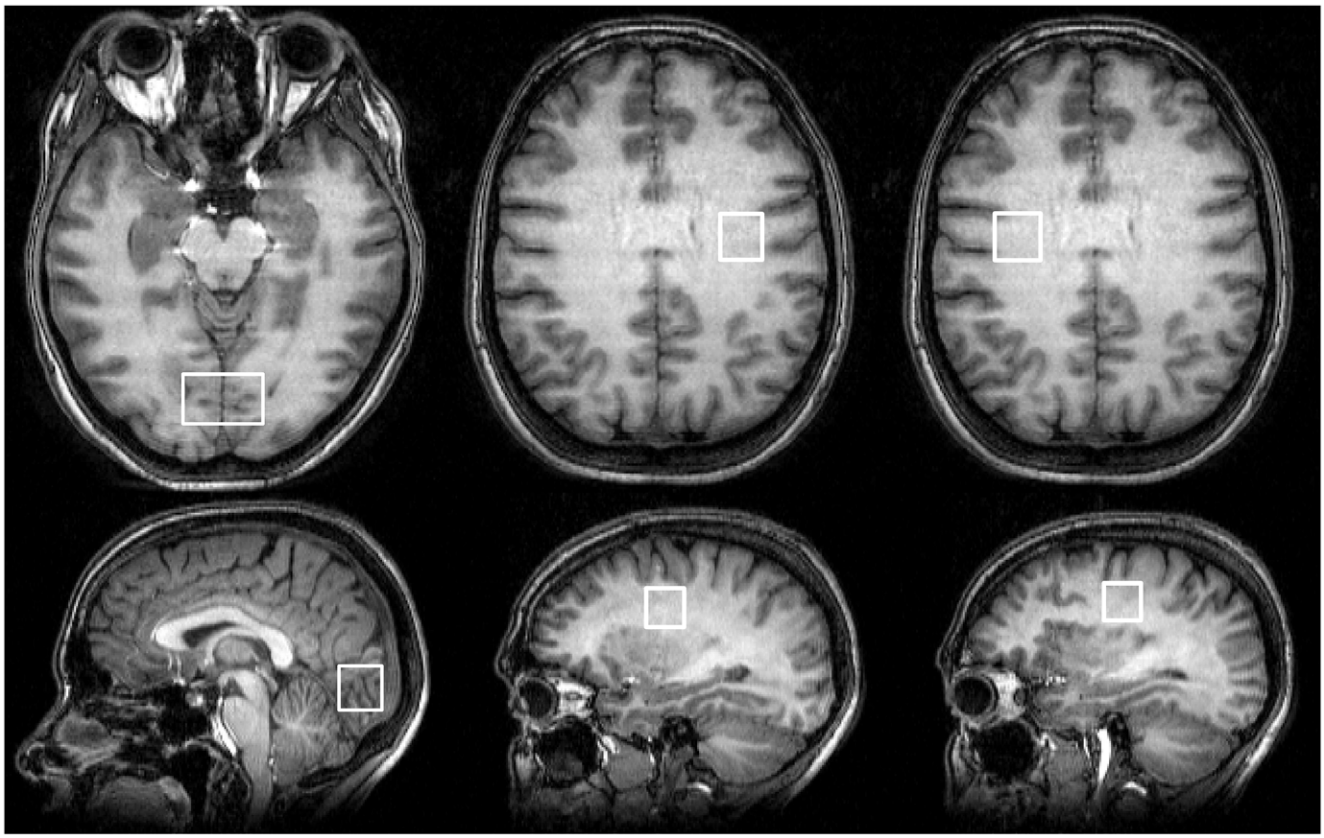

Figure 1. ^{1}H-MRS voxel placement. Visual representation of typical voxel placement for MR spectroscopy. In each patient, NAA concentrations were measured in a visual cortex voxel (VC) and two normal-appearing white matter voxels (NAWM).
doi:10.1371/journal.pone.0018019.g001

defined by history of optic neuritis there was a trend towards lower BPF in patients with previous episodes of optic neuritis (ANOVA, $p = 0.055$) (Figure 2A and B). Further statistical details, including a calculated standardized coefficient, are provided in Table 2.

RNFLT correlates with NAA concentration in the visual cortex but not in the normal-appearing white matter

We found a correlation between RNFLT and visual cortex NAA concentration (GEE, Confidence Interval (CI95%) low = 0.03 μm/(mmol/l), high = 5.61 μm/(mmol/l), $p = 0.047$). The subgroups regarding history of optic neuritis differed in their visual cortex NAA concentration, indicating that patients with previous unilateral or bilateral optic neuritis exhibited lower NAA levels than those without optic neuritis (ANOVA, $p = 0.046$), (Figure 2C and D). There was no correlation between RNFLT and NAA concentration in the normal-appearing white matter (GEE, CI95% low = −1.26 μm/(mmol/l), high = 2.44 μm/(mmol/l), $p = 0.531$), nor a difference in normal-appearing white matter NAA concentration between subgroups regarding the history of optic neuritis (ANOVA, $p = 0.429$) (Figure 2E and F). (Further statistical details, including a calculated standardized coefficient, are provided in Table 2.)

BPF and visual cortex NAA concentrations are independently associated with average RNFLT

Using visual cortex NAA concentration and BPF as independent variables in a multivariate GEE analysis, we found that both BPF (CI 95% low = 0.45 μm/%, high = 1.96 μm/%, $p = 0.002$) and visual cortex NAA concentration (CI 95% low = 0.10 μm/(mmol/l), high = 5.47 μm/(mmol/l), $p = 0.042$) are independently associated with RNFLT. Further statistical details, including a calculated standardized coefficient, are provided in Table 2.

Table 2. Statistical data for GEE and combined model GEE (NAWM = normal-appearing white matter, VC = visual cortex).

	Variable	Dependent Variable	B (Std. Error; 95% CI)	standardized B (Std. Error; 95% CI)	Chi-Square	*P* value
GEE 1	VC-NAA	RNFLT	2.823 (1.4238; .033–5.614)	.191 (.0964; .002–.380)	3.932	.047
GEE 2	BPF	RNFLT	132.907 (39.941; 54.625–211.190)	.269 (.0809; .111–.428)	11.073	.001
GEE Combined model	BPF	RNFLT	120.448 (38.3810; 45.223–195.673)	.244 (.0777; .092–.396)	9.848	.002
	VC-NAA	RNFLT	2.784 (1.3720; .095–5.473)	.188 (.0929;.006–.370)	4.117	.042

doi:10.1371/journal.pone.0018019.t002

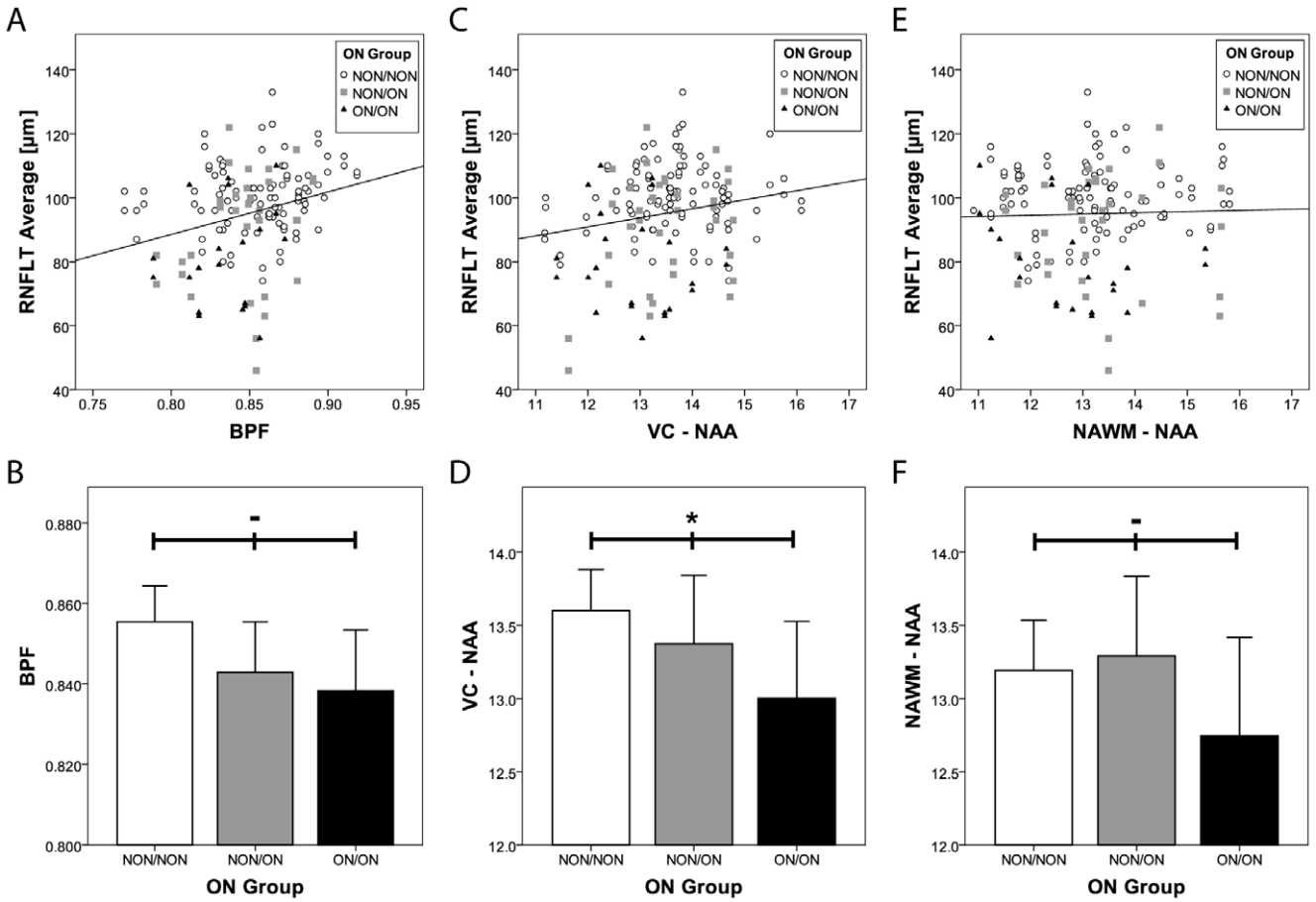

Figure 2. Correlation of RNFLT with BPF and ^{1}H-MRS parameters. a) Depicted is the average RNFLT, every symbol representing a single eye examined together with the corresponding BPF values. The symbols represent the patient's previous history of optic neuritis (open circles – no previous optic neuritis, grey squares - unilateral optic neuritis, black triangles – bilateral optic neuritis) A linear correlation function was calculated by a Generalised Linear Model to account for intra-individual inter-eye relationships ($p = 0.001$). b) Mean BPF was calculated for three groups that were defined based on their previous history of optic neuritis (white bar– no previous optic neuritis, grey bar - unilateral optic neuritis, black bar – bilateral optic neuritis). The (-) symbol indicates a trend, but a missing significant correlation of group differences as calculated by ANOVA ($p = 0.055$). Error bars represent 2× standard error of the mean (SEM). c) RNFLT averages are shown in relation to corresponding NAA concentrations in the visual cortex (VC). The symbols are coded as in a). The correlation is significant ($p = 0.047$). d) Mean visual cortex voxel (VC) NAA and the significance of group differences was calculated for optic neuritis groups as in b). The asterisk indicates statistically significant ($p = 0.046$) group differences. Error bars represent 2× standard error of the mean (SEM). e) RNFLT averages are shown in relation to corresponding NAA concentrations in normal-appearing white matter (NAWM). The symbols are coded as in a). No significant correlation was found ($p = 0.531$). f) Mean NAA in normal-appearing white matter (NAWM) and the significance of group differences was calculated for optic neuritis groups as in b) ($p = 0.429$). Error bars represent 2× standard error of the mean (SEM).
doi:10.1371/journal.pone.0018019.g002

Discussion

This cross-sectional study is the first to investigate MS-related axonal and neuronal damage in a large number of patients by three different imaging modalities including OCT, brain atrophy measurement by MRI, and ^{1}H-MRS of the visual cortex and normal-appearing white matter at 3 T. Our main findings are that (i) RNFLT is correlated with NAA concentration in the visual cortex but not in the normal-appearing white matter, (ii) visual cortex NAA concentrations are lower in patients with previous optic neuritis than in those without, (iii) both visual cortex NAA and BPF are independently associated with RNFLT, and (iv) BPF and RNFLT show a significant association.

The novel multimodal imaging approach merging OCT and MRI and the additional application of ^{1}H-MRS at 3 T, yielding an improved signal-to-noise ratio compared to 1.5 T[17] enabled us to investigate not only the relationship between brain atrophy and RNFL reduction which had already been assessed previously in smaller patient cohorts[13] [15] and by us in a larger patient cohort (Dörr et al., submitted), where we analyzed the association of RNFLT and the total macular volume with global brain atrophy, but to evaluate also the association between disease-related damage of the anterior part (RNFLT by OCT) and that of the retrogeniculate part of the visual pathway (NAA in the visual cortex by ^{1}H-MRS). Thus, our combined OCT and ^{1}H-MRS data may suggest an interconnection of MS-associated neurodegeneration in both parts of the visual pathway. The multivariate statistical model revealed that the correlation of RNFLT reduction with lower NAA concentrations is not a mere consequence of global brain tissue loss as one could assume given the correlation of RNFLT with BPF in both our work and that of others [13][15]. On the contrary, loss of NAA in the visual cortex appears to be associated with thinning of the RNFL independently from brain atrophy. These findings could indicate that – beyond an

undoubted diffuse neurodegenerative process in MS which is detectable by measurement of global brain tissue loss and also by RNFL measurements - additional progressive neurodegenerative damage may evolve in specific tracts or functional systems such as the visual pathway. This assumption is further supported by previous studies describing visual pathway damage in MS by means of voxel-based morphometry, diffusion tensor imaging or magnetization transfer ratio[33–35], and damage to other functional systems such as pathways involved in learning and memory [36–37]. The connection between the anterior and posterior visual pathway damage raises the question of a mutual interdependency of these alterations and implicates the possible existence of transsynaptic damage processes in the anatomical correlates of the visual pathway in MS. This concept has already been described recently for glaucoma[38–39] and in the context of amblyopia[40] and in congenital or acquired homonymous hemianopia [41]. In this regard, the lateral geniculate nucleus (LGN) as region of change-over from axons deriving from the anterior visual pathway to neurons from which axons forming the optic radiation emerge is of importance. Interestingly, a histopathological study of neuronal changes in the LGN by Evangelou et al. [42] strongly supports the concept of transsynaptic degeneration. In this context, Green et al. could show just recently by a larger histopathological study, that retinal atrophy and intraretinal inflammation may exceed previous assumptions, indicating that also other structures of the foremost part of the visual pathway, as the retinal inner nuclear layer, may be affected by transsynaptical axonal and neuronal degeneration, not only the retinal nerve fiber layer [43]. This hypothesis is further supported by our findings of lower RNFL thickness in patients with previous optic neuritis compared to those without, in line with earlier reports[12][44–45], and concordantly also lower visual cortex NAA concentrations in patients with previous optic neuritis. However, the underlying pathophysiological mechanisms remain to be elucidated, including the direction of damage cascades (anterograde, retrograde) and their temporal evolution. These mechanisms cannot be deduced from cross-sectional studies, as these only provide a description of the current status within a narrow time-frame.

A methodological limitation of our study is the lack of an additional MRS voxel containing mixed or gray matter in another brain region than the visual cortex. Preferably, a separate cortex voxel representative for an independent functional pathway, such as a voxel covering the motor cortex, could have been used as a more appropriate control region, which could not be applied in our study to limit scan time to an acceptable extent. The reduced cortical NAA concentrations and RNFL thinning may not be specific for the visual cortex but might also have been detected in other cortex regions, thus only indicating a relationship between two different parameters depicting global neurodegeneration. However, despite this limitation we believe that our findings from this exploratory study could support the hypothesis of tract specific damage to the visual pathway in MS as (i) NAA only in the visual cortex voxel comprising both gray and white matter but not NAA in the normal-appearing white matter voxel correlated with RNFLT, and (ii) our subgroup analysis showed that the extent of NAA reduction in the visual cortex voxel is related to the history of optic neuritis. Notwithstanding, future studies on visual pathway damage in MS should include additional cortical MRS voxel if possible. In addition, the advent of novel spectral-domain OCT devices with an improved spatial resolution and a better retest-reliability, that replace the current time-domain OCT devices in the future, will also contribute to a more accurate description of the pathology of visual pathway damage on a morphological level [46–47].

Acknowledgments

We thank our study nurses Antje Els, Franziska Lipske and Cordula Rudolph, and Susan Pikol for expert technical support.

Author Contributions

Conceived and designed the experiments: CFP AUB JTW FP. Performed the experiments: CFP FS MB BW HW JD FP. Analyzed the data: CFP AUB FS MB TS CM JTW. Contributed reagents/materials/analysis tools: AUB FS MB CM BI JTW. Wrote the paper: CFP AUB JTW FP. Collection of clinical data: CFP AUB MB JD JBS NWE FP. Study supervision: CFP AUB FP. Set-up of imaging procedures: FS MB BI JTW. Optical coherence tomography supervision: MB.

References

1. Charcot JM (1868) Histologie de la sclerose en plaques. Gazette des Hopitaux 141: 554–558.
2. Ropper AH, Brown RJ (2005) Adams and Victor's Principles of Neurology. 8th edition. McGraw-Hill Medical.
3. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, et al. (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338: 278–285.
4. Vogt J, Paul F, Aktas O, Müller-Wielsch K, Dörr J, et al. (2009) Lower motor neuron loss in multiple sclerosis and experimental autoimmune encephalomyelitis. Ann Neurol 66: 310–322.
5. Barkhof F (2002) The clinico-radiological paradox in multiple sclerosis revisited. Curr Opin Neurol 15: 239–245.
6. Miller DH, Barkhof F, Frank JA, Parker GJM, Thompson AJ (2002) Measurement of atrophy in multiple sclerosis: pathological basis, methodological aspects and clinical relevance. Brain 125: 1676–1695.
7. Bermel RA, Bakshi R (2006) The measurement and clinical relevance of brain atrophy in multiple sclerosis. Lancet Neurol 5: 158–170.
8. Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L (1999) Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. Neurology 53: 1698–1704.
9. Frohman EM, Fujimoto JG, Frohman TC, Calabresi PA, Cutter G, et al. (2008) Optical coherence tomography: a window into the mechanisms of multiple sclerosis. Nat Clin Pract Neurol 4: 664–675.
10. Barkhof F, Calabresi PA, Miller DH, Reingold SC (2009) Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. Nat Rev Neurol 5: 256–266.
11. Parisi V, Manni G, Spadaro M, Colacino G, Restuccia R, et al. (1999) Correlation between morphological and functional retinal impairment in multiple sclerosis patients. Invest Ophthalmol Vis Sci 40: 2520–2527.
12. Bock M, Brandt AU, Dörr J, Kraft H, Weinges-Evers N, et al. (2010) Patterns of retinal nerve fiber layer loss in multiple sclerosis patients with or without optic neuritis and glaucoma patients. Clin Neurol Neurosurg 112(8): 647–52.
13. Gordon-Lipkin E, Chodkowski B, Reich DS, Smith SA, Pulicken M, et al. (2007) Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology 69: 1603–1609.
14. Grazioli E, Zivadinov R, Weinstock-Guttman B, Lincoff N, Baier M, et al. (2008) Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis. J Neurol Sci 268: 12–17.
15. Siger M, Dziegielewski K, Jasek L, Bieniek M, Nicpan A, et al. (2008) Optical coherence tomography in multiple sclerosis: thickness of the retinal nerve fiber layer as a potential measure of axonal loss and brain atrophy. J Neurol 255: 1555–1560.
16. Toledo J, Sepulcre J, Salinas-Alaman A, García-Layana A, Murie-Fernandez M, et al. (2008) Retinal nerve fiber layer atrophy is associated with physical and cognitive disability in multiple sclerosis. Mult. Scler 14: 906–912.
17. De Stefano N, Filippi M (2007) MR spectroscopy in multiple sclerosis. J Neuroimaging 17(Suppl 1): 31S–35S.
18. Husted CA, Goodin DS, Hugg JW, Maudsley AA, Tsuruda JS, et al. (1994) Biochemical alterations in multiple sclerosis lesions and normal-appearing white matter detected by in vivo 31P and 1H spectroscopic imaging. Ann Neurol 36: 157–165.
19. Fu L, Matthews PM, De Stefano N, Worsley KJ, Narayanan S, et al. (1998) Imaging axonal damage of normal-appearing white matter in multiple sclerosis. Brain 121(Pt 1): 103–113.
20. Polman CH, Reingold SC, Edan G, Filippi M, Hartung H, et al. (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 58: 840–6.
21. Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33: 1444–1452.

22. Cheung CYL, Leung CKS, Lin D, Pang C, Lam DSC (2008) Relationship between retinal nerve fiber layer measurement and signal strength in optical coherence tomography. Ophthalmology 115: 1347–1351, 1351.e1-2.
23. Smith SM (2002) Fast robust automated brain extraction. Hum Brain Mapp 17: 143–155.
24. Smith SM, De Stefano N, Jenkinson M, Matthews PM (2001) Normalized accurate measurement of longitudinal brain change. J Comput Assist Tomogr 25: 466–475.
25. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, a u (2004) Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23(Suppl 1): S208–219.
26. Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, et al. (2002) Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage 17: 479–489.
27. Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17: 825–841.
28. Jenkinson M, Smith S (2001) A global optimisation method for robust affine registration of brain images. Med Image Anal 5: 143–156.
29. Zhang Y, Brady M, Smith S (2001) Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 20: 45–57.
30. Schubert F, Gallinat J, Seifert F, Rinneberg H (2004) Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla. Neuroimage 21: 1762–1771.
31. Elster C, Schubert F, Link A, Walzel M, Seifert F, et al. (2005) Quantitative magnetic resonance spectroscopy: semi-parametric modeling and determination of uncertainties. Magn Reson Med 53: 1288–1296.
32. Schubert F, Seifert F, Elster C, Link A, Walzel M, et al. (2002) Serial 1H-MRS in relapsing-remitting multiple sclerosis: effects of interferon-beta therapy on absolute metabolite concentrations. MAGMA 14: 213–222.
33. Sepulcre J, Goñi J, Masdeu JC, Bejarano B, Vélez de Mendizábal N, et al. (2009) Contribution of white matter lesions to gray matter atrophy in multiple sclerosis: evidence from voxel-based analysis of T1 lesions in the visual pathway. Arch.Neurol 66: 173–179.
34. Reich DS, Smith SA, Gordon-Lipkin EM, Ozturk A, Caffo BS, et al. (2009) Damage to the optic radiation in multiple sclerosis is associated with retinal injury and visual disability. Arch Neurol 66: 998–1006.
35. Audoin B, Fernando KTM, Swanton JK, Thompson AJ, Plant GT, et al. (2006) Selective magnetization transfer ratio decrease in the visual cortex following optic neuritis. Brain 129: 1031–1039.
36. Fink F, Eling P, Rischkau E, Beyer N, Tomandl B, et al. (2010) The association between California Verbal Learning Test performance and fibre impairment in multiple sclerosis: evidence from diffusion tensor imaging. Mult. Scler 16: 332–341.
37. Benedict RHB, Ramasamy D, Munschauer F, Weinstock-Guttman B, Zivadinov R (2009) Memory impairment in multiple sclerosis: correlation with deep grey matter and mesial temporal atrophy. J Neurol Neurosurg Psychiatr 80: 201–206.
38. Gupta N, Greenberg G, de Tilly LN, Gray B, Polemidiotis M, et al. (2009) Atrophy of the lateral geniculate nucleus in human glaucoma detected by magnetic resonance imaging. Br J Ophthalmol 93: 56–60.
39. Gupta N, Yücel YH (2003) Brain changes in glaucoma. Eur J Ophthalmol 13(Suppl 3): S32–35.
40. Barnes GR, Li X, Thompson B, Singh KD, Dumoulin SO, et al. (2010) Decreased gray matter concentration in the lateral geniculate nuclei in human amblyopes. Invest Ophthalmol Vis Sci 51: 1432–1438.
41. Jindahra P, Petrie A, Plant GT (2009) Retrograde trans-synaptic retinal ganglion cell loss identified by optical coherence tomography. Brain 132: 628–634.
42. Evangelou N, Konz D, Esiri MM, Smith S, Palace J, et al. (2001) Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. Brain 124: 1813–1820.
43. Green AJ, McQuaid S, Hauser SL, Allen IV, Lyness R (2010) Ocular pathology in multiple sclerosis: retinal atrophy and inflammation irrespective of disease duration. Brain 133: 1591–1601.
44. Trip SA, Schlottmann PG, Jones SJ, Altmann DR, Garway-Heath DF, et al. (2005) Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. Ann.Neurol 58: 383–391.
45. Costello F, Hodge W, Pan YI, Freedman M, DeMeulemeester C (2009) Differences in retinal nerve fiber layer atrophy between multiple sclerosis subtypes. J Neurol Sci 281: 74–79.
46. Bock M, Brandt AU, Dörr J, Pfueller CF, Ohlraun S, et al. (2010) Time domain and spectral domain optical coherence tomography in multiple sclerosis: A comparative cross-sectional study. Mult Scler 2010 Jul; 16(7): 893–6.
47. Talman LS, Bisker ER, Sackel DJ, Long DA, Galetta KM, et al. (2010) Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. Ann Neurol 67: 749–760.

Zimmermann H, Freing A, Kaufhold F, Gaede G, Bohn E, Bock M, Oberwahrenbrock T, Young KL, Dörr J, Wuerfel J, Schippling S, Paul F, Brandt AU.

Optic neuritis interferes with optical coherence tomography and magnetic resonance imaging correlations.

Mult Scler. 2013 Apr;19(4):443-50

http://dx.doi.org/10.1177/1352458512457844

Young KL*, **Brandt AU***, Petzold A, Reitz LY, Lintze F, Paul F, Martin R, Schippling S.

Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis.

Eur J Neurol. 2013 May;20(5):803-11

http://dx.doi.org/ 10.1111/ene.12070

Eur J Neurol. 2013 May;20(5):805

Eur J Neurol. 2013 May;20(5):806

Eur J Neurol. 2013 May;20(5):807

Eur J Neurol. 2013 May;20(5):808

Eur J Neurol. 2013 May;20(5):809

Eur J Neurol. 2013 May;20(5):810

Eur J Neurol. 2013 May;20(5):811

Lebenslauf

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

(30) Wieder L, Gäde G, Pech LM, Zimmermann H, Wernecke KD, Dörr JM, Bellmann-Strobl J, Paul F, **Brandt AU**. *Low contrast visual acuity testing is associated with cognitive performance in multiple sclerosis: a cross-sectional pilot study*. BMC Neurol. 2013 Nov 8;13(1):167. 2.56/ 0.0067

(29) Kaufhold F, Zimmermann H, Schneider E, Ruprecht K, Paul F, Oberwahrenbrock T*, **Brandt AU***. *Optic neuritis is associated with inner nuclear layer thickening and microcystic macular edema independently of multiple sclerosis.* PLoS One. 2013 Aug 6;8(8):e71145 3.73/ 0.78

(28) Schneider E, Zimmermann H, Oberwahrenbrock T, Kaufhold F, Kadas EM, Petzold A, Bilger F, Borisow N, Jarius S, Wildemann B, Ruprecht K, **Brandt AU**, Paul F. *Optical Coherence Tomography Reveals Distinct Patterns of Retinal Damage in Neuromyelitis Optica and Multiple Sclerosis.* PLoS One. 2013 Jun 21;8(6):e66151. 3.73/ 0.78

(27) Oberwahrenbrock T, Ringelstein M, Jentschke S, Deuschle K, Klumbies K, Bellmann-Strobl J, Harmel J, Ruprecht K, Schippling S, Hartung HP, Aktas O, **Brandt AU***, Paul F*. *Retinal ganglion cell and inner plexiform layer thinning in clinically isolated syndrome.* Mult Scler. 2013 May 23. [Epub ahead of print] 4.47/ 0.02

(26) Finke C, Kopp UA, Scheel M, Pech LM, Soemmer C, Schlichting J, Leypoldt F, **Brandt AU**, Wuerfel J, Probst C, Ploner CJ, Prüss H, Paul F. *Functional and structural brain changes in anti-NMDAR encephalitis.* Ann Neurol. 2013 May 20. [Epub ahead of print] 11.19/ 0.07

(25) Roth NM, Saidha S, Zimmermann H, **Brandt AU**, Oberwahrenbrock T, Maragakis NJ, Tumani H, Ludolph AC, Meyer T, Calabresi PA, Paul F. *Optical coherence tomography does not support optic nerve involvement in amyotrophic lateral sclerosis.* Eur J Neurol. 2013 Aug;20(8):1170-6. 4.16/ 0.02

(24) Young KL*, **Brandt AU***, Petzold A, Winkler L, Lintze F, Paul F, Martin R and Schippling S. *Spectral domain optical coherence tomography correlates with MRI white but not grey matter damage in early multiple sclerosis.* Eur J Neurol. 2013 May;20(5):803-11. 4.16/ 0.02

(23) Zimmermann H, Freing A, Kaufhold F, Gaede G, Bohn E, Bock M, Oberwahrenbrock T, Young KL, Dörr J, Wuerfel JT, Schippling S, Paul F, **Brandt AU**. *Optic neuritis interferes with optical coherence tomography and magnetic resonance imaging correlations.* Mult Scler. 2013 Apr;19(4):443-50. 4.47/ 0.02

(22) Sinnecker T, Bozin I, Dörr J, Pfueller CF, Harms L, Niendorf T, **Brandt AU**, Paul F, Wuerfel J. *Periventricular venous density in multiple sclerosis is inversely associated with T2 lesion count: a 7 Tesla MRI study.* Mult Scler. 2013 Mar;19(3):316-25. 4.47/ 0.02

(21) Mähler A, Steiniger J, Bock M, **Brandt AU**, Haas V, Boschmann M, Paul F. *Is metabolic flexibility altered in multiple sclerosis patients?* PLoS One. 2012;7(8):e43675. 3.73/ 0.78

(20) Oberwahrenbrock T, Schippling S, Ringelstein M, Kaufhold F, Zimmermann H, Keser N, Young KL, Harmel J, Hartung HP, Martin R, Paul F, Aktas O, **Brandt AU**. Retinal damage in multiple sclerosis disease subtypes measured by high-resolution optical coherence tomography. Mult Scler Int. 2012;2012:530305. n/a

(19) Finke C, Pech LM, Sömmer C, Schlichting J, Stricker S, Endres M, Ostendorf F, Ploner CJ, **Brandt AU**, Paul F. Dynamics of saccade parameters in multiple sclerosis patients with 3.58/ 0.026

fatigue. J Neurol. 2012 Dec;259(12):2656-63.

(18) Brandt AU, Zimmermann H, Kaufhold F, Promesberger J, Schippling S, Finis D, Aktas O, Geis C, Ringelstein M, Ringelstein EB, Hartung HP, Paul F, Kleffner I, Dörr J. *Characteristic Patterns of retinal damage facilitate differential diagnosis between Susac syndrome and MS.* PLoS One. 2012;7(6):e38741. 3.73/ 0.78

(17) Kaufhold F, Kadas EM, Schmidt C, Kunte H, Hoffmann J, Zimmermann H, Oberwahrenbrock T, Harms L, Polthier K, **Brandt AU***, Paul F*. *Optic nerve head quantification in idiopathic intracranial hypertension by spectral domain OCT.* PLoS One. 2012;7(5):e36965. 3.73/ 0.78

(16) Kadas EM, Kaufhold F, Schulz C, Paul F, Polthier K, **Brandt AU**. *3D Optic Nerve Head Segmentation in Idiopathic Intracranial Hypertension*. Bildverarbeitung für die Medizin 2012. Springer Berlin Heidelberg; 2012. p. 262–7. n/a

(15) Herz J, Niesner R, **Brandt AU**, Siffrin V, Leuenberger T, Paterka M, Glumm R, Zipp F, Radbruch H. *In vivo imaging of lymphocytes in the CNS reveals different behaviour of naïve T cells in health and autoimmunity.* J Neuroinflammation. 2011 Oct 6;8:131. 4.35/ 0.01

(14) Stricker S, Oberwahrenbrock T, Zimmermann H, Schroeter J, Endres M, **Brandt AU**, Paul F. *Temporal retinal nerve fiber layer loss in patients with Spinocerebellar Ataxia Type 1*. PLoS One. 2011;6(7):e23024. 3.73/ 0.78

(13) Brandt AU, Oberwahrenbrock T, Ringelstein M, Young KL, Tiede M, Hartung HP, Martin R, Aktas O, Paul F*, Schippling S*. *Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography.* Brain. 2011 Nov;134(Pt 11):e193. 9,92/ 0.098

(12) Pfueller C*, **Brandt AU***, Schubert F, Bock M, Walaszek B, Waiczies H, Schwenteck T, Dörr J, Bellmann-Strobl J, Mohr C, Weinges-Evers N, Ittermann B, Wuerfel J, Paul F. *Metabolic changes in the visual cortex are linked to retinal nerve fiber layer thinning in multiple sclerosis.* PLoS ONE 2011 Apr 6;6(4):e18019. 3.73/ 0.78

(11) Dörr J, Wernecke KD, Bock M, Gaede G, Wuerfel J, Pfueller C, Bellmann-Strobl C, **Brandt AU***, Paul F*. *Association of Retinal and Macular Damage with Brain Atrophy in Multiple Sclerosis.* PLoS ONE 2011 Apr 8;6(4):e18132. 3.73/ 0.78

(10) Bock M*, **Brandt AU***, Kuchenbecker, Dörr J, Pfueller C, Weinges-Evers N, Gaede G, Zimmermann H, Bellmann-Strobl J, Ohlraun S, Zipp F, Paul F. *Impairment of contrast visual acuity as a functional correlate of retinal nerve fibre layer thinning and total macular volume reduction in multiple sclerosis.* Br J Ophthalmol. 2011 Mar 3. 2.73/ 0.03

(9) Hohn O, Strohschein K, **Brandt AU**, Seeher S, Klein S, Kurth R, Paul F, Meisel C, Scheibenbogen C, Bannert N. *No evidence for XMRV in German CFS and MS patients with fatigue despite the ability of the virus to infect human blood cells in vitro.* PLoS One. 2010 Dec 22;5(12):e15632. 3.73/ 0.78

(8) Bock M*, **Brandt AU***, Dörr J, Kraft H, Weinges-Evers N, Gaede G, Pfueller CF, Herges K, Radbruch H, Ohlraun S, Bellmann-Strobl J, Kuchenbecker J, Zipp F, Paul F. *Patterns of retinal nerve fiber layer loss in multiple sclerosis patients with or without optic neuritis and glaucoma patients.* Clin Neurol Neurosurg. 2010 Oct; 112(8):647-52. 1.23/ 0.007

(7) Weinges-Evers N***, Brandt AU***, Bock M, Pfueller CF, Dörr J, Bellmann-Strobl J, Scherer P, Urbanek C, Ohlraun S, Zipp F, Paul F. *Correlation of self-assessed fatigue and alertness in* 4.47/ 0.02

multiple sclerosis. Mult Scler. 2010 Sept; 16(9):1134-40.

(6) Bock M*, **Brandt AU***, Dörr J, Pfueller CF, Ohlraun S, Zipp F, Paul F. *Time domain and spectral domain optical coherence tomography in multiple sclerosis: a comparative cross-sectional study*. Mult Scler. 2010 Jul;16(7):893-6. 4.47/ 0.02

(5) Herz J, Siffrin V, Hauser AE, **Brandt AU**, Leuenberger T, Radbruch H, Zipp F, Niesner RA. *Expanding two-photon intravital microscopy to the infrared by means of optical parametric oscillator.* Biophys J. 2010 Feb 17;98(4):715-23. 3.67/ 0.12

(4) Siffrin V*, **Brandt AU***, Radbruch H*, Herz J, Boldakowa N, Leuenberger T, Werr J, Hahner A, Schulze-Topphoff U, Nitsch R, Zipp F. *Differential immune cell dynamics in the CNS cause CD4+ T cell compartmentalization.* Brain. 2009 May;132(Pt 5):1247-58. 9.915/ 0.098

(3) Waiczies S, Bendix I, Prozorovski T, Ratner M, Nazarenko I, Pfueller CF, **Brandt AU**, Herz J, Brocke S, Ullrich O, Zipp F. *Geranylgeranylation but not GTP loading determines rho migratory function in T cells.* J Immunol. 2007 Nov 1;179(9):6024-32. 5.52/ 0.33

(2) Siffrin V, **Brandt AU**, Herz J, Zipp F. *New insights into adaptive immunity in chronic neuroinflammation.* Adv Immunol. 2007;96:1-40. 7.26/ 0.007

(1) Heintze C, Matysiak-Klose D, Krohn T, Wolf U, **Brandt AU**, Meisner C, Fischer I, Wehrmeyer H, Braun V. *Diagnostic Work up of Rectal Bleeding in General Practice - A Prospective Study About Treating Rectal Bleeding Within General Practice.* Br J Gen Pract. 2005 Jan;55(510):14-9. 1.83/ 0.007

**) gleichbeteiligte Autorenschaft*

Danksagung

An dieser Stelle möchte ich die Gelegenheit nutzen und Worte des Dankes an all jene richten, die mich während der Arbeit unterstützt und inspiriert haben.

Allen voran bedanke ich mich bei Prof. Dr. Friedemann Paul, mit dem zusammen ich die Ehre hatte das Thema Retina bei Multipler Sklerose und anderen Neurologischen Erkrankungen wissenschaftlich an der Charité etablieren zu dürfen.

Ganz besonderen Dank möchte ich an dieser Stelle den Mitgliedern des DIAL Teams äußern, die mich während der Promotion und meiner wissenschaftlichen Arbeit an der Charité begleitet haben. Dr. Alina Freing, Ella Maria Kadas, Timm Oberwahrenbrock und Hanna Zimmermann, ihr hattet immer großen Teil an meiner Inspiration und Freude an der Arbeit! Ich darf mich glücklich schätzen, mit so engagierten und wundervollen Wissenschaftlern zusammenzuarbeiten!

Nicht zuletzt gilt mein Dank meinen Coautoren und allen Mitarbeitern des NCRC und des inims, die an der Durchführung der Studien beteiligt waren. Ohne euch wären diese Arbeiten nicht entstanden!