Optical coherence tomography in multiple sclerosis

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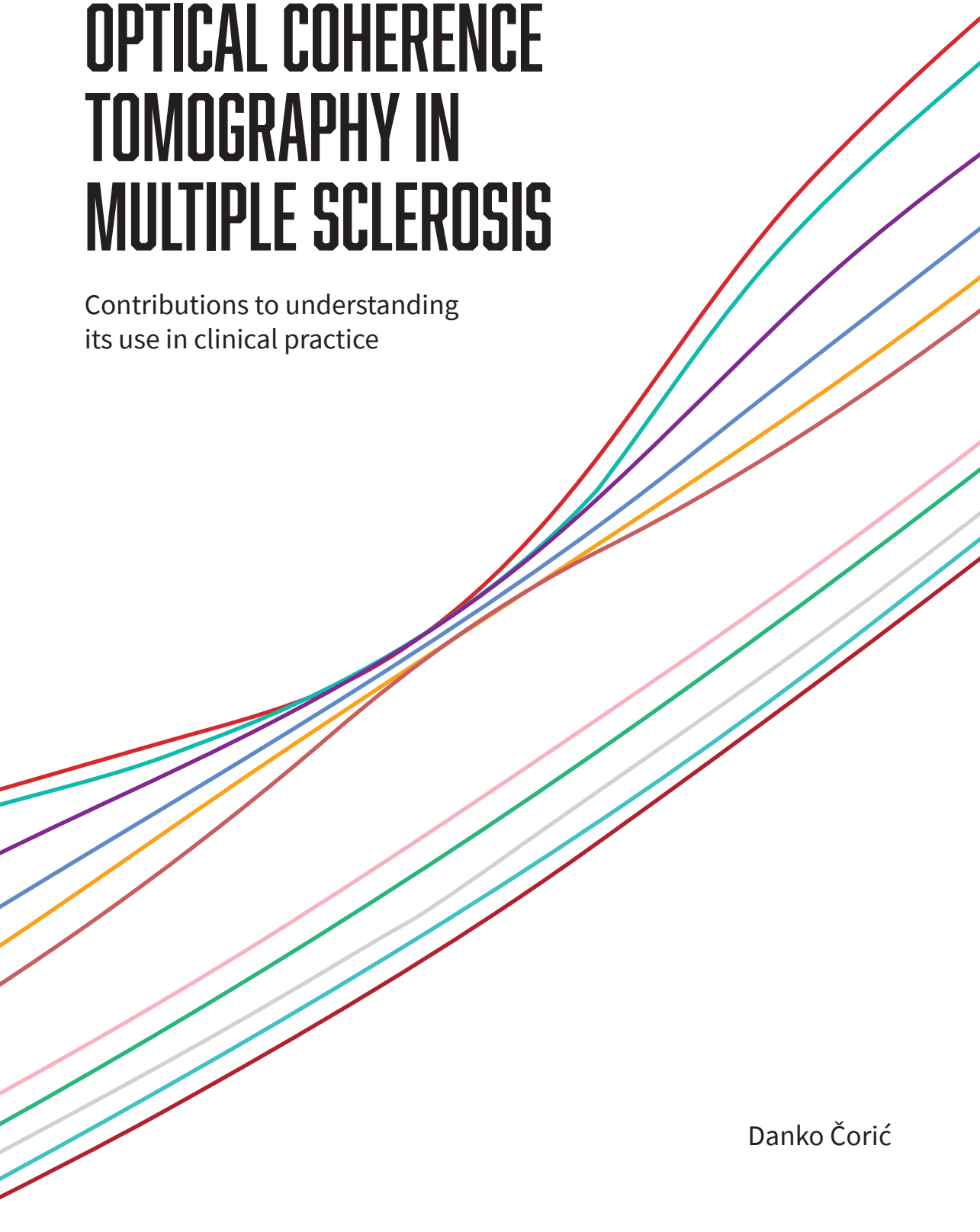
OPTICAL COHERENCE TOMOGRAPHY IN MULTIPLE SCLEROSIS

Contributions to understanding its use in clinical practice



Danko Čorić

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Contributions to understanding its use in clinical practice

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OPTICAL COHERENCE TOMOGRAPHY IN MULTIPLE SCLEROSIS

Contributions to understanding its use in clinical practice

ACADEMISCH PROEFSCHRIFT

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dr. L.J. Balk

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LIST OF ABBREVIATIONS

ART = automatic real time
AUC = area under the curve
AAR = annualized atrophy rate

BRB-N = Rao's brief repeatable battery of neuropsychological tests

CD = cognitive decline
CI = cognitively impaired
CIS = clinically isolated syndrome
CNS = central nervous system
CP = cognitively preserved
CS = cognitively stable
CST = concept shifting test

DIS = dissemination in time
DIT = dissemination in space
DMT = disease modifying therapy

EDI = enhanced depth imaging
EDSS = expanded disability status scale

FA = fractional anisotropy

(m)GCIPL = (macular) ganglion cell – inner plexiform layer
(m)GCL = (macular) ganglion cell layer
GEE = generalized estimating equations
GLM = generalized linear models

HC = healthy control

IAED = inter-eye absolute difference
IEPD = inter-eye percentage difference
IIH = increased intracranial hypertension
IMSVISUAL = International Multiple Sclerosis Visual System Consortium
(m)INL = (macular) inner nuclear layer
IRL = inner retinal layer
IQR = interquartile range

MCT = memory comparison test
MMO = microcystic macular oedema
MRI = magnetic resonance imaging
MS = multiple sclerosis
MSON = multiple sclerosis associated optic neuritis
MSNON /nonMSON = no history of multiple sclerosis associated optic neuritis

N = nasal
N/a = not available
NEI-VFQ-25 = National Eye Institute visual function questionnaire
NI = nasal inferior
NS = nasal superior

(SD) OCT = (spectral domain) optical coherence tomography
OCTA = optical coherence tomography angiography
OD = right eye
ODD = optic disc drusen
ODS right and left eye combined
ON = optic neuritis
OR = odds ratio
OS = left eye

PHOMS = peripapillary hyperreflective ovoid mass-like structure
PPMS = primary progressive multiple sclerosis

QC = quality control
QoL = quality of life

RCI = reliable change index
(p)RNFL = (peripapillary) retinal nerve fiber layer
ROC = receiver operator characteristics
RRMS = relapsing remitting multiple sclerosis

SCWT = Stroop color word test
SD = standard deviation
SDMT = symbol digit modalities test
SPMS = secondary progressive multiple sclerosis
SRT = selective reminding test

T = temporal
TI = temporal inferior
TS =temporal superior

(HC/LC) VA = (high contrast/low contrast) visual acuity
VEP = visual evoked potentials
VH = vitreous haze

WLGT = word list generation test

10/36 SRT = 10/36 spatial recall test
95%CI = 95% confidence interval

CHAPTER 1

| GENERAL INTRODUCTION

Parts of this introduction have also been published in the following review:
The role of optical coherence tomography and infrared oculography in assessing the visual pathway and CNS in multiple sclerosis.
D Coric, JA Nij Bijvank*, LJ van Rijn, A Petzold, LJ Balk*
**Both authors contributed equally to this manuscript*

Neurodegenerative Disease Management 2018;8:323-335 ■

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a disease of the central nervous system (CNS) affecting more than two million people worldwide, making it one of the most common neurological disorders in young people.[1] The exact cause of MS is unknown but both genetic predisposition and environmental risk factors play a role.[2] A clear geographical distribution exists with the highest prevalence (≥ 1:1000) found in North America and Western Europe and decreasing prevalence observed moving closer to the equator.[1] Apart from geographical latitude, risk factors include, among others female sex, smoking, obesity and infection with Epstein-Bar virus.[3]

Classically viewed as an inflammatory white matter disease it is now understood that MS is characterized by inflammatory as well as neurodegenerative components.[4] The exact interaction between inflammation and neurodegeneration (i.e. the loss of axons and neurons) however is not yet fully elucidated. In the early phase of the disease, peripheral immune cells cross the blood-brain barrier, infiltrate the brain tissue and typically form focal sclerotic plaques consisting of demyelination and gliosis with partial preservation of axons leading to a disruption of neuronal signaling and clinical relapses.[5,6] As the disease progresses, active demyelinating plaques decrease in number resulting in less relapses.[7] However, the earlier inflammatory changes trigger a pathogenic cascade causing global neurodegeneration later in the disease course. This neurodegeneration is considered to be the main contributing factor to permanent clinical disability.[6]

Typically, MS starts at the age of 20 to 40 with a first episode of focal neurological dysfunction, which is referred to as a clinically isolated syndrome (CIS).[8] For the majority of these patients, this is followed by recurrent episodes of neurological worsening (i.e. relapses) with a various degree of recovery and residual damage,[9] which defines the relapsing remitting phase of the disease (RRMS). Eventually, most of these patients will enter the secondary progressive phase (SPMS), characterized by slowly progressive and permanent disability. A small proportion (about 15%) of patients have a primary progressive disease course (PPMS). This is characterized by a progressive course from onset without having any acute relapses. In SPMS and PPMS, neurodegeneration is thought to play the main role.[10,11]

Patients experience a wide range of symptoms such as muscle weakness, sensory disturbances, ataxia, fatigue and bowel and bladder problems.[12] Cognitive dysfunction is also increasingly recognized as an important aspect of MS. Cognitive impairment is estimated to occur in 40-70% of patients and can occur at any stage of the disease.[13-16] It has a large impact on daily functioning and quality of life of MS patients.[17-19] Lastly, visual problems are a major cause of disability in MS as well. Up to 80% of MS patients experience visual disturbances during the course of the disease, including both afferent and efferent disorders.[20-23] These deficits often lead to visual disabilities in daily life and reduced vision-related quality of life.[23, 24] Afferent visual problems in MS most often occur in the form of optic neuritis.[12] Optic neuritis is characterized by inflammation of the optic nerve[25] and when caused by MS, it is typically referred to as MS associated optic neuritis (MSON). It is the presenting symptom in about 20% of MS

patients and approximately 50% of MS patients will experience one or more episodes of MSON during the course of their disease.[26] It is characterized by loss of vision, dyschromatopsia and pain on eye movements usually in one eye, that gradually worsens over the course of a few days to weeks before it (partially) recovers.[12] The diagnosis is based on clinical observations, but can be supported by ancillary tests like visual evoked potentials.

Regarding treatment of MS, a great deal of progress has been made since the introduction of the first disease modifying therapy (DMT) interferon-β in the early 1990s. Since then, about ten new drugs have been developed. While all have shown to decrease the number of relapses and some possibly even to slow down disability progression, they have all been reserved for use in RRMS.[27] Only recently did ocrelizumab gain approval by the Food and Drug Administration and European Medicines Agency for the treatment of PPMS patients, having shown to slow down clinical and MRI progression.[28] With an increasing number of treatment options available, it becomes more and more important to be able to predict disease outcome in individual patients. This is in part because more effective DMT carry greater risks of serious side effects.[29] For example natalizumab, although highly effective in the treatment of RRMS, it has a substantial risk of progressive multifocal leukoencephalopathy in John Cunningham virus seropositive patients.[30] The prediction of disease outcome in individual MS patients is, however, a major issue that needs to be solved.

THE PROBLEM AND A POSSIBLE SOLUTION

The disease course in MS, even among patients with the same type, is highly variable and still difficult to predict.[3] Numerous clinical features are known to be associated with a worse clinical outcome (e.g. male sex, high age at onset, high frequency of relapses in the early phase of the disease) but the prognostic value of these features for individual patients is limited at best.[31] Magnetic resonance imaging (MRI) is the cornerstone in the diagnosis of MS, being able to demonstrate demyelinating plaques and provide evidence for dissemination in space and dissemination in time, but has limited ability to predict disease outcome.[32-34] Brain atrophy measured on MRI, which is considered to be an estimate of neurodegeneration, does not necessarily reflect loss of neuron and axons and can be caused by loss of other, non-neuronal cells or pseudo-atrophy following the start of DMT.[35, 36] Likewise, a clear discrepancy exists between MRI markers of inflammation and degree of clinical disability.[34] Lastly, the often reported Expanded Disability Status Scale (EDSS)[37] also has its limitations. The EDSS score measures neurological disability in MS patients, but is heavily biased towards pyramidal dysfunction. Other common problems such as cognitive decline, fatigue but also visual functioning are relatively undervalued. Thus, there is a need for a sensitive and accurate biomarker to monitor disease activity and progression in patients with MS.

One sophisticated system that has the potential to overcome these aforementioned issues is the afferent visual pathway. Being easily assessable, the afferent visual system offers a convenient place to study the effects of MS in vivo by use of retinal optical coherence tomography (OCT). The optic nerve has the advantage of being the only part of the human CNS that contains

unmyelinated axons.[38] Studying the afferent visual system not only allows for assessment of damage caused by MS directly to the visual system but it also gives us insights in what is happening in the CNS on a more global scale.

RETINAL OPTICAL COHERENCE TOMOGRAPHY

First described in 1991, retinal OCT is a non-invasive imaging technique that uses near infrared light to generate high resolution cross-sectional or 3D images of the retina, enabling an 'optical biopsy'. Images are formed by analyzing the interference patterns of the back-scattered light, somewhat analogous to ultrasound but using light instead of sound.[39,40] Current spectral domain OCT devices have an axial resolution of 3-7 µm making it possible to distinguish each individual retinal layer.[41-43] Due to the high accuracy (1.14 – 2.39 µm), it is possible to detect small changes in thickness over time.[44] In MS, two layers are of particular interest: the peripapillary retinal nerve fiber layer (pRNFL) which consists of unmyelinated axons that will eventually form the optic nerve, and the combined macular ganglion cell - inner plexiform layer (mGCIPL) in which the retinal ganglion cells reside.[45] In recent years, the macular inner nuclear layer (mINL) has become a subject of interest for its potential as a surrogate marker for inflammation.[46] The pRNFL thickness is measured by performing a circular scan around the optic nerve head. The thickness of the mGCIPL and mINL is measured by performing a macular volume scan centered on the fovea. (Figure 1).

Atrophy of the pRNFL in MS patients was first described by Parisi et al. in 1991.[47] Since then, several studies have investigated the association between atrophy of the pRNFL and mGCIPL (the inner retinal layers), and disease characteristics in MS. In MSON, thinning of the pRNFL and mGCIPL is caused by retrograde degeneration of axons and ganglion cells in the retina and seems to stop at the INL.[48] The eventual amount of atrophy in eyes following an episode of MSON is on average 20.1 µm for the pRNFL and 16.4 µm for the mGCIPL, compared to healthy controls.[49]

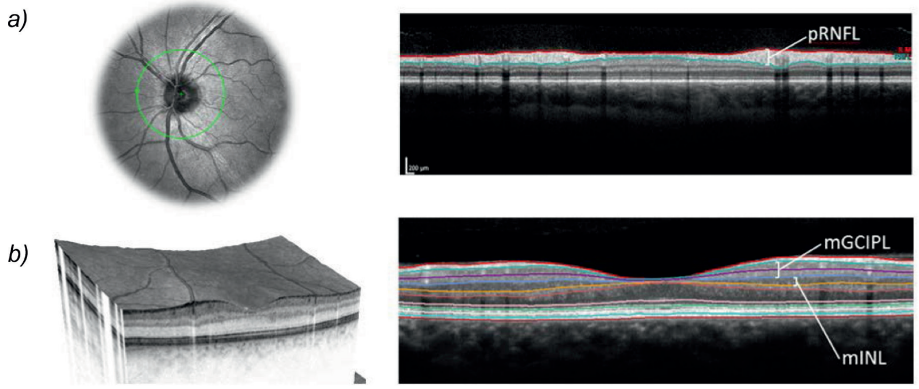

Figure 1. Peripapillary ring scan (a) and macular volume scan (b). The top figure shows the peripapillary ring scan which is used to measure pRNFL thickness. The bottom figure shows the macular volume scan (and one of the cross-sectional images) which is used to measure mGCIPL and mINL thickness.
pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer; mINL = macular inner nuclear layer

Figure 2 shows the typical changes on OCT following an episode of acute MSON. However, due to the wide inter-subject variability in retinal layer thickness, even among healthy subjects, a clear cut-off value for the diagnosis of a previous episode of MSON does not exist. Patients who have not experienced an episode of MSON (MSNON) also show atrophy of the mGCIPL and pRNFL, albeit to a lesser degree.[49] It is believed that in MSNON patients, the atrophy is a reflection of the neuro-axonal damage in the CNS.[50] Several hypotheses have been put forward as possible explanations for the retinal atrophy seen in MSNON patients. Retrograde, trans-synaptic degeneration is the most widely accepted and has been shown to occur within the visual system.[50-52] The association between atrophy of the pRNFL and mGCIPL and global measures of neurodegeneration would suggest that this mechanism extends beyond the visual system. Another explanation is the presence of a global process of neurodegeneration affecting the whole CNS including the optic nerve and retina.[50] A third possible explanation is that of local microinflammatory processes occurring within the optic nerve.[53] Independent of the causal process, it has been shown that atrophy of the two inner retinal layers is associated with brain volume loss and physical disability.[54,55] Interestingly, an increase in mINL thickness has been linked to inflammatory disease activity.[46,56] The exact mechanism leading to this thickening of the mINL is unknown, but it might have something to do with inflammation-related dynamic fluid shifts or Müller cell dysfunction as is discussed in **chapter 5.2** of this thesis.

All things considered, OCT has shown that it has the potential to become a useful surrogate marker for clinically relevant disease progression in patients with MS. However, there are still questions that need to be explored before OCT can be implemented in routine medical practice.

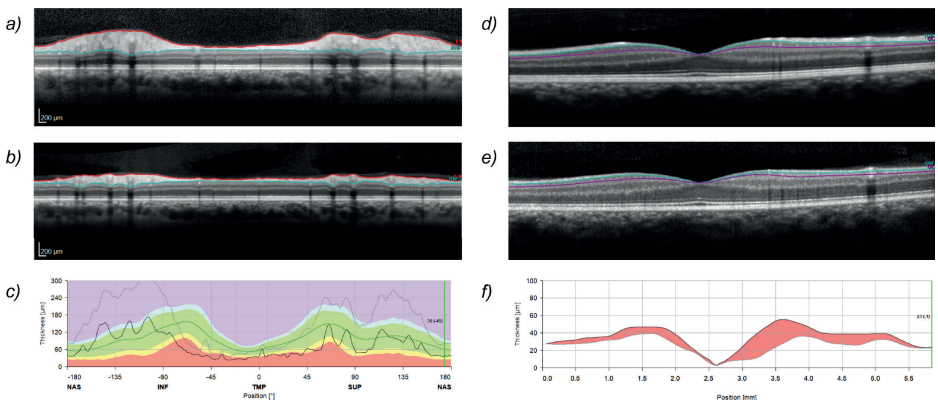

Figure 2: Peripapillary retinal nerve fiber layer (pRNFL) and macular ganglion cell layer (mGCL*) thickness changes in a 30-year old male patient with acute MSON. At the onset of MSON there is swelling of the pRNFL due to edema (a) followed by atrophy at 3 months post onset due to loss of axons (b). Figure 2c shows the difference in pRNFL thickness between onset (grey line, mean thickness 156 µm) and at three months (black line, mean thickness 74 µm). In contrast to the pRNFL, the mGCL does not show any thickening at onset (d) but, just like the pRNFL, shows atrophy three months later (e). The red shaded area (f) indicates the amount of mGCL loss in this cross section.
NAS = nasal; INF = inferior; TMP = temporal; SUP = superior
* Please note that this figure refers to the mGCL and not the composite ganglion cell - inner plexiform layer

In particular, little is known about the relationship between OCT and cognitive dysfunction or how OCT can aid in the assessment of inflammatory disease activity in MS. In addition, while OCT is highly suited to detect damage caused by MSON, clear diagnostic OCT criteria for MSON still do not exist. But before these questions can be answered, a methodological issue relating to longitudinal research has to be addressed. OCT technique and software are constantly being improved. Despite all the benefits of this, in a longitudinal setting this can also lead to bias.

AIMS AND OUTLINE OF THIS THESIS

The first aim of this thesis is to address methodological aspects of OCT in longitudinal research. In **chapter 2.1** we examine the influence of updates in OCT segmentation algorithms on longitudinal atrophy measurements. Secondly, the aims of this thesis are to provide more understanding on 1) how OCT can aid in the assessment of manifestations of MS directly related to the visual system (chapter 3) and 2) on how OCT can aid in the assessment of clinically relevant disease progression, in particular cognitive dysfunction and inflammatory disease activity (chapters 4 and 5), in patients with MS. These questions are addressed in the chapters mentioned below.

Chapter 3.1 investigates the optical cut-off point for, and diagnostic accuracy of, a new criterion, the inter-eye percentage difference, for the diagnosis of a previous episode of MSON. In **chapter 3.2** we study the relationship between retinal atrophy and visual function and vision related quality of life.

In **chapter 4.1** we investigate the relationship between inner retinal layer atrophy and cognitive impairment in patients with MS and the effect of MSON on this relationship. In **chapter 4.2** we expand on this study by prospectively investigating whether cross-sectional and longitudinal atrophy measurements are related to cognitive decline. In **chapter 4.3** the occurrence of peripapillary hyperreflective ovoid mass-like structures (PHOMS), a new phenomenon seen on OCT images, in MS is investigated longitudinally and related to disease characteristics.

In **chapter 5.1** the relationship between inflammatory disease activity and changes in mINL volume is investigated. **Chapter 5.2** describes the application of a novel technique, developed for the quantification of vitreous haze in uveitis, to study the link between vitreous inflammation and inflammatory disease activity in MS.

Finally, **chapter 6** provides a summary of the main results of this thesis. In addition, the clinical implications of the results and recommendations for future studies are discussed.

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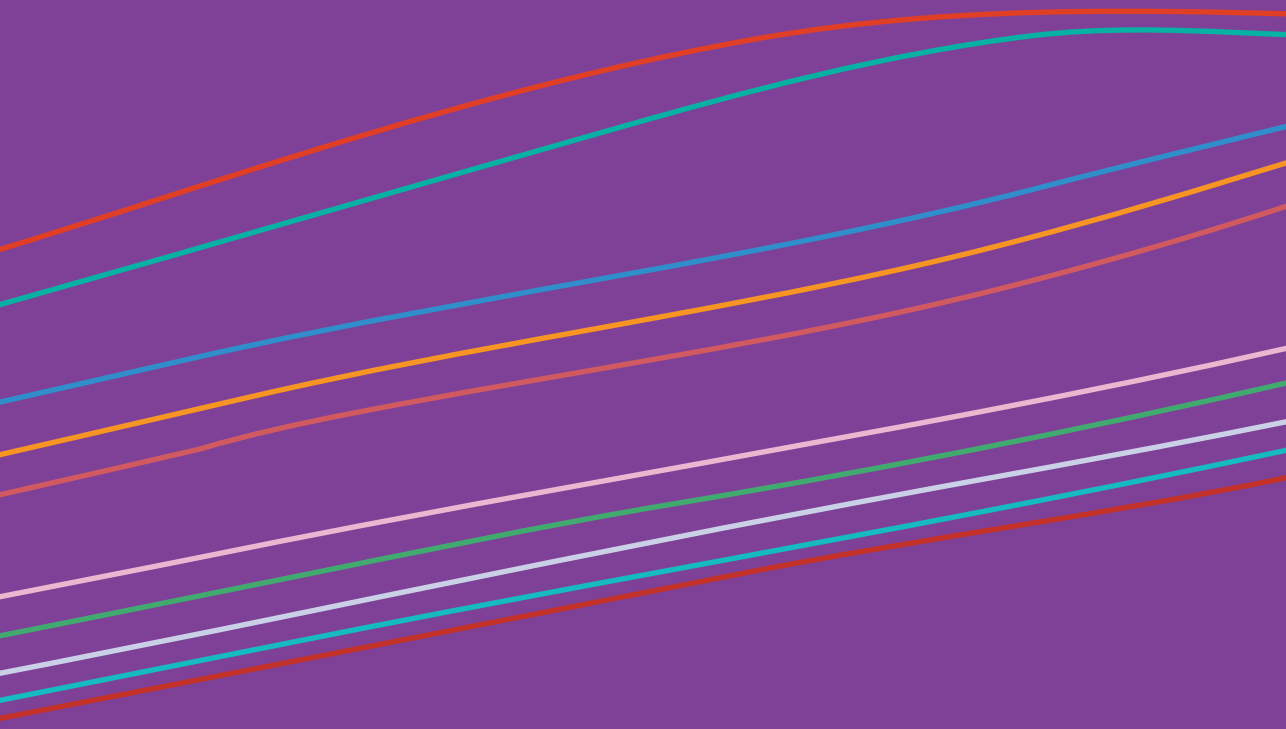
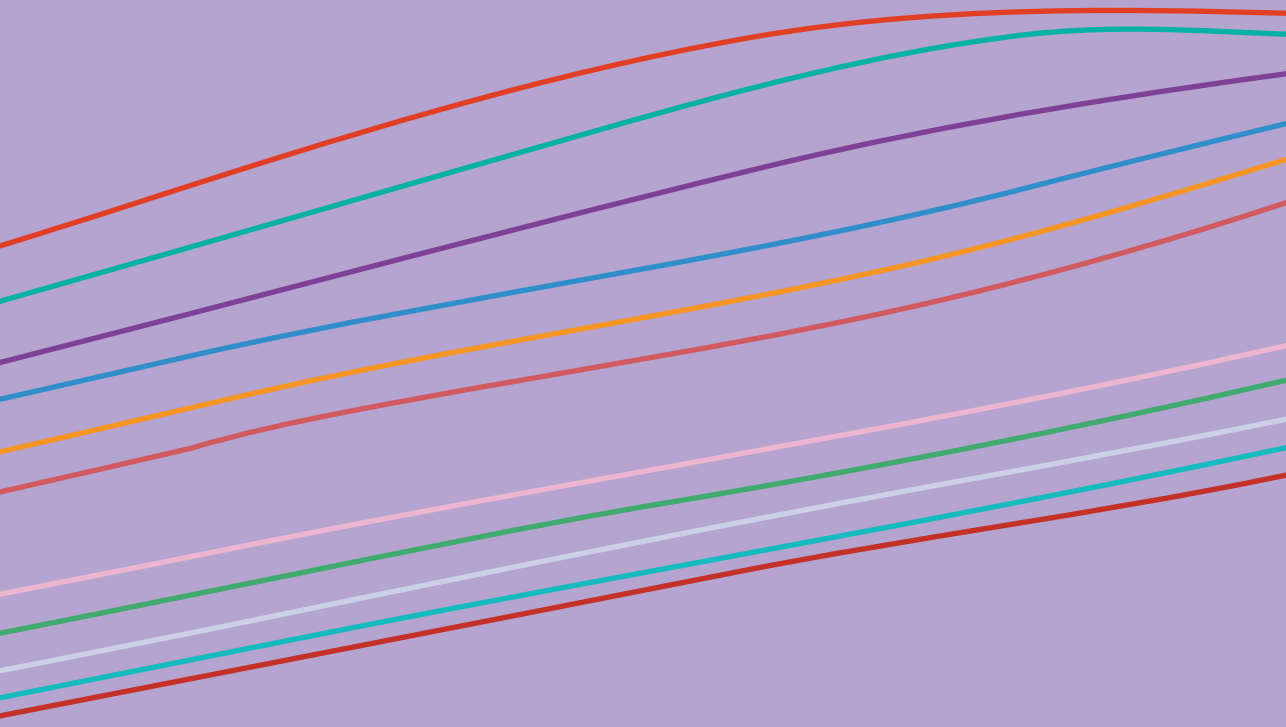


CHAPTER 2

METHODOLOGICAL CONSIDERATIONS



CHAPTER 2.1

SOFTWARE UPDATES OF OCT SEGMENTATION ALGORITHMS INFLUENCE LONGITUDINAL ASSESSMENT OF RETINAL ATROPHY

D Coric, A Petzold, BMJ Uitdehaag, LJ Balk

Journal of the Neurological Sciences 2018;387:16-20

ABSTRACT

Objective: To investigate whether there is a systematic difference in peripapillary retinal nerve fiber layer (pRNFL) thickness measurements between subsequent updates of pRNFL segmentation software provided by Heidelberg Spectralis optical coherence tomography (OCT).

Methods: In total, 838 pRNFL scans from 213 multiple sclerosis (MS) patients and 61 healthy controls were analyzed. All scans were performed on the same OCT device followed by automated segmentation (HRA 5.6.4.0) and data extraction. Subsequently, all scans were re-segmented with an updated software version (HRA 6.0.7.0). To assess level of agreement between the two algorithms, Bland-Altman Plots were constructed. Paired samples T-test and linear regression analyses were used to investigate for differences in mean thickness and proportional bias respectively.

Results: Overall, the updated version showed an overestimation of 0.16 µm [95%CI 0.097 – 0.23, p<0.001] for the global pRNFL thickness compared to the earlier version. The largest differences were found for the nasal inferior (mean Δ 0.29 µm, p<0.001) and temporal inferior (mean Δ 0.43 µm, p<0.001) sectors. Inspection of the Bland-Altman Plot revealed that the difference between the two versions could be up to 6 µm for the global mean. There was no proportional bias for the global mean (β = 0.003, p=0.245) nor for any of the separate sectors.

Conclusion: The data show a significant difference in pRNFL thickness measurements between two subsequent versions of the same segmentation software. Although the mean difference was relatively small, the differences within the individual subject could be considerably higher than the known atrophy rate of 1 µm/year in MS.

INTRODUCTION

Optical coherence tomography (OCT) is a non-invasive imaging technique that allows for detailed imaging and quantification of the thickness of individual retinal layers.[1,2] Having gained wide acceptance in the ophthalmology practice for the evaluation of various primary ocular disorders, OCT is employed more and more in the assessment of neurodegenerative disorders as well, particularly in multiple sclerosis (MS).[3,4] Cross-sectional studies in MS patients have demonstrated a link between atrophy of the peripapillary retinal nerve fiber layer (pRNFL), and various (para)clinical parameters of neurodegeneration including physical disability, cognitive impairment and brain atrophy.[5-7] Current studies focus on longitudinal atrophy measurements in the hope of being able to predict which MS patients will develop physical or cognitive disability.

The assessment of retinal atrophy has improved considerably with the introduction of spectral domain OCT (compared to the previously used time-domain OCT), allowing for reliable quantification of individual retinal layers.[8] In addition, automated retinal layer segmentation algorithms have not only replaced the time consuming and demanding task of manual segmentation but have also increased the accuracy of retinal layer thickness measurements. Subsequent updates in these algorithms aim at refining these aspects by decreasing the processing time and increasing accuracy.[9]

The Spectralis OCT by Heidelberg Engineering, which is frequently used for clinical and research purposes in MS, is a device that gets updated regularly with newer versions of the segmentation algorithm. Nevertheless, in a longitudinal setting there is the potential of biased measurements if not all subsequent scans are segmented using the same software version. This problem can occur due to the difference in the manner of segmentation (for example how the algorithm handles artefacts due to blood vessels). Therefore, the aim of this study was to investigate whether there is a systematic difference in pRNFL thickness measurement between subsequent updates of the pRNFL segmentation algorithm provided by the Heidelberg Spectralis OCT.

METHODS

PARTICIPANTS

For this cross-sectional study, scans were used from participants of the prospective Amsterdam MS Cohort. The inclusion criteria for this cohort have been described previously.[10] At baseline, this cohort consisted of 230 patients and 63 healthy controls (HCs). Patients were retested after a period of two years. Both the baseline and the follow-up scans were used in this study. All examinations (clinical, OCT etc.) were performed on the same day.

This study was approved by the medical ethics committee and scientific research committee of the VU University Medical Center and was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from every participant.

OCT ACQUISITION AND SEGMENTATION

OCT imaging was performed in all subjects on a Spectralis spectral domain OCT (Heidelberg Engineering, Heidelberg, Germany) with dual beam simultaneous imaging and the eye tracking function enabled.[11] All scans, baseline as well as the follow-up scans, were acquired using the same acquisition software (version 1.7.1.0.). In order to measure pRNFL thickness, a 12° ring scan (1536 A-scans, no predetermined automatic real time, ART) manually centered around the optic nerve head was performed. Follow-up scans were acquired using the automatic follow-up function in order to be sure the scans were made at the exact same location. The scans were segmented using software provided by the manufacturer, HRA / Spectralis Viewing Module 5.6.4.0 and the data was exported for statistical analysis. Subsequently, the scans were re-segmented using an updated version of the software, HRA / Spectralis Viewing Module 6.0.7.0, and again exported. After both segmentation procedures, quality control was performed according to the OSCAR-IB criteria[12] and scans were rejected if they failed one or more criteria. Special attention was paid to the algorithm failure criterion. Scans with obvious algorithm failures were rejected. In contrast, scans with small, debatable segmentation deviations (e.g. due to blood vessels), which would normally be accepted for any other study, were included. These scans were not corrected manually in order to be able to investigate the potential bias of the segmentation algorithms, instead of the bias caused by manual correction.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS version 22.0. Normality was assessed visually by means of histograms. Differences in subject characteristics were tested using student T-test (parametric variables), Mann-Whitney-U test (nonparametric variables) and chi square test (categorical variables). Bland-Altman plots were constructed by calculating the mean difference (systematic bias) and 95% limits of agreement of the thickness measured by the two software versions.[13] The thickness measured by version 5.6.4.0 was always subtracted from the thickness measured by version 6.0.7.0, meaning that a positive difference indicates that version 6.0.7.0 measured a thicker pRNFL. Mean differences in retinal layer thickness between the two versions were tested using the paired samples T-test. Proportional bias was tested by means of linear regression analyses. Linear regression was also used to test the association between the absolute difference in pRNFL thickness between the two versions and the number of averaged B-scans (expressed by the ART). Subgroup analyses were performed comparing the systematic bias in patients and HCs. Lastly, longitudinal changes in pRNFL thickness were calculated using both software versions. The difference in the amount of progressive atrophy between the two versions was tested using paired samples T-test. All analyses were performed for the mean thickness of the entire pRNFL (global mean) as well as the six individual sectors: temporal superior (TS), temporal (T), temporal inferior (TI), nasal inferior (NI), nasal (N) and nasal superior (NS). Statistical significance was set at $p<0.05$.

RESULTS

SUBJECT CHARACTERISTICS

For this cross-sectional study, 802 scans from 230 patients and 211 scans from 63 HCs were available for review. After quality control, 150 patient scans (18.7%) and 25 HC scans (11.8%) were rejected. This lead to the inclusion of 652 scans (353 baseline and 299 follow-up) from 213 MS patients and 186 scans (113 baseline and 73 follow-up) from 61 HCs. The baseline characteristics of these 213 patients and 61 HCs are shown in Table 1. Compared to patients HCs were younger (mean difference 3.6 years, p=0.001). Patients had a mean disease duration of 20.2 years and showed a considerable level of disability which is reflected in the median EDSS score of 4.0. Most patients had a relapsing remitting disease course. As expected patients showed considerably more atrophy of the pRNFL compared to HCs (mean difference 11.20 µm, p<0.001).

MEAN THICKNESS BY SECTOR

Table 2 shows the average of the global mean and the six separate sectors for all scans as calculated by both software versions. There was a significant difference for the global mean, with version 6.0.7.0 showing a thicker pRNFL compared to version 5.6.4.0 (mean difference 0.16 µm, p<0.001). The largest difference in mean thickness between the two versions was found for the NI and TI sectors (NI 0.29 µm, p<0.001; TI 0.43 µm, p<0.001). The N sector showed a significant difference as well, with a mean difference of 0.14 µm, p=0.015. On the contrary, there was no significant difference in mean thickness between the two software versions for the T, TS or NS sector. The median difference was 0.0 µm for the global mean as well as for all of the sectors.

Table 1: Baseline characteristics of the study cohort.

	Patients N = 213	**Healthy controls N = 61**	**p-value**
Age (years), mean (± SD)	53.8 (9.8)	50.2 (7.0)	0.001
Sex (female : male)	147 : 66	41 : 20	0.789
Disease duration (years), mean (± SD)	20.2 (6.9)	N/a	
EDSS, median [range]	4.0 [1.0 – 8.0]	N/a	
Type of MS		N/a	
RR	130 (61.0%)		
SP	56 (26.3%)		
PP	27 (12.7%)		
pRNFL thickness, mean (± SD)*	83.36 (7.65)	94.56 (14.14)	< 0.001

** measured with version 6.0.7.0*

SD = standard deviation; EDSS = expanded disability status scale; MS = multiple sclerosis; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive; pRNFL = peripapillary retinal nerve fiber layer

Table 2: Mean pRNFL thickness and proportional bias for the global mean and each of the six separate sectors as measured by the two software versions. Proportional bias was tested using linear regression analyses, resulting in regression coefficient β and corresponding p-value.

Sector	pRNFL thickness version 6.0.7.0 µm (± SD)	pRNFL thickness version 5.6.4.0 µm (± SD)	*p*-value	Proportional bias (β)	*p*-value
Global Mean	85.22 (13.87)	85.06 (13.83)	<0.001	0.003	0.245
Nasal Superior	92.55 (20.05)	92.47 (20.11)	0.114	-0.003	0.247
Nasal	63.68 (11.45)	63.55 (11.36)	0.015	0.006	0.120
Nasal Inferior	96.90 (23.15)	96.62 (23.24)	<0.001	-0.004	0.241
Temporal Inferior	124.37 (23.12)	123.94 (22.98)	<0.001	0.006	0.157
Temporal	59.60 (14.99)	59.62 (15.02)	0.402	-0.001	0.437
Temporal Superior	121.31 (21.50)	121.16 (21.32)	0.162	0.009	0.081

pRNFL = peripapillary retinal nerve fiber layer; SD = standard deviation

BLAND-ALTMAN PLOTS AND PROPORTIONAL BIAS

Figure 1 shows the Bland-Altman plot for the global mean of all scans combined. Patients are represented as blue dots, HCs as red triangles. The dotted lines represent the bias (mean difference) and 95% levels of agreement. As illustrated, most scans showed no or only a small difference of 1 or 2 µm. Nevertheless, a considerable part of the scans showed larger differences, with a maximum of up to 6 µm. There was no proportional bias (β = 0.003, p=0.245) for the global mean pRNFL thickness, meaning that the difference in thickness measured by the two segmentation software versions was independent of the thickness of the pRNFL.

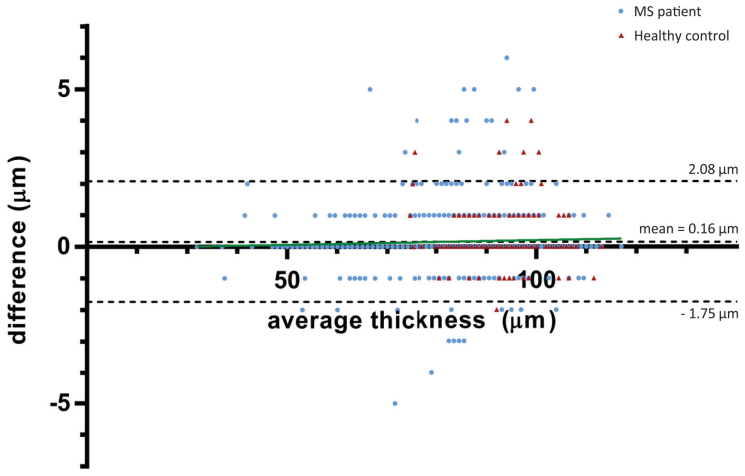


Figure 1: Bland-Altman plot for the global mean pRNFL thickness. The difference in pRNFL thickness between the two segmentation software versions (y-axis) is plotted against the average of the two measurements (x-axis). MS Patients (N = 652) are presented as blue dots and healthy controls (N = 186) as red triangles. The green line represent the regression line to test for proportional bias (β = 0.003, p=0.245). The middle broken line represents the mean difference (systematic bias) in pRNFL thickness for all scans combined. The top and bottom broken lines represent the 95% limits of agreement.

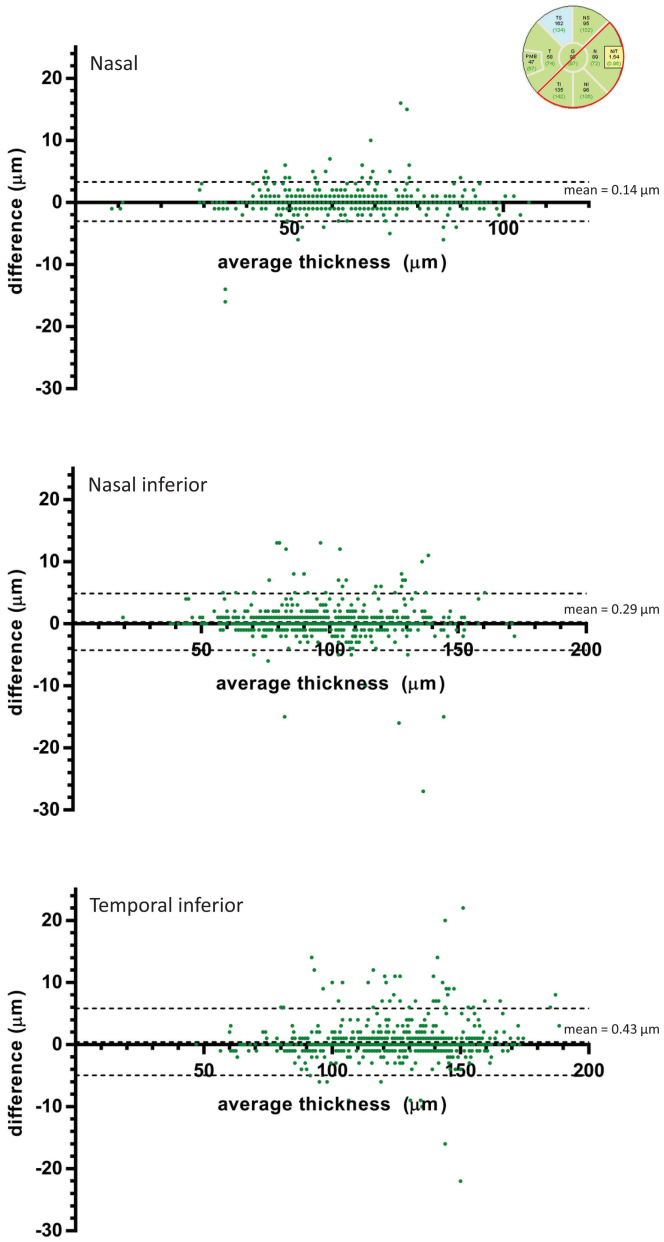


Figure 2: Bland-Altman plots of the three sectors that showed a significant difference in pRNFL thickness between the two segmentation software versions. The difference in pRNFL thickness between the two segmentation software versions (y-axis) is plotted against the average of the two measurements (x-axis). Data from all subjects (N = 838) is used. The middle broken line represents the mean difference (systematic bias) in pRNFL thickness for all scans combined. The top and bottom broken lines represent the 95% limits of agreement. Notice that the scale on the y-axis has been enlarged in comparison to Figure 1.

As described earlier, only the NI, TI and N sectors showed a significant difference in thickness measured by the two software versions. The Bland-Altman plots for these three sectors are displayed in Figure 2. All three sectors, and especially the NI and TI sectors, showed considerable variability in the difference in thickness measured, with outliers of up to -27 µm for the NI sector. This sector has a high density of retinal vessels. Again, there was no proportional bias for any of the sectors.

We also examined whether the systematic bias in pRNFL thickness measurement was different in patients compared to HCs. The patient scans showed a mean difference of 0.15 µm between the two versions and the HC scans showed 0.19 µm. This difference of 0.039 µm was statistically not significant (p=0.634).

The median ART of all scans combined was 31 with a range of 1 - 100. There was no significant association between the amount of B-scan averaging as measured by the ART and differences in pRNFL thickness measurements for the global mean (β = -0.001, p=0.451). The N and T sectors did however show a small, yet significant, inverse association (N β = -0,003, p=0.047; T β = -0.002, p=0.008). The other four sectors did not show any significant associations.

THE EFFECT ON LONGITUDINAL ATROPHY MEASUREMENTS

A total of 178 MS patients completed the two year follow-up. Using the initial software version (5.6.4.0) for both the baseline and follow-up visits, these patients showed a decline in pRNFL thickness of -0.95 µm (95%CI -0.67 – -1.22, p<0.001). If the later software version was used (6.0.7.0), the amount of atrophy was slightly less, -0.71 µm (95%CI -0.46 – -0.96, p<0.001). This difference of 0.18 µm was statistically significant (p=0.006).

The amount of atrophy in the (hypothetically) most extreme case was also calculated. In this scenario, where the baseline scans would be segmented with version 5.6.4.0 and the follow-up scans with version 6.0.7.0 the amount of atrophy that the patients showed was -0.63 µm (95%CI -0.37 – -0.89, p<0.001). This was significantly lower than the amount of atrophy when all scans were segmented with version 5.6.4.0 (mean difference 0.32 µm, p<0.001) but not when they were all segmented with version 6.0.7.0 (mean difference 0.08 µm, p=0.136).

DISCUSSION

This study shows that there is a significant, systematic difference in the pRNFL thickness measurement between two subsequent software updates of the retinal layer segmentation algorithm provided by the Heidelberg Spectralis OCT. Although the mean difference was relatively small (yet significant), the difference between the two algorithms in individual scans could be as high as 6 µm for the global mean pRNFL thickness. This last finding is an important one if we plan on using OCT as a surrogate marker for neurodegeneration in individual patients. The expected rate of pRNFL atrophy in MS patients is about 1-2 µm/year[5,14,15] and is even lower in patients with a longer disease duration.[16] A large part of our scans showed a degree of differ-

ence in pRNFL thickness measurement between the two versions that could mask or overestimate the true pRNFL atrophy, which is presumed to be caused by retrograde trans-synaptic degeneration.[17] In addition, the study shows that using different software versions does in fact influence longitudinal atrophy measurements. To our knowledge, this is the first study that has investigated this methodological issue. The findings are relevant and should be considered by regulatory authority for approval of OCT segmentation software. Producers will need to be aware of the need to potentially update their segmented normative databases.

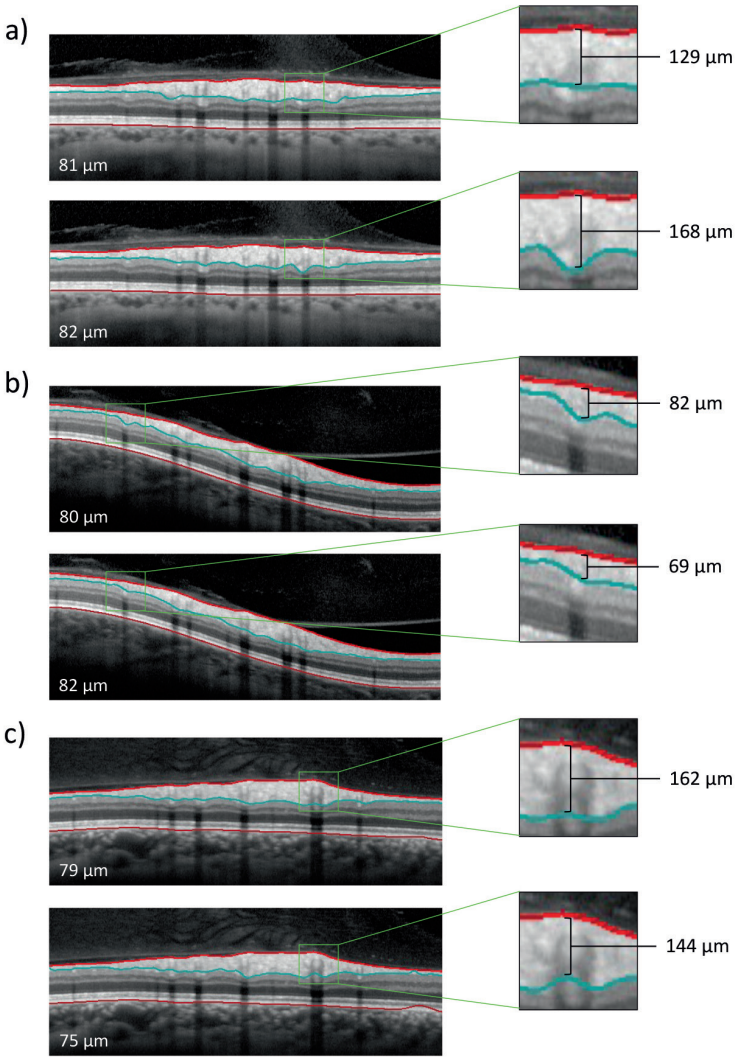


Figure 3: Three examples of differences in segmentation between the two software versions. The top image is segmented with version 5.6.4.0 and the bottom image with version 6.0.7.0. The mean thickness of the entire peripapillary retinal nerve fiber layer (global mean) is given in white.

Current Spectralis OCT machines provide pRNFL thickness measurements directly after scanning. This process is done automatically and according to the last installed segmentation algorithm. However, even when the follow-up scanning function is used, the previous (baseline) scan retains its previous segmentation. So, if the OCT device gets updated with a new version of the segmentation algorithm in between patient assessments, a measurement error can be introduced easily. It is therefore important to make sure that the baseline as well as each subsequent follow-up scan is segmented by the same software version. In the case of the Heidelberg Spectralis OCT this can be done quite easily and quickly by opening the previous scans and intentionally segmenting them again. In our experience, it is, in most scans, visible that the line between the RNFL and ganglion cell layer replaces when the scan is re-segmented. In contrast, the line indicating the inner limiting membrane remains very stable. For illustration, Figure 3 shows three examples of (obvious) differences in segmentation between the two software versions. All three scans were of high quality and the segmentation would be accepted in all cases. Nevertheless, there was a difference in pRNFL thickness between the two software versions.

There are other factors that can influence pRNFL thickness measurement and these need to be considered as well. Two of these factors are interobserver and intraobserver variability. However, it has been shown that both the inter- as well as intraobserver variability are generally high when only those scans that have passed the OSCAR-IB criteria are selected, as is the case in our study.[18] One specific confounder that is especially relevant in longitudinal studies is off-axis beam placement, which can introduce a large error.[19] To our knowledge, the potential error of off-center beam placement is not dependent on the software version.

One of the strengths of this study is the statistical robustness due to the large sample size. We chose to analyze scans from both patient as well as HCs. One could argue that patient scans are generally of poorer quality (due to problems with fixation for example) and are thus more prone to (small) segmentation errors leading to a difference in thickness measurements. However, we could not find a significant difference in the systematic bias between patient scans and HC scans. Although the ART did not affect the difference in global pRNFL thickness, there were small but significant associations for the N and T sectors, which may indicate that higher ART is associated with a smaller difference between the software versions. However, as all scans were of sufficient quality, we cannot say with certainty that the differences in segmentation occur more easily with low quality scans. Another strength of this study is that we performed OSCAR-IB quality control. We only included those scans that would pass the quality control if they were to be used in any other study or clinical practice.

A limitation of this study is the fact that we did not investigate whether this problem also occurs with OCT machines and segmentation software of other manufacturers. A recent meta-analysis by Petzold et al. showed that of all the studies investigating pRNFL atrophy in MS patients with and without optic neuritis, seventeen have been performed with the Spectralis OCT, fourteen with the Cirrus OCT by Carl Zeiss Meditec and four with the 3D-OCT-1000 / 2000

by Topcon Corporation.[20] A literature review on longitudinal SD-OCT studies revealed that six have been performed with the Spectralis OCT[16,21-25] and five with the Cirrus OCT.[15,26-29] It is plausible that this problem might occur with these OCT devices as well, but this still remains to be investigated and these groups are well placed to investigate this point.

In conclusion, this study has demonstrated that updates in pRNFL segmentation software lead to a significant difference in pRNFL thickness measurement, especially when analyzing individual patient scans. Therefore, we recommend that all scans are segmented with the same software version when assessing pRNFL atrophy in a longitudinal setting.

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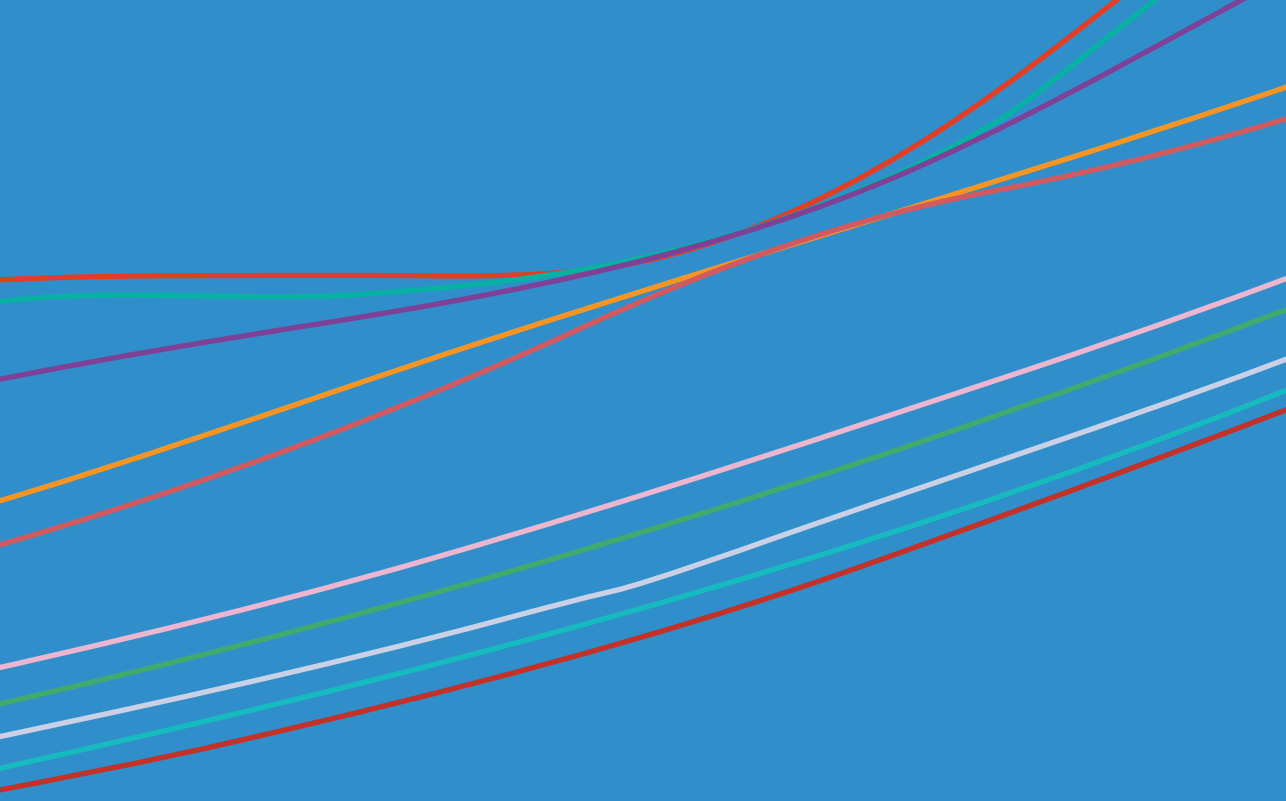
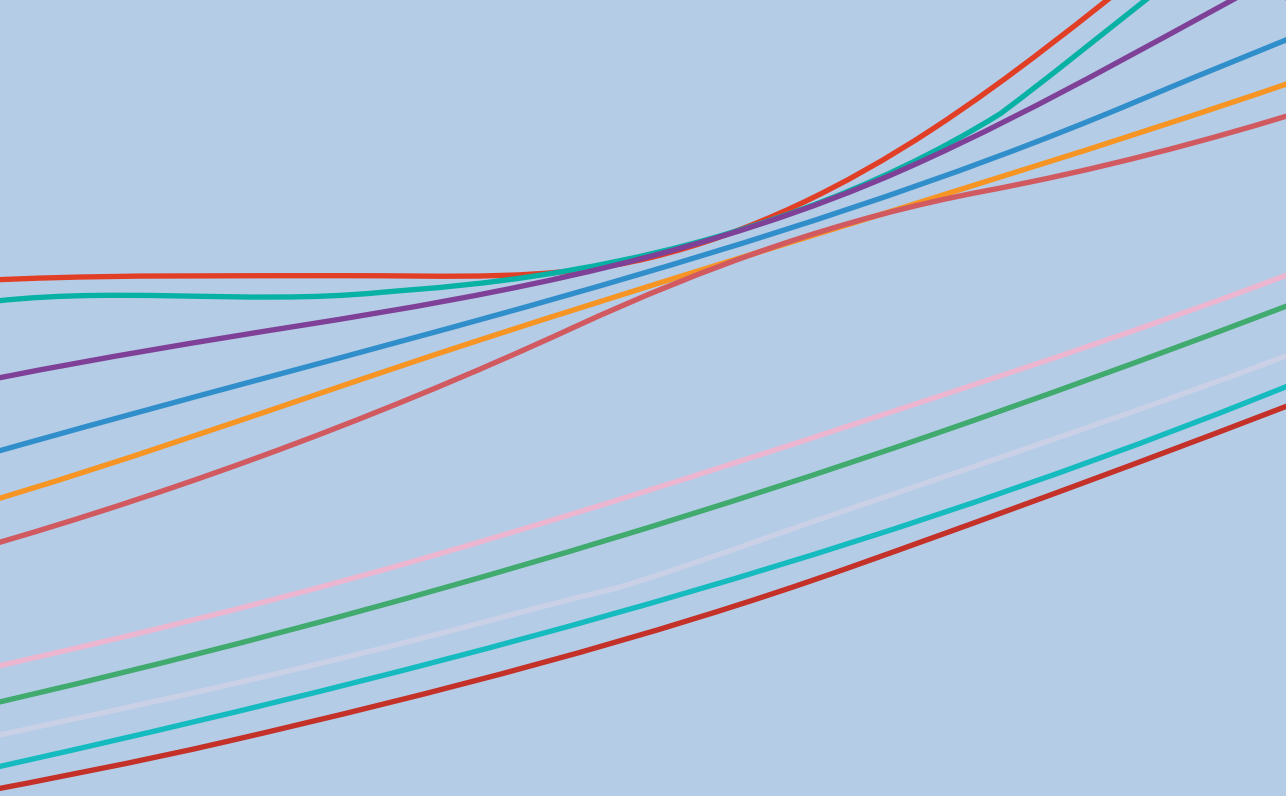


CHAPTER 3

| OCT AND THE VISUAL SYSTEM IN MS



CHAPTER 3.1

DIAGNOSTIC ACCURACY OF OPTICAL COHERENCE TOMOGRAPHY INTER-EYE PERCENTAGE DIFFERENCE FOR OPTIC NEURITIS IN MULTIPLE SCLEROSIS

D Coric, LJ Balk, BMJ Uitdehaag, A Petzold

European Journal of Neurology 2017;24:1479-1484

ABSTRACT

Background: Multiple sclerosis (MS) associated optic neuritis (MSON) causes atrophy of the inner retinal layers, which can be quantified by optical coherence tomography (OCT). It has been suggested that the Inter-Eye Percentage Difference (IEPD) of atrophy may be of diagnostic value in MSON.

Methods: Prospective, cross-sectional study in MS patients and healthy controls (HC). Spectral-domain OCT of both eyes was performed, followed by automated retinal layer segmentation of the peripapillary retinal nerve fibre layer (pRNFL) and macular ganglion cell and inner plexiform layer (mGCIPL). Receiver Operator Characteristics Curves were plotted and the area under the curve (AUC) was calculated for group comparisons of the IEPD of the pRNFL and mGCIPL.

Results: There were 39 patients with bilateral MSON, 62 patients with unilateral MSON, 106 patients without MSON and 63 HC. Diagnostic accuracy (AUC) of the IEPD was 0.73-0.86 for the pRNFL and 0.75-0.94 for the mGCIPL. The diagnostic sensitivity of the mGCIPL IEPD was 70% with a specificity of 97% for distinguishing unilateral MSON from HC. For the comparison of bilateral MSON with HC, sensitivity was 86% with a specificity of 97%.

Conclusion: The IEPD of the pRNFL and more so the IEPD of the mGCIPL is an useful diagnostic measure for MSON. The IEPD is a dimensionless unit and may therefore contribute to overcome device and proprietary segmentation algorithm limitations.

INTRODUCTION

The intimate relationship between optic neuritis (ON) and multiple sclerosis (MS) has long been recognised.[1] In fact, one of the most frequent clinically isolated syndromes remains MS associated ON (MSON).[2] The diagnosis for both conditions has been clinical[3,4] and in the case of MS is based on finding evidence for dissemination in space (DIS) and dissemination in time (DIT).[5] With the increasing recognition of the diagnostic sensitivity of MRI for DIS and DIT the need for clinical evidence has declined in the past two decades.[6-8]

There are cases however, either in clinical or research setting, in which there is a need to establish the presence of a previous (subclinical) episode of MSON but can be challenging. Clinically recommended,[9,10] the art of using an ophthalmoscope in practice has declined with emergence of new diagnostic techniques such as optical coherence tomography (OCT). In recognition of this development it was proposed to use a 20% inter-eye percentage difference (IEPD) of the peripapillary retinal nerve fibre layer (pRNFL) as a diagnostic criterion for MSON.[10] There are several advantages to a dimensionless variable such as the IEPD, one of which is that it is less likely that differences between devices and segmentation algorithms will matter. Because of class III evidence only for recommended consensus of a 20% IEPD cut-off of the pRNFL on OCT this study aimed to define optimised cut-off ranges for the IEPD of the pRNFL and combined ganglion cell and inner plexiform layer (mGCIPL) for making a diagnosis of MSON.

METHODS

This study was approved by the Medical Ethical Committees of the VU University Medical Center in Amsterdam, the Netherlands and is in accordance with the 1964 Declaration of Helsinki. Written informed consent was obtained from all subjects before study inclusion.

STUDY DESIGN AND PATIENT POPULATION

Patients with MS[8] and healthy control subjects (HC) were enrolled from the VU University Medical Center Amsterdam (VUmc).

All subjects underwent clinical and OCT assessments, with both assessments taking place on the same day. The assessment of MSON was based on medical history and always preceded the OCT measurement, making sure that the physician who established the diagnosis of MSON did not have the OCT data available at the time of assessment. Disease duration was defined as the time from the first MS symptoms. The expanded disability status scale (EDSS) was obtained by a certified examiner. As potential swelling of the pRNFL during the acute stages of MSON may confound OCT measurements, patients were excluded if they had experienced symptomatic MSON three months prior to either OCT measurement, according to a consensus protocol.[10] Patients were classified into those without MSON (nonMSON), unilateral MSON and bilateral MSON.

OPTICAL COHERENCE TOMOGRAPHY

Spectral Domain OCT (SD-OCT, Spectralis, Heidelberg Engineering, Heidelberg, Germany) was performed in all subjects as described previously.[11] Scans were excluded from the analyses if manual correction for algorithm segmentation failures was not possible or if they did not meet the remaining six quality control criteria (OSCAR-IB).[12] This led to a rejection rate of 12.8%.

STATISTICAL ANALYSES

Data were assessed for normality using the Shapiro-Wilk test followed by graphical inspection in SAS (version 9.4). According to distribution, non-parametric (Mann Whitney U-test) or parametric (independent sample T-test) tests were used for comparison of two groups or ANOVA and GLM followed by post-hoc analyses for comparison of more than two groups. For OCT data, all analyses performed on eye-level (two separate eyes per subject) were done using generalized estimation equations (GEE), with an exchangeable correlation matrix and adjustments for intra-subject inter-eye correlations. Analyses for OCT and visual acuity (VA) data averaged from both eyes were done by GLM. Additional adjustments for confounding factors such as age, sex and disease type were performed as indicated. The IEPD was calculated for the pRNFL and mGCIPL for each patient. Receiver Operator Characteristics (ROC) curves were used to calculate the Area Under the Curve (AUC) to describe the level of diagnostic accuracy.[13] The diagnostic value was rated as 'no or low discriminatory power' for an AUC 0.5-0.7, as of 'moderate discriminatory power' for an AUC of 0.7-0.9 and of 'high discriminatory power' for an AUC >0.9.[14] The ROC were plotted to determine graphically optimised IEPD cut-off values as the shortest distance from the top left corner to the ROC curve.[15] p-values of <0.05 were accepted as significant.

RESULTS

In total 296 subjects were assessed. Of these 26 had to be excluded because a diagnosis of previous MSON could not be established with certainty or because of visual symptoms due to Uhthoff's phenomenon or eye movement problems.[10] The overall prevalence of MSON was 48.8%. The highest prevalence was found in patients with a secondary progressive (SP) disease course, followed by those with a relapsing remitting (RR) disease course. In patients with SP MS a previous episode of MSON was established in the right eye of 11/55 (20.0%), in the left eye of 9/55 (16.4%) and bilateral in 13/55 (23.6%). In patients with RR MS a previous episode of MSON was established in the right eye of 20/123 (16.3%), in the left eye of 21/123 (17.1%) and bilateral in 24/123 (19.5%). Of the patients suffering from primary progressive MS 26/29 (89.7%) had no history of MSON, but there was convincing evidence for MSON in 3 patients. The characteristics of the patients with and without MSON and HC are summarised in Table 1.

The HC were younger compared to patients without MSON (p=0.0006), but there was no age difference between HC, uni- or bilateral MSON (p>0.05). Likewise, there was a difference in disease duration between groups (p=0.023), which was longer in patients with bilateral MSON compared to those without MSON (p=0.008). The VA averaged from both eyes was worse in pa-

tients with bilateral MSON compared to patients with unilateral MSON (p=0.0006) or nonMSON (p=0.024). There was no significant difference between groups for the EDSS (Table 1).

Table 1: Characteristics of the study cohort. The mean ± SD, median (interquartile range) are shown.

		Patients with MS		
	HC	**Non-MSON**	**Unilateral MSON**	**Bilateral MSON**
N	63	106	62	39
Age (years)	50.5 ±7.2[a]	55.6 ±10.1[a]	53 ±9.5	52.7 ±9.7
Gender (F:M)	41:22	66:40	45:17	27:12
Disease type (N)	N/a			
RR MS		58	41	24
SP MS		22	20	13
PP MS		26	1	2
Disease duration (years)	N/a	19.7 ±7.0[b]	21.0 ±6.8	22.0 ±7.0
DMT (past/present)	N/a	33[c]	33[d]	21[e]
EDSS	N/a	4 (3-7)	4 (3-6)	4 (3-6)
VA[f]	N/a	0.85 ±0.17[g]	0.82 ±0.16	0.74 ±0.21
pRNFL thickness[f] (µm)	91.7 ±6.6[h]	85.9 ±10.0	81.2 ±9.8	75.0 ±9.5
mGCIPL thickness[f] (µm)	94.2 ±5.6[i]	84.8 ±13.3	76.8 ±14.8	72.7 ±16.6
pRNFL IEPD (%)	2.0 (3.0)	3.0 (4.0)	7.5 (9.0)	6.5 (10.0)
mGCIPL IEPD (%)	1.0 (1.0)	4.0 (7.0)	14.0 (17.5)	10.5 (19.0)

a Overall age difference between groups (p=0.006) with post-hoc analyses indicating patients with nonMSON being significantly older than healthy control subjects (p=0.0006). There was no significant difference in age between HC, unilateral MSON and bilateral MSON for each comparison.
b Overall difference of disease duration between groups (p=0.023) with post-hoc analyses indicating patients with bilateral MSON having a longer disease duration compared to patients with nonMSON (p=0.008).
c 33 patients with nonMSON were or had been previously on DMT of which 4 GA, 3 FTY, 22 IFN, 4 NTZ.
d 33 patients with unilateral MSON were or had been previously on DMT of which 1 AZT, 5 GA, 20 IFN, 2 MTX, 1 TER, 4 NTZ.
e 21 patients with bilateral MSON were or had been previously on DMT of which 1 ATM-027, 3 GA, 15 IFN, 1 MTX, 1 NTZ.
f The mean values from both eyes are shown in this table and used for statistical analyses.
g There was a significant group difference in the VA (p=0.027) with post-hoc analyses revealing a significantly poorer VA in patients with bilateral MSON compared to patients without MSON (p=0.0006) or patients with unilateral VA (p=0.024).
h There was a significant group difference of the pRNFL (p<0.001) with post-hoc analyses revealing significant difference for each paring of group comparisons. p<0.0001 for bilateral MSON vs HC and nonMSON; for HC vs unilateral MSON. p<0.001 for nonMSON vs HC. p<0.01 for bilateral MSON vs unilateral MSON; for unilateral MSON vs nonMSON.
i There was a significant group difference of the mGCIPL (p<0.001) with post-hoc analyses revealing significant difference of p<0.0001 for bilateral MSON vs HC and nonMSON; for HC vs unilateral MSON and nonMSON. p<0.001 for nonMSON vs unilateral MSON.
HC = healthy controls, MSON = multiple sclerosis associated optic neuritis, RR = relapsing remitting, SP = secondary progressive, PP = primary progressive, DMT = disease modifying therapy, EDSS = expanded disability status scale, VA = visual acuity, pRNFL = peripapillary retinal nerve fibre layer, mGCIPL = macular ganglion cell and inner plexiform layer, AZT = azathioprine, 027 = ATM-027, FTY = fingolimod, MTX = mithoxantrone, GA =glatriamer acetate, IFN = interferon beta 1a and 1b, TER = teriflunomide, NTZ = natalizumab.

INTER-EYE PERCENTAGE DIFFERENCES (IEPD)

The results of the ROC curves for the IEPD are summarised in Table 2. The AUCs and optimised cut-off values are presented for the IEPD of the pRNFL and mGCIPL. For each group comparison the optimised IEPD cut-off values were below 10%. The highest AUC (0.94) was found for the mGCIPL for the comparison of patients with bilateral MSON and HC with a cut-off as small as 5% inter-eye difference.

The IEPD of either the pRNFL or mGCIPL was of no diagnostic value for comparing unilateral with bilateral MSON. Whilst the IEPD of the mGCIPL did distinguish between HC and nonMSON (AUC 0.77) the IEPD of the pRNFL did not (0.56).

For further illustration the raw data for the IEPD of the pRNFL and mGCIPL are shown in Figure 1. The IEPD was highly significantly ($p<0.0001$) larger in patients with either unilateral or bilateral MSON compared to patients with nonMSON or HC. The IEPD for the mGCIPL but not the IEPD for the pRNFL was significantly ($p<0.01$) higher in patients with nonMSON compared to HC.

DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF IEPD

The diagnostic sensitivity and specificity levels of the IEPD were calculated for those comparisons where the AUC indicated a 'high discriminative power' (AUC >0.9).[15] This was the IEPD for the mGCIPL comparing patients with unilateral and bilateral MSON to HC (see Table 2). The ROC optimised cut-off value was compared to the consensus cut-off value of 20%.[10]

Using the consensus cut-off value of 20%, the diagnostic sensitivity of the IEPD for the mGCIPL for unilateral MSON compared to HC was 34% with a specificity of 100%. Using the optimised cut-off value of 6% (see Table 2), this changed to a sensitivity level of 70% and specificity level of 97%.

Table 2: Results of the ROC curve analyses giving the Area Under the Curve (AUC) and optimised IEPD cut-off values. The grey shaded cells are for the peripapillary retinal nerve fiber layer and the white cells for the macular ganglion cell and inner plexiform layer.

IEPD	MSON			
	HC	**Non**	**Unilateral**	**Bilateral**
HC	-	0.56 (4%)	0.86 (9%)	0.78 (4%)
Non	0.77 (3%)	-	0.81 (5%)	0.73 (6%)
Unilateral	0.91 (6%)	0.75 (9%)	-	0.55 (10%)
Bilateral	0.94 (5%)	0.77 (9%)	0.50 (9%)	-

IEPD = inter-eye percentage difference, HC = healthy controls, MSON = multiple sclerosis associated optic neuritis.

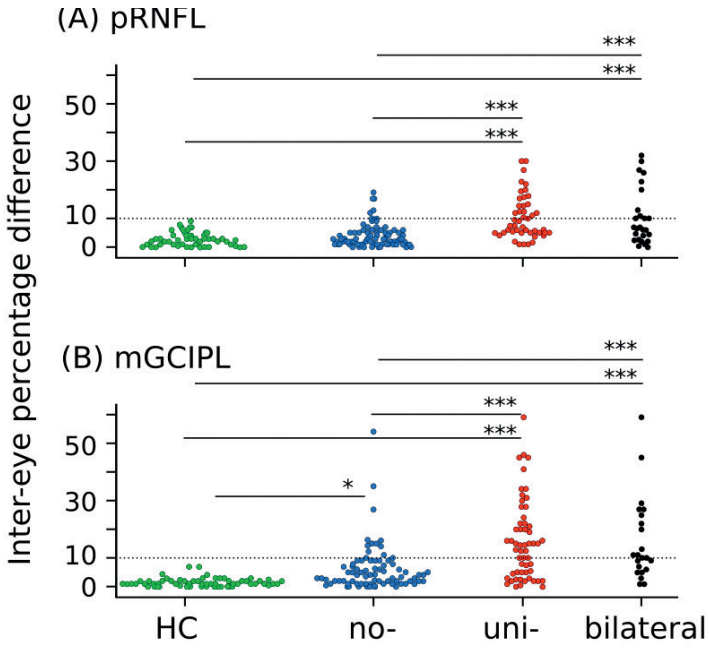


Figure 1: Inter-eye percentage difference (IEPD) for the peripapillary retinal nerve fibre layer (pRNFL) and macular ganglion cell and inner plexiform layer (mGCIPL) are shown per subject and per group. Levels of significance are indicated as p<0.0001 (***) and p<0.01 (*).
HC = healthy controls

Using the consensus cut-off value of 20%, the diagnostic sensitivity of the IEPD for the mGCIPL for bilateral MSON compared to HC was 36% with a specificity of 100%. Using the optimised cut-off value of 5% (see Table 2) this changed to a sensitivity level of 86% and specificity level of 97%.

DISCUSSION

This study showed that the IEPD of the pRNFL and the IEPD of the mGCIPL were of diagnostic value for MSON. Detailed ROC analyses suggest that the published consensus on a 20% inter-eye difference for the pRNFL[10] was overly conservative. While inter-eye analysis of OCT data is not new, previous studies have looked at the absolute difference between the two eyes[16,17] and the 20% IEPD consensus for the pRNFL is based on expert opinion only. There are yet no published data on the inter-eye percentage difference for the mGCIPL. The present study suggests that a 5% IEPD of the mGCIPL is of excellent diagnostic sensitivity and specificity for bilateral MSON in the setting investigated.

These findings are relevant because one advantage of the IEPD is that the dimensionless value may help to overcome limitations to pool data from different devices and segmentation algorithms. As many new OCT devices enter the market adding to heterogeneity between center equipment,[18] there is a need to start thinking about strategies to facilitate future data analyses.

The IEPD could potentially be a valuable secondary outcome measure in ON treatment trials, pending independent validation.

There are statistical advantages to the IEPD too. As correctly pointed out, the statistical analyses of OCT data needs to consider inter-eye interactions.[19] Consequently, a concerted effort developed OCT reporting guidelines outlining the use of GEE of binocular OCT data.[20] The robustness of the model is a strength, but does not lend itself for modelling of associations with single outcome variables.[11] Clinical outcome measures such as the EDSS, Multiple Sclerosis Functional Composite, cognition, relapse rate and treatment response are all relevant.[21] Statistical evaluation of these data makes use of multivariate Cox models, Kaplan-Meyer curves, the many flavours of GLM, forms of multimodal modelling, principal component analysis and many more[22,23] to which the IEPD may have advantages over device and eye dependent absolute values of OCT data.

Finally, the IEPD may overcome present exclusion criteria due to high hypermetropia or myopia as well as ethnic retinal layer thickness differences and thickness variations due to physiological variability.

A likely limitation of the IEPD will be a low diagnostic specificity for separating MSON from other forms of ON such as isolated optic neuritis, chronic relapsing isolated optic neuropathy and neuromyelitis optica ON. None of these patient groups were investigated in the present study, which is a limitation of the study.

Another limitation of the present study is that the IEPD has not been tested in parallel on different devices. With the advent of many new OCT devices,[18] testing this will be resource demanding. The IEPD is also unlikely to overcome pertinent quality control issues.[12,20]

There were two unexpected findings in this study. First, the IEPD of the mGCIPL was of high diagnostic accuracy for bilateral MSON as well as for the expected unilateral MSON. Second, the IEPD of the mGCIPL was superior to the IEPD of the pRNFL. There is a likely anatomical explanation for both results. The large variability of the optic disc appearance between patients will cause a larger degree of variability of pRNFL data. In contrast, the more homogeneous appearance of the macula lutea will make the mGCIPL data more consistent between eyes and patients. It is well known that MSON can cause any type of visual field defect, the anatomical basis for which is loss of retinal axons and their ganglion cells.[2,10] With bilateral MSON the visual field defect is frequently asymmetric between eyes. Therefore the diffuse, asymmetric damage caused is likely to affect the IEPD of the mGCIPL independent to whether the damage was due to unilateral or bilateral MSON.

CONCLUSION

This study suggest that the IEPD of the pRNFL and the IEPD of the mGCIPL may prove a useful variable for interpretation of OCT data. Advantages of the IEPD are simplicity for clinical practise, the possibility to become a cross OCT device measure, the suitability for many statistical models on established MS outcome measures and a potentially robust and useful secondary outcome measure for treatment trials. There is a need for further validation of the IEPD.

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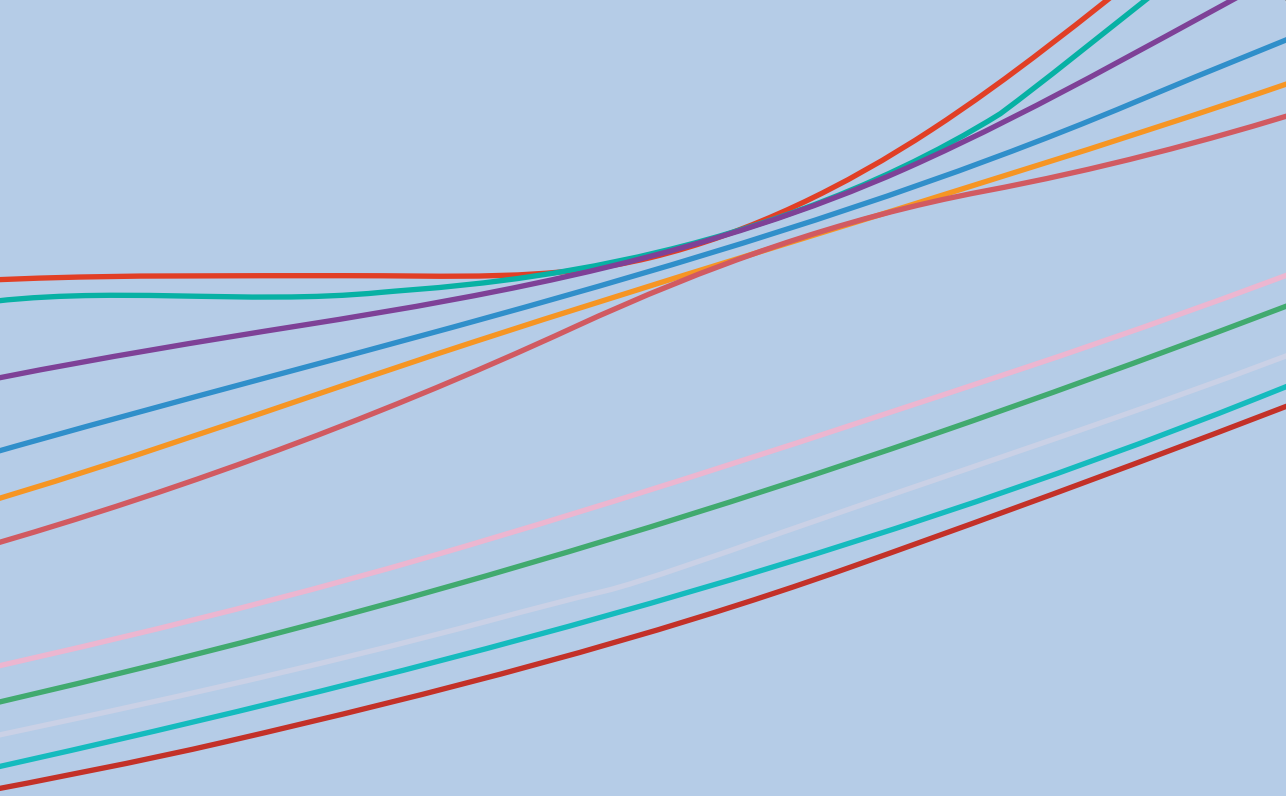

CHAPTER 3.2

RETINAL ATROPHY IN RELATION TO VISUAL FUNCTIONING AND VISION-RELATED QUALITY OF LIFE IN PATIENTS WITH MULTIPLE SCLEROSIS

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ABSTRACT

Background: Inner retinal layer atrophy in patients with Multiple Sclerosis (MS) has been validated as a structural imaging biomarker for neurodegeneration.

Objective: To determine how retinal layer thickness relates to high (HC) and low contrast (LC) visual acuity (VA) and vision-related quality of life (QoL) and to investigate the effect of previous episodes of optic neuritis (MSON).

Methods: Spectral-domain OCT was performed in 267 patients with MS. Images were segmented for the peripapillary retinal nerve fibre layer (pRNFL) and the macular ganglion cell inner plexiform layer (GCIPL). Ophthalmological evaluations included history on MSON, HCVA, LCVA and vision-related QoL.

Results: Independent of MSON, HCVA and LCVA were significantly associated with pRNFL and GCIPL thickness. Vision-related QoL was positively associated with pRNFL (β=0.92, p=0.06) and GCIPL (β=0.93, p=0.02) thickness. These associations were independent of MSON. Not only binocular, but also monocular atrophy of the inner retinal layers was associated with lower vision-related QoL.

Conclusion: This study showed that retinal atrophy has a significant impact on visual functioning in patients with MS. OCT may therefore give useful insight in patients with visual dysfunction and our findings support including OCT and vision-related QoL measures into optic neuritis treatment trials.

INTRODUCTION

Atrophy of the inner retinal layers, as observed with the use of optical coherence tomography (OCT) is a common observation in multiple sclerosis (MS) patients with a history of MS associated optic neuritis (MSON). However, even in patients without a history of MSON, substantial thinning of the inner retinal layers is observed.[1] This retinal atrophy is thought to be caused by retrograde trans-synaptic degeneration,[2,3] although other mechanisms, such as local microinflammatory processes in the optic nerve,[4] have also been suggested. Retinal atrophy has shown to be associated with clinical disability,[1,5,6] gray and white matter atrophy[7,8] and possibly also cognitive functioning.[9] Although SD-OCT has been suggested as a structural outcome measure for neuroaxonal degeneration,[5] it should be noted that the clinical meaningfulness of this outcome is not always selfevident. A large proportion of patients with MS (up to 80%) will experience visual disability at some point during their course of disease. The most common being MSON, decreased high-contrast visual acuity (HCVA) and low-contrast visual acuity (LCVA) and eye movement disorders.[10-12] This poor visual functioning has a major impact on quality of life (QoL) as good visual function is highly valued by patients. Importantly, Heesen *et al.* demonstrated that MS patients reported visual functioning as the second most important body function affecting QoL, after lower limb function.[13] Despite this, the visual system is not generally included as outcome measure. Even the commonly used MS functional composite does not include an objective assessment of visual functioning.[14]

Previous studies have investigated the relationship between peripapillary retinal nerve fibre layer (pRNFL) thickness and visual dysfunction,[15-17] or general measures of QoL[18] in patients with MS. The assessment of general QoL in patients with MS is however strongly influenced by the mobility of the patient. The vision-related QoL measures the specific influence of visual disability and visual symptoms on different QoL domains, and is therefore not biased by ambulation or other non-visual symptoms. These visionspecific measures may provide information on the clinical meaningfulness of retinal atrophy. Therefore, the objective of this study was to investigate how inner retinal layer thickness relates to LCVA, HCVA and vision-related QoL and to determine whether previous MSON affects this relationship.

METHODS

STUDY DESIGN AND PATIENT POPULATION

For this observational cross-sectional study, patients were enrolled from the Amsterdam MS Cohort (MS Center Amsterdam, VU University Medical Center, the Netherlands). This study was approved by the Medical Ethical Committee on Human Research of the VU University Medical Center in Amsterdam, the Netherlands. Written informed consent was obtained from all subjects before study inclusion.

All included subjects were diagnosed with clinically definite MS following the revised McDonald criteria[19] and were part of an ongoing observational cohort study (the Amsterdam MS Cohort) of which previous assessments have been described.[20-22] All subjects were required to be between 18 and 80 years of age and had a diagnosis of either a relapsing remitting (RR), secondary progressive (SP) or primary progressive (PP) disease course at the time of their assessment.[23] Patients were excluded if they fulfilled any of the following criteria: pregnancy; received a course of steroids or had a relapse within six weeks prior to inclusion; HIV or other immunodeficiency syndrome or history of substance abuse (drug or alcohol). Patients were also excluded if they had experienced symptomatic MSON within six months preceding the OCT assessment, because thickening of the pRNFL during the acute stages of MSON may confound the OCT measurement. All assessments (clinical, OCT and questionnaires) were performed on the same day.

SD-OCT

Spectral domain OCT (SD-OCT, Spectralis, Heidelberg Engineering, Heidelberg, Germany) was performed in all subjects, with eye-tracking function enabled. All OCT scans were obtained at the same site, by three different experienced technicians. Room light conditions were dimmed and pupil diameter was sufficient for obtaining high quality OCT images, such that pharmacological pupil dilation was not required in any of the cases. Data on global pRNFL thickness (μm) was obtained using a 12° ring scan, manually placed around the optic disc. Data on the mean GCIPL thickness (μm) in the macular area was acquired using a macular volume scan (20×20° field, 49 B-scans, vertical alignment) centered on the fovea, averaging thickness for all but the central sector of the 1, 2.22, 3.4 mm grid. Automated segmentation of the pRNFL and GCIPL was performed (Heidelberg Engineering, software version 1.9.10.0). Scans were excluded from the analyses if they did not fulfill the revised quality control criteria (OSCAR-IB).[24]

CLINICAL AND OPHTHALMOLOGICAL OUTCOME MEASURES

Disease duration was defined as the time from the first MS symptom. The Expanded Disability Status Scale (EDSS)[25] was obtained by a certified examiner.

The assessment of history of symptomatic MSON was based on medical history, according to a standard protocol.[26] Visual acuity was tested using Sloan letter charts (100% for HCVA, 2.5% for LCVA),[27] placed on a retro-illuminated cabinet, at a 2m distance. Each eye was tested individually on both contrast levels, with best possible correction for refractive errors. VA scores were quantified as the number of letters correctly read by the patient.

Vision-related QoL was assessed using the National Eye Institute Visual Function questionnaire (NEI-VFQ-25), which is a validated tool to assess self-reported visual disability and vision-targeted health status.[28] The NEI-VFQ-25 is widely used in ophthalmological research and has shown to be sensitive and useful in patients with MS.[29] The NEI-VFQ-25 consists of 25 vision targeted questions representing 11 vision-related constructs (global vision rating, difficulty with near vision activities, difficulty with distance vision activities, limitations in social functioning

due to vision, role limitations due to vision, dependency on others due to vision, mental health symptoms due to vision, driving difficulties, limitations with peripheral vision, limitations with colour vision and ocular pain). Next, all 11 sub-scores are converted to a 0-100 scale and overall composite score is calculated by averaging the weighted subscale scores. This overall score is therefore also on a scale from 0 (lowest possible score) to 100 (highest possible score).[30]

STATISTICAL ANALYSES

Linear regression analyses were used to analyze group differences and associations with clinical outcome measures assessed on patient level. When data on visual acuity was analyzed on patient-level, the *mean* value of both eyes was used, in accordance with the APOSTEL guidelines.[31] Vision-related QoL was compared between groups with the non-parametric Mann-Whitney-U test. Generalized estimating equations (GEE), with an exchangeable correlation matrix and adjustments for intra-subject inter-eye dependency were used for analyses and comparisons when the outcome was assessed on eye-level (retinal thickness, VA). All linear regression and GEE analyses were additionally adjusted for relevant confounders (age, sex, disease duration, history of MSON, use of disease modifying treatment, VA) as indicated.

In order to investigate the effect of binocular, monocular and no retinal atrophy on vision-related QoL, patients were divided in three groups, based on the level of atrophy in each eye (group 1: patients with binocular atrophy, group 2: patients with monocular atrophy and group 3: patients with no retinal atrophy in either eye). The presence of retinal atrophy was defined as a pRNFL thickness ≤ 75 µm, which was based on the findings of Costello *et al.*, who demonstrated a threshold of pRNFL thickness of 75 µm as the "point of no return" for predicting visual recovery after optic neuritis.[32] There is no published cut-off for the GCIPL. For this reason we decided to use the same percentile corresponding to a pRNFL of less than 75 µm, which corresponded to the 25th percentile, for the GCIPL. The 25th percentile for the GCIPL thickness resulted in a cut-off of ≤68 µm. Patients with missing data for at least one eye were excluded from these sub-analyses. Groups were compared using linear regression analyses with dummy variables. Statistical analyses were performed using SPSS V.22.0, with a two-sided statistical significance level of 0.05.

RESULTS

DESCRIPTIVES

A total of 267 patients with MS were included in this cross-sectional observational study. Demographic and clinical characteristics of all included MS patients, and stratified by history of MSON are shown in Table 1. In order to avoid the introduction of noise by pooling MSON eyes and MSNON eyes within the same patient, only patients with the same history of both eyes (i.e. bilateral MSON and bilateral MSNON) were included in the right part of Table 1. Patients had a mean disease duration of 19.1 years (±7.4), but this differed between disease types, as SPMS and PPMS patients (22.6±8.5 and 23.1±7.7 years respectively) had a considerable longer disease duration compared to the RRMS patients (17.7±6.5).

Table 1: Demographic and clinical characteristics of all included MS patients, and stratified by history of MSON.

	All subjects	Subjects with MSNON	Subjects with bilateral MSON
	N=267	*N=156*	*N=33*
Gender (N, female, %)	184 (69%)	100 (64.1%)	25 (75.8%)
Age (years)	52.3 (± 10.5)	53.1 (±10.7)	52.1 (±9.9)
Disease duration (years)	19.1 (± 7.4) (range 8.7-48.0)	18.5 (±7.2)	23.5 (±7.2)
EDSS (median [range])	3.5 [0-8.5]	3.5 [0-8.5]	4.0 [1.0-8.0]
Disease type			
RR MS	184	99	24
SP MS	53	31	9
PP MS	27	24	0
Unclassifiable	3	2	0
Disease modifying treatment			
Current	90 (33.7%)	43 (27.6%)	11 (33.3%)
β-*interferon/glatiramer acetate*	*66*	*32*	*9*
Natalizumab	*9*	*4*	*2*
*Other**	*15*	*7*	*0*
Past	55 (20.6%)	27 (17.3%)	11 (33.3%)
Never	122 (45.7%)	86 (55.1%)	11 (33.3%)
HCVA (mean ODS)	52.6 (± 8.8)	53.4 (± 8.0)	50.5 (±9.5)
LCVA (mean ODS)	27.2 (± 11.5)	26.9 (± 10.5)	24.0 (±13.9)

** fingolimod, dimethylfumarate and teriflunomide*
MSNON = no history of MS associated optic neuritis; MSON = MS related optic neuritis; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive; HCVA = high contrast visual acuity; LCVA = low contrast visual acuity; ODS = right (OD) and left (OS) eye combined

The majority of patients (68%) had a RR disease course. More than half of all patients (N=156, 58.4%), of which 27 were PPMS, had never experienced a clinically identified episode of MSON. Of all patients with a history of MSON (N=97, 36.4%), 64 patients had a unilateral MSON and 33 bilateral MSON. For 5% of patients, the MSON history was ambiguous. Nearly half of the patients never received disease modifying treatment (45.7%). Among the patients who were treated at the time of their assessment, the majority used β-interferon or glatiramer acetate (73%).

Furthermore, mean visual function (both HCVA and LCVA) was decreased in patients with bilateral MSON (see Table 1). Patients with no MSON history showed a difference of 2.8 letters for HCVA and 2.9 letters for LCVA compared with patients with bilateral MSON (p=0.108 and p=0.322 respectively).

RETINAL THICKNESS AND VISUAL FUNCTIONING IN MS, WITH AND WITHOUT MSON

All OCT scans were checked for quality control criteria by two independent raters (LB and DC), which led to a rejection rate of 14% (149/1068 scans).

Figure 1 shows the pRNFL and GCIPL thickness for all included eyes, and also stratified by history of MSON. When all eyes of MS patients were included (N=485 eyes) the pRNFL showed a mean thickness of 84.0 µm (±15.1) and the GCIPL a thickness of 76.9 µm (±14.8). Previous episodes of MSON had a significant effect on both retinal layers, with significant thinning in eyes with a history of MSON (pRNFL: 75.1 µm (±15.6) vs 87.1 (±13.7), GCIPL: 67.7 (±14.6) vs 79.9 (±13.7), p<0.001 for both comparisons, see Figure 1). After adjustments for disease duration, these differences between MSON and MSNON remained significant (pRNFL difference 9.6 µm, p<0.001, GCIPL difference 10.9 µm, p<0.001).

Table 2 shows the associations between retinal layer thickness and VA. Independent of MSON, VA was significantly associated with pRNFL and GCIPL thickness. Although both LCVA and HCVA showed significant associations, stronger associations were observed for LCVA. Every 10 µm reduction in pRNFL thickness corresponded with a reduction in HCVA score of 2.0 letters (p<0.001) and 2.9 letters for LCVA (p<0.001). Likewise, for the GCIPL a 10 µm reduction corresponded to a lower HCVA score of 3.1 letters (p<0.001) and 4.7 letters for LCVA (p<0.001, all GEE models accounting for age, history of MSON, use of disease modifying treatment and inter-eye dependency, see Table 2).

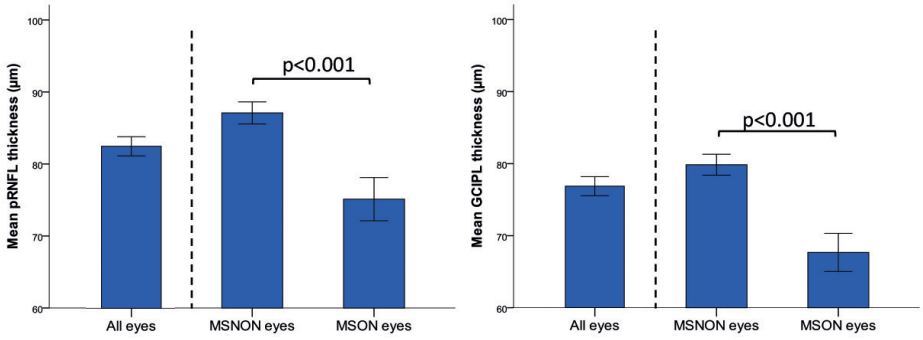

Figure 1: Retinal layer thickness (with 95% confidence interval) in all (N = 485), MSNON (N = 343), and MSON (N = 119) eyes. Note that history of MSON was ambiguous in 23 eyes which were excluded from further analyses.

MSON = MS related optic neuritis; MSNON = no history of MSON

Table 2: Association between retinal layer thickness and visual acuity. Data reported as β (95% confidence interval, p-value)

	HCVA	LCVA
pRNFL global (per 10 µm)	2.0 (1.0 to 3.0, p<0.001)*	2.9 (1.3 to 4.5, p<0.001)*
GCIPL (per 10 µm)	3.1 (2.1 to 4.2, p<0.001)*	4.7 (3.4 to 6.1, p<0.001)*

**GEE, adjusted for age, history of MS related optic neuritis, use of disease modifying treatment and inter-eye dependency.*

RETINAL THICKNESS AND VISION-RELATED QOL IN MS

The MS patients reported a median overall vision-related QoL score of 90.4 (interquartile range [IQR] 11.9). When the effect of MSON on overall vision-related QoL was investigated, it was shown that bilateral MSON patients had a lower vision-related QoL (median 88.5, IQR 15.3) compared to MSNON patients (median 91.7 (IQR 9.3), p=0.111, see Figure 2).

The overall vision-related QoL score was positively associated with pRNFL and GCIPL thickness, showing higher vision-related QoL scores in patients with less retinal atrophy (Figure 3A and B).

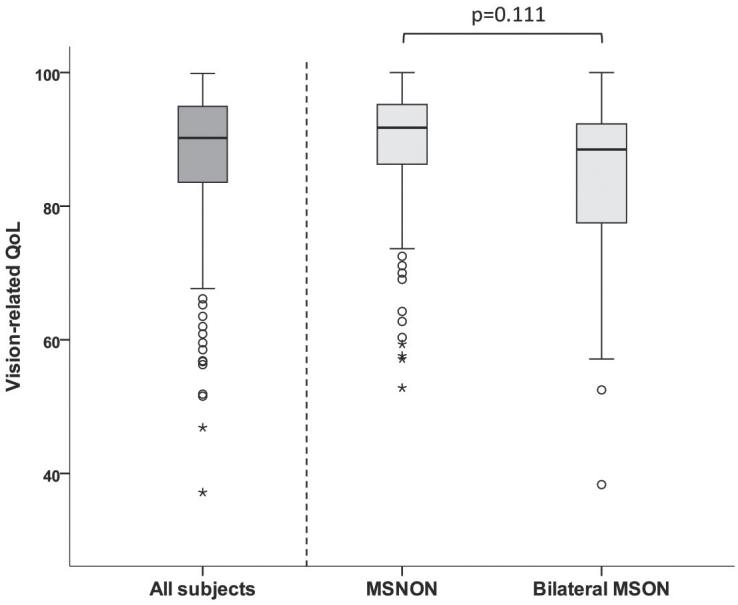

Figure 2: Box-and-whisker plot showing that the median vision-related quality of life is lower in patients with bilateral MSON (median: 88.5), compared to MSNON (median: 91.7) patients (p = 0.111).
MSON = MS related optic neuritis; MSNON = no history of MSON

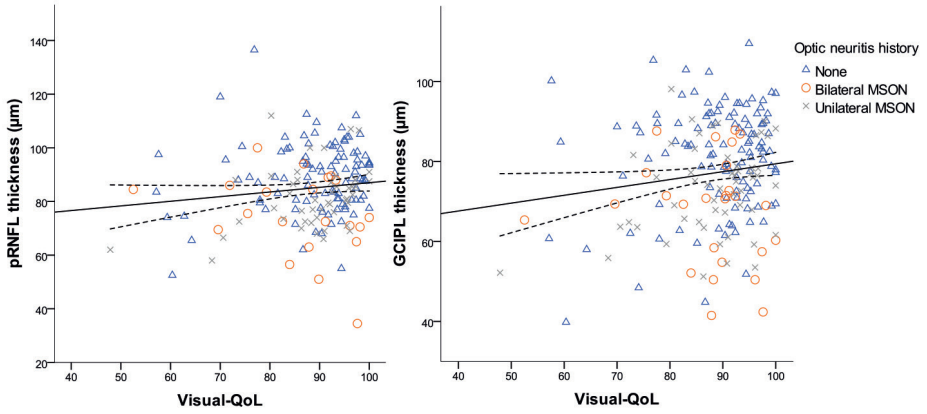

Figure 3: Scatter plot and fitted linear regression line (with 95% confidence curves) demonstrating the association between (a) pRNFL and (b) GCIPL thickness and the overall visual quality of life score (NEI-VFQ-25). Data are shown for patients without MSON (blue triangle), bilateral MSON (red circle), and unilateral MSON (gray cross).
MSON = MS related optic neuritis; QoL = quality of life; pRNFL = peripapillary retinal nerve fiber layer; GCIPL = ganglion cell – inner plexiform layer

When this association was adjusted for age, sex, use of DMT and history of MSON, the effect slightly decreased, but remained significant for GCIPL thickness (see Table 3). The goodness of fit (based on scale parameter) of the adjusted models were 10% for pRNFL and 9% for GCIPL.

Table 3: GEE analyses demonstrating the association between vision-related QoL (per 5 points) and pRNFL and GCIPL thickness. Adjustments for age, sex, use of DMT and history of MSON had minimal effect. Data shown as β (95% confidence interval, p-value).

	pRNFL β (95%CI)	p-value	GCIPL β (95%CI)	p-value
Vision-related QoL (unadjusted)	1.03 (0.01 to 2.01)	0.038	1.12 (0.26 to 1.99)	0.011
Vision-related QoL (adjusted for age, sex, use of DMT)	0.96 (0.01 to 1.91)	0.048	1.08 (0.28 to 1.87)	0.008
Vision-related QoL (adjusted for age, sex, use of DMT and MSON)	0.92 (-0.04 to 1.87)	0.060	0.93 (0.15 to 1.71)	0.020

All analyses were corrected for inter-eye dependency.
QoL = quality of life; pRNFL = peripapillary retinal nerve fiber layer; GCIPL = ganglion cell – inner plexiform layer; DMT = disease modifying treatment; MSON = MS related optic neuritis

Additionally, the GEE analyses were performed for the VFQ subscales (see Table 4). All analyses were adjusted for age, sex, use of DMT and MSON. Of the 11 subscales, the largest effects were observed for 'distance activities' (pRNFL: β=0.97, p=0.004 and GCIPL β=0.87, p=0.008), 'social functioning' (pRNFL: β=1.39, p=0.002 and GCIPL β=1.31, p=0.005) and colour vision (pRNFL: β=1.05, p=0.043).

Table 4. GEE analyses demonstrating the association (β [95% confidence interval]) between the overall vision-related quality of life (QoL) and all 11 subscales (per 5 points) and pRNFL and GCIPL thickness.

	pRNFL*	p-value	GCIPL*	p-value
Overall vision-related QoL	0.92 (-0.04 to 1.87)	0.060	0.93 (0.15 to 1.71)	0.020
Subscales				
General vision	0.37 (-0.19 – 0.94)	0.196	0.47 (-0.07 – 1.00)	0.088
Ocular pain	-0.35 (-1.03 – 0.34)	0.326	-0.18 (-0.63 - 0.28)	0.445
Near activities	0.76 (0.11 – 1.41)	0.022	0.79 (0.18-1.39)	0.011
Distance activities	0.97 (0.31 – 1.63)	0.004	0.87 (0.23 – 1.52)	0.008
Social functioning	1.39 (0.54 – 2.33)	0.002	1.31 (0.40 – 2.21)	0.005
Mental health	0.33 (-0.16 – 0.81)	0.186	0.21 (-0.24 – 0.65)	0.361
Role difficulties	0.19 (-0.30 – 0.67)	0.453	0.15 (-0.27 – 0.55)	0.494
Dependency	0.33 (-0.13 – 0.78)	0.156	0.22 (-0.08 – 0.52)	0.141
Driving	0.29 (-0.52 – 1.10)	0.484	0.37 (-0.29 – 1.04)	0.271
Colour vision	1.05 (0.03 – 2.06)	0.043	0.93 (-0.12 – 1.98)	0.082
Peripheral vision	0.43 (-0.09– 0.94)	0.101	0.52 (0.03 – 1.00)	0.038

**All analyses were adjusted for age, sex, use of disease modifying therapy and MS related optic neuritis*
QoL = quality of life; pRNFL = peripapillary retinal nerve fiber layer; GCIPL = ganglion cell – inner plexiform layer

MONO- VS BINOCULAR ATROPHY

In order to investigate the effect of mono- or binocular retinal thinning on vision-related QoL, patients were divided into three groups, based on the level of retinal atrophy (group 1: patients with binocular atrophy, group 2: patients with monocular atrophy and group 3: patients with no retinal atrophy in either eye).

Patients with binocular atrophy of the pRNFL showed the lowest vision-related QoL score (82.7±13.3) followed by patients with monocular pRNFL atrophy (85.1±13.3) and patients with no pRNFL atrophy (90.0±8.2). The difference in vision-related QoL between patients with mono- and binocular atrophy (2.4 points) was not statistically significant (p=0.363, see Table 5).

Regarding the GCIPL, a similar situation was observed as patients with binocular GCIPL atrophy showed the lowest vision-related QoL score (84.0±11.9), followed by patients with monocular atrophy (87.3±11.4) and patients with no retinal atrophy (89.4±8.5, see Table 5).

Table 5: Vision-related QoL in patients with binocular, monocular or no retinal atrophy

	Binocular atrophy	Monocular atrophy	No retinal atrophy	p-value$ binocular vs monocular	p-value$ binocular vs no atrophy	p-value$ monocular vs no atrophy
pRNFL*						
Vision-related QoL	82.7 (13.3)	85.1 (12.8)	90.0 (8.2)	0.363	0.001	0.013
GCIPL^						
Vision-related QoL	84.0 (11.9)	87.3 (11.4)	89.4 (8.5)	0.152	0.005	0.232

** pRNFL atrophy cut-off at 75 µm (binocular atrophy N=30, monocular atrophy N=35, no atrophy N=129)*
^ GCIPL atrophy cut-off at 68 µm (binocular atrophy N=35, monocular atrophy N=44, no atrophy N=148)
$ Linear regression analyses. Adjustments for MS related optic neuritis did not change the results.
QoL = quality of life; pRNFL = peripapillary retinal nerve fiber layer; GCIPL = ganglion cell – inner plexiform layer

DISCUSSION

This study showed that retinal atrophy has a significant impact on visual functioning in patients with MS. Both visual acuity and vision-related QoL were decreased in patients with atrophy of the inner retinal layers, independent of previous MSON.

Consistent with current literature, the present data showed significant thinning of the pRNFL and GCIPL in eyes with a history of MSON compared to unaffected eyes (difference of about 12 µm for both layers) as a result of retrograde degeneration.[1] Furthermore, the presence of MSON also influenced the VA of the patient, showing lower HC and LC VA for patients with bilateral MSON. While the retinal thickness and VA were both affected by history of MSON, the association between the two was similar for MSON and MSNON eyes (no effect modification by MSON). The data suggests that VA reduces with decreasing pRNFL or GCIPL thickness. Every 10 µm reduction in pRNFL thickness corresponded with a reduction in HCVA score of 2.0 letters and 2.9 letters for LCVA. Likewise, for the GCIPL, a 10 µm reduction corresponded to a lower HCVA score of 3.1 letters and 4.7 letters for LCVA. The clinical meaningfulness of these reductions in VA have previously been defined as >5 letters for HCVA, and >7 letters for LCVA at 2.5%.[33,34] Assuming a linear relationship, this would correspond to 24 µm (LCVA) or 25 µm (HCVA) for the pRNFL and 15 µm (LCVA) or 16 µm (HCVA) for GCIPL thickness. This suggests that although the observed effect was larger in LCVA, the clinical impact of retinal thinning on HC and LC VA seems to be quite similar. Our findings on the association between retinal atrophy and VA are consistent with previous studies, although comparing outcomes is difficult due to methodological differences. Nevertheless, the majority of studies showed that thinning of both pRNFL and GCIPL are associated with VA,[15-17,34,35] whereas some only found significant associations with LCVA.[36]

Overall vision-related QoL was positively associated with inner retinal layer thickness. Patients with a higher vision-related QoL score showed less atrophy of both the pRNFL and GCIPL. Adjustment for confounding factors such as age, sex, use of DMT, and also MSON, only resulted in minor changes of the effect. Importantly, the association between QoL and retinal atrophy is mediated by VA. This was supported by the strong reduction of the effect when LCVA was added to the model (pRNFL: β=0.07 [95%CI -1.6 to 1.85, p=0.941] and for GCIPL: β=0.15 [95%CI -0.95 to 1.25, p=0.791). Our findings build upon a previous study by Walter *et al.*, reporting similar associations between vision-related QoL and GCIPL thickness of 0.9 µm and 1.0 µm on pRNFL thickness per 5 points on the NEI-VFQ25 scale (using GEE models accounting for age and within-patient inter-eye correlations).[17] Furthermore, a study by Longbrake *et al.* showed that vision-related QoL correlated with average pRNFL thickness, but only below a critical threshold of 75 µm. Above 75 µm, no relationship between pRNFL thickness and vision-related QoL was observed.[37] In contrast, a relatively small study (N=54) by Garcia-Martin *et al.*, did not show any relationship between pRNFL (Spectralis) and overall MSQOL-54 score (r=0.08), but they did report significant correlations with the physical health composite (r=0.23, p<0.05) and fatigue (r=0.30, p<0.05).[18] It should however be noted that in this study a non-specific QoL measure was used (MSQOL-54), which may explain the lack of correlation with other items than the physical health composite and fatigue.

Retinal atrophy is undoubtedly assessed on eye-level, as both eyes are scanned individually. Although some relevant outcomes are also eye-specific (history of MSON, VA), many relevant research questions include clinical outcome measures assessed on a patient level (EDSS score, cognition, QoL). Besides the fact that this discrepancy in level of assessment results in methodological challenges, as the suggested approach (GEE with adjustments for inter-eye dependency) is methodologically not correct in such situations, it also raises the question whether inter-eye differences are clinically relevant to a patients visual functioning. In the present study we have investigated the effect of mono- or binocular retinal atrophy on vision-related QoL and demonstrated that having atrophy of the pRNFL in only one eye, resulted in a significantly lower vision-related QoL score compared to having two unaffected eyes. When both eyes showed atrophy of the pRNFL, this only further decreased the vision-related QoL score minimally (difference 2.4 points, p=0.363). Regarding the GCIPL a more stepwise situation was observed, with binocular GCIPL atrophy showing the lowest vision-related QoL score, followed by patients with monocular atrophy and finally patients with no retinal atrophy. These findings suggest that monocular atrophy of the inner retinal layers already has significant impact on the vision-related QoL of a patient. This phenomenon may be a result of binocular inhibition (when the best eye has better acuity than both eyes together), which is present in patients with MSON,[38] but this was not further investigated as it was beyond the scope of this study and no data on binocular vision were available.

One limitation of this study is its cross-sectional design, which does not permit to hint on causality. We are therefore in the process of re-investigating all patients after two more years of follow-up.

In conclusion, this study showed that retinal atrophy has a significant impact on visual functioning in patients with MS. Both VA and vision-related QoL were decreased in patients with atrophy of the macular ganglion cells. Retinal OCT gives useful insight to patients with visual dysfunction and our findings support including OCT and visual functioning measures into optic neuritis treatment trials.

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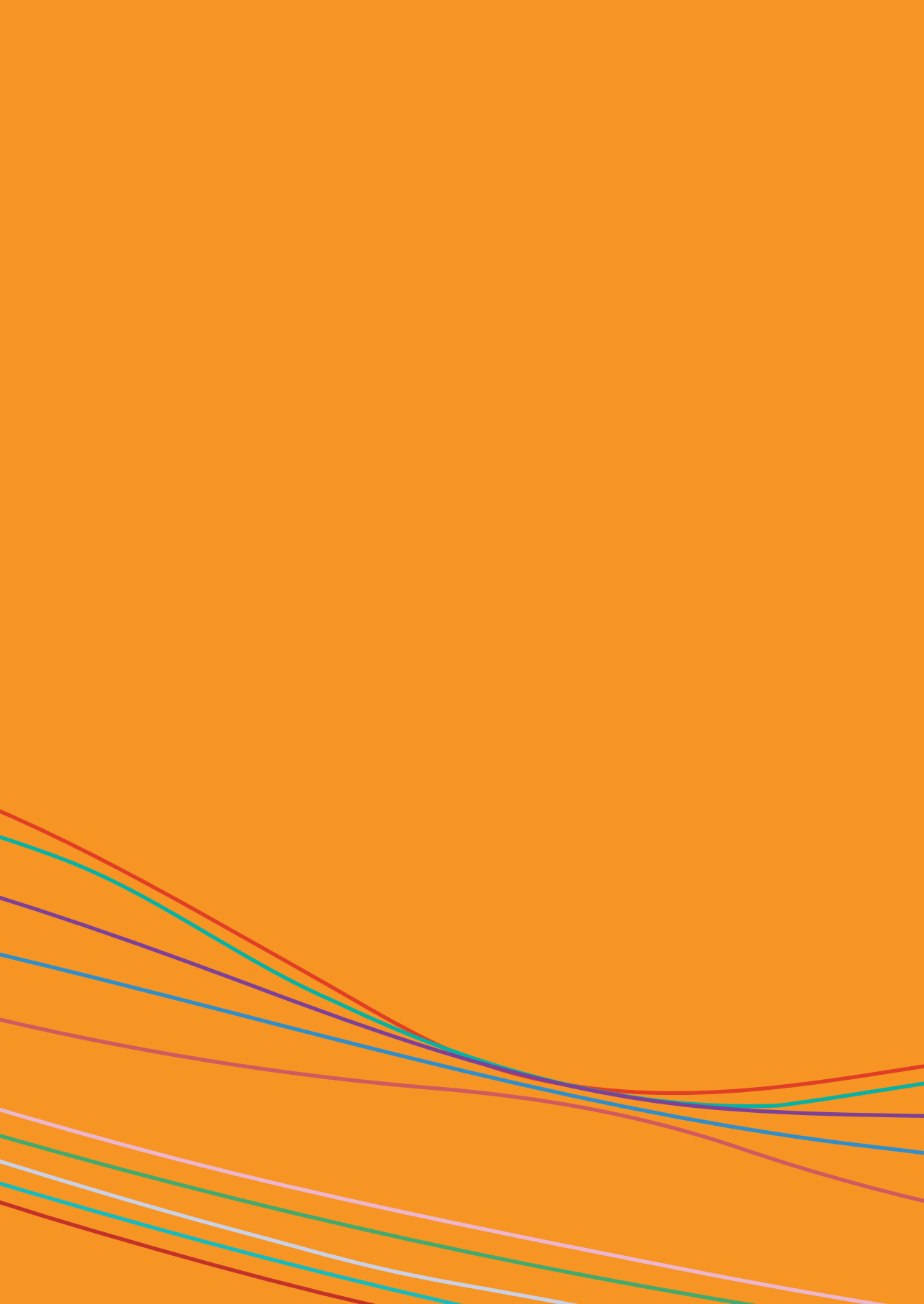
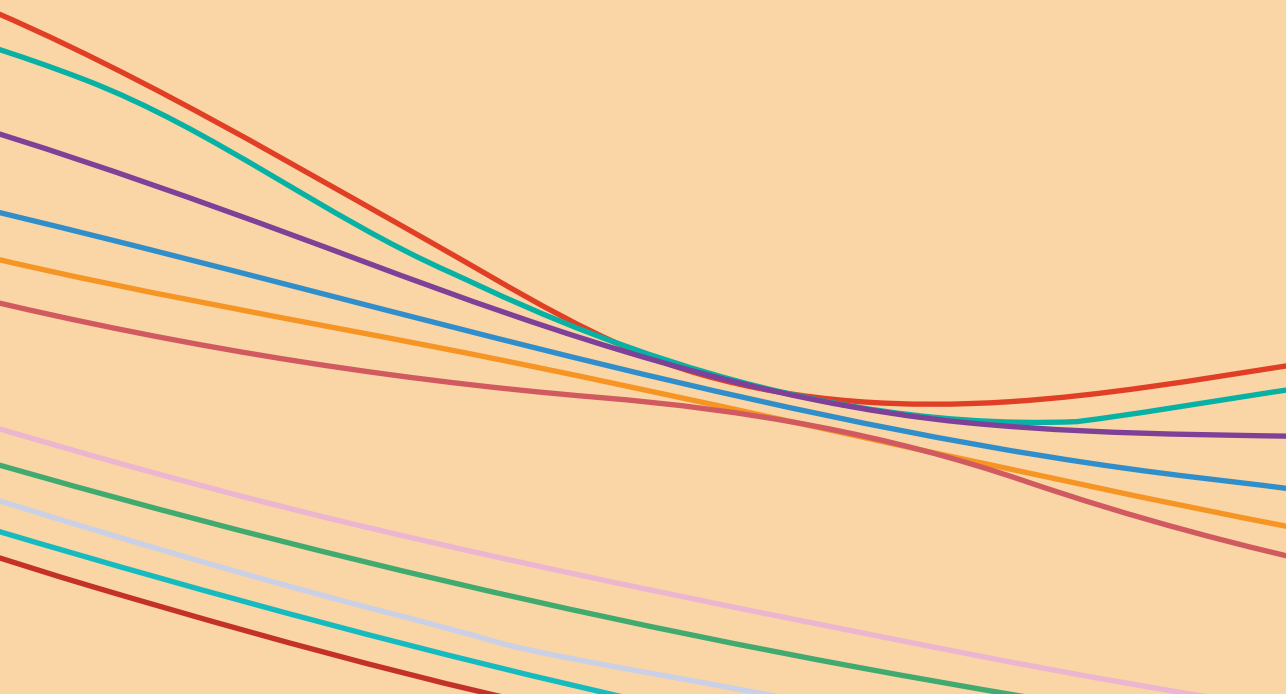


CHAPTER 4

OCT AS A MARKER OF NEURODEGENERATION IN MS



CHAPTER 4.1

COGNITIVE IMPAIRMENT IN PATIENTS WITH MULTIPLE SCLEROSIS IS ASSOCIATED WITH ATROPHY OF THE INNER RETINAL LAYERS

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Multiple Sclerosis 2018;24:158-166 ■

ABSTRACT

Background: Inner retinal layer (IRL) atrophy is a potential biomarker for neurodegeneration in multiple sclerosis (MS).

Objective: To investigate the relationship between cognitive impairment and IRL atrophy in MS.

Methods: Cross-sectional study design, including 217 patients and 59 healthy controls. Subjects were investigated clinically, underwent retinal optical coherence tomography (OCT) and comprehensive cognitive assessments. The association between these modalities was evaluated by regression analyses.

Results: Of the patients, 44.2% was cognitively impaired. In the absence of MS associated optic neuritis (MSON), cognitively impaired patients had a significantly lower mean peripapillary retinal nerve fiber layer (pRNFL, Δ 8.13 µm, $p < 0.001$) and mean macular ganglion cell-inner plexiform layer (mGCIPL, Δ 11.50 µm, $p < 0.001$) thickness compared to cognitively preserved patients. There was a significant association between the presence of cognitive impairment and pRNFL (OR 1.11 [95%CI 1.04 -- 1.18, $p = 0.001$]) and mGCIPL (OR 1.11 [95%CI 1.05 -- 1.18, $p < 0.001$]) atrophy. This association was masked by the severe retinal atrophy seen following MSON.

Conclusion: The strong relationship between cognitive impairment across multiple cognitive domains and atrophy of the pRNFL and mGCIPL in patients who never suffered from MSON suggests that OCT is useful in assessing central nervous system neurodegeneration in MS.

INTRODUCTION

Cognitive dysfunction and multiple sclerosis associated optic neuritis (MSON) have long been recognized as relevant to a patient's disability in multiple sclerosis (MS).[1] Cognitive impairment is present in 40 to 70% of patients and contributes to a significant decrease in quality of life.[1-3] Frequently observed problems include deficits in information processing speed, long-term memory and executive functioning.[1,4] Brain imaging studies have demonstrated that cognitive impairment in MS is related to brain atrophy, an important sign of neurodegeneration.[2,4-6]

The anterior visual system offers a suitable model to study MS related disease mechanisms.[7] Importantly, the association of MSON with retinal optical coherence tomography (OCT) has been exhaustively investigated and is now well established.[8,9] In recent years, retinal OCT has also been used as a sensitive and more practical alternative to MRI for the analysis of the process of neurodegeneration in MS.[8,10] The relationship between cognitive impairment and inner retinal layer (IRL) atrophy, however, is less well known. One single study found a possible link between cognitive impairment and peripapillary nerve fiber layer (pRNFL) atrophy[11] but definitive results are lacking. Moreover, advancements in OCT technology and software have led to more reliable segmentation of individual macular layers, most importantly the macular ganglion cell – inner plexiform layer (mGCIPL).

Therefore, the aim of this study was to test if MS disease-related atrophy of pRNFL and mGCIPL, measured with spectral domain OCT, is associated with cognitive impairment as assessed across several cognitive domains using a battery of validated psychometric tests in a large cohort of patients with MS.

METHODS

STUDY POPULATION

In this observational, cross-sectional study patients were included from the Amsterdam MS Cohort at the VU University Medical Center Amsterdam. This cohort has been described previously.[12] In brief, all patients included in the present study had a diagnosis of relapsing remitting (RR), secondary progressive (SP) or primary progressive (PP) MS[13] and were required to be between 18 and 80 years of age at time of inclusion. Exclusion criteria were a relapse or corticosteroid treatment one month prior to inclusion, pregnancy, any other previous neurological or neuropsychiatric disorders, a history of alcohol or drug abuse or central nervous system (CNS) comorbidity showing on MRI which could not be attributed to MS. The same exclusion criteria applied to the healthy control (HC) group which consisted of subjects who had to be between 40 and 60 years of age, without any neurological or psychiatric disease and who were not related (within the first or second degree of consanguinity) to a patient with MS. All subjects with high refractive errors (> -6.0 or +6.0 dpt.) or ocular diseases affecting the retina were excluded.

This study was approved by the medical ethics committee (protocol number 2010/336) and the scientific research committee (protocol number CWO/10-25D) of the VU University Medical Center. Written informed consent was obtained from all participants.

OCT IMAGING

OCT imaging was performed by four trained technicians on a spectral domain OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany, software version 1.7.1.0.), with dual beam simultaneous imaging and the eye tracking function enabled for optimal measurement accuracy.[14] Room lighting conditions were dimmed and no pharmacological pupil dilation was used. A 12° peripapillary ring scan (1536 A-scans, 1 B-scan, no predetermined automatic real-time (ART)) manually centered around the optic nerve head and a 20° x 20° macular volume scan (512 A scans, 49 B-scans, vertical alignment, ART 16) manually centered around the fovea were performed. Individual retinal layer thicknesses were obtained by automated segmentation software provided by the manufacturer (HRA / Spectralis Viewing Module version 5.6.4.0). Quality control (QC) was performed according to validated international consensus quality control criteria (OSCAR-IB)[15] by two co-authors (LJB, AP) who were blind to the results of the neuropsychological examination. On QC a small proportion of scans were identified with algorith failures which could readily be manually corrected. Scans where algorithm failures were not due to automated image post-processing, but violation of other OSCAR-IB criteria, were excluded from further analyses.

For pRNFL thickness, the global mean of the entire pRNFL was used. For the macular scan the software provides a thickness map for every retinal layer on a 1mm, 3mm, 6mm grid (as is defined by the Early Treatment Diabetic Retinopathy study[16]). Because of the low contrast between the ganglion cell layer and the inner plexiform layer, these two layers were combined to form the mGCIPL.[10] For the mGCIPL the mean thickness of the inner four quadrants of the grid (corresponding to the 3 mm ring, excluding the 1 mm center ring) was used.

COGNITIVE AND CLINICAL ASSESSMENT

All subjects received an extensive neuropsychological examination consisting of Rao's Brief Repeatable Battery of Neuropsychological Tests (BRB-N) and three additional cognitive tests, which have been described previously.[17] Each test corresponds to a different cognitive domain. The examination was administered by three trained research assistants according to a standardized protocol, who were blind to the results of the OCT. The BRB-N consists of the Selective Reminding Test (SRT, measuring verbal memory), Symbol Digit Modalities Test (SDMT, measuring information processing speed), Word List Generation Test (measuring verbal fluency) and 10/36 Spatial Recall Test (10/36 SPRT, measuring visuospatial memory).[18] The Paced Auditory Serial Addition Test was excluded due to multiple disadvantages.[19] The three additional test comprised the Concept Shifting Test (CST, measuring executive functioning), Stroop Color Word test (measuring attention) and Memory Comparison Test (MCT, measuring working memory). The raw test scores were corrected for the effects of age, sex and level of education using a linear regression model in the same way as Amato *et al.*[20], correcting only

for those factors that had a significant effect on the test score. Z scores were calculated for each individual domain. Consistent with previous publication from our center, a patient was considered cognitively impaired if he or she scored at least 1.5 SD ($Z \leq 1.5$) below the average of the HCs on two or more domains.[17]

A history of MSON was determined per consensus protocol.[21] The degree of physical disability was assessed by trained and certified physicians using the Expanded Disability Status Scale (EDSS). All examinations, including OCT, were performed on the same day.

STATISTICAL ANALYSIS

Because of the large effect MSON has on IRL thickness[9] patients were grouped accordingly. The binocular average pRNFL and mGCIPL thickness was calculated for each subject. This approach was mandatory because the outcome measure, which is the presence or absence of cognitive impairment, was measured on patient level and not eye level. This approach is also in accordance with reporting guidelines, which specifically mention exceptions to frequently used models for adjustment of inter-eye correlations, such as generalized estimating equations.[10] In order to avoid the introduction of noise by pooling MSON eyes and MSNON eyes (eyes without a history of MSON) within the same patient, only patients with the same history of MSON in both eyes (so bilateral MSON and bilateral MSNON) were analyzed. At no point was data from MSON and MSNON eyes combined as a mean. Results for the MSON and MSNON group are shown separately.

Normal data distribution was assessed graphically. Differences in demographic variables between patients and HCs and between cognitively impaired and cognitively preserved patients were analyzed using chi square test for categorical variables, two-tailed T test for parametric continuous variables and Mann-Whitney U test for non-parametric continuous variables. Differences in IRL thickness between the aforementioned groups were tested using multiple linear regression analyses with cognitive status (HC, cognitively preserved, cognitively impaired) as a categorical variable, adjusting for age and sex.

In order to investigate whether IRL atrophy is associated with the presence of cognitive impairment we performed binary logistic regression analyses, with adjustment for age and sex. Two models were used, one with IRL thickness as a continuous variable and one with IRL thickness as a categorical variable by dichotomizing the subjects according to median thickness. In the MSNON group this resulted in a group with pRNFL thickness ≤ 85.0 µm and pRNFL thickness > 85.0 µm. Likewise, patients were divided in a group with mGCIPL thickness ≤ 88.1 µm and mGCIPL thickness > 88.1 µm. In the MSON group patients were dichotomized in a group with pRNFL thickness ≤ 75.0 µm and pRNFL thickness > 75.0 µm and mGCIPL thickness ≤ 73.0 µm and mGCIPL thickness > 73.0 µm. Correlations between IRL thickness and scores on cognitive subtests were analyzed using partial correlation coefficients (r), adjusting for age and sex. All analyses were performed using SPSS version 22.0. Statistical significance was set at $p < 0.05$.

RESULTS

The Amsterdam MS Cohort consists of a total of 230 MS patients and 63 healthy control subjects. Five patients were excluded due to uncertain diagnosis or suspected alcohol and/or drug abuse. Eight patients and four HCs were excluded due to insufficient data on the neuropsychological examination. The presented results concern the remaining 217 MS patients and 59 HCs. Following QC 193/1104 (17.5%) of the OCT scans were rejected.

Table 1: Characteristics of patients with multiple sclerosis and healthy control subjects. The mean ± SD, median [range] and frequency (percentage) are presented. Data are presented for all patients, as well as for the two clinical subgroups (MSNON, MSON) and the HCs.

	All patients N = 217	**MSNON patients N = 102**	**MSON patients N = 35**	**Healthy controls N = 59**	**p-value Patients vs HCs**	**p-value MSNON vs MSON**
Age (years), mean (±SD)	54.30 (±9.96)	55.91 (±10.00)	53.18 (±9.75)	50.39 (±7.28)	0.001	0.133
Sex (Male : Female)	67 : 150	37 : 65	11 : 24	22 : 37	0.350	0.604
Disease duration (years), mean (±SD)	20.34 (± 6.99)	19.34 (±7.05)	22.78 (±7.34)	N/A	N/A	0.015
EDSS, median [range]	4.0 [1.0 – 8.0]	3.75 [1.0 – 7.5]	4.0 [1.5 – 7.5]	N/A	N/A	0.813
Type of MS						
RR	133 (61.3%)	56 (54.9%)	22 (62.9%)	N/A	N/A	0.043
SP	56 (25.8%)	21 (20.6%)	11 (31.4%)			
PP	28 (12.9%)	25 (24.5%)	2 (5.7%)			
Use of disease modifying therapy				N/A	N/A	< 0.001
Current	61 (28.1%)	24 (23.5%)	10 (28.6%)			
Past	40 (18.4%)	9 (8.8%)	12 (34.3%)			
Never	116 (53.5%)	69 (67.6%)	13 (37.1%)			
Cognitive impairment (CP : CI)	121 : 96	61 : 41	20 : 15	55 : 4	< 0.001	0.782
pRNFL thickness (µm), mean (±SD)	83.16 (±11.18)	85.62 (±10.29)	75.04 (±10.33)	91.67 (±6.82)	< 0.001	< 0.001
mGCIPL thickness (µm), mean (±SD)	82.66 (±14.89)	86.12 (±12.29)	69.86 (±16.88)	94.21 (±6.08)	< 0.001	< 0.001

MSNON = no history of multiple sclerosis associated optic neuritis; MSON = multiple sclerosis associated optic neuritis; HCs = healthy controls; EDSS = expanded disability status scale; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive; CP = cognitively preserved; CI = cognitively impaired; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell-inner plexiform layer

CHARACTERISTICS OF THE PATIENTS AND HEALTHY CONTROL SUBJECTS

Table 1 shows the characteristics of the patients (all patients as well as the two clinical subgroups) and the 59 HCs. Compared to HCs, patients were older (mean difference 3.91 years, p = 0.001) and were slightly more likely to be female, though the latter was statistically not significant. Patients had a mean disease duration of over 20 years and a median EDSS score of 4.0 [range 1.0 -- 8.0]. Most patients had a relapsing remitting course (61.3%), followed by a secondary progressive (25.8%) and a primary progressive (12.9%) course. Overall, patients showed significant thinning of both the pRNFL and the mGCIPL compared to HCs (mean differences 8.51 µm, p < 0.001 and 11.55 µm, p < 0.001), with MSON patients showing more atrophy than MSNON patients (table 1).

In total, 44.2% (96/217) of the patients was classified as cognitively impaired, compared to 6.8% (4/59) of the HCs (p < 0.001). Cognitively impaired patients were older (mean difference 3.99 years, p = 0.003) and had a longer disease duration (mean difference 2.50 years, p = 0.011). In addition, cognitively impaired patients had a higher median EDSS score (4.5 vs. 3.0, p < 0.001), Supplementary Table 1.

Subsequently, patients were stratified according to MSON history resulting in 102 (47.0%) bilateral MSNON patients and 35 (16.1%) bilateral MSON patients. 61 (28.1%) patients had a history of unilateral MSON and in 19 (8.8%) patients the history of MSON was unclear.

Table 2: Characteristics of the MSNON patients. Characteristics of the cohort of patients with MS who never experienced an episode of MS associated optic neuritis dichotomized into those who were cognitively impaired and those who were cognitively preserved. The mean ± SD, median [range] and frequency (percentage) are presented.

	Cognitively impaired N = 41	Cognitively preserved N = 61	p-value
Age (years), mean (±SD)	57.66 (± 9.59)	54.73 (± 10.17)	0.149
Sex (Male : Female)	19 : 22	18 : 43	0.083
Disease duration (years), mean (±SD)	20.55 (± 7.97)	18.52 (± 6.29)	0.154
EDSS, median [range]	4.5 [3.5 – 6.5]	3.0 [2.5 – 5.0]	< 0.001
Type of MS			0.029
RR	16 (39.0%)	40 (65.6%)	
SP	12 (29.3%)	9 (14.8%)	
PP	13 (31.7%)	12 (19.7%)	
Use of disease modifying therapy			0.515
Current	10 (24.4%)	14 (23.0%)	
Past	2 (4.9%)	7 (11.5%)	
Never	29 (70.7%)	40 (65.6%)	
pRNFL thickness (µm), mean (±SD)	80.59 (± 8.49)	88.72 (± 10.14)	< 0.001
mGCIPL thickness (µm), mean (±SD)	79.13 (± 13.53)	90.63 (± 9.02)	< 0.001

EDSS = expanded disability status scale; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer

IRL THICKNESS IN MSNON AND MSON PATIENTS

MSNON patients Table 2 shows the characteristics of the MSNON group in which 41 patients (40.2%) were classified as cognitively impaired and 61 (59.8%) as cognitively preserved. There was no significant difference in mean age or mean disease duration between the two groups. Cognitively impaired patients did however show a higher degree of disability (median EDSS score 4.5 vs. 3.0, $p < 0.001$) and were more likely to suffer from a progressive form of the disease. Patients scored the worst on the SDMT and MCT (for more details see Supplementary Table 2).

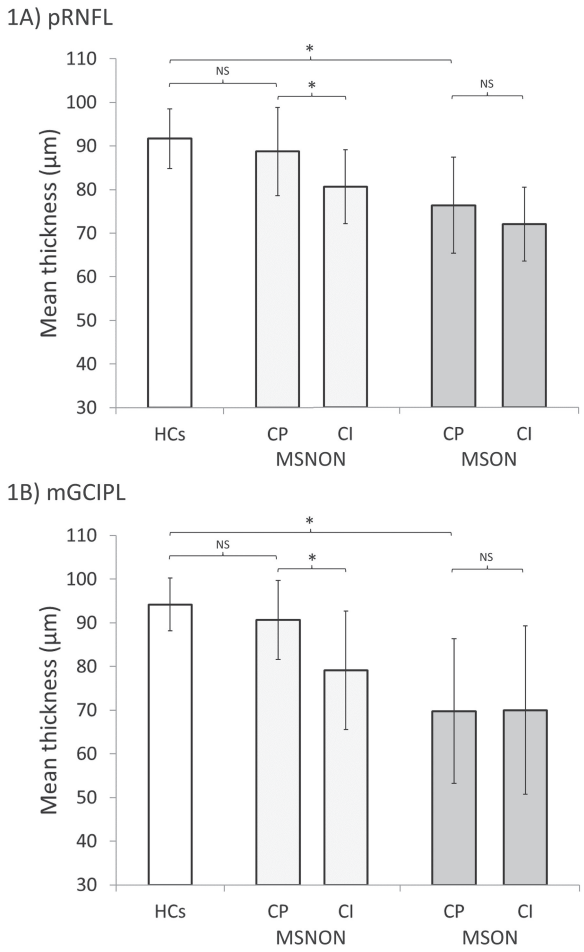

Figure 1: Mean pRNFL and mGCIPL thickness in HCs, MSNON patient and MSON patients. Patients are dichotomized according to cognitive status. Data are presented for the pRNFL (A) and mGCIPL (B). Error bars represent standard deviations. Differences in retinal layer thickness were tested using multiple linear regression analyses, adjusting for age and sex. * = $p < 0.001$, NS = Not Significant.
pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell-Inner plexiform layer; HCs = healthy controls; CP = cognitively preserved; CI = cognitively impaired; MSNON = no history of multiple sclerosis associated optic neuritis; MSON = multiple sclerosis associated optic neuritis

Cognitively impaired MSNON patients showed a large and statistically significant degree of atrophy compared to cognitively preserved patients for both the pRNFL and the mGCIPL (mean difference 8.13 µm, $p < 0.001$ and 11.50 µm, $p < 0.001$, Table 2 and Figure 1). Cognitively preserved patients showed a small difference in pRNFL and mGCIPL thickness compared to HCs but this was statistically not significant (mean differences 2.95 µm, $p = 0.098$ and 3.58 µm, $p = 0.119$).

MSON patients In the MSON group 15/35 patients (42.9%) were classified as cognitively impaired. Cognitively preserved MSON patients showed substantial atrophy of both the pRNFL and the mGCIPL compared to HCs (mean difference 15.29 µm, $p < 0.001$ and 24.42 µm, $p < 0.001$) but there were no differences in either pRNFL or mGCIPL thickness between the cognitively impaired and cognitively preserved MSON patients (mean difference 4.38 µm, $p = 0.159$ and -0.23 µm, $p = 0.879$), Figure 1. For detailed demographic data and cognitive test results of this subgroup see Table 3 and Supplementary Table 2.

ASSOCIATION BETWEEN IRL THICKNESS AND COGNITIVE IMPAIRMENT

MSNON patients pRNFL thickness showed a significant, inverse association with cognitive impairment with an odds ratio (OR) of 1.09 (95%CI 1.03 -- 1.15, $p = 0.002$) indicating that thinning of the pRNFL by 1 µm increases the odds of being cognitively impaired by 1.09. Likewise, the mGCIPL showed a similar significant association with cognitive impairment, with an OR of 1.10 (95%CI 1.04 -- 1.16, $p < 0.001$). Both associations remained significant after adjusting for age and sex, resulting in an OR for pRNFL of 1.11 (95%CI 1.04 -- 1.18, $p = 0.001$) and an OR for mGCIPL of 1.11 (95%CI 1.05 -- 1.18, $p < 0.001$), Figure 2A.

Table 3: Characteristics of the MSON patients. Characteristics of the cohort of patients with bilateral MS associated Optic Neuritis dichotomized into those who were cognitively impaired and those who were cognitively preserved. The mean ± SD, median [range] and frequency (percentage) are presented.

	Cognitively impaired N = 15	Cognitively preserved N = 20	p-value
Age (years), mean (±SD)	55.97 (± 10.27)	51.10 (± 9.05)	0.146
Sex (Male : Female)	5 : 10	6 : 14	0.833
Disease duration (years), mean (±SD)	24.40 (± 7.64)	21.56 (± 7.06)	0.263
EDSS, median [range]	4.5 [4.0 – 6.0]	3.0 [2.5 – 4.5]	0.008
Type of MS			0.112
RR	7 (46.6%)	15 (75.0%)	
SP	6 (40.0%)	5 (25.0%)	
PP	2 (13.3%)	0 (0.0%)	
Use of disease modifying therapy			0.853
Current	5 (33.3%)	5 (25.0%)	
Past	5 (33.3%)	7 (35.0 %)	
Never	5 (33.3%)	8 (40.0%)	
pRNFL thickness (µm), mean (±SD)	72.00 (± 8.46)	76.38 (± 11.03)	0.159
mGCIPL thickness (µm), mean (±SD)	70.02 (± 19.27)	69.79 (± 16.54)	0.879

EDSS = expanded disability status scale; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer

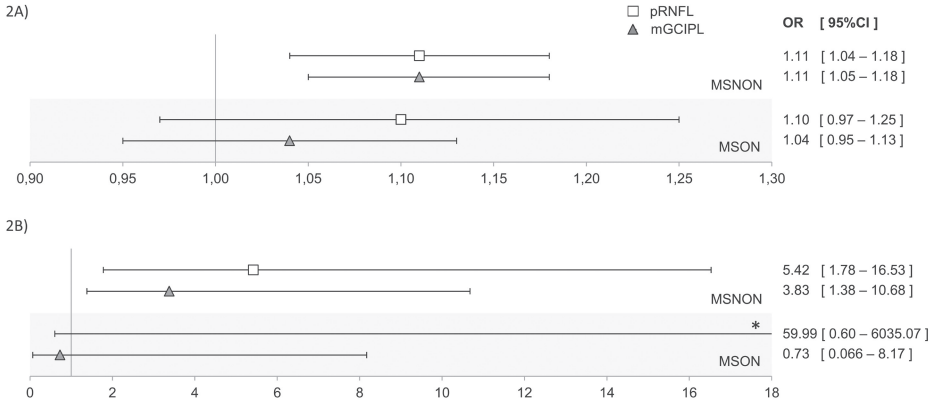

Figure 2: Adjusted odds ratios for pRNFL and mGCIPL to determine cognitive impairment in patients with MS. Data are presented for clinical subgroups (MSNON and MSON). The OR and 95%CI were significant and comparable in effect size when data were analysed both on a rational scale (continuous data, 2A) and categorical scale (dichotomized data, 2B). * OR and 95%CI exceed graph axis limits.
pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell-Inner plexiform layer; MSNON = no history of multiple sclerosis associated optic neuritis; MSON = multiple sclerosis associated optic neuritis; OR = odds ratio; 95%CI = 95% confidence interval

In order to further investigate the effect of severe IRL atrophy we dichotomized the patients according to IRL thickness. Patients with pRNFL thickness equal to or less than 85.0 µm had a significantly increased odds of being cognitively impaired compared to patients with pRNFL thickness greater than 85.0 µm, OR 4.63 (95%CI 1.69 -- 12.70, p = 0.003). Similarly, patients with a mGCIPL thickness equal to or below 88.1 µm had a significantly increased odds of being cognitively impaired (OR 3.66 [95%CI 1.36 -- 9.86, p = 0.010]) compared to patients with a mGCIPL thickness above 88.1 µm. Again, the results remained significant after adjusting for age and sex with an OR of 5.42 (95%CI 1.78 -- 16.53, p = 0.003) for the pRNFL and an OR of 3.83 (95%CI 1.38 -- 10.68, p = 0.010) for the mGCIPL (Figure 2B). Adjusting the data for the use of disease modifying treatment (never, past, current use) did not have any effect on the observed effect. pRNFL thickness was only significantly correlated with the SDMT ($r = 0.34$, p = 0.004) and MCT score ($r = 0.24$, p = 0.042), whereas mGCIPL thickness correlated with every test score and most strongly with the CST ($r = 0.46$, p < 0.001) and 10/36 SPRT ($r = 0.42$, p < 0.001), Table 4.

Table 4: Partial correlation coefficients (*r*) between pRNFL and mGCIPL thickness and test scores of separate cognitive tests.

	MSNON				MSON			
	pRNFL		mGCIPL		pRNFL		mGCIPL	
	r	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*
SRT	0.21	0.075	0.32	0.007	0.580	0.007	0.35	0.174
SDMT	0.34	0.004	0.29	0.014	0.26	0.225	0.20	0.433
WLGT	0.21	0.079	0.24	0.039	0.013	0.954	-0.068	0.789
10/36 SPRT	0.18	0.137	0.42	< 0.001	0.256	0.262	0.20	0.422
CST	0.22	0.062	0.46	< 0.001	0.211	0.358	0.30	0.221
SCWT	0.12	0.304	0.25	0.037	0.16	0.493	0.24	0.354
MCT	0.24	0.042	0.26	0.032	0.011	0.962	0.27	0.282

MSNON = no history of multiple sclerosis associated optic neuritis; MSON = multiple sclerosis associated optic neuritis; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer; SRT = selective reminding test; SDMT = symbol digit modalities test; WLGT = word list generation test; 10/36 SPRT = 10/36 spatial recall test; CST = concept shifting test; SCWT = Stroop color word test; MCT = memory comparison test

MSON patients In the MSON group there was no significant association between pRNFL (OR 1.04 [95%CI 0.96 -- 1.14, p = 0.350]) nor mGCIPL (OR 1.00 [95%CI 0.94 -- 1.06, p = 0.978]) thickness and cognitive impairment. After adjusting for age and sex this did not change (pRNFL OR 1.10 [95%CI 0.97 -- 1.25, p = 0.122]; mGCIPL OR 1.04 [95%CI 0.95 -- 1.13, p = 0.399]), Figure 2A. Patients with a pRNFL thickness equal to or less than 75.0 µm had an increased odds of being cognitively impaired, but this was statistically not significant (unadjusted OR 4.17 [95%CI 0.61 -- 28.62, p = 0.147]; adjusted OR 59.99 [95%CI 0.60 -- 6035.07, p = 0.082]). There was no association between the dichotomized mGCIPL thickness and cognitive impairment (unadjusted OR 1.00 [95%CI 0.15 -- 6.77, p = 1.00]; adjusted OR 0.73 [95%CI 0.066 -- 8.17, p = 0.801]), Figure 2B. Again, adjusting for use of disease modifying treatment did not alter the results. There were no significant correlations between test scores and IRL thickness, apart from a correlation between pRNFL thickness and the SRT score (*r* = 0.58, p = 0.007), Table 4.

DISCUSSION

This study provides strong evidence for a relationship between atrophy of the pRNFL and mGCIPL and cognitive impairment in patients with MS. The results demonstrate that cognitively impaired MSNON patients show substantial and highly significant thinning of both the pRNFL and the mGCIPL compared to cognitively preserved MSNON patients. More importantly, in this same patient group, atrophy of the pRNFL and mGCIPL was significantly associated with an increased odds of being cognitively impaired, taking other disease related factors into account. As expected a history of MSON caused severe IRL atrophy which masked any relationship to cognitive function. This masking effect of MSON has been described before.[22] The extremely high OR for the adjusted, dichotomized pRNFL is caused by the small number of patients.

Our findings confirm and extend on the time-domain OCT data by Toledo et al.[11] in which the authors found a trend towards a lower average pRNFL thickness in cognitively impaired MS patients. They also found significant correlations between the average and temporal pRNFL thickness and test scores on some subtests of Rao's BRB-N, particularly the SDMT. In contrast, three other studies failed to demonstrate a relationship between pRNFL thickness and test scores on various neuropsychological tests.[23-25] There are important differences between these studies and our study with regard to the study populations, which were of shorter disease duration, and the pooling of data from MSNON and MSON eyes.

There have been no previous studies on the relationship between cognitive impairment and mGCIPL thickness, but the association found is anatomically logical. The retinal ganglion cell residing in the mGCIPL and the axon residing in the RNFL form one anatomical unit. The first to third order neurons of the optic pathways are known to intimately share their fate through retrograde axonal degeneration.[26, 27] The finding that in absence of MSON, the main contributor to retrograde axonal degeneration, atrophy of the IRL was strongly associated with cognitive impairment implies that a more systemic degree of neurodegeneration is at play. This interpretation extends on the anatomically restrictive definition of axonal degeneration in the visual system alone. This argument is strengthened by studies showing a relationship with physical disability measured by EDSS and disease course.[28-32]

One limitation of this study is its cross-sectional design, we are therefore in the process of re-investigating all patients after a four year interval. Another limitation is the long disease duration, which could introduce a potential bias towards patients with more severe neurodegeneration affecting cognitive function.[1] Likewise, the potential of a "plateau effect" of IRL atrophy in the later disease course needs to be considered.[33] The latter two concerns underline the importance for longitudinal studies in patients with early disease. Because of the deleterious effect of MSON such studies should focus on patients with clinically isolated syndromes other than MSON. With respect to the neuropsychological examination, there is a lack in agreement regarding the criteria of cognitive impairment in MS patients. For internal consistency we have therefore strictly adhered to the multi-cognitive domain 1.5 SD criterion consistently used in previous publications from our center. We note the difference in age between patients and HCs but we corrected this by adjusting all analyses for age (among others). Another limitation is the fact that the assessment of MSON was merely based on clinically confirmed episodes and patient-reported history. Although this approach is consistent with other studies in the field, subclinical episodes may have gone unnoticed.

A strength of this study is its large sample size which, among others, made it possible to conduct the research in patients with bilateral MSNON eyes. Previous OCT studies often included the unaffected contralateral eyes of unilateral MSON patients as well. We chose to exclude patients with a history of an unilateral episode of MSON, and thus limiting our sample size, because the contralateral unaffected eyes of MSON eyes are known to show more atrophy than bilateral MSNON eyes.[9]

In summary, in this paper we provide for the first time evidence for a strong relationship between atrophy of the pRNFL and mGCIPL and cognitive impairment in patients with MS, suggesting that retinal OCT might be useful in assessing systemic neurodegeneration in MS.

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Supplementary Table 1: Characteristics of the complete cohort of MS patients. Characteristics of all patients with MS dichotomized into those who were cognitively impaired and those who were cognitively preserved. The mean ± SD, median [range] and frequency (percentage) are presented. For inner retinal layer thickness only patients with the same history of MSON in both eyes were used.

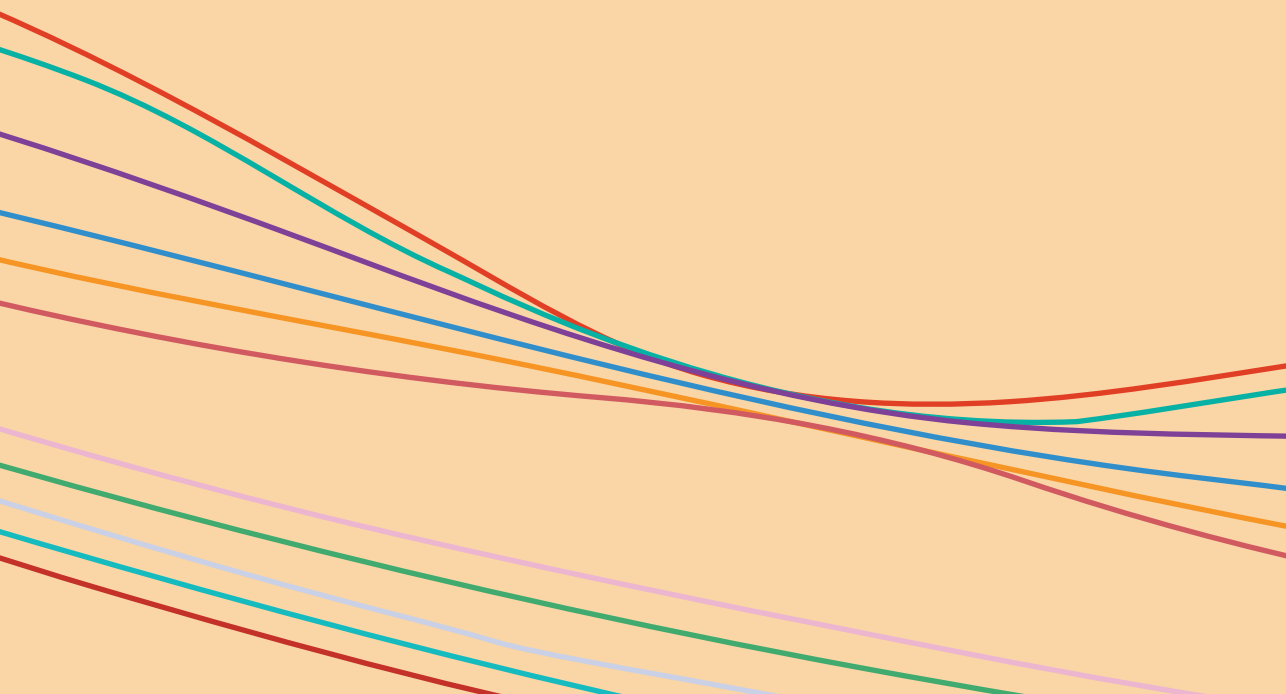
	Cognitively impaired N = 96	**Cognitively preserved N = 121**	**p-value**
Age (years), mean (±SD)	56.52 (± 9.78)	52.53 (± 9.78)	0.003
Gender (Male : Female)	34 : 62	33 : 88	0.197
Disease duration (years), mean (±SD)	21.73 (± 7.85)	19.23 (± 6.03)	0.011
EDSS, median [range]	4.5 [3.5 – 6.5]	3.0 [2.5 – 4.0]	<0.001
Type of MS			<0.001
RR	44 (45.8%)	89 (73.6%)	
SP	36 (37.5%)	20 (16.5%)	
PP	16 (16.7%)	12 (9.9%)	
History of MSON			
Bilateral MSNON	41 (42.7%)	61 (50.4%)	
Bilateral MSON	15 (15.6%)	20 (16.5%)	
Unilateral MSON	30 (31.3%)	31 (25.6%)	
Unknown	10 (10.4%)	9 (7.4%)	
Use of disease modifying therapy			0.941
Current	28 (29.2%)	33 (27.3%)	
Past	17 (17.7%)	23 (19.0%)	
Never	51 (53.1%)	65 (53.7%)	
pRNFL thickness (µm), mean (±SD)	78.92 (± 9.05)	85.59 (± 11.62)	0.003
mGCIPL thickness (µm), mean (±SD)	77.56 (± 14.75)	85.68 (± 14.24)	0.008

EDSS = expanded disability status scale; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive; MSNON = no history of multiple sclerosis associated optic neuritis; MSON = multiple sclerosis associated optic neuritis; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell-inner plexiform layer

Supplementary Table 2: Mean Z scores of separate cognitive tests and number of subjects that failed each test. Data are presented for the MSNON and MSON patients and the HCs.

	Z score (mean)			Number of subjects failed (%)		
	MSNON N = 102	**MSON N = 35**	**HCs N = 59**	**MSNON N = 102**	**MSON N = 35**	**HCs N = 59**
SRT	-0.57	-0.51	0.00	10/101 (9.9%)	3/34 (8.8%)	2/59 (3.4%)
SDMT	-1.03	-1.15	0.00	32/101 (31.7%)	13/35 (37.1%)	4/59 (6.8%)
WLGT	-0.43	-0.37	0.00	11/102 (10.8%)	6/35 (17.1%)	4/59 (6.8%)
10/36 SPRT	-0.59	-0.55	0.00	22/102 (21.6%)	9/35 (25.7%)	5/59 (8.5%)
CST	-0.76	-1.23	0.00	18/100 (18.0%)	10/34 (29.4%)	3/59 (5.1%)
SCWT	-0.52	-0.81	0.00	12/100 (12.0%)	5/33 (15.2%)	3/59 (5.1%)
MCT	-1.10	-1.56	0.00	34/99 (34.3%)	12/35 (34.3%)	2/59 (3.4)%

MSNON = no history of multiple sclerosis associated optic neuritis; MSON = multiple sclerosis associated optic neuritis; HCs = healthy controls; SRT = selective reminding test; SDMT = symbol digit modalities test; WLGT = word list generation test; 10/36 SPRT = 10/36 spatial recall test; CST = concept shifting test; SCWT = Stroop color word test; MCT = memory comparison test



CHAPTER 4.2

PROGRESSIVE MACULAR ATROPHY IS RELATED TO COGNITIVE DECLINE IN MULTIPLE SCLEROSIS

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ABSTRACT

Objective: To determine if retinal optical coherence tomography (OCT) can predict cognitive decline (CD) in multiple sclerosis (MS), by testing whether CD is related to baseline thickness as well as longitudinal changes of the peripapillary retinal nerve fiber layer (pRNFL) and macular ganglion-cell inner plexiform layer (mGCIPL).

Methods: Retinal OCT was performed at baseline and after two and four years of follow-up in 151 MS patients and 27 healthy controls. Cognitive assessment at baseline and at four-year follow-up was available in 122 patients. CD was evaluated in seven domains by calculating the reliable change index.

Results: Patients (mean disease duration 20.8 years) showed an annualized atrophy rate of -0.28 µm/year ($p<0.001$) for the pRNFL and -0.27 µm/year ($p<0.001$) for the mGCIPL. In total, 40 out of 122 patients (32.8%) declined cognitively. Compared to cognitively stable patients, CD patients showed significantly faster rates of mGCIPL atrophy during the first two years (mean difference 0.20 µm/year, $p=0.024$) and over the entire four-year period (mean difference 0.24 µm/year, $p<0.001$). Moreover, the amount of mGCIPL atrophy during the first two years and during the whole four-year period was associated with CD (OR per 1 µm mGCIPL loss 1.64, $p=0.043$ and 1.78, $p=0.005$ respectively). Baseline pRNFL and mGCIPL thickness did not predict CD.

Conclusion: The rate of mGCIPL atrophy and not the extent of baseline atrophy was related to CD in patients with longstanding MS. This strongly suggests that the rate of mGCIPL atrophy is a clinically relevant surrogate marker for disease progression in MS.

INTRODUCTION

Cognitive dysfunction is frequent in multiple sclerosis (MS) and has a large, negative impact on patients' quality of life.[1-3] All cognitive domains can be affected, but most often affected domains include information processing speed, episodic memory and executive functioning.[4] Early recognition of cognitive decline (CD) is essential for the selection of the most appropriate treatment. Validated neuropsychological test batteries to assess cognitive status exist, but these are time consuming and results can be influenced by depression and fatigue.[5] There is urgent need for a rapid, easily obtainable, non-invasive biomarker for predicting CD. Retinal optical coherence tomography (OCT) may serve this purpose.[6,7]

Changes in the inner retinal layers, as assessed by retinal OCT, mirror neurodegenerative changes in the CNS of MS patients.[8] We and others have previously demonstrated that thinning of the peripapillary retinal nerve fiber layer (pRNFL) and macular ganglion cell-inner plexiform layer (mGCIPL) is related to cognitive impairment in MS.[6,9] In addition, Bsteh *et al.* have shown that low pRNFL thickness at baseline predicted a decline in information processing speed, highlighting the potential value of retinal OCT as a biomarker for CD in MS.[10] However, this study focused only on the pRNFL and only on one cognitive domain. Therefore, in the current study we examined the predictive value of both baseline pRNFL and mGCIPL thickness and longitudinal changes in the thickness of these layers on CD, measured across multiple cognitive domains, in patients with MS.

METHODS

STUDY DESIGN AND POPULATION

This prospective study is the four-year follow-up on our previously published study in which we investigated the cross-sectional association between inner retinal layer atrophy and cognitive impairment in MS.[6] Patients with MS and healthy control subjects (HCs) were recruited from the prospective Amsterdam MS Cohort (MS Center Amsterdam, Amsterdam University Medical Center, location VU Medical Center, the Netherlands). Subjects were eligible for inclusion if they had OCT assessment at baseline and at four-year follow-up. The in- and exclusion criteria have been described in detail previously.[6] All patients were diagnosed with clinically definite MS following the revised 2010 McDonald criteria and had either a relapsing remitting (RR), secondary progressive (SP) or primary progressive (PP) disease course.[11,12]

Study visits were conducted at baseline and after two and four years of follow-up. Demographic data (disease duration, age, sex, level of education, disease type, history of MS associated optic neuritis [MSON]) were recorded at baseline. Level of disability (Expanded Disability Status Scale [EDSS] score)[13] and OCT assessments were performed at each visit, whereas extensive cognitive testing was only performed at baseline and after four years. All assessments during a visit were performed on the same day.

This study was approved by the Medical Ethical Committee on Human Research of the Amsterdam UMC, location VUmc in Amsterdam, the Netherlands. Written informed consent was obtained from all subjects before study inclusion.

OPTICAL COHERENCE TOMOGRAPHY

All OCT images were acquired using a spectral domain OCT device (Spectralis, Heidelberg Engineering, Heidelberg, Germany). All OCT scans were obtained at the same site, by experienced and certified technicians. Room light conditions were dimmed and pharmacological pupil dilation was not required in any of the cases. Peripapillary and macular images were obtained using a 12° ring scan (1536 A-scans, 1 B-scan, no predetermined automatic real time [ART]), manually placed around the optic disc and a macular volume scan (20x20°, 512 A-scans, 49 B-scans, vertical alignment, ART 16) centered on the fovea, respectively. Follow-up scans were performed with the follow-up function, ensuring placement was identical to the baseline scan.[14] Automated segmentation of the pRNFL and mGCIPL was performed using the same updated algorithm for all scans (HRA / Spectralis Viewing Module 6.9.5.0). Scanning was followed by OCT quality control (OSCAR-IB).[15] For pRNFL thickness measurements (in µm) the global mean of the entire pRNFL was used. For mGCIPL thickness measurements the average thickness (in µm) of all but the central sector of the 1, 2.22, 3.4-mm EDTRS grid was used. Annualized atrophy rates (AARs) for the first two years and the whole four-year period were calculated for each eye in all subjects.

COGNITIVE ASSESSMENT

To assess cognitive decline, all participants were subjected to a seven domain cognitive test battery at baseline and after four years, as described previously.[6] Examinations at both time points were administered according to a standardized protocol by trained research assistants who were blinded to all other data. The complete neuropsychological examination consisted of Rao's Brief Repeatable Battery of Neuropsychological Tests (BRB-N) and three additional cognitive tests, each covering a different cognitive domain. This examination included the Selective Reminding Test (verbal learning and memory), Symbol Digit Modalities Test (SDMT, information processing speed), Word List Generation Test (semantic verbal fluency) and 10/36 Spatial Recall Test (visuospatial learning and memory), Concept Shifting Test (executive functioning), Stroop Color Word test (attention) and the Memory Comparison Test (working memory).[16] The raw test scores were adjusted for age, sex and education, based on a sample of healthy controls, as described in previous publications.[17,18]

As described previously,[17] the number of cognitively impaired (CI) and cognitively preserved patients (CP) at baseline were determined using domain-specific z-scores based on the means and standard deviations (SDs) of the HCs. In order to determine these HCs reference scores, the cognitive assessment data of an addition 33 HCs from the same cohort, but who did not have OCT data, were used. Patients were subsequently classified as CI if they scored more than 1.5 SDs below the HCs on two or more domains. The remainder was classified as CP. For longitudinal analysis of CD we used the validated, modified practice adjusted reliable

change index (RCI).[17,19] The RCI indicates the change in scores between baseline and four-year follow-up corrected for practice effects observed in HCs. To control for the variation in time-interval between baseline and follow-up, a yearly RCI score was computed by dividing the RCIs by the individual patient's time interval. Patients were classified as CD if they had a yearly RCI score of at least -0.25 on two or more domains, which has previously been shown to optimally separate CD patients from cognitively stable (CS) patients and HCs.[17] All other patients were classified as CS.

STATISTICAL ANALYSES

Normality of data distribution was assessed visually. For all analyses on subject level (e.g. age and EDSS score), differences in continuous variables between two groups (e.g. between patients and HCs and between CD and CS patients) were tested using independent samples T-test (parametric distribution) or Mann-Whitney-U test (non-parametric distribution) and differences in categorical variables were tested using chi-squared test. Differences in baseline retinal layer thickness between the aforementioned groups and between eyes with a history of MSON and without (MSNON eyes) were tested using generalized estimating equations (GEE) with an exchangeable correlation matrix.

GEE models were also used to analyze differences in AARs between patients and HCs and to analyze associations between AARs and clinical measures (e.g. history of MSON, disease type). In addition, patients were categorized into four groups according to their age and disease duration (lowest/shortest to highest/longest quartiles) in order to assess the relationship between AARs and both age and disease duration. Again, GEE models were used to test the differences in AARs between the quartiles.

In order to determine the predictive value of OCT parameters on CD logistic regression analyses were used. Three different parameters were tested: baseline thickness, AAR, and the absolute amount of atrophy. In the case of baseline thickness, only MSNON eyes were used (mean value of both eyes in bilateral MSNON patients or only the MSNON eye in unilateral MSON patients) because of the large effect of MSON on retinal thickness.[6] Baseline pRNFL and mGCIPL thickness were considered both as a continues and as a dichotomous predictor (with median thickness as cutoff). When testing the association between the two- and four-year AAR and two- and four-year absolute atrophy and CD MSON and MSNON eyes were pooled.

All analyses were adjusted for age and sex and some were adjusted for additional factors as well (as indicated). Analyses were performed in SPSS version 22.0, with alpha set at 0.05.

RESULTS

OCT data at baseline and four-year follow-up was available in 151 MS patients and 27 HCs, leading to the inclusion of 178 subjects in this study. Their baseline characteristics are shown in Table 1. Of these 178 subjects, 131 patients and 21 HCs also had OCT assessment at two-

year follow-up. Mean total follow-up time was similar in patients and HCs (both 4.5 years on average). After OSCAR-IB quality control of OCT scans, 12/108 (11.1%) baseline and 19/108 (17.6%) four-year follow-up scans were rejected in HCs. In patients, 132/604 (21.9%) baseline and 138/604 (22.8%) four-year follow-up scans were rejected. Complete longitudinal cognitive assessment (baseline and year four) was available in 122 of 151 patients.

At baseline, MS patients had a mean disease duration of 20.8 years (SD 6.5, range 9.2-45.9). Patients were more often female and were older compared to the HCs. On the whole, 40.3% of patients was classified as CI at baseline compared to 0% in HCs. As expected, both inner retinal layers were significantly thinner in patients than in HCs, even when only MSNON eyes were considered.

Table 1: Baseline characteristics of the cohort.

	Patients N = 151	**Healthy controls N = 27**	**p-value**
Age (years), mean (±SD)	53.8 (9.6)	52.2 (5.4)	0.234
Sex (% female)	65.6%	44.4%	0.037
Disease duration (years), mean (±SD)	20.8 (6.5)	N/a	
Type of MS			
RRMS	98 (64.9%)	N/a	
SPMS	34 (22.5%)		
PPMS	19 (12.6%)		
History of MSON			
Bilateral MSNON	73 (48.3%)	N/a	
Unilateral MSON	50 (33.1%)		
Bilateral MSON	28 (18.6%)		
EDSS, median [range]	3.5 [1.0 – 8.0]	N/a	
Use of DMT			
Current	44 (29.1%)	N/a	
Past	30 (19.9%)		
Never	77 (51.0%)		
Cognitive function (CP : CI) [a]	86 : 58	25 : 0	<0.001
pRNFL thickness (µm), mean (±SD)[b]			
Overall	84.1 (14.1)	93.5 (7.6)	<0.001
MSNON eyes	88.4 (11.3)		0.008
MSON eyes	75.7 (15.3)		<0.001
mGCIPL thickness (µm), mean (±SD)[b]			
Overall	77.3 (14.0)	92.7 (5.9)	<0.001
MSNON eyes	82.0 (11.7)		<0.001
MSON eyes	68.0 (13.6)		<0.001

[a] Seven patients and two healthy controls had an incomplete/invalid neuropsychological examination
[b] adjusted for age and sex
SD = standard deviation; N/a = not available; RR = relapsing remitting; SP = secondary progressive, PP = primary progressive; MSON = MS associated optic neuritis; MSNON = no history of MSON; EDSS = expanded disability status scale; DMT = disease modifying therapy; CP = cognitively preserved; CI = cognitively impaired; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer

LONGITUDINAL RETINAL ATROPHY RATES

Seven eyes in six patients developed a new episode of MSON during follow-up and these eyes were excluded from further pooled analyses. Table 2 shows the mean AARs over the course of four years of the pRNFL and mGCIPL in patients, HCs and different patient subgroups. Patients showed a statistically significant AAR of the pRNFL (-0.28 µm/year, p<0.001) and mGCIPL (-0.27 µm/year, p<0.001) during the four-year follow-up, while HCs only showed a significant AAR of the mGCIPL (-0.27 µm/year, p<0.007) but not the pRNFL (-0.14 µm/year, p=0.196). While patients did show a higher rate of pRNFL atrophy compared to HCs, this was not statistically significant (mean difference 0.14 µm/year, p = 0.110). There was no difference in the AAR of the mGCIPL between patients and HCs (mean difference 0.0 µm/year, p=0.985).

Eyes with a history of MSON prior to baseline assessment showed a significantly thinner pRNFL and mGCIPL at baseline compared to MSNON eyes (mean difference 12.7 µm, p<0.001 and 14.0 µm, p<0.001 respectively). However, eyes with and without a prior history of MSON showed the same atrophy rate of the pRNFL and mGCIPL over time (-0.32 vs. -0.25 µm/year, p=0.251 and -0.23 vs. -0.28 µm/year, p=0.922 respectively).

Table 2: Annualized atrophy rates over the four-year follow-up period. The reference for statistical comparisons is indicated as 'Ref.'

	pRNFL µm/year (95%CI)	p-value	mGCIPL µm/year (95%CI)	p-value
MS patients[a]	-0.28 (-0.34;-0.21)	Ref.	-0.27 (-0.32; -0.21)	Ref.
Healthy controls	-0.14 (-0.33; 0.05)	0.110	-0.27 (-0.37; -0.17)	0.985
Type of MS[b]				
RRMS	-0.33 (-0.41; -0.26)	Ref.	-0.25 (-0.30; -0.19)	Ref.
SPMS	-0.14 (-0.29; 0.02)	0.595	-0.24 (-0.37; -0.10)	0.894
PPMS	-0.20 (-0.44; 0.05)	0.808	-0.41 (-0.59; -0.23)	0.460
History of MSON[c]				
MSON	-0.32 (-0.43; -0.21)	Ref.	-0.23 (-0.32; -0.14)	Ref.
MSNON	-0.25 (-0.34; -0.17)	0.251	-0.28 (-0.35; -0.22)	0.922
Use of DMT[d]				
Current	-0.31 (-0.43; -0.19)	Ref.	-0.21 (-0.29; -0.11)	Ref.
Past	-0.18 (-0.33; -0.02)	0.326	-0.22 (-0.34; -0.10)	0.823
Never	-0.30 (-0.39; -0.21)	0.737	-0.32 (-0.39; -0.24)	0.251
Cognitive function[e]				
Preserved	-0.20 (-0.28; -0.11)	Ref.	-0.25 (-0.31; -0.19)	Ref.
Impaired	-0.34 (-0.44; -0.24)	0.017	-0.29 (-0.36; -0.22)	0.514

a Adjusted for age and sex
b Adjusted for age, sex and use of disease modifying therapy
c Adjusted for age, sex, type of MS and use of disease modifying therapy
d Adjusted for age, sex and type of MS
e Adjusted for age, sex, type of MS and use of disease modifying therapy
pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer; 95%CI = 95% confidence interval; RR = relapsing remitting; SP = secondary progressive, PP = primary progressive; MSON = MS associated optic neuritis; MSNON = no history of MSON; DMT = disease modifying therapy

For this reason, MSON and MSNON eyes were pooled when using longitudinal OCT parameters as predictors of CD. The seven eyes that developed a new episode of MSON during follow-up showed more absolute atrophy of the inner retinal layers over the four-year period compared to the eyes of the rest of the patients, but this was not statistically significant for the pRNFL (pRNFL -7.20 vs. -1.25 µm, p=0.208; mGCIPL -8.70 vs. -1.17 µm, p=0.040). Patients who were CI at baseline showed a higher AAR of the pRNFL compared to CP patients (mean difference 0.14 µm/year, p=0.017). No differences in AARs were found for the other clinical subgroups (see Table 2).

Figure 1 shows the AARs (with 95% confidence intervals) in patients in different age and disease duration quartiles. Patients in the highest age quartile showed a significantly lower pRNFL AAR compared to patients in the other three quartiles. No such relationship was found between age and the mGCIPL AAR nor between disease duration and the pRNFL or mGCIPL AARs.

COGNITIVE DECLINE

Of the 122 patients with complete cognitive assessment 40 (32.8%) had worsened at least -0.25 SD per year on two or more tests and were classified as CD. The other 67.2% of patients was considered CS. Table 3 shows the baseline characteristics of the patients that declined and of those that remained stable. The patients that declined cognitively, were more often diagnosed with PP (p=0.001) or SP (p<0.001) MS and had a higher median EDSS score (p=0.001) at baseline.

There was no difference in baseline pRNFL or mGCIPL thickness between patients who declined cognitively and those who did not, see Table 3. However, patients who were classified as CD did show a higher AAR of the mGCIPL in the first two years (mean difference 0.20 µm/year, p=0.024) and over the whole follow-up period (mean difference 0.24 µm/year, p<0.001) compared to the CS patients. As for the pRNFL, no difference was found in the AARs between the CD and the CS group, see Table 3.

PREDICTION OF COGNITIVE DECLINE

Baseline pRNFL and mGCIPL thickness were considered both as continuous and as dichotomous predictors (with median thickness as cutoff), see Table 4. For these analyses, only MSNON eyes were used. Baseline pRNFL and mGCIPL thickness did not predict cognitive decline at four-year follow-up (OR respectively 1.03 [95%CI 0.98 – 1.08], p=0.239 and 1.00 [95%CI 0.95 – 1.04], p=0.858). Patient in the lower half of pRNFL (≤87.0 µm) and mGCIPL (≤81.1 µm) thickness had a lower odds ratios of showing CD compared to patients in the top half, but this failed to reach statistical significance (respectively OR 0.35 [95%CI 0.11 – 1.14], p=0.082 and OR 0.85 [95%CI 0.28 – 2.53], p=0.766).

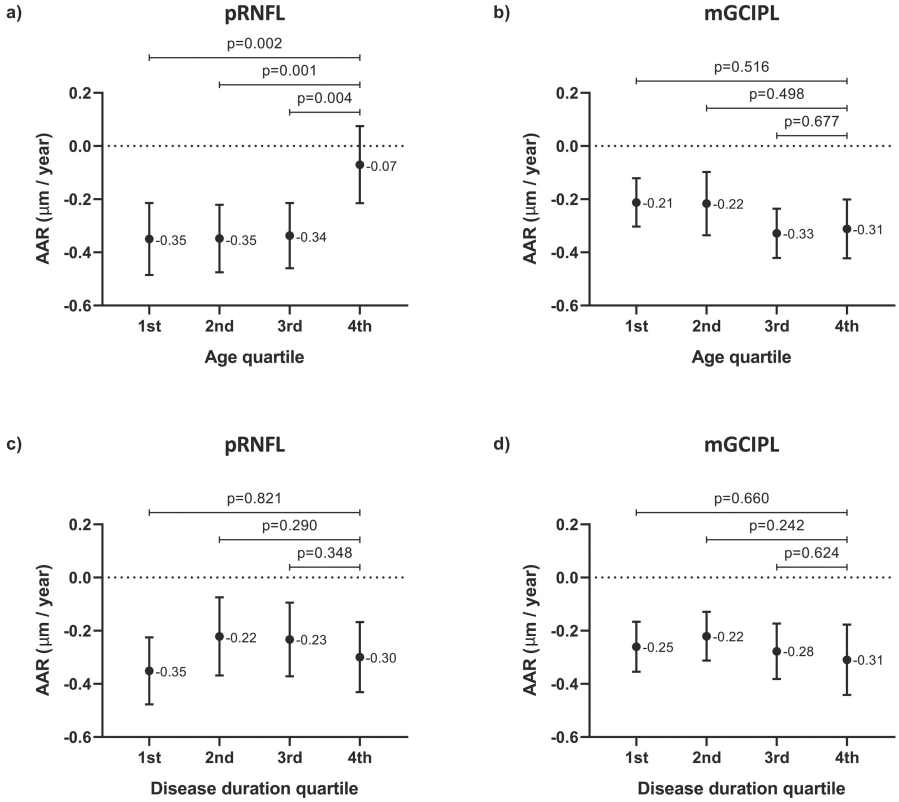

Figure 1: Four-year annualized atrophy rates (AARs) and 95% confidence intervals of the peripapillary retinal nerve fiber layer (pRNFL) and macular ganglion cell-inner plexiform layer (mGCIPL) per quartile of age and disease duration in patients. All analyses are adjusted for sex, type of MS and use of disease modifying therapy.

For the analysis of longitudinal atrophy as a predictor of CD, MSON and MSNON eyes were pooled. The absolute amount of mGCIPL loss in the first two years of follow-up was associated with CD with an OR of 1.64 (95%CI 1.02 – 2.65, p=0.043), meaning the odds of CD at year four increased by 1.64 for every micrometer of mGCIPL lost during the first two years of follow-up.

Likewise, the amount of mGCIPL atrophy during the whole four-year period was related to an increased odds of CD (OR 1.78 [95%CI 1.19 – 2.67, p=0.005]). Similar results were found for the mGCIPL when using the AAR instead of the absolute amount of retinal atrophy, see Table 4. As for the pRNFL, neither the total amount of atrophy nor the AAR was related to CD at four-year follow-up.

Table 3: Baseline characteristics of the patients that declined cognitively and those that remained stable at four-year follow-up.

	Cognitively declined N = 40	Cognitively stable N = 82	p-value
Age (years), mean (±SD)	54.6 (9.3)	52.9 (9.4)	0.350
Sex (% female)	60.0%	67.1%	0.443
Disease duration (years), mean (±SD)	21.2 (5.4)	20.7 (6.8)	0.719
Type of MS			
RRMS	16 (40.0%)	64 (78.0%)	<0.001
SPMS	15 (37.5%)	12 (14.6%)	
PPMS	9 (22.5%)	6 (7.3%)	
History of MSON			
Bilateral MSNON	18 (45.0%)	36 (43.9%)	0.912
Unilateral MSON	15 (37.5%)	29 (35.4%)	
Bilateral MSON	7 (17.5%)	17 (20.7%)	
EDSS, median [range][a]	4.0 [2.0 – 8.0]	3.0 [1.5 – 7.5]	0.001
Use of DMT			
Current	8 (20.0%)	25 (30.5%)	0.240
Past	7 (17.5%)	19 (23.2%)	
Never	25 (62.5%)	38 (46.3%)	
Baseline pRNFL thickness (µm), mean (±SD)[a]			
Overall	83.6 (16.5)	83.5 (13.7)	0.986
MSNON eyes	89.3 (14.0)	87.4 (10.3)	0.329
MSON eyes	72.7 (15.6)	76.4 (16.1)	0.724
Baseline mGCIPL thickness (µm), mean (±SD)[a]			
Overall	75.4 (14.3)	77.4 (14.1)	0.372
MSNON eyes	80.8 (11.7)	82.2 (11.4)	0.659
MSON eyes	63.8 (12.8)	68.9 (14.3)	0.363
AAR pRNFL (µm/year), mean (95%CI)[b]			
Two-year period	-0.30 (-0.62; 0.01)	-0.23 (-0.39; -0.06)	0.825
Four-your period	-0.30 (-0.44; -0.16)	-0.24 (-0.33; -0.15)	0.320
AAR mGCIPL (µm/year), mean (95%CI)[b]			
Two-year period	-0.34 (-0.49; -0.20)	-0.14 (-0.25; -0.04)	0.024
Four-your period	-0.41 (-0.51; -0.32)	0.17 (-0.23; -0.11)	<0.001

a Adjusted for age and sex

b Adjusted for age, sex, type of MS and use of disease modifying therapy

RR = relapsing remitting; SP = secondary progressive, PP = primary progressive; MSON = MS associated optic neuritis; MSNON = no history of MSON; EDSS = expanded disability status scale; DMT = disease modifying therapy; CP = cognitively preserved; CI = cognitively impaired; AAR = annualized atrophy rate; 95%CI = 95% confidence interval; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer

Table 4: Odds ratios of OCT parameters for predicting cognitive decline in patients with MS. All analyses are adjusted for age, sex, disease course and use of disease modifying therapy.

	pRNFL OR (95%CI)	p-value	mGCIPL OR (95%CI)	p-value
Baseline thickness (per µm thinning)[a]	1.03 (0.98 – 1.08)	0.239	1.00 (0.95 – 1.04)	0.858
Baseline thickness (lower vs. upper half)[a, b]	0.35 (0.11 – 1.14)	0.082	0.85 (0.28 – 2.53)	0.766
Two-year absolute loss (per µm)	1.03 (0.79 – 1.34)	0.832	1.64 (1.02 – 2.65)	0.043
Four-year absolute loss (per µm)	1.08 (0.84 – 1.39)	0.566	1.78 (1.19 – 2.67)	0.005
Two-year AAR (per µm/year loss)	1.08 (0.61 – 1.92)	0.788	3.00 (1.04 – 8.66)	0.042
Four-year AAR (per µm/year loss)	1.51 (0.48 – 4.72)	0.483	13.31 (2.21 – 80.21)	0.005

a Analysis only in MSNON eye

b Cutoff pRNFL = ≤87.0 µm and cutoff mGCIPL = ≤81.1 µm

OR = odds ratio; 95%CI = 95% confidence interval; OCT = optical coherence tomography; pRNFL = peripapillary retinal nerve fiber layer; mGCIPL = macular ganglion cell – inner plexiform layer; AAR = annualized atrophy rate

DISCUSSION

In this prospective, longitudinal study, we investigated the prognostic value of retinal OCT parameters on future cognitive decline as measured across multiple cognitive domains. The main finding was that the rate and amount of mGCIPL atrophy was strongly related to cognitive decline in patients with longstanding MS. Baseline (cross-sectional) data on pRNFL or mGCIPL atrophy were not predictive of future cognitive decline. This study provides support for the usefulness of longitudinal retinal OCT measurements in monitoring and predicting clinically relevant disease progression in MS patients, beyond what can be achieved with well-established scales which focus predominantly on the pyramidal system.

Atrophy of the pRNFL and mGCIPL in MS is thought to occur due to retrograde trans-synaptic degeneration, or may be a reflection of a more global mechanism causing neuroaxonal damage to the brain and to the visual system.[20,21] The cross-sectional associations between thinning of the inner retinal layers and prevalent cognitive impairment and physical disability have been demonstrated.[6,9,22,23] Low baseline pRNFL thickness has also been shown to predict future physical deterioration.[24] Longitudinal studies investigating the predictive value of retinal OCT on CD in MS are scarce, but are needed if OCT is to become useful as a prognostic marker in clinical setting. To date, only one other study has investigated the predictive value of retinal OCT on CD in MS. The study by Bsteh *et al.* found that RR MS patients with pRNFL thickness ≤ 88 µm at baseline had a 2.7-fold increased risk of CD, defined as a sustained decrease of ≥ 4 points or ≥ 10% in SDMT score, after a follow-up period of three years compared to patients with pRNFL thickness > 88 µm.[10] Two other large, community-based studies have investigated the predictive value of OCT on cognitive worsening, although outside of MS. The large (n=32038) community based study by Ko *et al*. in people not suffering from a neurodegenerative disease found that a thinner macular RNFL was not only associated with worse cognitive performance

at baseline but also that people in the two lowest quintiles of macular RNFL thickness had twice the likelihood of cognitive worsening over a three-year period compared to people in the highest RNFL quintile.[7] In another, smaller (n=3289), community-based study, low mGCIPL thickness was associated with prevalent dementia, whereas low pRNFL thickness was associated with an increased risk of developing dementia.[25] Taken together, these statistically well powered studies strongly suggest a progressive neurodegenerative link between the brain and the eye which can be captured by inner retinal layer atrophy.

An important difference between the aforementioned studies and the present study is the smaller statistical power of our single center cohort compared to the community based cohort studies. This may in part explain why we did not find a statistically significant association between baseline inner retinal layer thickness and an increased risk of CD in our cohort. Another might be the longer disease duration in our patients compared to the patients in the other studies. The study by Bsteh *et al*.[10] was performed in MS patients early in the disease course (mean disease duration 5.8 years) and the participants in the two community-based studies[7,25] were included in the 'pre-clinical' stage (i.e. before they had any symptoms). The patients in the present study had a mean disease duration of over twenty years. Balk *et al*. have shown that the rate of pRNFL and mGCIPL atrophy is highest in the early phase and declines with longer disease duration.[26] This observation is strengthened by the fact that the highest pRNFL AARs (of 1-2 µm/year) have been found in patients with a short disease duration and low age,[27-30] whereas lower AARs of about 0.3 µm/year have been found in studies that included patients with a long disease duration and high age.[31, 32] The pRNFL and mGCIPL atrophy rates in our cohort best match the atrophy rates of the latter. We did not find a relationship between the rate of pRNFL or mGCIPL atrophy and disease duration, but this is probably due to the fact that the shortest disease duration in our cohort was 9.2 years.

Structural brain damage is thought to occur early in the disease course but cognitive impairment manifests itself only when a certain threshold is crossed, at which cognitive reserves cannot compensate for the amount of damage any more. After this point cognition steadily declines.[33,34] The hypothetical relationship between disease duration, cognitive dysfunction and inner retinal layer atrophy is depicted in Figure 2. If we assume this model is true, then, due to the presumed floor effect of inner retinal layer atrophy and the high risk of cognitive decline in the later stages of disease, a single thickness measurement is not able to distinguish patients who will decline cognitively from those who will remain stable. Perhaps at this point, longitudinal atrophy measurements are more informative when aiming to predict CD, as the present study shows.

Another possible advantage of longitudinal atrophy measurement is lower inter-subject variability. While several studies have demonstrated a relationship between inner retinal layer thickness and disability on a group level, the applicability of retinal OCT in individual patients in routine medical practice has been limited by high inter-subject variability.

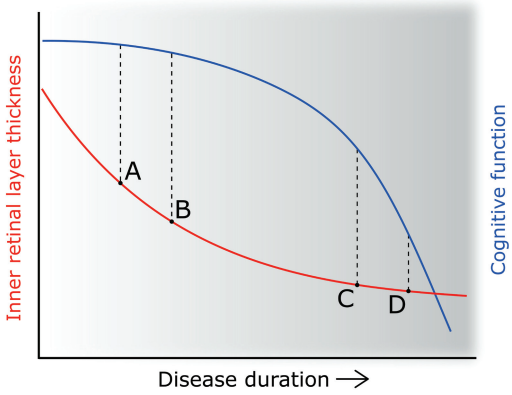

Figure 2: Summary on the longitudinal relationships between rate of mGCIPL atrophy and cognitive decline. The rate of inner retinal layer atrophy (red line) is highest in the early phase of the disease and slowly attenuates leading to a floor effect. Cognitive functioning (blue line) on the other hand remains relatively stable until a certain amount of damage has occurred after which it progressively declines. In this proposed model, inner retinal layer thickness indicates at which point in the neurodegenerative process a patient is. Patients are indicated by dots and letters. Here, patient A and B both have a short disease duration but patient B has a higher risk of cognitive decline (the slope of the cognitive dysfunction line after point B is steeper compared to the slope after point A). In the later stages of the disease a lot of damage to the inner retinal layers has occurred but due to the floor effect the ability to distinguish patients C and D is low while the risk of cognitive decline is high in both. In this stage of the disease progressive loss of the inner retinal layers is more informative of the risk of cognitive decline (inspired *by Schoonheim et al. [33]*).

Therefore it would be interesting to investigate the prognostic value of longitudinal atrophy measurements in a cohort of patients with short disease duration, or in patients across a wide range of disease durations.

Lastly, longitudinal atrophy measurements also overcome the masking effect of MSON typically affecting cross-sectional data. This masking effect on cross-sectional associations between inner retinal layer thickness and disability has complicated the use of OCT as a biomarker in MS patients with a previous history of bilateral MSON. The same problem arises when using baseline thickness as a predictor of CD. In line with previous studies,[32,35,36] the present study shows that although MSON eyes demonstrate a significantly thinner pRNFL and mGCIPL at baseline compared to MSNON eyes, the rate of atrophy of these two layers over time is comparable in both eye types. This implies that longitudinal data on atrophy rates from MSON and MSNON eyes can be pooled for a whole range of prediction models. The only remaining limitation is to exclude eyes with a new episode of MSON during the observation period.

This study found that the amount and rate of mGCIPL atrophy were associated with CD at follow-up but this effect did not reach statistical significance for the pRNFL. This might at first glance appear inconsistent. At second glance, it becomes apparent that the variability of optic discs between subjects is much larger than what is observed for the perimacular rim. Therefore the data scatter for the pRNFL is larger compared to the mGCIPL (see error bars in Figure 1). This makes data from the mGCIPL a statistically more attractive metric for future studies.

The main strength of this study, the long disease duration, can also be seen as its main limitation and might have influenced our results as discussed previously. In addition, the long disease duration has resulted in low AARs overall and has perhaps limited the ability to reveal associations between AARs and certain disease characteristics. Another limitation is the loss to follow-up, which might have led to the selection of less affected or more stable patients (i.e. more affected patients are less likely to return to the hospital for a follow-up visit). This potential selection bias is inherent to most longitudinal studies, but may play a more important role in studies where a large portion of patients are in a progressive disease stage. Strengths of this study include the extensive battery of cognitive tests enabling the assessment of cognitive functioning across multiple cognitive domains and the use of the RCI for CD thereby adjusting for practice effects. Bsteh *et al*.[10] assessed CD by means of the SDMT. Information processing speed is indeed one of the most commonly affected domains in MS and the SDMT is a validated instrument to assess deficits in this domain,[4] but it does not capture all the cognitive problems a MS patient might suffer from.

In conclusion, the present study shows that in the later stages of MS longitudinal mGCIPL atrophy is strongly associated with cognitive decline. Longitudinal atrophy measurements have the additional advantage of lower inter-subject variability and permit the pooling of data from MSON and MSNON eyes. Being a rapid, inexpensive and practical instrument, if future research can confirm and build upon the findings in this study, retinal OCT will become an essential aid in the assessment of cognitive, in addition to physical, deterioration in patients with MS.

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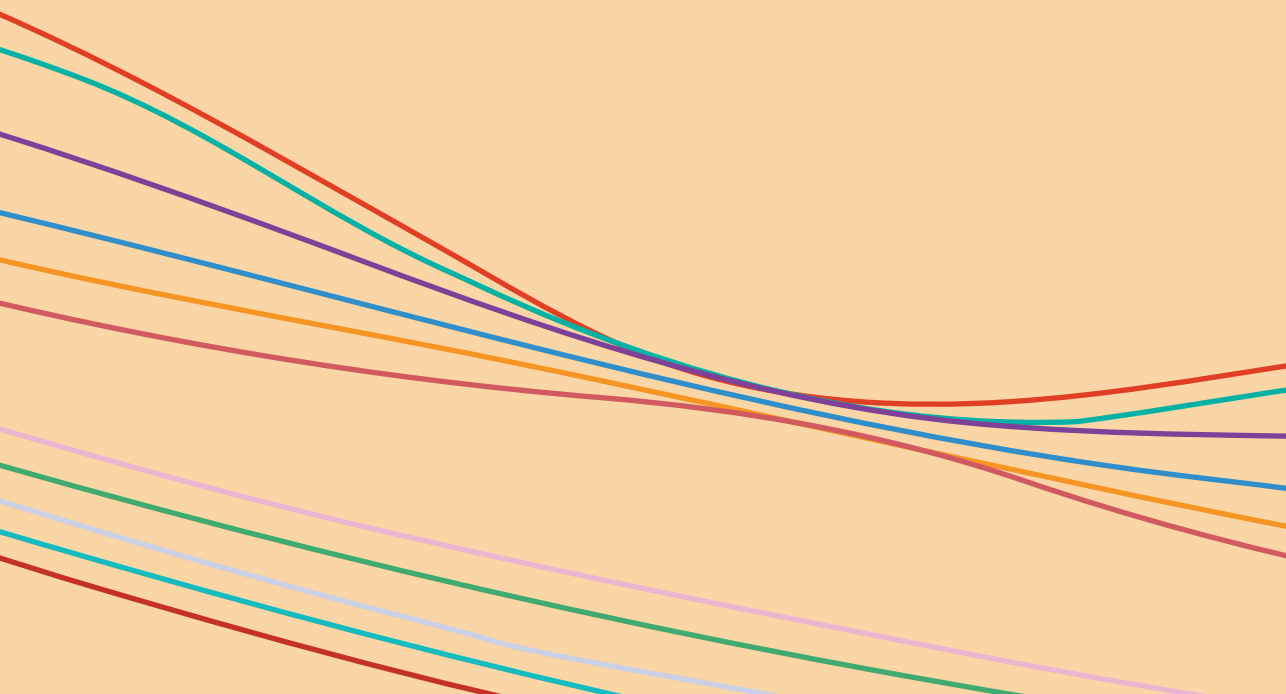

CHAPTER 4.3

LONGITUDINAL DEVELOPMENT OF PERIPAPILLARY HYPER-REFLECTIVE OVOID MASSLIKE STRUCTURES SUGGESTS A NOVEL PATHOLOGICAL PATHWAY IN MULTIPLE SCLEROSIS

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ABSTRACT

Objective: Peripapillary Hyperreflective Ovoid Mass-like Structures (PHOMS) are a new spectral domain Optical Coherence Tomography (OCT) finding.

Methods: Prospective, longitudinal study. Patients (n=212) with MS (n=418 eyes), 59 healthy controls (HC, n=117 eyes) and 267 non–MS disease controls (534 eyes). OCT and Diffusion Tensor Imaging.

Results: There were no PHOMS in HC eyes (0/117, 0%). The prevalence of PHOMS was significantly higher in patients with MS (34/212, p=0.001) and MS eyes (45/418, p=0.0002) if compared to HC (0/59, 0/117). The inter–rater agreement for PHOMS was 97.9%, kappa 0.951. PHOMS were present in 16% of patients with relapsing remitting, 16% of patients with progressive and 12% of patients with secondary progressive disease course (2% of eyes). There was no relationship of PHOMS with age, disease duration, disease course, disability or disease modifying treatments. The fractional anisotropy of the optic radiations was lower in patients without PHOMS (0.814) if compared to patients with PHOMS (0.845, p=0.03). The majority of PHOMS remained stable, but increase in size and de novo development of PHOMS were also observed. In non–MS disease controls, PHOMS were observed in intracranial hypertension (62%), ODD (47%), anomalous optic discs (44%), isolated optic neuritis (19%) and optic atrophy (12%).

Interpretation: These data suggest that PHOMS are a novel finding in MS pathology. Future research is needed to determine if development of PHOMS in MS is due to intermittently raised intracranial pressure or an otherwise impaired “glymphatic” outflow from eye to brain.

INTRODUCTION

The optic disc has been of interest in multiple sclerosis (MS) ever since the original description of structural changes observed following optic neuritis.[1,2] Early post–mortem histological observations of the optic disc were that "nerve–fibres showed numerous spindle–shaped swellings [...]. Adhering to the nerve–fibres were very numerous ovoid flattend nuclei [...].".[3] This was interpreted to represent "nutritive hyperplasia" supposedly of "increased activity of the protoplasm".[3]

Longitudinal study of these ovoid structures has been challenging because they are buried below the nerve fibre layer and the need for histology. With the introduction of retinal optical coherence tomography (OCT) it has become possible to study retinal structures in much more detail than previously possible.[4] These ovoid structures have recently been termed Peripapillary Hyperrefelective Ovoid Mass–like Structures (PHOMS) by an international consensus panel.[5] Current evidence suggests that these PHOMS originate from axoplasmatic stasis or congestion in the prelaminar optic nerve head.[6] The occurrence of PHOMS in MS has not yet been studied with studies almost exclusively focusing on quantitative assessment of the peripapillary retinal nerve fibre layer (pRNFL) from an OCT ring scan rather then qualitative assessment of optic disc volume scans.[4,7]

In this longitudinal, prospective study we investigated the occurrence and development of PHOMS and optic disc drusen (ODD) in patients with MS and healthy control subjects.[8] We also studied how this novel observation related to non–MS disease controls and MS specific clinical data and integrity of the visual pathways on magnetic resonance brain imaging (MRI).

METHODS

This prospective study was approved by the ethics committee of the Amsterdam University Medical Center (protocol number 2010/336) and the scientific research committee (protocol number CWO/10-25D). The study is in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to study inclusion.

STUDY DESIGN AND PATIENTS

MS disease population All subjects were recruited at the MS Center Amsterdam between March 2011 and August 2012. Patients were assessed at baseline and after two years.

Inclusion criteria were a diagnosis of MS according to the 2010 revision of the McDonald diagnostic criteria.[9] Exclusion criteria were pregnancy, a relapse or a course of steroids in the last four weeks, a diagnosis of HIV or other immunodeficiency, substance abuse in the past five years or MRI findings that could interfere with evaluation. For healthy controls additional exclusion criteria were any other neurological, ophthalmological or psychiatric disease or a first or second degree relative with a diagnosis of MS. Episodes of MS optic neuritis (MSON) were

identified through patient history and confirmed clinically using a standard care protocol.[10] Subjects with ODD were excluded as per OSCAR-IB criteria.[11] At baseline screening a total of 53 eyes were excluded due to opacities in the visual pathways, abnormal retinal or optic disc findings or other problems.[12] In order to be consistent with these previous publications on this cohort[12,13] the disease course was classified into relapsing remitting (RR), secondary progressive (SP) and primary progressive (PP) according to the Lublin *et al.* 1996 classification.[14]

Non MS disease control population The retrospective case note review was approved by the Research and Development Department of Moorfields Eye Hospital, London (protocol number ROAD17/030). The diagnostic groupings were: optic atrophy (OA), isolated optic neuritis (ION), referrals for assessment of an incidentally detected anomalous optic disc, increased intracranial hypertension (IIH), ODD, medical retinal diseases, headaches, non embolic transient visual field loss (neTMVL) and those who experienced entoptic phenomena.

The retrospective control population was added after the MS cohort study had finished. Therefore only descriptive statistics were performed to illustrate the general distribution of PHOMS as may be encountered in clinic.

OCT PROTOCOL

All OCT images were obtained with a SD-OCT (Heidelberg Spectralis, Heidelberg Engineering, Germany [software version 1.1.6.3]) with eye tracking function enabled for best accuracy.[15] Data were collected from an optic disc volume scan (15x15 degrees, 37 B-scans), peripapillary ring scan (12 degrees, 1 B-scan) and a macular volume scan (20x20 degrees, 49 B-scans). We could not include enhanced depth imaging (EDI) because this feature was added at a later stage to the software.

Automated segmentation was performed with the manufacturer's software (HEYEX version 1.10.2.0, Viewing Module version 6.9.5.0). All scans underwent a rigorous quality control (QC) check.[11] Algorithm failures were corrected by hand. The peripapillary retinal nerve fibre layer (pRNFL), macular ganglion cell inner plexiform layer (GCIPL) and macular inner nuclear layer (INL) thicknesses were exported for statistical analysis. All OCT terminology used follows consensus guideline recommendations.[16] The ODD consortium definition of PHOMS for OCT was used based on consensus.[5] We did not perform ultrasound.

MRI PROTOCOL

Structural magnetic resonance imaging (MRI) was performed on a 3T whole body system (GE Signa HDxt, Milwaukee, WI, USA). The detailed acquisition parameters have been described previously as well as an example of the 3T MRI.[12,13] In brief, normalised grey and white matter volumes and lesion volumes were quantified automatically using k nearest neighbour classification with tissue type priors (KNN-TTP), and SIENAX (part of the FMRIB Software Library (FSL) 5.0.4, http://www.fmrib.ox.ac.uk/fsl). Lesion filling was applied to minimise the effect of lesions on atrophy measurements.

EXTERNAL VALIDATION

External validation of the PHOMS rating was performed (S.H.). The inter-rater agreement for rating of PHOMS has been investigated using Fleiss kappa statistics. The inter–rater kappa for the two independent raters of PHOMS in the present study (A.P., S.H.) was 0.811 in the multirater study of the ODD consortium.[17]

STATISTICAL ANALYSIS

The statistical analyses were performed in SAS (version 9.4). First, normality in measurements was tested graphically and using Shapiro-Wilk statistics. Non-parametric tests were used for non-normal or skewed data and parametric tests for normally distributed data. Median (interquartile range, IQR) or mean ± standard deviation (SD) are shown. Differences between two groups were analysed using the Chi-square test for categorical variables, the two-tailed t-test for parametric continuous variables and the Mann-Whitney test for non-parametric continuous variables. General linear models (GLM) were used for comparison of data from more than two groups. Correlation analyses were performed using Pearson's *r* for normally distributed and Spearman's rho for non-Gaussian data. Bonferroni method was used to correct for multiple correlations. Differences for segmented retinal layer thickness data between groups was analysed using generalised estimation equations (GEE) as recommended;[16] these were adjusted for intra subject inter-eye correlations, repeated measurements, and employed an exchangeable correlation structure. Inter–rater agreement on rating of PHOMS was assessed using Cohen's kappa. Missing data were handled as such and indicated in the footnotes to the tables. A p-value of 0.05 was accepted as statistically significant.

RESULTS

The baseline data of the 227 patients with MS and 62 control subjects are summarised in Table 1. Patient with MS were only slightly older on average compared to the control subjects (p=0.0105; 95% mean difference = 3.5 years, 95% confidence interval = 1 to 6 years).

Figure 1 shows the appearance of a normal optic disc in comparison to an optic disc with PHOMS. The disc shown in Figure 1B represents one of the two cases where PHOMS did co-exist with ODD. Because ODD were an exclusion criterion, these two cases were excluded from all further statistical analyses.

The inter–rater agreement for PHOMS was 97.9% with a kappa of 0.951. The proportion of PHOMS was significantly higher in patients with MS (16%) if compared to healthy control subjects (0%). Statistical significance increased further for comparison of the proportion of affected eyes (45 in MS and 0 in controls, p<0.0001). There was no significant age difference between patients with MS who had PHOMS and those who had not (p=0.54). There was no association between presence of PHOMS and the clinical disease course or disease duration (p=0.26, p=0.46, respectively).

Table 1: Subject characteristics. Mean (standard deviation) or n (%) is shown.

	Controls	Patients	*p*
Subjects	62	227	
Eyes	117	418	
Gender, F:M	41:21	155:72	ns
Age, yr	50.6 (7.1)	54.1 (10.0)	0.01[a]
Disease duration, yr	N/a	20.4 (6.9)	
Follow-up, mo	27.5 (2.9)	26.0 (2.7)	ns
EDSS	N/a	4.2 (1.7)	
EDSS progression	N/a	0.3 (0.7)	
Disease course	N/a	139 RR, 28 PP, 60 SP	
MSON	None	32 right, 30 left, 39 bilateral, 126 never	
DMT	N/a	IFN, 65; FTY, 4; AZT, 1; MTX, 3; 1, ATM-027; NTZ, 10; GA, 15; teriflunomide, 1	
ODD	1 (2%)	1 (1%)	ns
PHOMS	0 (0%)	34 (16%) all MS, 21 (16%) RR, 6 (12%) SP, 7 (16%) PP	<0.001[b]

[a]*The Bonferroni adjusted p value for multiple comparisons (n = 5) is 0.01.*
[b]*The statistical significance of this finding increases to p < 0.0001 if the number of eyes (45/373 vs 0/117) instead of the number of patients (as shown in the table) is taken for comparison.*
AZT = azathioprine; DMT = disease-modifying treatment; EDSS = Expanded Disability Status Scale; F = female; FTY = fingolimod; GA = glatiramer acetate; IFN = interferon beta 1a and 1b; M = male; MS = multiple sclerosis; MSON = multiple sclerosis associated optic neuritis; MTX = mitoxantrone; N/a = not applicable; ns = not significant; NTZ = natalizumab; ODD = optic disc drusen; PHOMS = peripapillary hyper-reflective ovoid masslike structure; PP = primary progressive; RR = relapsing remitting; SP = secondary progressive

Disease progression on the EDSS was mild (Table 1). There was no statistical relationship between progression on the EDSS and presence or absence of PHOMS (p=0.23).

Management with disease modifying treatments (DMTs) was not associated with presence of PHOMS (Chi–square test, p=0.83). Of the four patients with fingolimod only one had PHOMS.

The longitudinal images demonstrated three patterns of PHOMS. First, PHOMS remained stable over the two year observation period as illustrated for a small PHOMS (Figure 2A) and a larger PHOMS (Figure 2B). Second, there was *de novo* development of PHOMS (Figure 2C). Finally, existing small PHOMS could increase in size (Figure 2D).

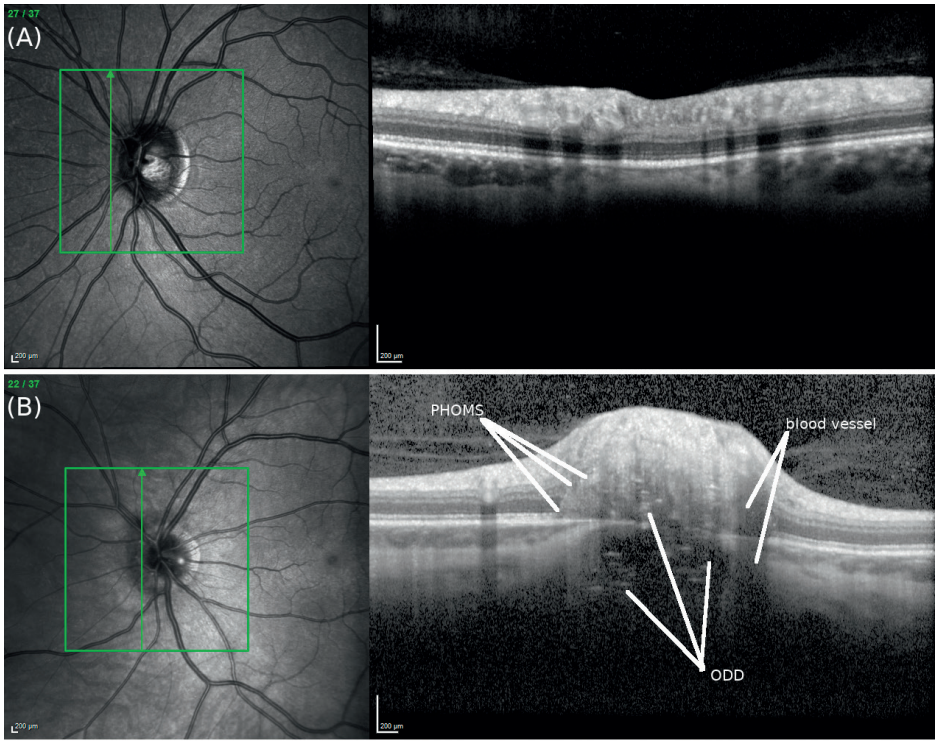

Figure 1: A normal optic disc is shown in (A) and an optic disc with PHOMS and ODD in (B). The hyperintense PHOMS typically transverse several of the retinal layers, in this case from the RNFL down to the basal membrane. The ODD are located above and below Bruch's membrane and impress as conglomerates of low intensity signal with intermingled hyperintense small horizontal lines. This appearance is very different to the vertical shadow cast by blood vessel artefacts. The confocal laser scanning ophthalmoscopy (cSLO) is shown to the left. The green line indicates the location of the OCT B–scan shown to the right.

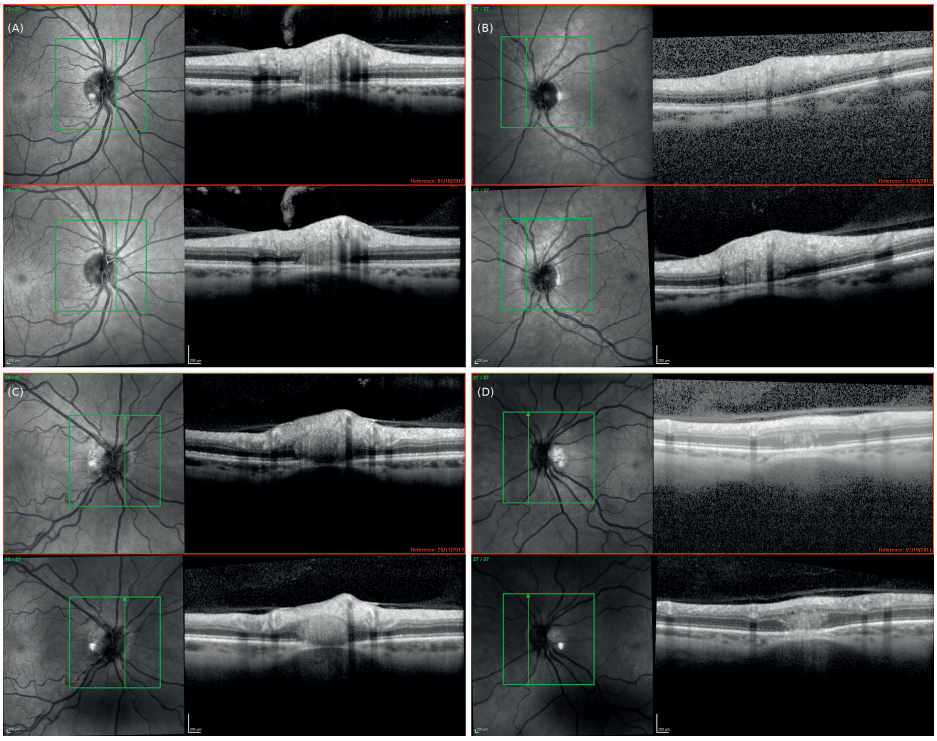

Figure 2: Examples of PHOMS in patients with MS. (A) a small PHOMS which remained stable in size over the 2 year observation period. The baseline image is shown to the top (red frame) and the follow up image to the bottom. (B) A larger PHOMS which also remained stable over time. (C) A de novo PHOMS which has developed. (D) A small PHOMS at baseline which has increased in size over two years. The confocal laser scanning ophthalmoscopy (cSLO) is shown to the left. The green line indicates the location of the OCT B–scan shown to the right.

PHOMS AND MSON

An episode of MSON had occurred in 144 eyes. The location was on the right in 74 and on the left in 70 eyes. Percentage of PHOMS in eyes affected by MSON was 10% on the right and 8% on the left. The percentage of PHOMS in eyes never affected by MSON was 14% on the right and 12% on the left. Overall the presence of PHOMS was not related to a history of MSON (Chi–square test >0.05). The patterns of PHOMS (stable, de novo, increase) were not related to MSON.

PHOMS AND VISUAL PATHWAY INTEGRITY

Table 2 summarised data on visual pathway integrity in subjects with and without PHOMS. In all eyes there was progressive atrophy of the pRNFL and GCIPL over the two year observation period. At baseline, eyes from patients with PHOMS did have a mildly thinner pRNFL and GCIPL if compared to patients without PHOMS but this did not reach statistical significance. Progression of atrophy was more marked in eyes without PHOMS compared to eyes with PHOMS for both layers, again without reaching statistical significance.

Table 2: PHOMS and visual pathway integrity in multiple sclerosis.

	PHOMS+	PHOMS-	*p*
Eyes[a]			
n	427	40	
pRNFL baseline, µm	86.34 ± 13.91	84.83 ± 12.96	ns
pRNFL follow–up, µm	84.03 ± 14.44	84.57 ± 12.48	ns
Δ pRNFL, µm	−0.77 ± 1.98	−0.09 ± 2.22	ns
GCIPL baseline, µm	80.07 ± 14.58	79.53 ± 13.49	ns
GCIPL follow–up, µm	78.64 ± 14.92	78.06 ± 13.49	ns
Δ GCIPL, µm	−0.65 ± 1.51	−0.15 ± 1.19	ns
Patients[b]			
n	173	33	
Optic radiations			
MD	1.905 ± 0.197	1.863 ± 0.169	ns
FA	0.814 ± 0.084	0.845 ± 0.073	0.03[c]
Visual cortex			
V1	3.56 ± 0.25	3.52 ± 0.21	ns
V2	4.10 ± 0.23	4.06 ± 0.23	ns

[a]There were n = 5 eyes of patients with PHOMS and n = 33 eyes of patients without PHOMS who failed quality control for quantitative data for either the pRNFL or GCIPL.
[b]The magnetic resonance imaging metrics were not available from 1 patient with PHOMS and n = 49 patients without PHOMS.
[c]The Bonferroni-adjusted p value for multiple comparisons (n = 4) is 0.0125.
FA = fractional anisotropy; GCIPL = ganglion cell-inner plexiform layer; MD = mean diffusivity; ns = not significant; PHOMS = peripapillary hyperreflective ovoid masslike structure; pRNFL = peripapillary retinal nerve fiber layer.

In patients without PHOMS, the fractional anisotropy (FA) of the optic radiations was lower compared to patients with PHOMS and this appeared to be statistically significant (p=0.0363). This difference was no longer statistically significant after Bonferroni correction for multiple comparisons. Consistent with this finding, the mean diffusivity (MD) was higher in the optic radiations of patients with PHOMS compared to patients without PHOMS, but this did not reach statistical significance. Likewise there was no statistically significant difference in the degree of atrophy of the occipital cortex either for V1 or V2 comparing the two groups (Table 2).

The patterns of PHOMS (stable, de novo, increase) were not related to these visual pathway data.

Table 3: Subject characteristics of the retrospective non–multiple sclerosis disease control cohort (n = 267). Mean (SD) or n (%) is shown.

	ION	OA	Disc	Cog	Opt	Pain	IIH	MR	ODD	TMVL
Subjects	16	49	81	4	17	19	13	20	38	10
Eyes	32	98	162	8	34	38	26	40	76	20
Gender, F:M	10:6	22:27	53:28	2:2	12:5	15:4	12:1	9:11	21:17	6:4
Age, yr	44.5 (19.1)	53.2 (16.6)	34.0 (14.4)	40.8 (7.7)	38.4 (11.9)	36.0 (13.1)	29.7 (9.2)	44.7 (18.4)	38.4 (15.7)	43.5 (15.1)
ODD	1 (6%)	3 (6%)	6 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	38 (100%)	0 (0%)
PHOMS	3 (19%)	6 (12%)	36 (44%)	1 (1%)	0 (0%)	3 (16%)	8 (62%)	1 (1%)	18 (47%)	1 (10%)

Cog = cognitive; Disc = anomalous discs; F = female; IIH = increased intracranial hypertension; ION = isolated optic neuritis; M = male; MR = medical retinal disease; OA = optic atrophy; ODD = isolated optic disc drusen; Opt = entoptic phenomena; Pain = headaches not due to IIH; PHOMS = peripapillary hyper-reflective ovoid masslike structure; TMVL = nonembolic transient monocular visual field loss.

PHOMS IN NON-MS DISEASE CONTROLS

An additional retrospective case note review was performed on 267 patients who had for their routine clinical work up OCT optic nerve head volume imaging. Table 3 summarises the subject characteristics for the disease groups. Overall, PHOMS were more frequent than ODD. Conditions associated most frequently with PHOMS were IIH (62%), ODD (47%) and anomalous optic discs (44%). The percentage of PHOMS in patient with an isolated optic neuritis (19%) and optic atrophy (12%) was comparable to the patients with MSON (18% of MSON eyes, see above).

DISCUSSION

There are four main findings from this study. First, PHOMS can clearly be seen in patients with MS using a routine optic nerve head OCT volume scan with an excellent inter–rater kappa of 0.951. Second, PHOMS are significantly more frequently present in patients with MS compared to healthy controls. Third, in patients with PHOMS the optic radiations have a significantly higher FA compared to patients without PHOMS. Both findings are relevant because they provide indirect evidence for impaired axoplasmatic flow in the visual pathways of patients with MS (Figure 3). Finally, there are three types of PHOMS, (i) PHOMS who remain stable; (ii) PHOMS which increase in size; (iii) PHOMS which develop *de novo*.

It is important to note that presence of PHOMS is an observation independent to biases by key clinical features. This makes PHOMS an interesting new object to study an hitherto unknown aspect of pathology in MS. Specifically, it has been excluded that PHOMS were observed more frequently in patients with MSON as compared to those who never experienced MSON.[10] This observation was confirmed in the non–MS cohort for isolated optic neuritis. This is relevant because PHOMS can be observed in the course of a whole range of aetiology leading to optic

disc swelling (personal observation, AP). Next, PHOMS were not related to the degree of either pRNFL or GCIPL atrophy. Neither were degree of atrophy of the pRNFL or GCIPL associated with PHOMS. Longitudinally PHOMS, even if developing, were not related to progression of atrophy. Again, the finding was confirmed by the data from the non–MS cohort for optic atrophy. This is an observation which for example can be made with ODD causing progressive visual field defects.[18] The known association between more severe retinal inner layer atrophy and progression of disability on the EDSS[19] cannot be shown for PHOMS. There was no relationship between PHOMS and use of DMTs. This is relevant because for example fingolimod has been identified as a cause for macular oedema.[20] Likewise there was no association of PHOMS with demographic data or disease duration. The latter can however also be interpreted as a limitation of the study because we cannot comment on PHOMS during the early disease course of MS. Patients in this study had a long disease duration of about 20 years. A strength of the study is however, the longitudinal data with an averaged follow–up period of 26 to 27 months.

After having ruled out an association of PHOMS with demographic or clinical data it is possible to return to the observation with an unbiased mind. In MS there are very few patients with PHOMS and in these patients one can observe a significant increase of the FA in the optic radiations. Anatomically, the first observation locates to the anterior and the second to the posterior optic pathways. What could be an explanation connecting the two?

There are at least three potential explanations to discuss. First, axoplasmatic stasis and localised aggregate formation. Second, the glymphatic system. Third, the trans-laminar pressure gradient at the optic disc.

The first argument builds on an earlier indirect observation of impaired axonal transport and aggregate formation.[21] In MS some axons, particularly those adjacent to MS lesions show signs of increased neurofilament compactness and aggregation in the axolemma. Neurofilaments are key component of the axonal cytoskeleton.[22,23] Therefore, the observation of axonal swellings indicating a reversible form of axonal damage is intriguing.[24] The images shown in this elegant study demonstrate local accumulation of neurofilament proteins in axonal swelling.[24] Could PHOMS be a late sign for reversible axonal damage in MS? Future immuno-histochemical post–mortem studies of PHOMS in the retina of patients with MS will be needed to clarify whether or not neurofilament proteins or myelin products can be found in PHOMS. Such future studies should also investigate the role of phosphorylation of neurofilaments and other proteins which significantly affects MRI metrics.[25] One question arising from the observation of the increased FA is whether or not proton mobility in the optic pathways (Figure 3A) of patients with PHOMS is influenced by accumulation, aggregation and phosphorylation of proteins, all contributing to impaired axonal flow. This line of argumentation may be useful for helping to explain the otherwise paradoxical observation of an increase of the RNFL over time in some patients with MS and from experimental models.[26,27]

The second hypothesis builds on the observation of impairment of the glymphatic system.[28] Hamann *et al.* have demonstrated that water channel (aquaporin) distribution in the eye provides an excellent anatomical basis for a presumed ocular glymphatic system.[29,30] Therefore it could be possible that an impaired glymphatic system through aquaporins in MS reduces the ability to remove extracellular waste products such as compounds extravasted during axoplasmatic stasis (Figure 3C).[31]

There have been independent lines of argumentation of the past five years raising the likelihood of such a retinal glymphatic system.[32–35] The longitudinal data from this study show that the increase of size of PHOMS had been very mild over a two year period. Such a slow dynamic would be more consistent with a glymphatic problem, rather than being a consequence of more acute pathology. Particularly after relevant demographic and clinical factors have been ruled out.

The third hypothesis is related to the second and offers a mechanistic approach to the previous two. The trans–laminar pressure gradient at the optic disc (Figure 3B) has been suggested as a relevant factor in driving neurodegeneration in a chronic optic neuropathy, glaucoma.[36] We are not aware of data on PHOMS in the glaucoma literature, but another example for transient change of the trans-laminar pressure gradient at the optic disc is IIH. The development of PHOMS in patients with IIH has been observed, as well as PHOMS regression after treatment[37] (personal observation AP). Recent reports on an elevated lumbar opening pressure in MS come from the paediatric literature.[38-39] But the observation had been made reliably by experienced neuro-ophthlamologists anecdotally in adults.[40] All of their three patients reported headaches and lumbar puncture opening cerebrospinal fluid pressures were > 29 cm H2O, 42 cm H2O and 25 cm H2O.[40] Contemporary routine examination of the cerebrospinal fluid in patients with MS does not include measurement of the opening pressure.[41] Future studies investigating the hypothesis that there could be intermittent intracranial pressure elevation in MS (Figure 3B) are advised to follow a well–designed protocol for calibrated pressure measurements of the eye and brain.[42] This will be of particular interest in patients in whom PHOMS develop *de novo* or increase in size over time. These observations provide indirect evidence for a glymphatic system which connects the eye with the brain.

Above interpretations of our data also highlight the most relevant shortcomings. There are no recent histological data showing PHOMS in MS which shed light on the underlying pathology. The published immuno-histochemical images of post–mortem optic discs in MS by Green *et al.* show degrees of axonal atrophy, but these samples did not contain eyes from patients with PHOMS.[43] Arguably the three cases presented historically by Norris did not present optic neuritis as defined by von Graefe and Nettleship.[1,2] Instead he described optic disc oedema in the context of other pathology.[3] Nevertheless these authors reported an important histological observation which, given the alternative pathology and the findings in our non–MS cohort, remind us that PHOMS are not specific for MS. Similar to what has been reported for other new OCT based observations in MS, PHOMS can be found with a whole range of clinical pathologies and, in a clinical context, are most frequently misinterpreted as pseudopapilloedema (personal observation AP).

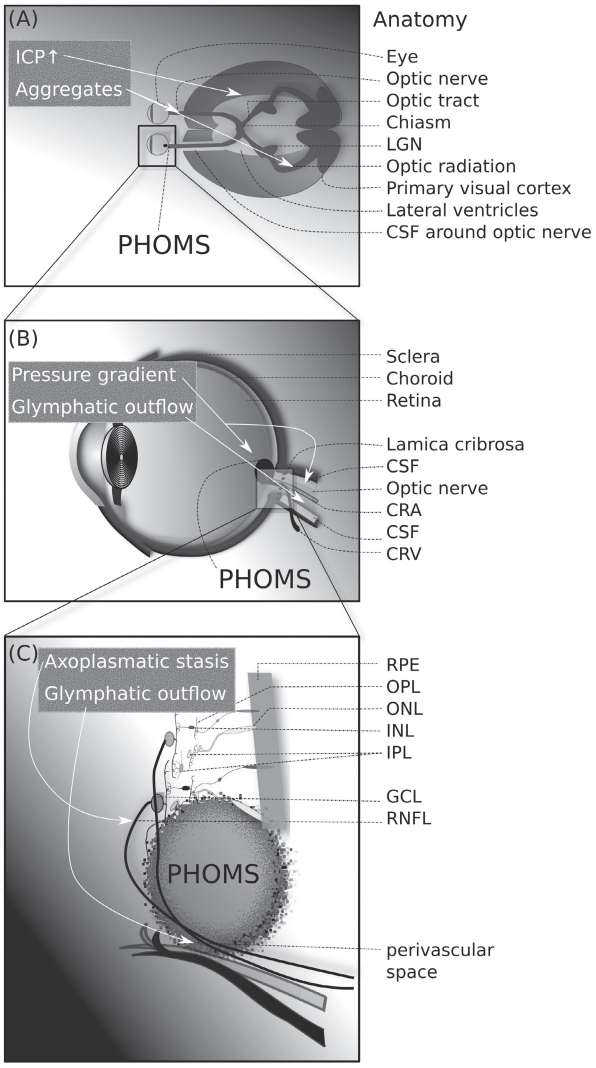

Figure 3: Development of PHOMS in MS. (A) The anatomy of the visual pathways in relation to the brain and cerebrospinal fluid (CSF) spaces. Increased intracranial pressure and aggregate formation in the optic pathways may result in development of PHOMS (white arrows). (B) PHOMS are located in the peripapillary area where they are in close anatomical proximity to axons leaving the eye to form the optic nerve, the CSF in the optic nerve sheet, the central retinal artery and central retinal vein. A change of the trans-laminar pressure gradient (pre- / post-lamina cribrosa) may result in axoplasmatic stasis and reduced glymphatic outflow (white arrows) resulting in PHOMS. (C) PHOMS have a local mass effect which typically displaces content from several retinal layers. This can give the impression of pseudopapilloedema. Axoplasmatic stasis and impaired glymphatic outflow through the perivascular space of the optic nerve may contribute to build up of PHOMS (white arrows).

LGN = lateral geniculate ganglion; CRA = central retinal artery; CRV = central retinal vein; RPE = retinal pigment epithelium; OPL = outer plexiform layer; ONL = outer nuclear layer; INL = inner nuclear layer; IPL = inner plexiform layer; GCL = ganglion cell layer; RNFL = retinal nerve fibre layer.

Technical limitations of the study are related to software updates. Very small ODD below Bruch's membrane can escape detection without use of EDI. The ODD consortium has therefore developed a highly sensitive ODD imaging protocol which should be employed in future studies on PHOMS in MS (see Table 1 in reference [5]). Likewise, we did not quantitatively assess the relative afferent pupillary defect (RAPD) which would be an interesting additional metric for visual function to be correlated to PHOMS.[44,45]

Clinical limitations of the study are related to the retrospective nature of the non–MS cohort. The retrospective cohort does, however, permit examining in more general terms the association of PHOMS with other diseases. This is clinically relevant because of the differential diagnosis of IIH. If PHOMS are misinterpreted clinically as true disc swelling, than there is a risk of overestimating IIH. In 16% of cases with a primary headache disorder, presence of PHOMS will give the impression of pseudopapilloedema. The difficulty interpreting PHOMS as a cause for pseudo–papilloedema is also reflected in the high referral pattern to clinic of patients with what has been classified as an "anomalous disc". In 44% this was due to PHOMS. The presence of PHOMS in 19% of patients with an isolated optic neuropathy and 12% of patients with optic atrophy will require future research. For example, the ODD Consortium has identified PHOMS as a novel, independent risk factor for young onset, < 50 years, non–ateritic ischaemic optic neuropathy (manuscript in preparation). Likewise almost half of all patients with ODD also harbour PHOMS. Taken together the clinical limitations of the post–hoc retrospective addition of the non–MS cohort does still add valuable clinical information.

It is worthwhile to reflect on the study limitations in a broader context. To date work on the glymphatic system largely relies on histological data from tracer studies in rodents.[46,47] There is a need for other methodological approaches suitable for longitudinal *in vivo* human studies. This is required in order to study any presumed relationships to disease processes. The hypothesis to be investigated further in MS research is whether impairment of the glymphatic system could contribute to explaining reduced clearance of potentially immunogenic compounds from the paravascular space. Approaches in this direction are needed to explain the pathognomic but enigmatic perivascular compartmentalization of lymphocytes in the brain of patients with MS.[48] A further limitation is that the only MRI parameter we found to be associated with presence of PHOMS, FA, does not have pathological specificity. Lower FA is typically thought to represent bundle atrophy. FA can increase as a result of restricted perpendicular diffusivity, facilitated parallel diffusivity, or some combination of the two. Future studies may benefit from advanced multi-modal brain including PET with novel dynamic tracers.[49] Other limitations of our study relate to the regular update of consensus criteria. For internal consistency with our previous publications[12,13] we adhered to the 2010 revision of the McDonald criteria and the 1996 Lublins classification. All of our patients also met the 2017 revision of the McDonald criteria.[41] However, there have been relevant changes to the disease course in the revision to the disease course.[50] The main difference relates to 'active' and 'non active' disease. In this context, observed development of PHOMS over time may be interrogated as an alternative approach to recognise disease activity which may be of interest for future revisions

of such classifications. The probably most relevant limitation comes, however, from a recent debate.[51–54] The existence of a glymphatic transport system from the eye to the brain has just been demonstrated experimentally in rodents.[55]

In conclusion, this study shows that a small proportion of patients with MS harbour PHOMS. In these patients PHOMS can slowly increase in size over time or develop *de novo*. There are plausible mechanism which can explain this development. Presence of PHOMS may be caused by axoplasmatic stasis, impairment of a presumed glymphatic system from the eye through the optic nerve, or change of the trans-laminar pressure gradient at the optic disc. Taken together PHOMS are a novel finding in MS which might be useful for study of a hitherto unexplored pathway in MS, the glymphatic system.

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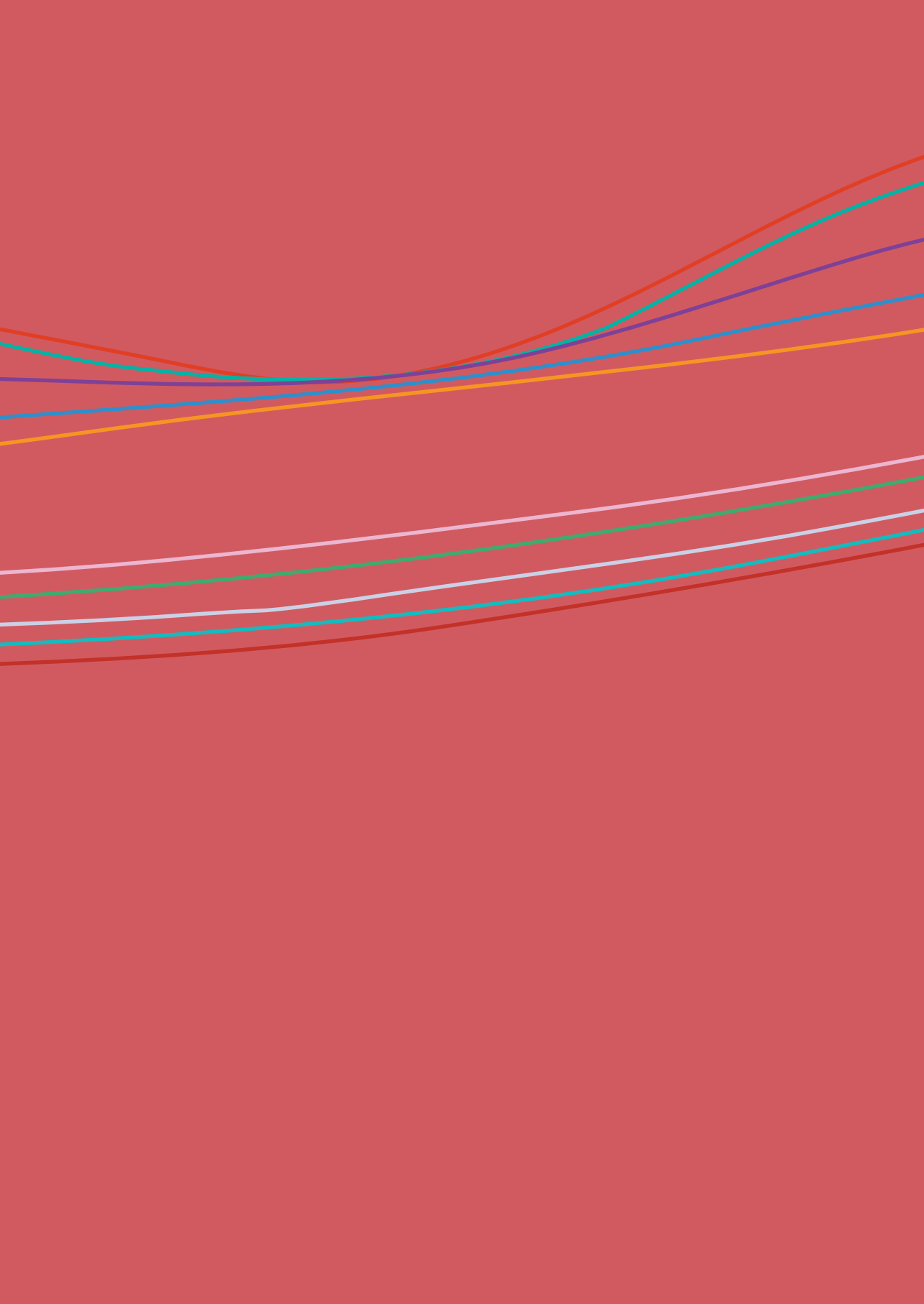
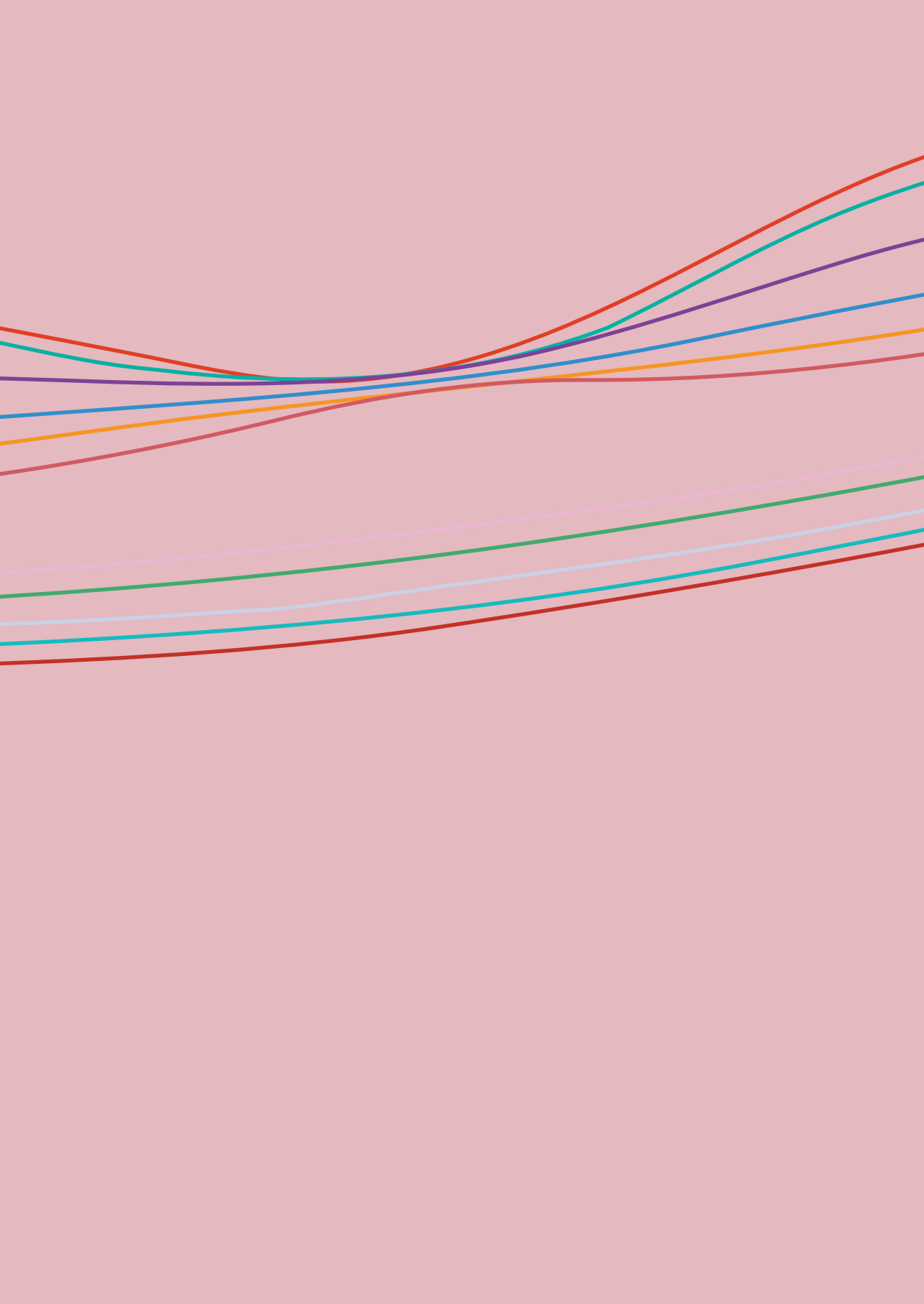


CHAPTER 5

OCT AS A MARKER OF INFLAMMATION IN MS



CHAPTER 5.1

RETINAL INNER NUCLEAR LAYER VOLUME REFLECTS INFLAMMATORY DISEASE ACTIVITY IN MULTIPLE SCLEROSIS; A LONGITUDINAL OCT STUDY

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ABSTRACT

Background: The association of peripapillary retinal nerve fiber layer (pRNFL) and ganglion cell-inner plexiform layer (GCIPL) thickness, with neurodegeneration in MS is well established. The relationship of the adjoining inner nuclear layer (INL) with inflammatory disease activity is less well understood.

Objective: To investigate the relationship of INL volume changes with inflammatory disease activity in MS.

Methods: In this longitudinal multi-center study, optical coherence tomography (OCT) and clinical data (disability status, relapses, MS optic neuritis [MSON]) were collected in 785 patients with MS (68.3% female) and 92 healthy controls (63.4% female) from eleven MS centers between 2010 and 2017 and pooled retrospectively. Data on pRNFL, GCIPL and INL were obtained at each centre.

Results: There was a significant increase in INL volume in eyes with new MSON during the study (N=61/1562, β=0.01 mm^3, $p<0.001$). Clinical relapses (other than MSON) were significantly associated with increased INL volume (β=0.005, p=0.025). INL volume was independent from disease progression (β=0.002 mm^3, p=0.474).

Conclusion: Our data demonstrate that an increase of INL volume is associated with MSON and the occurrence of clinical relapses. Therefore, INL volume changes may be useful as an outcome marker for inflammatory disease activity in MSON and MS treatment trials.

INTRODUCTION

Thinning of the inner retinal layers, as observed with the use of optical coherence tomography (OCT) is a common finding in multiple sclerosis (MS) patients.[1] Retinal OCT has been suggested as a structural imaging biomarker for neuroaxonal degeneration, as reduced thickness of both the peripapillary retinal nerve fiber layer (pRNFL, consisting of axons) and the combined thickness of the ganglion cell layer and inner plexiform layer (GCIPL, consisting of mainly ganglion cells) have shown to be associated with grey and white matter atrophy in patients with MS.[2-6] Although the association of pRNFL and GCIPL thickness with neurodegeneration in MS is well established, a more complex situation is observed for the adjoining inner nuclear layer (INL). A histological study demonstrated that the INL, representing a neuronal network of bipolar, amacrine and horizontal cells, shows signs of atrophy but the presence of inflammatory cells was also described.[7] Nevertheless, the INL seems not to be susceptible to retrograde degeneration caused by MS related optic neuritis (MSON), as it does not show the extensive neuro-axonal injury in eyes with MSON as observed in the pRNFL and GCIPL.[1,8] Rather than reflecting neurodegeneration, like the innermost pRNFL and GCIPL, the INL may be a biomarker for inflammatory processes. In 2012, Gelfand *et al.* first described the presence of microcystic macular oedema (MMO) in the INL and the relationship with disability.[9] Furthermore, a retrospective study by Saidha and colleagues reported that increased thickness of the combined inner nuclear and outer plexiform layer (OPL) was associated with disease activity in MS.[10] More recently, Knier *et al.* reported that successful treatment with disease modifying treatment (DMT) is associated with sustained reduction of INL volume,[11] suggesting that the INL could serve as a biomarker to monitor CNS inflammation. Therefore, the aim of this study was to investigate the relationship of INL volume changes over time with local and global inflammatory disease activity in a large cohort of patients with MS.

METHODS

STUDY DESIGN AND PARTICIPANTS

We used longitudinal data from the International Multiple Sclerosis Visual System Consortium (IMSVISUAL) database (www.imsvisual.org). Patients were recruited from 11 centers in the Netherlands (Amsterdam N=165), Germany (Berlin N=81, Düsseldorf N=15, Munich [Universität München] N=11, Munich [Technische Universität München] N=169), Kuwait (Kuwait-City N=98), Spain (Barcelona CEMCAT N=39, Barcelona IDIBAPS N=69), Italy (Milan N=56), the USA (New York City N=10) and France (Lille N=72). Healthy control subjects (HCs) were recruited from three centers (Amsterdam N=41, Munich N=17 and Berlin N=34). All patients and HCs participated in local observational studies and provided written informed consent for participation in their respective studies. Data was pooled retrospectively. All data from reported and ongoing cohort studies at multiple sclerosis centers were stored in the IMSVISUAL repository. The raw dataset is available from IMSVISUAL on request.

Data were collected between 2010 and 2017. MS patients were included if they were aged between 16 and 80 years, had a diagnosis of clinically isolated syndrome (CIS) or MS (including relapsing-remitting [RR], secondary progressive [SP], and primary progressive [PP] subtypes) according to the revised 2010 McDonald Criteria.[12] HCs were included if they were aged between 18 and 80 years and had no history of any neurological disease or ophthalmologic reason for retinal pathology. Regarding the OCT assessments, subjects were included if they had at least two OCT measurements (baseline and at least 1 follow-up) with INL volume available for at least one eye (minimum follow-up period of 6 months). Patients were excluded if they had experienced symptomatic MSON within six months preceding the OCT assessment (baseline or follow-up), or if history of MSON was ambiguous or unknown. In- and exclusion of subjects is shown in the flow chart in Figure 1.

OPTICAL COHERENCE TOMOGRAPHY

Retinal OCT was performed at each center by use of spectral-domain OCT with Spectralis (Heidelberg Engineering, Heidelberg, Germany, N=10) or OCT-2000 (Topcon Corp., Itabashi, Japan, N=1). Data on the INL and GCIPL volume (mm^3) in the macular area was acquired using a macular volume scan centred on the fovea, using a 6mm ring area.

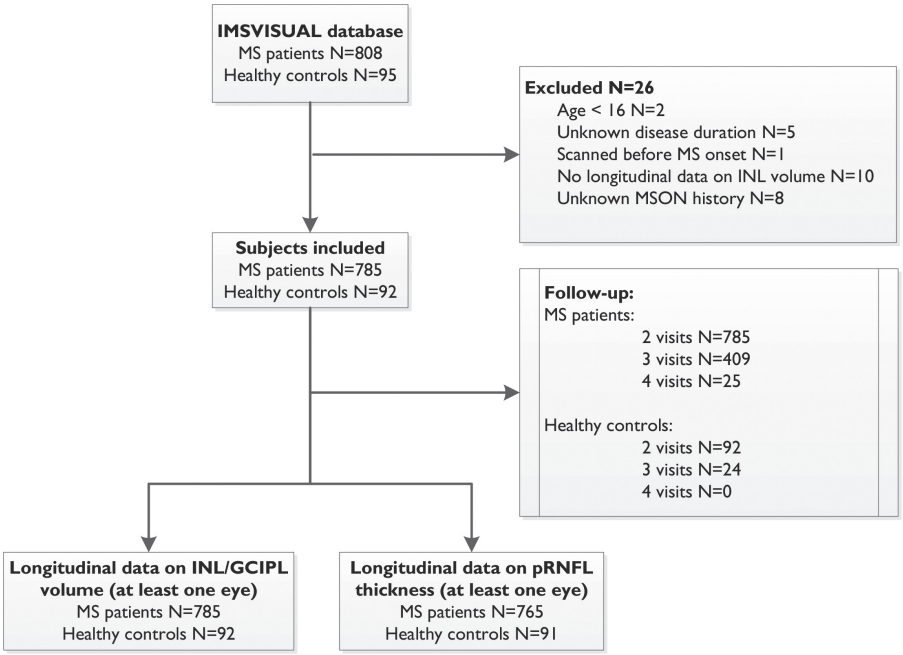

Figure 1: Figure 1. Flowchart of study design. Of the 903 individuals in the initial database, 785 patients and 92 healthy controls were included in this study. All participants had at least two visits, and a subset also had a third or fourth visit. Longitudinal data on inner nuclear layer (INL) and ganglion cell-inner plexiform layer (GCIPL) volume (at least two visits, minimum follow-up >6 months) was available for all included individuals and peripapillary retinal nerve fiber layer (pRNFL) for 765 patients and 91 healthy controls. *IMSVISUAL = International Multiple Sclerosis Visual System Consortium; MSON = MS related optic neuritis.*

Data on global pRNFL thickness (μm) was obtained using a 12° ring scan (corresponding to a 3.4 mm diameter), manually placed around the optic disc. At each center, automated segmentation of OCT scans and quality control (including the assessment whether eyes had signs of MMO) was performed.[13,14] Importantly, the scanning device and protocols were kept identical for all longitudinal measurements within each center.[15]

CLINICAL AND OPHTHALMOLOGICAL OUTCOME MEASURES

Demographic data included data on sex, age at baseline and disease duration (from disease onset). Clinical data was collected longitudinally and included MS subtype, the occurrence of relapses between visits, Expanded Disability Status Scale (EDSS) score, history of previous MSON and occurrence of new episodes of MSON between visits, presence of MMO and use of DMT. The assessment of history of symptomatic MSON (based on medical history, according to a standard protocol),[16] EDSS score and data on clinical relapses were provided by the individual centers.

Importantly, given the longitudinal design of this study, we made a clear distinction between episodes of MSON before the study (referred to as 'pre-study MSON') and episodes of MSON during the follow-up of the study (referred to as 'MSON during follow-up').

EDSS assessment was performed by a certified examiner and in the absence of acute relapses. Disability progression was defined by an increase in EDSS score of 1.0 point in case EDSS score was <5.5 at baseline, or an increase of 0.5 if EDSS score was ≥5.5 at baseline. This approach is consistent with previous IMSVISUAL collaborative projects.[17]

STATISTICAL ANALYSES

Annualised changes in retinal layer thickness or volume were calculated for every follow-up period. Subsequently, the annualised change scores were averaged over the complete observation period, resulting in one average annualised rate of change for every eye. All analyses were therefore performed on eye level, using generalised estimation equation (GEE) models with a correlation matrix structure that treats the eye-measurements as exchangeable in order to adjust for intra-subject inter-eye dependency.[15] All GEE models were additionally adjusted for relevant confounders (baseline OCT value, pre-study episodes of MSON, disease duration, use of disease modifying treatment) as indicated. Figures showing longitudinal changes in retinal layer thickness were produced using relative annualised change scores (i.e. baseline was set as 100%).

Regarding the associations between annualised change in retinal thickness and the occurrence of relapses or disease progression, all eyes with a history of MSON were excluded. Both short term effects (clinical event and retinal change assessed within same follow-up period) and long-term effects (time-lag analyses, clinical event between baseline and first follow-up visit and change in retinal layer thickness between first and second follow-up visit) were investi-

gated. Consequently, only subjects with at least three visits were included in these analyses. All analyses were adjusted for their respective baseline retinal layer thickness.

Correlations between the different layers were calculated with standardised regression coefficients in GEE models, and are therefore also adjusted for inter-eye dependency. Statistical analyses were performed using SPSS V.22.0 (IBM Corp, Armonk, NY, US) and Stata V.14.1 (StataCorp LP, College Station, TX, US) with a two-sided statistical significance level of 0.05.

RESULTS

BASELINE

In total, 1570 eyes from 785 MS patients (68.3% female) and 184 eyes from 92 HCs (63.4% female) were included (Figure 1). MS patients had a median disease duration of 6.4 years (IQR 1.9 – 15.0). The majority of patients (80.3%) had a RR disease course. More than half of all patients (N=419, 53.4%) had never experienced a clinically confirmed MSON before baseline. Of all patients with a history of at least one confirmed episode of pre-study MSON (N=366), 281 (77%) patients had a unilateral MSON and 85 (23%) a history of MSON in both eyes (not necessarily simultaneously). MMO was present in 2.4% of patients (15/638) and in 1.4% (18/1275) of eyes. An overview of the baseline characteristics is shown in Table 1.

Table 1: Baseline characteristics

	All subjects N=785	**Healthy controls N=92**
Gender (female, N, %)	536 (68.3%)	59 (63.4%)
Age (years)	41.0 (±12.6)	43.4 (±11.5)
Disease duration (years, median [IQR])	6.4 [1.9 – 15.0]	
EDSS (median [IQR])	2.0 [1.0 – 3.0]	
Disease type		
CIS	45 (5.7%)	
RR MS	630 (80.3%)	
SP MS	74 (9.4%)	
PP MS	36 (4.6%)	
MSON before baseline, N (%)		
No previous MSON		
MSON	419 (53.4%)	
Unilateral MSON	281 (35.8%)	
Bilateral MSON	85 (10.8%)	
MMO before baseline (N=638)		
MMO-	623 (97.6%)	
MMO+	15 (2.4%)	

Table 1: Continued

	All subjects N=785	Healthy controls N=92
Disease modifying treatment at moment of baseline (N=743)		
None	343 (46.2%)	
Interferon beta	172 (23.2%)	
Glatiramer acetate	72 (9.7%)	
Natalizumab	61 (8.3%)	
Fingolimod	53 (7.1%)	
Dimethyl fumarate	20 (2.7%)	
*Other**	21 (2.8%)	

**Rituximab, teriflunomide, azathioprine, mitoxantrone, cyclophosphamide, alemtuzumab, and mycophenolate mofetil.*
IQR = interquartile range, reported as 25th and 75th percentile; EDSS = expanded disability status scale; MSON = MS related optic neuritis; CIS = clinically isolated syndrome; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive; MMO = microcystic macular oedema

At baseline, MS patients showed significantly higher INL volume compared to HCs (difference of 0.02 mm^3, p=0.018) and lower GCIPL volume and pRNFL thickness (difference of -0.18 mm^3 and -4.4 µm respectively, p<0.001 for both comparisons). Eyes with pre-study episodes of MSON showed a higher INL volume compared to eyes without (0.99±0.08 mm^3 and 0.97±0.08 mm^3 respectively, p=0.001), whereas both GCIPL volume and pRNFL thickness were lower in eyes with pre-study MSON compared to eyes without (Table 2).

CHANGE OVER TIME IN INL, GCIPL AND pRNFL THICKNESS AND THE EFFECT OF MSON

The median follow-up duration was 2.1 years (range 0.5 to 5.2) for MS patients and 2.0 years (range 0.6 to 4.6 years) for HCs. When all eyes of MS patients were analysed together, the INL showed a non-significant average annualised rate of change of -0.0003 mm^3 (p=0.553, Figure 2A). HCs also showed no significant change (annualised rate of change 0.006 mm^3, p=0.245).

Table 2: Retinal layer thickness at baseline

	All eyes N=1570	MSON before BL N=451	No MSON before BL N=1119	HC N=184	p-value* MSON vs HC	p-value no MSON vs HC	p-value* No MSON vs MSON
INL (mm^3)	0.98 (0.08)	0.99 (0.08)	0.97 (0.08)	0.96 (0.09)	0.001	0.066	0.001
GCIPL (mm^3)	1.79 (0.26)	1.62 (0.25)	1.86 (0.23)	1.97 (0.19)	<0.001	<0.001	<0.001
pRNFL (µm)	91.4 (15.8)	81.4 (17.5)	95.2 (13.2)	95.8 (9.1)	<0.001	0.106	<0.001

**GEE analyses, unadjusted,*
MSON = MS related optic neuritis; BL = baseline visit; HC = healthy control; INL = inner nuclear layer, GCIPL = ganglion cell-inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer;

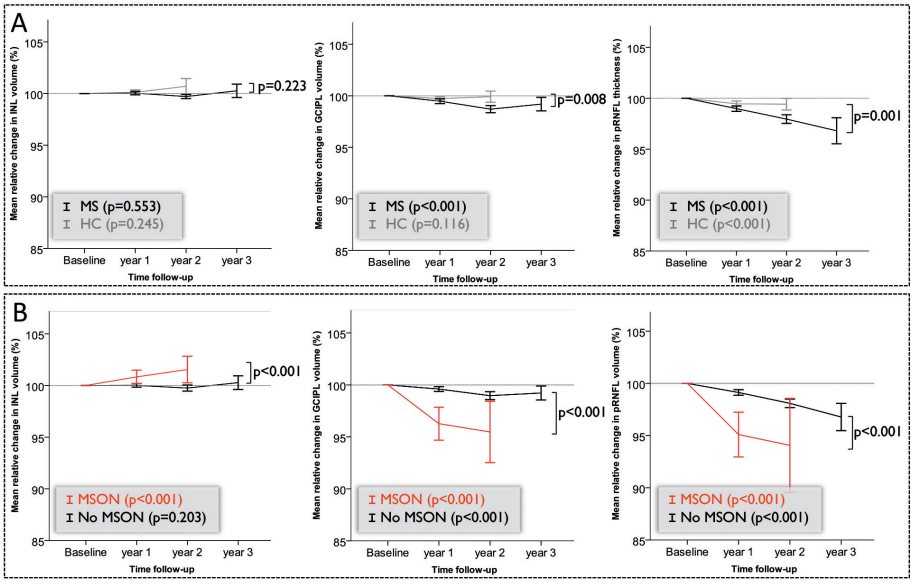

Figure 2. Relative change in retinal layer thickness with 95% confidence interval (based on generalized estimation equation model) for (a) all multiple sclerosis (MS) and healthy control (HC) eyes and (b) stratified by multiple sclerosis optic neuritis (MSON).

INL = inner nuclear layer, GCIPL = ganglion cell-inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer

Regarding the effect of MSON, there was a clear difference between pre-study MSON (i.e. before the baseline OCT assessment) and MSON occurring during the follow-up period. Pre-study MSON did not affect the rate of change in INL thickness significantly. Eyes with and without pre-study MSON showed similar rates of change of the INL (β=0.001, p=0.219). In contrast, any episode of MSON during the observation period strongly affected INL volume. In eyes with MSON during follow-up (N=61/1562), INL volume showed a significant annualised increase of 0.01 mm^3 (p<0.001). In contrast, in eyes without MSON during the observation period, no significant annualised change in INL was observed (β=-0.001 mm^3, p=0.203, Figure 2B). Exclusion of patients with a progressive disease type, or adjustments for use of DMT, disease duration or participating center did not change these results (data not shown).

The annualised rate of change for GCIPL in MS patients was -0.012 mm^3 (p<0.001), which was significantly more than observed in HCs (-0.004 mm^3, p=0.116, p-value for comparison 0.008). The pRNFL showed significantly more thinning in MS patients (-0.97 µm, p<0.001), compared to HCs (0.42 µm, p<0.001, p-value for comparison 0.001, Figure 2A). For both layers, eyes with episodes of MSON during the follow-up period showed significantly more thinning than unaffected eyes (Figure 2B).

THE SHORT AND LONG-TERM EFFECTS OF CLINICAL DISEASE ACTIVITY ON RETINAL LAYER THICKNESS

Table 3 demonstrates the effects of new episodes of MSON during follow-up, other clinical relapses and disease progression, on annualised change in INL and GCIPL volume and pRNFL thickness. Both the short term (clinical event and retinal change assessed within same follow-up period, table 3A) and long-term (time-lag analyses, clinical event between t0 and t1 and change in retinal layer thickness between t1 and t2, table 3B) effects are reported. The median duration of t0-t1 was 1.1 year (IQR 1.01.9), and for t1-t2 the median duration was 1.0 year (IQR 1.0-1.7).

Table 3: Short (A) and long term (B) effects of MSON, clinical relapses (other than MSON) and disability progression on annualised change in INL and GCIPL volume and pRNFL thickness.

A	**β (95%CI) short term**	**p-value***
	MSON (N=26 eyes) vs no MSON (N=1039 eyes)	
INL	0.01 (0.006 to 0.020)	<.001
GCIPL	-0.13 (-0.18 to -0.08)	<.001
pRNFL	-7.61(-10.8 to -4.3)	<.001
	Relapse (N=214 eyes) vs no relapse (N=789 eyes)	
INL	0.000 (-0.004 to 0.004)	.868
GCIPL	-0.10 (-0.18 to -0.002)	.012
pRNFL	-0.54 (-1.14 to 0.07)	.082
	Progression (N=223 eyes) vs no progression (N=673 eyes)	
INL	0.001 (-0.004 to 0.005)	.774
GCIPL	0.001 (-0.006 to 0.008)	.764
pRNFL	-0.13 (-0.66 to 0.41)	.646
B	**β (95%CI) long term (time-lag model)**	**p-value***
	MSON (N=11 eyes) vs no MSON (N=581 eyes)	
INL	-0.006 (-0.026 to 0.013)	.535
GCIPL	0.023 (-0.065 to 0.112)	.604
pRNFL	-1.124 (-3.78 to 1.53)	.406
	Relapse (N=148 eyes) vs no relapse (N=440 eyes)	
INL	0.005 (0.001 to 0.01)	.025
GCIPL	-0.005 (-0.015 to 0.005)	.307
pRNFL	-0.40 (-1.57 to 0.77)	.501
	Progression (N=97 eyes) vs no progression (N=409 eyes)	
INL	0.001 (-0.004 to 0.007)	.609
GCIPL	-0.006 (-0.02 to 0.006)	.329
pRNFL	-0.65 (-0.69 to 1.99)	.342

**GEE model adjusted for inter-eye dependency and baseline retinal thickness.*
β = regression coefficient; 95%CI = 95% confidence interval; INL = inner nuclear layer, GCIPL = ganglion cell-inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer; MSON = MS related optic neuritis

Clinical episodes of MSON during follow-up only demonstrated a shortterm effect on INL (thickening) and GCIPL and pRNFL (thinning). In the time-lag analyses investigating the long-term effects, these effects disappeared. Exclusion of patients with a progressive disease course did not change the statistical findings.

Clinical relapses (other than MSON) during follow-up were present in 24.4% of patients. The occurrence of clinical relapses during the first follow-up was not related to change in INL within the same period (median 1.1 years from baseline, β=0.000, 95%CI -0.004 to 0.004, p=0.868), but was significantly associated with an increase in INL volume in the subsequent follow-up (median 2.2 years from baseline, β=0.005, 95%CI 0.001 to 0.01, p=0.025). This effect was similar when only patients with a relapsing disease course were included (N=508 eyes, β=0.005 (95%CI 0.00 to 0.01, p=0.049)). For GCIPL volume and pRNFL thickness, this effect was more pronounced in the short term (i.e. relapse and retinal volume change within the same follow-up period, Table 3A).

Disability progression was observed in 17.2% (during the entire follow-up period). Annualised change in INL volume was independent of disability progression both on short and long term. Likewise, disability progression was not significantly associated with annualised changes in GCIPL or pRNFL (Table 3A and 3B).

THE EFFECT OF MMO, DISEASE TYPE AND DMT ON RETINAL CHANGES

In the 1.4% of eyes with MMO before or during study (18/1275 eyes), the INL volume at the last visit was 0.07 mm^3 higher compared to eyes without MMO (p=0.006, adjusted for new episodes of MSON). Likewise, the average annualised rate of change of INL volume was significantly higher in eyes with MMO compared to eyes without (β=0.01, p=0.011, adjusted for baseline INL and episodes of MSON during follow-up), showing a significant annualised increase over time in MMO eyes (0.01±0.02 mm^3), but no change in eyes without (-0.0002±0.02 mm^3).

Just over half of the patients (53.8%) used DMT during the study. Although the annualised change in INL volume was not influenced by use of DMT, the absolute INL volume was significantly higher in patients using fingolimod compared to RRMS patients who did not use any DMT, independent of history of pre-study MSON and MMO, EDSS at baseline and disease duration (difference 0.03 mm^3, p=0.004). Other therapies did not show significant differences in INL volume.

INTERRELATIONSHIP BETWEEN LAYERS

All analyses regarding the interrelationships between the layers demonstrated effect modification by presence of a new episode of MSON during follow-up and are therefore stratified. In eyes with MSON during follow-up, an increase in INL volume was related to a decrease in GCIPL volume (standardised β=-0.42, p=0.006, black line in Supplementary Figure 1A) and to a lesser extent (although not statistically significant) to a decrease in pRNFL (standardised β=-0.15, p=0.148, black line in Supplementary Figure 1B). In eyes without new MSON, no sig-

nificant association with change in INL volume was observed (grey lines in Supplementary Figures 1A and 1B). In contrast, GCIPL and pRNFL show positive correlations in both eyes with new MSON (standardised β=0.41, p<0.001) and without MSON (standardised β=0.26, p=0.004, see Supplementary Figure 1C).

DISCUSSION

This longitudinal multi-center study demonstrates that thickening of the INL as measured with spectral domain OCT reflects adjacent inflammation of the optic nerve. Besides this association with local inflammation, the INL seems to reflect some degree of global disease activity, as the occurrence of clinical relapses in any functional system was significantly associated with subsequent increase in INL volume. However, this effect was relatively small (difference of 0.005 mm^3) and should be interpreted with caution, as the sensitivity might be limited.

These findings build upon previous findings from other studies, demonstrating the relationship between thickening of the INL and physical disability[9] and disease activity.[10] Saidha *et al.* reported that INL/OPL thickening at baseline was predictive of clinical relapses, new T2 and contrast-enhancing lesions on MRI and disability progression during follow-up. In contrast, in the present study we did not observe any predictive value of INL thickening on clinical relapses or disability progression. Our data only demonstrated an association between INL volume and clinical relapses in the time-lag model where clinical relapses preceded INL thickening. This would suggest that INL thickening occurred subsequent to inflammatory disease activity. A predictive effect of baseline INL volume on the occurrence of relapses or disability progression, as described by Saidha *et al.*, was not observed in the present study.

When thickening of the INL was first described, it was directly linked to the presence of MMO.[9] Although MMO is present in MS and is related to increased disability,[9,10,18] it is not specific for MS and may vary over time in individual patients.[19,20] Importantly, we have previously demonstrated that MMO was transient in 84% of cases.[19] In the present study, MMO was present in 2.4% of patients, which is consistent with previous findings.[9-11,21] In the present study we observed an increased INL volume in eyes with MMO, which is also consistent with existing literature.[1] Moreover, eyes with MMO also showed a significant increase in INL volume over time. Nevertheless, the findings of the present study did not change when MMO eyes were excluded, suggesting that thickening of INL can occur in the absence of visually detectable MMO.

The underlying mechanism responsible for thickening of the INL remains unknown. The findings of this study would imply that previously suggested mechanisms such as inflammation related dynamic fluid shifts and Müller cell dysfunction are more likely than other non-inflammatory mechanisms such as traction and retrograde trans-synaptic degeneration.[21,22] Dynamic retinal layer volume changes can be explained by fluid shifts due to a combination of osmotic and hydrostatic gradients, the retinal glymphatic system.[23-26] The INL is embedded between the superficial vascular plexus and the deep capillary plexus, which can be clearly visualized on

OCT angiography.[24] Typically, fluid reaches the retina through the internal limiting membrane and both plexuses, whereas both plexuses and Müller cells can absorb the interstitial fluid. In case of inflammation, diffusion of fluid from the retinal blood vessels increases, leading to an increase of INL volume. Another suggested mechanism is pathology of Müller cells, which would impair the absorption of interstitial fluid, also resulting in increased INL volume. Other non-inflammatory suggested mechanisms such as traction or retrograde trans-synaptic degeneration are also plausible, but less well supported by our data, given the clear and direct increase of INL volume following MSON. One approach to further elucidate the underlying mechanism would be the investigation of the retinal vessels using OCT angiography.

Previously, we and others have described the limited susceptibility of the INL to retrograde degeneration caused by MSON.[1] This is in line with our current findings, where the pRNFL and GCIPL clearly showed significant thinning in eyes with previous episodes of MSON, whereas for the INL no thinning, but rather an increase in volume was observed. The opposing effects of local inflammation on the INL on the one hand and pRNFL/GCIPL on the other, are clearly demonstrated by the negative correlation between the layers. This further substantiates the potential of pRNFL/GCIPL as a measure for neurodegeneration, whereas INL volume may be a valuable parameter for reflecting inflammatory activity.

A recent study by Knier *et al.* reported that effective treatment with DMT in patients with MS is associated with sustained reduction of INL volume in absence of MSON and they suggested that INL volume may be a response marker for successful treatment of inflammation.[11] Building upon these findings, we investigated the effect of DMT on INL volume changes. Although the absolute INL volume was significantly higher in patients using fingolimod compared to RRMS patients who did not use any DMT, which corroborates previous findings on retinal effects of this drug,[27,28] the annualised change in INL volume was not influenced by use or type of DMT. However, it should be noted that DMT data was only available at baseline for the majority of patients, and that data on exact duration of treatment or previous DMTs was not available. This lack of detailed information did not permit a thorough investigation of direct effects of DMT or replication of previous results.

Another limitation of the study was that the data did not permit to determine how acute new episodes of MSON were. A systematic assessment of the early time course of acute MSON would be extremely valuable and there will need to be a consent as to what will be defined as onset of an acute episode of MSON. Furthermore, disease activity was only recorded by clinical relapse activity. Data on radiological disease activity (new T2 and/or Gd-enhancing lesions during follow-up) were not available. Therefore, no conclusions could be made regarding the relationship of INL volume and radiological disease activity. A common limitation of multi-center studies is the difference in methodology between the participating centers.[29,30] The OCT device and software was the same for all centers (Spectralis, Heidelberg) but one (Topcon). The data on retinal layer thickness of this particular center was not significantly

different from the other centers and additional adjustment for a potential center-effect did not change any of the results (data not shown).

In summary, our data demonstrate that an increase of the INL volume is strongly associated with inflammation of the optic nerve, and to a lesser degree with other clinical relapses. Therefore, INL volume may be a valuable parameter for capturing inflammatory disease activity and may be considered as an outcome measure for MS and MSON treatment trials.

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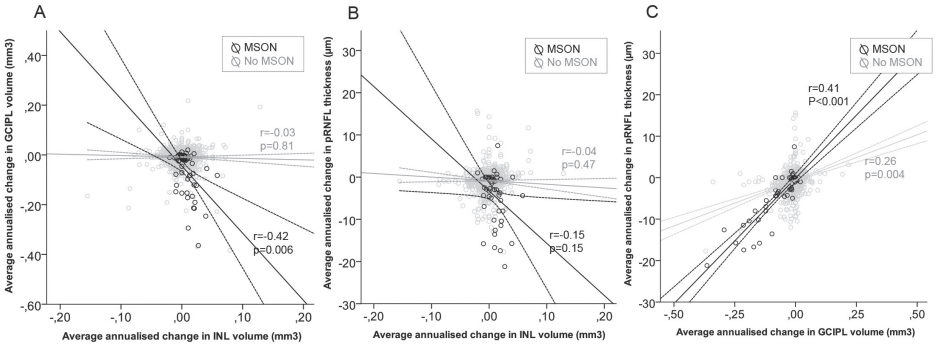
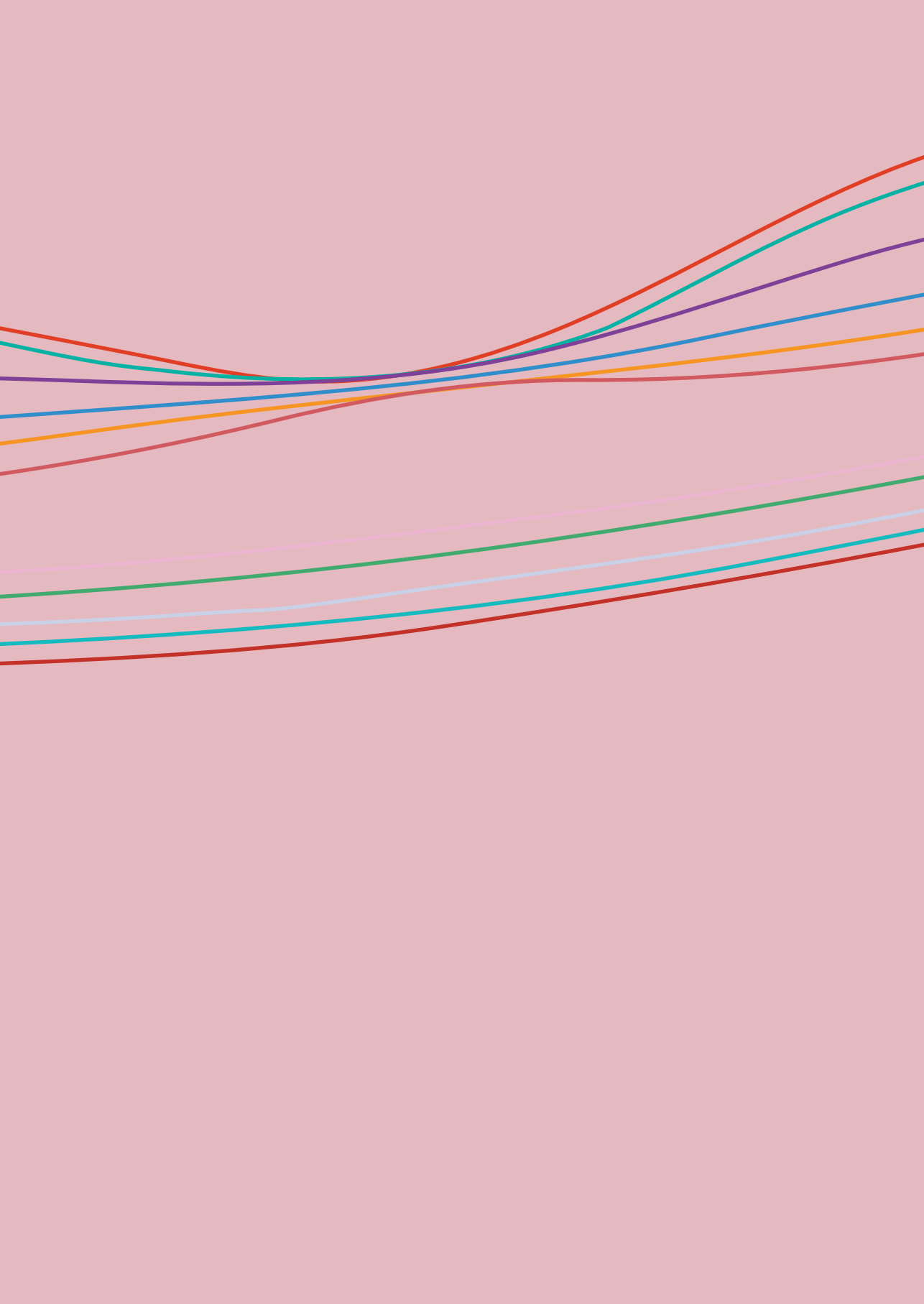


Supplementary Figure 1: Scatterplot showing the associations (with regression line and mean 95% confidence intervals) between (A) INL and GCIPL volume, (B) INL volume and pRNFL thickness and (C) GCIPL volume and pRNFL thickness, stratified by episodes of MSON during follow-up. Reported effects are standardized beta's with p-value, based on GEE models.

INL = inner nuclear layer, GCIPL = ganglion cell-inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer, MSON = MS related optic neuritis



CHAPTER 5.2

OBJECTIVE QUANTIFICATION OF VITREOUS HAZE ON OPTICAL COHERENCE TOMOGRAPHY SCANS: NO EVIDENCE FOR RELATIONSHIP BETWEEN UVEITIS AND INFLAMMATION IN MULTIPLE SCLEROSIS

D Coric, G Ometto, G Montesano, PA Keane, LJ Balk, BMJ Uitdehaag, A Petzold, DP Crabb, AK Denniston

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ABSTRACT

Background and purpose: The occurrence of intermediate uveitis, which is characterized by the presence of vitreous haze (VH), in patients with multiple sclerosis (MS) may be a sign of coexistent inflammatory central nervous system (CNS) disease activity. Using an automated algorithm to quantify VH on optical coherence tomography (OCT) scans, the aim was to investigate whether VH in MS patients is associated with signs of inflammatory CNS disease activity.

Methods: Vitreous haze was quantified on OCT macular volume scans of 290 MS patients and 85 healthy controls (HCs). The relationship between VH and clinical, retinal OCT and MRI parameters of inflammatory disease activity was investigated using generalized estimating equations.

Results: Mean VH scores did not differ between patients and HCs (p=0.629). Six patients (2.1%) showed values higher than the highest of the HCs. VH scores did not differ between the different disease types or between eyes with and without a history of optic neuritis (p=0.132). VH was not associated with inner nuclear layer volume on OCT (p=0.233), cerebral T2 lesion load on MRI (p=0.416) or the development of new relapses (p=0.205).

Conclusion: In this study OCT-based automated VH estimation did not detect increased vitreous inflammation in MS patients compared to HCs and did not find an association with CNS inflammatory burden.

INTRODUCTION

A link between uveitis and multiple sclerosis (MS) exists.[1] Compared to the general population, patients with uveitis are more likely to develop MS.[1-3] Likewise, uveitis is more common in patients with MS. Nevertheless, reported prevalence of uveitis in MS patients vary greatly, with numbers ranging from 0.65% to 36.7%.[4] Some authors have suggested that the occurrence of uveitis in a patient with MS indicates inflammatory central nervous system (CNS) disease activity.[5,6]

Intermediate uveitis, in particular, which is characterized by inflammation of the vitreous, is thought to be associated with MS.[2,7] In intermediate uveitis, blood-ocular-barrier breakdown results in the accumulation of protein-rich fluid and inflammatory cells in the vitreous which is seen clinically as vitreous haze (VH).[8] VH is graded by indirect ophthalmoscopy.[9] This method, however, has several limitations, which in turn may contribute to the aforementioned variability in reported prevalence data. Grading is subjective and data are noncontinuous with a poor sensitivity for detecting faint VH.[10] Recently, a new algorithm has been developed for the objective quantification of VH on optical coherence tomography (OCT) scans.[11]

It was hypothesised that this algorithm could potentially detect (subclinical) ocular inflammation reflected in increased VH in active MS patients. Therefore, our aim was to investigate whether patients with MS show higher levels of VH compared to healthy controls (HCs) and whether this VH is associated with signs of inflammatory CNS disease activity.

METHODS

STUDY POPULATION AND CLINICAL EVALUATION

Subjects were recruited from the Amsterdam MS Cohort.[12] All patients and HCs who had received an OCT scan at baseline assessment were eligible for inclusion (a total of 316 MS patients and 87 HCs). For the purpose of this study, subjects with ocular pathology were excluded expect for patients with uveitis. However, none of the subjects had symptoms indicative of uveitis at the moment of assessment. After excluding patients due to ocular pathology (all non-uveitic) or poor scan quality, 290 MS patients and 85 HCs in whom at least one eye was available were included in this study. A subset of subjects underwent additional OCT after two years of follow-up. Clinical follow-up of at least one year (including the occurrence of new relapses after baseline) was available for the majority of the cohort. The in- and exclusion process is shown in Figure 1.

Clinical assessment was performed by a trained physician and included, among other things history taking on use of disease modifying therapy (DMT) and the occurrence of clinical relapses. Disease duration was defined as the time from first symptoms until assessment. History of MS associated optic neuritis (MSON) was established according to a consensus protocol.[13] Level of disability was assessed by certified physicians by means of the Extended Disability Status Scale (EDSS).[14]

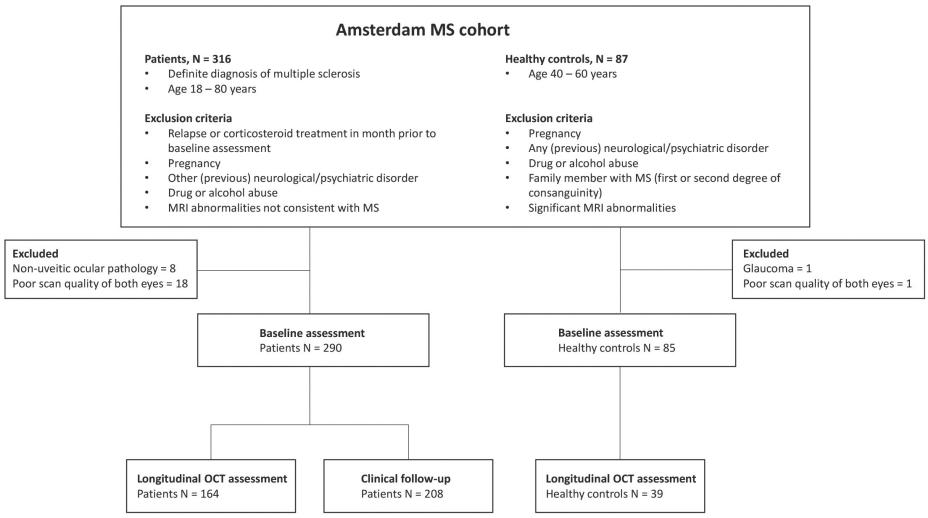

Figure 1: Flowchart illustrating the in- and exclusion of study subjects.

Baseline MRI data was available for the majority of subjects (230 patients and 63 HCs). MRI was performed on a 3T whole body scanner (GE Signa HDxt, Milwaukee, Wisconsin, USA) using an eight-channel phased array head coil. The MRI protocol, acquisition settings and white matter lesion segmentation have been described previously.[12]

This study was approved by the medical ethics committee (protocol number 2010/336) and the scientific research committee (protocol number CWO/10-25D) of the VU University Medical Center. Written informed consent was obtained from all participants.

OCT AND VH MEASUREMENT

OCT scans were performed with a spectral domain OCT device (Spectralis, Heidelberg Engineering, Heidelberg, Germany; acquisition software version 1.7.1.0) with acquisition settings and segmentation as previously described (see supplementary material for more details).[15] Follow-up scans were acquired with the automatic follow-up function making sure the placement of the follow-up scans was identical to the first scan.

VH measurements were calculated from the raw images recorded by the Spectralis rather than from the contrast-corrected, standard OCT images, as described in Keane et al.[11] Raw images were exported from the Heyex software (Heidelberg Engineering, Heidelberg, Germany) as VOL files. The measurement used in this work was obtained as the ratio of the mean intensity of all pixels of the vitreous (i.e. pixels above the segmented inner limiting membrane) and the mean values below the vitreous (Figure 2). This ratio was calculated separately for each A-scan in the B-scans and then averaged. The reliability of this algorithm has recently been validated.[16]

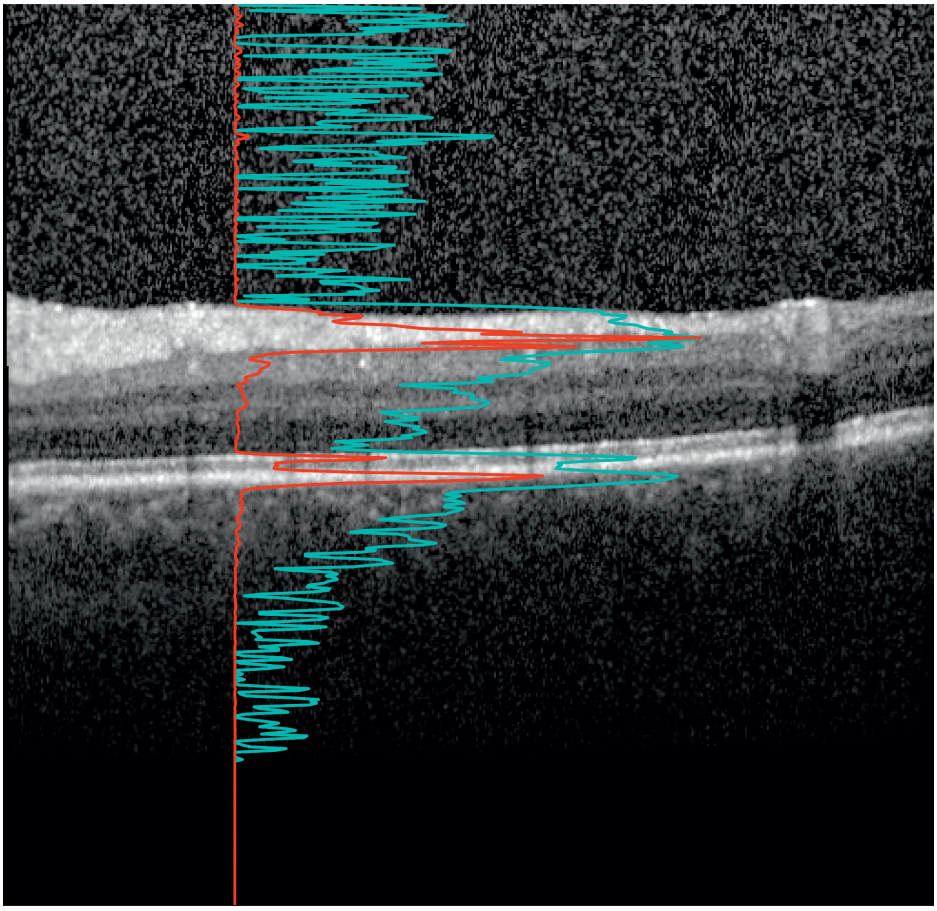

Figure 2: Algorithm used for measuring vitreous haze on OCT scans. An optical coherence tomography B-scan of the macula is shown. This image is optimized for viewing on the screen, clearly rendering the retinal layers visible. The red line shows the A-scan intensity profile in RAW format of the underlying vertical column in the image. Notice how the sharp peaks correspond to the inner segment/outer segment junction and retinal pigment epithelium - Bruch's membrane complex. This signal was used to calculate the ratio used in our measurement. The light blue line with sharper peaks in the vitreous and below the retina represents the same profile after contrast adjustment for visualization. Notice how the vitreous signal is artificially increased compared to the retinal signal.

STATISTICAL ANALYSIS

Normality of data distribution was assessed graphically by means of histograms. Because the distribution of VH scores was skewed to the right all VH scores were log transformed, which led to a normal distribution. All VH scores are reported as log VH scores. Differences in continuous variables between patients and HCs were tested using two-tailed T-test (parametric) or Mann-Whitney U test (non-parametric). Categorical variables were compared using chi-square test. For the longitudinal analyses, annualized VH change scores were calculated. For all analyses performed on eye level (VH and retinal layer thickness), generalized estimating equations

(GEE) with an exchangeable correlation matrix were used to correct for intra-subject inter-eye dependency. Logistic regression analysis was used to test the predictive value of baseline VH scores (mean value of both eyes) on future relapses. All analyses were performed using SPSS version 22.0. Statistical significance was set at $p<0.05$.

RESULTS

VITREOUS HAZE SCORES AND CLINICAL MEASURES

The baseline characteristics of the cohort are shown in Table 1. Patients were slightly older compared to HCs. As expected, the eyes of MS patients (eyes with and without a history of MSON combined) showed more atrophy of the macular ganglion cell-inner plexiform layer and peripapillary retinal nerve fiber layer, but a thickening of the macular inner nuclear layer (INL), compared to HCs eyes.

Vitreous haze scores were negatively associated with age ($\beta = -0.007$, $p=0.001$) and disease duration ($\beta = -0.009$, $p=0.004$) in MS patients, meaning a higher age or longer disease duration were associated with lower VH scores. VH scores in HCs were not associated with age ($\beta = 0.005$, $p=0.129$). In order to account for the effect of age, all subsequent analyses were corrected for age and sex, unless otherwise stated.

Overall, VH scores (reported as log VH scores) in MS patients ranged from -2.17 to 0.30 with a mean VH score of -0.96 (±0.41). HCs had a mean VH score of -0.93 (±0.33, range -1.76 to -0.06), which did not differ significantly from that of the MS patients (mean difference -0.03, $p=0.629$), see Figure 3a. RRMS patients showed significantly higher VH scores compared to PPMS (mean difference 0.14, $p=0.043$) and SPMS (mean difference 0.13, $p=0.032$) patients when age was not taken into account. However, after adjusting for age, these differences in VH score between RRMS patients and PPMS ($p=0.201$) and SPMS ($p=0.113$) patients were no longer significant, see Figure 3b. Eyes with a history of MSON showed the same amount of VH compared to patient eyes without a history of optic neuritis (mean difference = -0.04, $p=0.132$), see Figure 3c.

DISEASE MODIFYING THERAPY

Out of the 290 patients, 93 were using some form of DMT at time of assessment. Beta interferons were used most often (N=51), followed by glatiramer acetate (N=12), natalizumab (N=12), fingolimod (N=11), dimethyl fumarate (N=5), teriflunomide (N=1) and azathioprine (N=1). 20.0% of the patients had used one or more DMTs in the past but was not on any therapy at the moment of assessment whilst 47.9% had never used any form of DMT. After correcting for type of MS in addition to age and sex, patients who were on DMT showed significantly less vitreous haze compared to patients who had never been on DMT (mean difference 0.11, $p=0.032$), see Figure 3d.

Table 1: Baseline characteristics of the cohort.

	MS patients N= 290	HCs N= 85	p-value
Age (years), mean (±SD)	51.5 (±10.1)	49.3 (±8.2)	0.039
Sex (Female : Male)	195 : 95	53 : 32	0.402
Disease duration (years), mean (±SD)	17.9 (±7.0)	N/a	
Type of MS			
RRMS	200 (69.0%)	N/a	
SPMS	59 (20.3%)		
PPMS	31 (10.7%)		
EDSS, median [range]	3.5 [0 – 8.0]	N/a	
History of MSON		N/a	
No MSON	157 (54.1%)		
Unilateral MSON	81 (27.9%)		
Bilateral MSON	39 (13.4%		
Unknown	13 (4.5%)		
Use of disease modifying therapy		N/a	
Current	93 (32.1%)		
Past	58 (20.0%)		
Never	139 (47.9%)		
Relapses in year prior to assessment		N/a	
Yes	34 (11.7%)		
No	256 (88.3%)		
mGCIPL thickness (µm), mean (±SD)	77.5 (±14.3)	92.2 (±6.0)	<0.001*
pRNFL thickness (µm), mean (±SD)	84.6 (±14.4)	95.1 (±7.9)	<0.001*
INL thickness (µm), mean (±SD)	40.4 (±3.3)	39.4 (±2.9)	0.003*
Vitreous haze score, mean (±SD)	-0.96 (±0.41)	-0.93 (±0.33)	0.629*

** Adjusted for age and sex*
HCs = healthy controls; RRMS = relapsing remitting MS; SPMS = secondary progressive MS; PPMS = primary progressive MS, MSON = MS associated optic neuritis; MSNON = no history of MSON; EDSS = expanded disability status scale; mGCIPL = macular ganglion cell – inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer; INL = inner nuclear layer

CLINICAL RELAPSES

Only a small proportion of MS patients had experienced a relapse in the year prior to baseline assessment (11.7%, Table 1). There was no significant difference in the VH score of patients who did and patients who did not experience a relapse in the year prior to baseline (mean difference 0.00, p=0.867), see Figure 3e. Clinical follow-up was available in 208 patients. Of these patients, fourteen (6.7%) had experienced a new relapse in the year following baseline assessment. Although these fourteen patients did show a higher VH score compared to those patients who remained stable (-0.80±0.43 and -0.95±0.41 respectively), this was statistically not significant (p=0.159), see Figure 3f. VH score at baseline was not predictive of a MS relapse in the year following baseline assessment (odds ratio per 0.1 increase in VH score: 1.11, p=0.205).

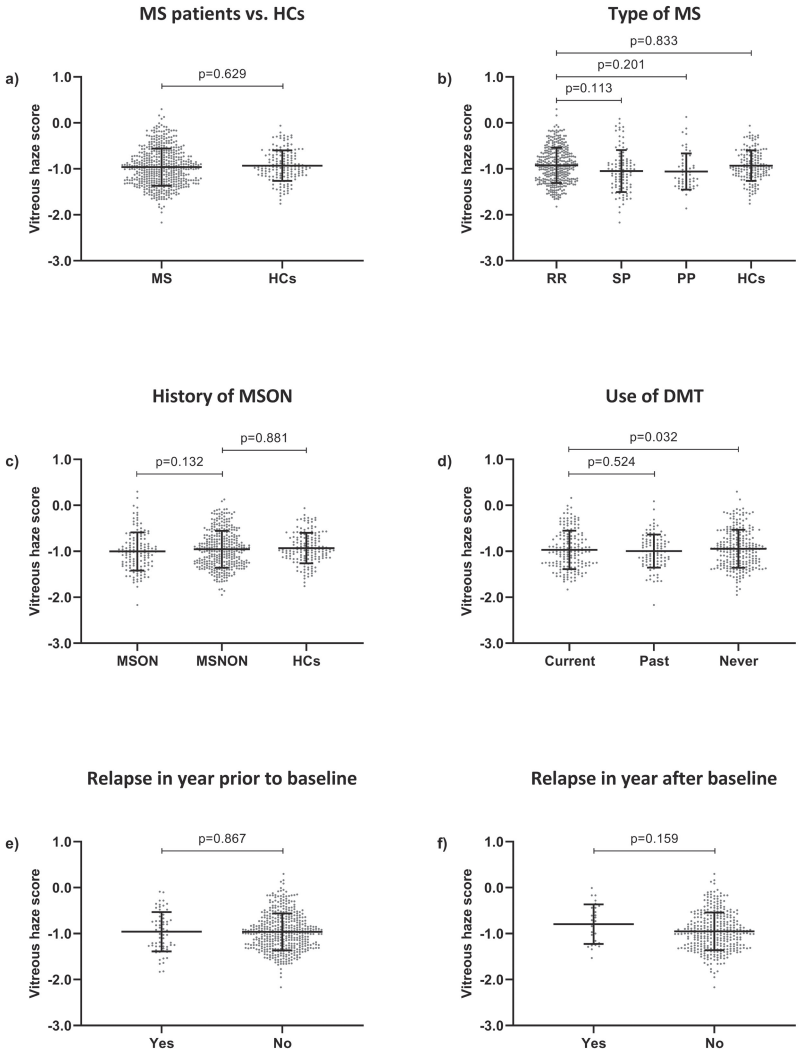

Figure 3: Mean vitreous haze scores (±SD) in all MS patients and HCs (a) and in different patient subgroups (b-f). All analyses were adjusted for age and sex. The relationship between vitreous haze and use of DMT was additionally adjusted for type of MS (disease course).
HCs = healthy controls; RR = relapsing remitting; SP = secondary progressive; PP = primary progressive, MSON = MS associated optic neuritis; MSNON = no history of MSON; *DMT = disease modifying therapy*

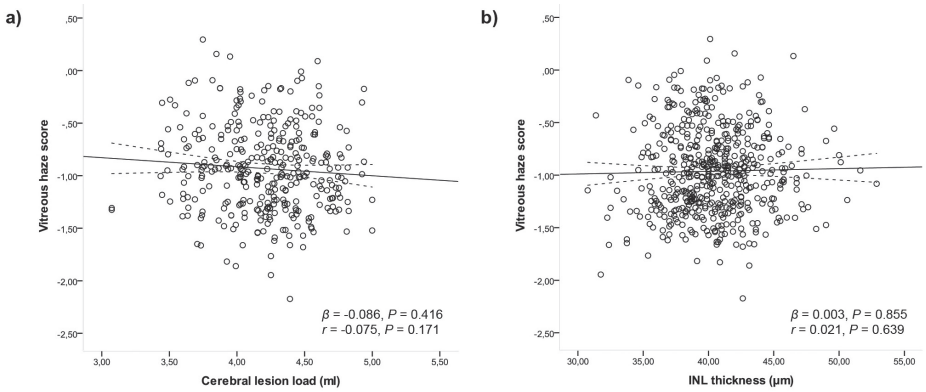

Figure 4: Scatter plot and fitted linear regression line (with 95% confidence interval) demonstrating the association between vitreous haze and cerebral lesion load (a) and inner nuclear layer (INL) thickness (b) in MS patients.
β = regression coefficient; r = Pearson correlation coefficient

5.2

IMAGING PARAMETERS

Magnetic resonance imaging of the brain was available in a subset of MS patients (N=230). In these patients, VH scores were not associated with cerebral T2 weighted lesion load (β = -0.086, p=0.416), see Figure 4a. Baseline macular INL thickness was available for the total cohort. Cross-sectionally, VH was not related to macular INL thickness measured on OCT in MS patients (β = 0.003, p=0.855, Figure 3b), see Figure 4b.

LONGITUDINAL CHANGES IN VH SCORE

Longitudinal OCT data was available in 164 patients and 39 HCs. On average, these subjects had been followed up for a period of 2.19 years. During this follow-up period, three patients experienced a clinical episode of MSON in an eye that had not been previously affected. The affected eyes of these patients were excluded from the longitudinal analyses.

Table 2 shows the annualized VH changes in MS patients and HCs. MS patients showed a small but statistically significant decline in VH score of -0.019/year (p=0.006) while HCs showed no significant changes over time (-0.0026/year, p=0.852). No difference in longitudinal VH changes were found among the different subgroups. Annualized changes in INL thickness were not related to annualized changes in VH scores in patients (β = 0.16, p=0.083).

Table 2: Annualized vitreous haze changes.

	Mean annualized vitreous haze change score (95% confidence interval)		p-value for subgroup analysis
MS patients, overall	-0.019 / year	(-0.031 – -0.007)	Reference
Healthy controls	-0.0026 / year	(-0.028 – 0.023)	0.256
Type of MS			
Relapsing Remitting MS	-0.014 / year	(-0.028 – -0.0002)	Reference
Secondary Progressive MS	-0.021 / year	(-0.053 – -0.011)	0.626
Primary Progressive MS	-0.044 / year	(-0.081 – -0.007)	0.076
History of MSON			
MSON eyes	-0.016 / year	(-0.037 – 0.005)	Reference
MSNON eyes	-0.021 / year	(-0.036 – -0.006)	0.850
Use of disease modifying therapy			
Current use	-0.007 / year	(-0.026 – 0.012)	Reference
Past	-0.027 / year	(-0.053 – -0.0003)	0.208
Never	-0.023 / year	(-0.042 – -0.005)	0.198
Relapses in the year following baseline assessment			
Yes	-0.035 / year	(-0.079 – 0.009)	Reference
No	-0.018 / year	(-0.030 – -0.005)	0.604

MSON = MS associated optic neuritis; MSNON = no history of MSON

DISCUSSION

In this study we postulated that MS patients might show signs of increased ocular inflammation, reflected in increased VH, as a sign of inflammatory CNS disease activity. However, using an objective measure to quantify VH on OCT scans, we could not find evidence of increased levels of VH in MS patients compared to HCs. In addition, we did not observe any association between VH and measures of CNS inflammation.

The absence of higher VH in the MS population might suggest that uveitis in MS is not as common as previously thought. Studies investigating the prevalence of uveitis in MS patients have yielded a wide range of results.[4,17] Many of the smaller studies were performed in centers specialized in uveitis possibly leading to selection bias and probably an overestimation of the actual prevalence. It is noteworthy that the large, population based studies show the lowest prevalence figures of ~1%.[3,18-20] In our study, six out of 520 eyes, all in different patients, showed VH values that were higher than the highest value of the HCs (prevalence 2.1%). This percentage corresponds to the low prevalence found in the large, population based studies. It is also possible that the relatively long disease duration in our cohort of MS patients contributed to the low VH values due to a burnt-out stage of the disease. The finding of the inverse association between disease duration and VH score further strengthens this argument. Therefore, there is a need for future studies with patients who present early with a clinically isolated syndrome, including MSON.

One difference between our study and most studies that investigate the prevalence of uveitis, is that our OCT-based approach only considers vitreous inflammation. It is therefore focused on detecting intermediate uveitis but may not detect other anatomically isolated cases of uveitis, notably anterior or posterior uveitis. However, almost all studies report intermediate uveitis to be the most common form in MS patients.[18, 21, 22] One study reported posterior uveitis to be the most common anatomical subtype, although they do not provide further details and that study was conducted prior to the release of the Standardization of Uveitis Nomenclature (SUN) Working Group guidelines for anatomical classification.[20]

Whilst this study is the first to investigate the direct relationship between VH and signs of inflammatory CNS disease activity in MS, it expands on previous work on the association between other aspects of uveitis and increased CNS inflammatory activity. Retinal vascular abnormalities have been shown to be associated with increased MS activity. Lightman *et al.* reported that optic neuritis patients with retinal vasculitis (and vitreous cells) were more likely to subsequently develop MS compared to optic neuritis patients without.[1] Two other studies have shown a significant association between retinal periphlebitis and inflammatory CNS disease activity.[5,6] The evidence for the association is, however, conflicting with some studies showing no differences in MS course or prognosis between patients with and without uveitis (all types of uveitis),[20,23] and one study even finding a favorable MS prognosis in patients with uveitis.[18] Since the patients in our cohort did not undergo a specialist ophthalmological examination as part of their routine assessment, we cannot rule out whether some patients in our cohort may have had another form of uveitis in which VH might be normal (eg. anterior or posterior uveitis). This lack of an accompanying ophthalmological examination is an important limitation of the study and such an examination should be included in similar studies in the future. Yet we would argue that since most of these assessments are subjective, it would be better to extend the imaging protocol to provide a more comprehensive objective assessment of ocular inflammatory status. For example, the protocol could use automated image-based measures of anterior chamber cell count and chorioretinal lesions. The development of such markers is a major focus of our group's work. Additionally, it would be beneficial to have longitudinal MRI data, to provide a better estimation of inflammatory disease activity. Conversely, we do have longitudinal data on INL thickness. INL volume changes are thought to reflect inflammatory disease activity.[24] In our study we found no association between INL thickness and VH changes.

The ability of the algorithm to objectively quantify the amount of VH on OCT scans is a strength of this study. The resulting value is continuous and allows for detection of small differences between subjects. Patients with intermediate uveitis can sometimes present with minimal complaints and establishing the presence clinically can be challenging. Another strength of this study is the large and heterogeneous cohort of MS patients.

The occurrence of uveitis in a patient with MS might cause the clinician to doubt whether the patient's current treatment is appropriate. The fact that no relationship between VH and measures of CNS inflammation (most importantly relapses) was found, might provide support and

reassurance for continuation of the already implemented treatment in a MS patient suffering from intermediate uveitis, who otherwise does not have any other clinical or radiological sign of disease activity. This in turn might help to avoid unnecessary switch to another form of DMT. The present study found lower VH scores in patients who were on DMT compared to those who had never been on DMT, possibly suggesting VH might serve as a biomarker for therapy efficacy. Nevertheless, further studies need to be conducted to support this claim.

In conclusion, this study found no evidence of increased ocular inflammation in MS patients compared to HCs when applying an automated algorithm to objectively measure VH on OCT scans. Moreover, there was no evidence for any association between VH and signs of inflammatory CNS disease activity. Although the clinical correlate of the OCT-based VH measurement is limited to intermediate uveitis, the findings of the present study suggest that the link between (subclinical) uveitis and MS is less evident than previously assumed.

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SUPPLEMENTARY MATERIAL

RETINAL OCT PROTOCOL

OCT scanning was performed with dual beam simultaneous imaging and the eye-tracking function enabled for optimal measurement accuracy.[1] Room-lighting conditions were dimmed and no pharmacological pupil dilation was needed no obtain the OCT images. After acquisition, all scans were segmented using software provided by the manufacturer (HRA / Spectralis Viewing Module 6.0.7.0). VH measurements and macular ganglion cell – inner plexiform layer (mGCIPL) and inner nuclear layer (INL) thickness were derived from a macular volume scan centred around the fovea (20x20°, 512 A-scans, 49 B-scans, vertical alignment, automatic real time 16). mGCIPL and INL thicknesses were calculated by averaging the thickness for all 8 sectors of the 1.0 mm, 2.22 mm and 3.4 mm grid excluding the central 1.0 mm circle. Peripapillary retinal nerve fiber layer (pRNFL) thickness was derived from a 12° circular scan (1536 A-scans, no predetermined automatic real time) centred around the optic nerve head.

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CHAPTER 6

| GENERAL DISCUSSION

Parts of this discussion have also been published in the following review:
The role of optical coherence tomography and infrared oculography in assessing the visual pathway and CNS in multiple sclerosis.
D Coric, JA Nij Bijvank*, LJ van Rijn, A Petzold, LJ Balk*
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Neurodegenerative Disease Management 2018;8:323-335. ■

The general aim of this thesis is to provide more understanding on how OCT can aid in the assessment of clinically relevant disability in patients with MS, in both the visual system and on a global, central nervous system (CNS) level. The studies that make up this thesis build upon previous research on how inner retinal layer thickness measurements may serve as a marker of disability and inflammation in MS. In addition, new OCT parameters that have the potential to serve as a structural biomarker for neurodegeneration or inflammation are introduced. This includes vitreous haze and a new phenomenon called peripapillary hyperreflective ovoid mass-like structures (PHOMS). In this last chapter, a summary of the previous chapters is provided and their main findings are critically discussed. Lastly, we provide recommendations for future research.

METHODOLOGICAL CONSIDERATIONS IN LONGITUDINAL OCT RESEARCH

Several of the studies described in this thesis involve longitudinal OCT research. OCT is highly suitable for longitudinal research due to its ability to detect small changes in retinal thickness over time.[1] When performed by experienced raters and when quality control criteria have been applied, OCT shows high levels of inter- and intra-rater reliability.[2,3] In MS research, the three most frequently investigated retinal layers are the peripapillary retinal nerve fiber layer (pRNFL), the macular ganglion cell inner – plexiform layer (mGCIPL) and the macular inner nuclear layer (mINL). pRNFL thickness is measured using a ring scan centered around the optic nerve head, whereas mGCIPL and mINL thickness is measured at the macular region using a volume scan. The Heidelberg Spectralis OCT device used in the studies described in this thesis (and frequently used in other studies as well) segments the pRNFL directly upon scanning according to the last installed algorithm, providing immediate pRNFL thickness measurements. In contrast, volume scans need to be processed after acquisition before data on mGCIPL and mINL thickness can be extracted. While the immediate availability of pRNFL thickness measurements is highly convenient in routine medical practice, clinicians and researchers need to be aware of some potentially confounding factors, especially in longitudinal research. For instance, a previous study by Balk *et al*. has shown that off-axis placement of the OCT measurement beam can have a profound effect on pRNFL thickness measurements (averaging at 9 µm).[4] In **chapter 2.1** we investigated whether updates in a retinal layer segmentation algorithm influence pRNFL thickness measurements. The results show that there is a statistically significant difference in pRNFL thickness measurements between two consecutive versions of the same segmentation algorithm. While the pooled analyses showed small (yet significant) differences in pRNFL thickness between the two versions, the difference in pRNFL thickness between the two versions in individual scans could be as high as 6 µm. This far exceeds the annual pRNFL loss in MS patients, which is around 1-2 µm/year and even lower in MS patients with longstanding disease and healthy controls. This potential bias introduced by software improvements was independent of pRNFL thickness or scan quality. This issue might seem minor, since it is easily corrected without need to recall a patient. However, if the operator who is performing the scan is not aware of this problem, it can lead to a significant over- or underestimation of true pRNFL atrophy. Studies investigating the agreement between, for instance, different OCT devices or vertically or horizontally obtained macular volume scans

have yielded similar results showing sufficient agreement on a group level, but low agreement on the individual patient level.[5,6] Therefore, we recommend that clinicians and researchers be aware of these confounding factors and should make sure all parameters, including the software version, are the same throughout all measurements, when acquiring and analyzing OCT data in a longitudinal setting, especially in individual patients.

OCT AND THE VISUAL SYSTEM IN MS

Visual symptoms, either due to afferent or efferent problems, are common in MS and lead to significant difficulties in daily life.[7,8] The link between MS and optic neuritis in particular is well established (MS associated optic neuritis, MSON).[9] Previous episodes of MSON lead to substantial atrophy of the pRNFL and mGCIPL.[10] Nonetheless, the amount of atrophy varies between MSON patients and depends on several factors, including severity of the episode. In addition, the retinal layers show some degree of inter-subject variability, even among healthy individuals.[11] Taking both points into consideration, it means that absolute values of pRNFL or mGCIPL thickness cannot indicate previous episodes of MSON with certainty.

Given that the two eyes of healthy control subjects (HCs) are more or less equal in thickness with regard to the inner retinal layers, it was suggested that the inter-eye asymmetry might be useful in the diagnosis of MSON.[12] In **chapter 3.1** we aimed to define optimized 'inter-eye percentage difference' (IEPD) cut-off values for the pRNFL and mGCIPL for the diagnosis of a previous episode of MSON. The results show excellent discriminatory power for the IEPD of the mGCIPL and moderate to good discriminatory power for the IEPD of the pRNFL for differentiating HCs from unilateral MSON and bilateral MSON. All optimal cut-off values were below 10%. An mGCIPL IEPD of only 5-6% had high sensitivity and specificity for discriminating HC eyes from unilateral and bilateral MSON eyes.

Since the publication of our study, several other studies have been published that have investigated the diagnostic value of inter-eye asymmetry for establishing optic nerve involvement.[13-17] They show comparable results to our study, although it must be noted that there are differences between these studies and our study. Most studies have only investigated the inter-eye *absolute* difference (IEAD) and not the IEPD.[13-16] Likewise, the reference group to which the ON group is compared varies between studies (e.g. HC or MSNON patients). In accordance with our study, all studies demonstrate that the mGCIPL IEPD/IEAD outperforms the pRNFL IEPD/IEAD. The study by Behbehani *et al.* shows near perfect sensitivity (100%) and specificity (98%) for an IEAD of 3.5 µm for the mGCIPL (corresponding to an IEPD of about 5%) for differentiating between unilateral ON patients and HCs.[13] A multi-center study by Nolan-Kennedy *et al.* investigating the optimal IEAD for differentiating between MS patients with and without a history of unilateral MSON found a similar optimal cut-off for the pRNFL as our study but a slightly lower optimal cut-off for the mGCIPL (6% versus 9% in our study).[16] Outteryck *et al.* investigated the optimal IEPD and IEAD for differentiating between CIS patients with and without optic nerve lesions on MRI. For symptomatic lesions they found similar optimal IEPD for the pRNFL and mGCIPL (with similar sensitivity and specificity as our study as well for

the latter). For asymptomatic lesions, the optimal IEPD and IEAD were lower than for symptomatic lesions.[17] The potential of our optimized cut-off value of the mGCIPL to diagnose MS was investigated by Petzold *et al.* in a large, population-based study among 72,120 subjects (prevalence of MS 0.02%). Using a 4% cut-off for the mGCIPL IEPD resulted in low sensitivity (51.7%) but high specificity (82.8%). The researchers found that higher age and number of co-morbidities lowered the diagnostic accuracy of the mGCIPL, suggesting that the mGCIPL IEPD was particularly useful in young patients without co-morbidity.[18]

In 2016 MAGNIMS, a European collaborative research network that studies MRI in MS, recommended that involvement of the optic nerve, whether it be a lesion in the optic nerve on MRI or atrophy of the pRNFL on OCT, should be added to the criteria for dissemination in space as an additional site, next to periventricular, cortical/juxtacortical, infratentorial and spinal.[19] However, optic nerve involvement was not incorporated in the 2017 revisions of the 2010 McDonald criteria as the expert panel felt there was insufficient data on the diagnostic sensitivity and specificity of MRI, visual evoked potentials (VEP) or optical coherence tomography to demonstrate optic nerve lesions in patients without a clear history or clinical evidence of optic neuritis. Studies investing this were deemed high priority.[20] The findings of our study and the other studies provide a definition for optic nerve involvement on OCT, which future studies can use to study the added value of optic nerve involvement in establishing dissemination in time and dissemination in space. The IEPD and IEAD could be especially of use for the demonstration of optic nerve involvement in patients experiencing their first clinical episode (CIS patients) that is not MSON and who do not yet meet criteria for dissemination in space, with the goal of establishing an earlier diagnosis of MS. According to our results, the IEPD could not discriminate between unilateral and bilateral MSON, but if applied for the diagnosis of MS this is irrelevant.

Aside from being a diagnostic marker for MSON, atrophy of the pRNFL and mGCIPL is also associated with a decrease in visual functioning.[21,22] However, the clinical relevance of this, i.e. how does it affect a patient's daily life, is not yet fully clear. Previous studies have assessed the association between retinal layer atrophy and *general* quality of life (QoL). Nevertheless, tools measuring general QoL focus primarily on lower limb function, which is surprising since MS patients value their visual functioning only second to ambulation.[23] In **chapter 3.2** we investigated the association between pRNFL and mGCIPL atrophy and visual functioning and vision-related QoL in 267 patients with MS. In accordance with previous research we found a significant inverse relationship between atrophy of the inner retinal layers and high contrast and low contrast visual acuity (HCVA respectively LCVA). In addition, pRNFL and mGCIPL atrophy were associated with lower vision-related QoL, with the largest effects found for the subdomains "distance activities", "social functioning" and "colour vision". The associations between retinal atrophy and both visual acuity and vision-related QoL were independent of a previous episode of MSON. It is important to note that the relationship between retinal atrophy and vision-related QoL was mediated by VA. Vision-related QoL did not differ between patients with unilateral and bilateral pRNFL (defined as pRNFL thickness ≤ 75 µm) or mGCIPL (defined

as mGCIPL thickness ≤ 68 µm) atrophy, suggesting that monocular atrophy of the inner retinal layers already has significant impact on the vision-related QoL in patients.

The results of our study are in line with previous work showing an association between retinal atrophy and decreased vision-related QoL.[24,25] Shortly after the publication of the present study, the work by Sanchez-Dalmau *et al*. was published, which showed that decreased vision-related QoL in MS patients was mainly dependent on decreased colour vision and HCVA. A decrease in colour vision and LCVA (both measures of visual functioning) was most strongly related to mGCIPL atrophy.[26]

Taking all of the above into account, retinal atrophy as measured by OCT seems to be a valid marker of both loss of visual functioning and decreased vision-related QoL (albeit the latter indirectly). This makes OCT not only a suitable, but also a clinically relevant outcome measure in trials investigating new MSON therapies.

OCT AS A MARKER OF NEURODEGENERATION IN MS

To understand the interplay between different disease mechanisms which contribute to (irreversible) disability in MS, focus has shifted from investigating MS as a white matter disease, to a broader perspective in which gray matter pathology and network dysfunction receive more attention. Brain atrophy, and especially thalamic atrophy, is strongly related to physical disability and cognitive decline, even in early phases of disease.[27-29] Furthermore, functional MRI and magnetoencephalography studies have shown that changes in functional connectivity of the brain, which are often interpreted as compensatory mechanisms for structural deficits, [30,31] play a crucial role in early deterioration in clinical and cognitive status.[32-34] The increasing focus of this type of MS research on cognitive decline besides physical disability is essential as cognitive dysfunction is increasingly recognized as an important aspect of MS. The prevalence is estimated at 40–70% and it can be apparent at early stages of disease. [34-37] It has a great impact on daily functioning and quality of life of MS patients.[27,38,39] It is difficult to localize lesions or disease processes which cause cognitive dysfunction, due to the complex nature of cognitive functioning, which require communication between a broad range of brain regions.[40] The ultimate goal is to study the brain in total as a functional and structural network. However, the exact interplay between different pathogenic mechanisms, as atrophy and disruption of cortical networks, is unknown. There is need for noninvasive methods to investigate these interactions that contribute to (irreversible) disability, especially cognitive dysfunction. OCT has the potential to serve this purpose.

In **chapter 4.1** we describe a cross-sectional study investigating the association between inner retinal layer atrophy and cognitive impairment (measured across multiple cognitive domains) in patients with MS. The results demonstrate that in bilateral MSNON, cognitively impaired patients show considerably more atrophy of the pRNFL (mean difference 8.1 µm, $p<0.001$) and mGCIPL (mean difference 11.5 µm, $p<0.001$) compared to cognitively preserved patients. Furthermore, pRNFL and mGCIPL atrophy was strongly associated with a higher

odds of being cognitively impaired. In the bilateral MSON group, no relationships between cognitive impairment and inner retinal layer atrophy was found. This last finding is likely due to the masking effect caused by the large amount of atrophy following MSON. The findings in this study are in line with other research showing a relationship between inner retinal layer atrophy and cognitive and physical disability[41-43] and support the claim that inner retinal layer atrophy in MS is a marker of neurodegeneration. However, the use of cross-sectional thickness measurements for this purpose is restricted to patients who have not experienced a previous episode of MSON. Seeing as more than 50% of MS patients experience one or more episodes of MSON this limits the usefulness considerably.

In **chapter 4.2**, we followed up on our study described in chapter 4.1, prospectively investigating the relationship between retinal layer atrophy and cognitive decline (CD) over the course of four years. Overall, yearly pRNFL and mGCIPL atrophy rates in patients were relatively low but comparable to atrophy rates of other studies in MS patients with long disease duration. An important finding was that the rate of atrophy between patients with and without a previous history of MSON did not differ, permitting pooled analyses of MSON and MSNON eyes. Contrary to expected, baseline pRNFL and mGCIPL thickness did not predict CD at four-year follow-up. A possible explanation for these findings might be the long disease duration in our cohort of patients meaning higher levels of atrophy at baseline and thereby possibly a lower discriminatory power. The study by Martinez-Lapiscina *et al*.[44] did show a higher risk of disability worsening in patients with low pRNFL thickness, but the median disease duration in this study was much lower than in our study (median disease duration 6.5 years vs. 20.4 years). Interestingly, in our study the *rate* of mGCIPL (but not pRNFL) atrophy during the first two years and the whole four-year period was positively associated with CD.

Taken together, the results suggest that OCT is well capable of capturing the neurodegenerative state in patients with MS across all stages, but finding low pRNFL or GCIPL thickness might only be *predictive* of cognitive (and physical) decline in patients earlier in the disease course. Perhaps looking at the rate of atrophy, by performing serial scans over the course of a certain time period, might be more informative than a single measurement when aiming to predict future disability. Studying the rate of atrophy has the additional advantage of not having to exclude patients with a previous episode of MSON (expect for new episodes of MSON during the follow-up period) and possibly less inter-subject variability. However, because of the lack of evidence to date, these claims remain mere assumptions and we would advise future researchers to investigate the relationship between progressive inner retinal layer atrophy and cognitive and physical decline.

Until now we have utilized OCT to study changes in the thickness of retinal layers in the hopes of unraveling disease mechanisms in MS. In **chapter 4.3** we attempt to expand the usefulness of OCT by studying a new phenomenon recognized on OCT images called peripapillary hyperreflective ovoid mass-like structures (PHOMS).[45] These structures have been defined by the International Optic Disc Drusen Consortium.[46] We prospectively investigated the prevalence

of PHOMS in our cohort of MS patients and related then to imaging and disease characteristics. PHOMS were significantly more prevalent in MS patients compared to healthy controls (16% vs. 0%). They were however not related to age, disease duration, disease course, use of disease modifying therapy or progression of psychical disability. PHOMS were not specific to MS as they were also observed in patients with intracranial hypertension, optic disc drusen and other ocular diseases. This clinically relevant observation has since been corroborated independently.[45] The exact nature of PHOMS is still unclear, but evidence suggests that they are causes by axoplasmic stasis or congestion in the prelaminar optic nerve head.[45] Alternatively they may build up due to an impaired glymphatic system from the eye to the brain. This interpretation of our data has been endorsed by the group of Maiken Nedergaard[47] among others.[48] As the histological features of this novel OCT observation become clearer, future translational research will show if the longitudinal dynamics of PHOMS demonstrated by us can be used as a model for studying the dynamics of the human glymphatic system in vivo. This would be relevant to the field because evidence until now comes mainly from experimental tracer studies in rodents.[47]

OCT AS A MARKER OF INFLAMMATION IN MS

Up to now, OCT research in MS has primarily focused on thickness of the pRNFL and mGCIPL as a measure of neurodegeneration and the relationship between atrophy of these two layers and disability is becoming increasingly better established. In recent years, however, the INL is gaining more and more interest as a marker of inflammatory disease activity in MS. As previously demonstrated, the INL acts as a barrier halting the further spread of retrograde (trans-synaptic) degeneration in the retina. The INL consists of a neuronal network of bipolar, amacrine and horizontal cells which all together form a 'synaptic tree'. The neuroplasticity resulting from the extensive intercellular connections of this synaptic tree is a possible explanation for how the INL is capable of blocking further degeneration.[49] Interestingly, no atrophy of the INL is found on OCT but instead, a thickening of the INL is observed in MS patients with a previous history of MSON.[10] A histopathological study by Green *et al*.[50] demonstrated the presence of inflammatory cells in the INL of MS patients, raising the question whether changes in INL thickness reflect inflammatory disease activity.

In **chapter 5.1** we report the results of a multi-center study investigating the relationship between mINL volume changes and the occurrence of MSON and clinical relapses. In total, 585 MS patients and 92 HCs were investigated with a median follow-up period of two years. MS patients showed significantly higher mINL volumes compared to HCs. In addition, mINL volume was significantly higher in eyes with a previous history of MSON compared to MSNON eyes. Overall, there were no significant changes in mINL volume over the course of the follow-up period. However, the occurrence of MSON *during* follow-up strongly affected mINL volume leading to an annualized increase of 1.0% of the initial mINL volume ($p<0.001$). Time-lag analyses demonstrated that this change in mINL thickness was only a short-term effect, i.e. the mINL volume increase occurred only in the same time period as the MSON episode whereas no mINL volume changes were observed in the period following the MSON. In contrast, the

occurrence of clinical relapses other than MSON was not associated with mINL volume changes within the same period, but was associated with an increase in mINL volume in the subsequent follow-up period. Disability progression (defined as an increase in EDSS score) was not related to changes in mINL volume, not in the short term nor in the long term. The results show that changes in the mINL reflect not only adjacent inflammation in the optic nerve, but to some degree also global central nervous system (CNS) inflammatory disease activity. The findings are in accordance with previous research showing a relationship between INL volume changes and higher relapse rates and radiological evidence of inflammatory disease activity in MS.[51-55]. In addition, the study by Knier *et al*. also demonstrated that patients who were treated with disease modifying therapy (DMT) appropriately and exhibited no signs of disease activity showed a reduction in INL volume, providing support for INL reduction as an indicator of therapy response in MS.[52] Not all studies, however, have shown a relationship between changes in INL thickness and signs of inflammatory disease activity.[56,57] In our study, we could not find a predictive value of baseline mINL thickness on future MSON or other relapses. This may imply that transient thickness changes may be related to acute inflammation and physiological variation, including functioning of the retinal glymphatic system.

Thickening of the INL, when first discovered, was linked to microcystic macular oedema (MMO).[58] Our data showed higher annualized mINL increase rates as well as a higher mINL volume at the end of follow-up in MMO eyes compared to eyes without MMO. Nevertheless, a significant increase in mINL thickness was observed in non-MMO eyes as well leading to the suggestion that thickening of the mINL can also occur in the absence of MMO. The data suggests that the most likely mechanisms responsible for thickening of the mINL are inflammation-related dynamic fluid shifts or Müller cell dysfunction.

All things considered, the mINL has the potential to become a marker of inflammatory disease activity in individuals with MS and optic nerve involvement. Nevertheless, a few hurdles have to be overcome before it can be applied in routine medical practice. Like the pRNFL and mGCIPL the mINL shows a degree of physiological variability.[11] In addition, the mINL is thinner than the other inner retinal layers and longitudinal changes are therefore smaller. For instance, in our study mean annualized increase in mINL volume in patients showing relapses other than MSON was 0.005 mm^3, corresponding to an increase in thickness of approximately 0.2 µm (which is comparable to previously reported rates of change[59]). Current OCT devices are not sensitive enough to detect changes this small in individual patients. However, the current evidence supports the usefulness of INL changes as an outcome measure on a group level, making it suitable for trials investigating new MSON therapies or DMT targeting inflammatory disease activity.

Previously found associations between MS and uveitis, in particular intermediate uveitis, have hinted that another ocular location might also provide evidence of inflammatory disease activity in MS.[60,61] Reported prevalence rates of uveitis in MS and vice versa vary greatly, but large population-based studies have indicated prevalence rates of approximately 1% for

both.[62] The development of a new algorithm for the objective quantification of vitreous haze (VH) on OCT scans,[63] reflecting the degree of inflammation in intermediate uveitis, allowed us to investigate the association between vitreous inflammation and inflammatory disease activity in patients with MS. In **chapter 5.2** we postulated that (subclinical) VH was associated with clinical an imaging signs of CNS disease activity. In order to answer this question we performed a longitudinal study in 290 MS patients and 85 HCs. In the cross-sectional analyses, no difference in VH scores between MS patients and HCs was found. There were also no associations between disease characteristics and VH scores (i.e. disease course, history of MSON and clinical relapses in the year before or after baseline assessment). VH scores were not associated with T2 lesion load on MRI or mINL thickness. In the longitudinal analyses, VH scores at baseline did not predict clinical relapses in the following year. Only 2.1% of patients showed VH scores that were higher than the highest of the HCs, which is in line with the low prevalence rates of uveitis in MS found in population-based studies. Previous research on the relationship between uveitis and MS has been conflicting with some studies showing an association between uveitis associated abnormalities and MS disease activity[60,64,65] and other studies showing no such relationship or even a favorable MS prognosis in patients with uveitis.[66-68] So the link between the two remains inconclusive. A possible explanation for our negative results might be the long disease duration in our MS cohort, leading to a burnt-out stage of the disease where neurodegeneration is much more prominent than inflammation. Therefore, it would be interesting to investigate the association between VH and inflammatory disease activity in a young cohort of MS patients including CIS and acute MSON patients. Interestingly, we did find an inverse association between VH and age and disease duration, supporting this claim. For now, our data show no relationship between vitreous inflammation and inflammatory CNS disease activity suggesting that the link between uveitis and MS is not as evident as previously assumed. The findings of the study might provide reassurance to clinicians that they are unlikely to miss subclinical uveitis. Therefore, rather than a neurologist missing uveitis, the burden remains on the ophthalmologist who cares for individuals with uveitis not to miss MS because some uveitis drugs (TNF-α inhibitors) are contraindicated in MS.

GENERAL CONCLUSIONS

Taken together, this thesis has shown that OCT is able to provide valuable information on disease characteristics that are relevant to a patient with MS by demonstrating that:

- the IEPD is an useful metric for establishing optic nerve involvement in patients with MS;
- atrophy of the pRNFL and mGCIPL is not only associated with decreased visual functioning but also with decreased vision-related quality of life;
- atrophy of the pRNFL and mGCIPL reflects cognitive impairment and progressive mGCIPL atrophy mirrors cognitive decline;
- thickening of the mINL not only reflects adjacent inflammation in the optic nerve but also inflammation on a more global CNS level.

In addition, this thesis has introduced two new OCT parameters in MS research, PHOMS and VH, opening up avenues for new hypotheses to be tested on a hot topic relevant to the broader spectrum of neurodegenerative and inflammatory pathology.

RECOMMENDATIONS FOR FUTURE RESEARCH

Over the years, OCT has gained more and more ground in routine medical practice for the clinical assessment of patients with MS. Being an inexpensive, non-invasive and quick tool, it renders itself exceptionally useful for this purpose. Some clinics have implemented OCT in their standard battery of diagnostic and monitoring tests. Evidence is accumulating rapidly on how to use and which parameters to use best in monitoring disease status in MS patients.

The ultimate goals is to be able to monitor and even predict disease progression in patients with MS, whether it be physical or cognitive decline. In order to achieve this goal, longitudinal research is essential. The studies described in this thesis highlight one important aspect. While pRNFL and mGCIPL atrophy is most profound in the late stages of MS, the rate of *progressive* atrophy is highest in the early stages. So, for the purpose of longitudinal research, a cohort of patients with short disease duration is most suitable. Future studies should investigate the predictive value of OCT on physical and cognitive decline in a cohort of patients early in the disease course, ideally CIS patients or newly diagnosed MS patients. Serial measurements in the early phase should be performed to see if they have higher predictive value for disease progression than a single, baseline measurement. Measurements of mINL thickness should be included as well, particularly in the case of acute MSON, to determine their predictive value on long-term (visual) disability. A major challenge, but an important one, will be the defining of cut-off points that indicate clinically meaningful baseline or progressive atrophy (or thickening) and thereby warrant start or change of DMT. In all likelihood, the clinical applications of OCT will be primarily in the early stage of the disease, which is also the stage in which the disease course can be influenced the most, by means of DMT.

There are multiple factors that can potentially influence retinal layer thickness measurement, for instance age/disease duration, long-term effects of MSON, ethnicity, MS type and prior use of DMT. The exact impact of some of these factors on retinal thickness is still unclear, which can complicate the clinical use of OCT. Future studies should investigate the extent to which these confounders influence retinal layer thickness measurements. Future research should also focus on the development of new segmentation algorithms. With the current focus on multicenter studies and different centers using different OCT devices, it is important to have reliable automated segmentation algorithms which can measure retinal thickness with high agreement among different OCT devices. Ideally, these segmentation algorithms should also have high agreement between consecutive versions of a particular OCT device. The development of such segmentation algorithms is crucial for longitudinal research, but also for clinical practice, in order to prevent prior scans (e.g. baseline scans) from becoming useless.

Since the introduction of the 2017 revisions to the 2010 McDonald criteria, only a few studies have investigated the added value of optic nerve involvement to the diagnostic criteria.[69-71] Two of these studies, in which optic nerve involvement was assessed clinically or by VEP, showed a slight improvement of the diagnostic performance.[69,70] Studies utilizing the IEPD or IEAD are lacking, despite their higher convenience compared to VEP. Therefore, there is need for new prospective studies in CIS patients investigating whether the inclusion of OCT-defined optic nerve involvement increases diagnostic performance compared to the current criteria.

As demonstrated in this thesis, the use of OCT is not limited to measurement of retinal thickness and atrophy. A relatively new and exciting development is the introduction of OCT angiography (OCTA), enabling the visualization of the retinal and choroidal vasculature and providing a measure of vascular density.[72] Hypoxia and hypoperfusion have been demonstrated in the CNS of patients suffering from MS and are increasingly recognized as important contributing factors to the pathophysiology of MS.[73] To date, a small number of OCTA studies have been performed in MS patients, all showing reduced vascular density in the macular or peripapillary regions of MS patients compared to HCs. Some studies have also shown correlations between OCTA and clinical outcome measures, but results have been inconsistent.[74] Further studies are needed in order to elucidate the relationship between OCTA metrics and clinical outcome measures, both neurodegenerative and inflammatory. As glaucoma research has revealed, OCTA is able to detect progressive damage even in the late stages of the disease.[75] If this finding is confirmed in MS, it would extend the usefulness of the technique to MS patients with a long disease course.

This thesis describes the first study in which an objective measure has been used to study the association between uveitis and inflammation in MS. Our study was performed in patients with a long disease duration and the study needs to be replicated in a young cohort of MS patients. Other automated OCT-based markers of uveitis are currently being developed, for instance automated measurement of anterior chamber cell count and chorioretinal lesions. It would be interesting to study the relationship between these new markers and inflammatory disease activity, in order to finally clarify the relationship between uveitis and MS.

In conclusion, since the first application in MS three decades ago, OCT has come a long way. There is enough evidence to support the usefulness of OCT in the clinical assessment of issues that are relevant to a patient with MS. Future studies will aim at refining some aspects that need further clarification and if they succeed, OCT will become an indispensable tool for the clinical evaluation of patients with MS.

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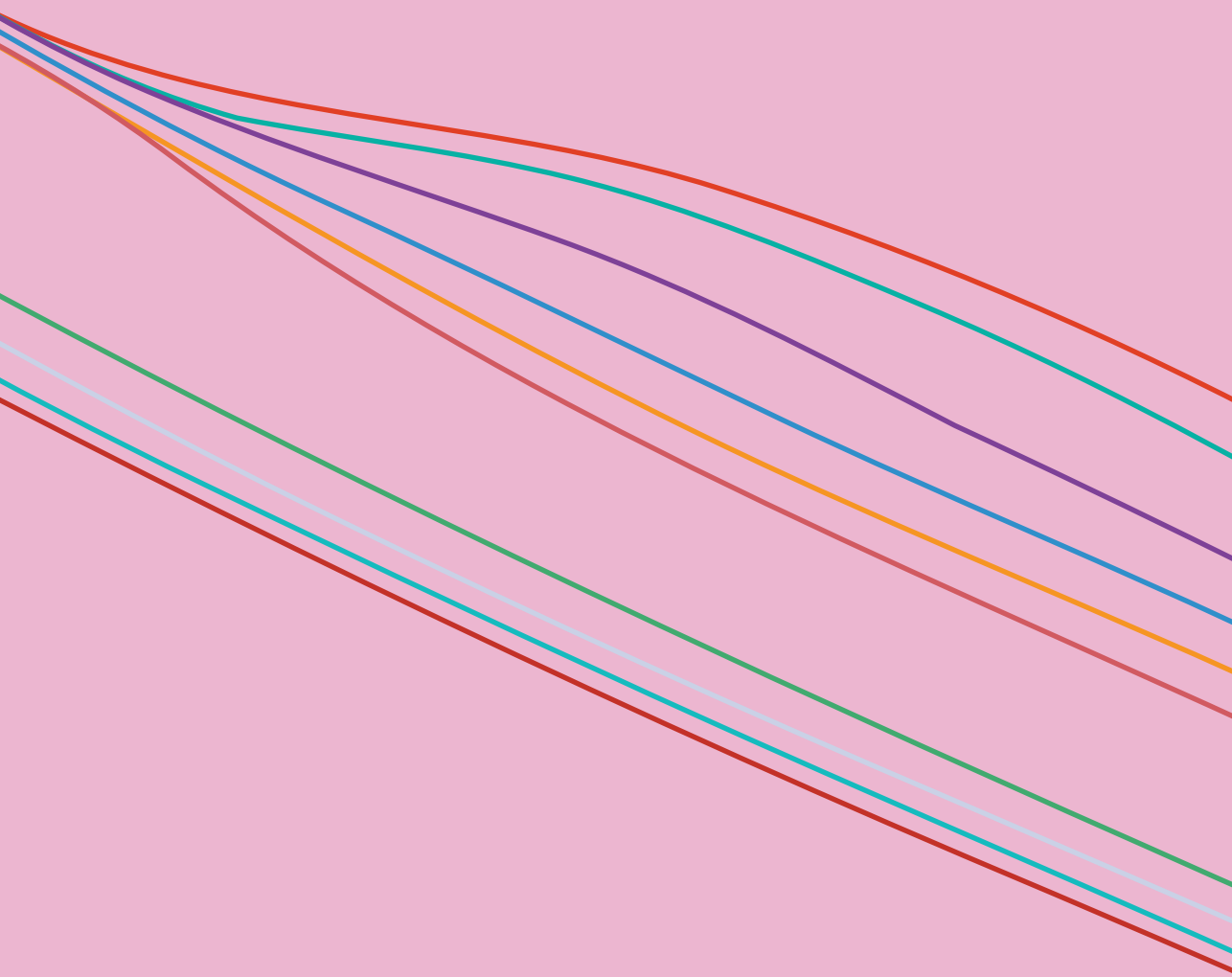

APPENDIX

NEDERLANDSE SAMENVATTING

OPTISCHE COHERENTIE TOMOGRAFIE IN MULTIPLE SCLEROSE: HOE KAN HET ONS HELPEN IN DE KLINISCHE PRAKTIJK?

MULTIPLE SCLEROSE

Multiple sclerose (MS) is een chronische aandoening van het centraal zenuwstelsel (hersenen, ruggenmerg en oogzenuwen) en is een van de meest voorkomende neurologische aandoeningen bij jonge mensen. In Nederland komt de ziekte bij ongeveer 1 op de 1000 mensen voor en openbaart deze zich meestal bij jongvolwassenen (20-40 jaar). Bij MS ontstaan er ontstekingen (inflammatie) in het centraal zenuwstelsel die de myelineschede, de isolerende laag rondom de uitlopers van zenuwcellen, beschadigen. Signalen tussen zenuwcellen worden hierdoor minder goed overgebracht, waardoor patiënten klachten ontwikkelen. Omdat de myelineschede door het lichaam weer hersteld kan worden, kunnen deze klachten ook weer verdwijnen. Naast de schade aan de myelineschede, ontstaat er echter ook schade aan de zenuwcellen zelf (neurodegeneratie). Dit gebeurt voornamelijk in de latere fase van de aandoening en leidt tot blijvende klachten. Afhankelijk van de locatie van de schade ontstaan verschillende klachten zoals krachtsverlies of gevoelsstoornissen van de ledematen, moeite met de coördinatie, loopstoornissen en blaasproblemen. Ook komen cognitieve stoornissen (stoornissen in o.a. het verwerken van informatie en het geheugen) en problemen met het zien vaak voor. Ongeveer 50% van de patiënten met MS zal gedurende het ziektebeloop een ontsteking van de oogzenuw, neuritis optica genaamd, doormaken. Dit gaat gepaard met een daling van het zicht, pijn achter het oog en verminderd kleurenzien.

MS kan worden onderverdeeld in drie verschillende typen. Bij verreweg de meeste patiënten (ca. 80%) begint de ziekte met de relapsing-remitting vorm, waarbij er episoden met neurologische klachten optreden (een schub of relapse genaamd). Deze episoden duren meestal enkele weken en de klachten herstellen vaak gedeeltelijk of volledig. Het merendeel van de relapsing-remitting MS patiënten zal uiteindelijk overgaan in de secundair progressieve fase waarbij er geen duidelijke schubs meer zijn, maar waar sprake is van een geleidelijke achteruitgang. In ongeveer 10-20% van de gevallen openbaart de ziekte zich niet met schubs, maar is er vanaf het begin een progressieve achteruitgang. In dit geval wordt gesproken van primair progressieve MS.

Tegenwoordig is bekend dat de processen die ten grondslag liggen aan zowel inflammatie als neurodegeneratie al vanaf het begin van de ziekte aanwezig zijn. Toch blijkt het lastig om het ziektebeloop bij patiënten met MS te voorspellen. Het beloop kan erg verschillend zijn, zelfs bij patiënten die dezelfde vorm hebben. Goede voorspellende factoren ontbreken. Dit moeilijk te voorspellen beloop vormt een probleem, mede omdat het ziektebeloop van invloed is op de therapiekeuze. De middelen die het meest effectief zijn, maar over het algemeen ook

meer ernstige bijwerkingen hebben, moeten worden voorbehouden aan patiënten met een agressiever ziektebeloop.

MRI is een belangrijk instrument voor het stellen van de diagnose MS. Echter, de voorspellende waarde van MRI ten aanzien van het ziektebeloop is tot nu toe beperkt gebleken. Zo is er bijvoorbeeld geen duidelijke relatie tussen het aantal afwijkingen dat te zien is op de MRI-scan en de hoeveelheid klachten die een patiënt ervaart. Daarnaast is het maken van een MRI-scan tijdrovend, duur en onprettig voor een patiënt. Daarom wordt er gezocht naar nieuwe manieren om het ziektebeloop bij MS-patiënten te kunnen voorspellen. Optische coherentie tomografie (OCT) zou hieraan kunnen bijdragen.

DOEL VAN HET PROEFSCHRIFT

OCT is een beeldvormende techniek waarmee het netvlies en de verschillende lagen waaruit dit bestaat, heel gedetailleerd kunnen worden afgebeeld. De binnenste twee lagen van het netvlies bestaan uit de zenuwcellen (de macular ganglion cell – inner plexiform layer, mGCIPL) en hun uitlopers (de peripapillary retinal nerve fiber layer, pRNFL) die uiteindelijk de oogzenuw zullen vormen. De oogzenuw, zoals eerder genoemd ook onderdeel van het centraal zenuwstelsel, staat in directe verbinding met de hersenen. Eerder onderzoek heeft laten zien dat veranderingen in de hersenen en ruggenmerg van MS-patiënten gepaard gaan met veranderingen in de oogzenuw. Deze veranderingen aan de oogzenuw kunnen op hun buurt weer zichtbaar worden gemaakt door naar het netvlies te kijken door middel van OCT. Schade aan de oogzenuw vertaalt zich namelijk in het dunner worden, atrofie genaamd, van de binnenste twee lagen van het netvlies (de pRNFL en mGCIPL).

In dit proefschrift wordt onderzocht hoe OCT kan bijdragen aan het monitoren en voorspellen van het ziektebeloop bij patiënten met MS in de klinische praktijk. Er wordt gekeken naar klachten van het visuele systeem (**hoofdstuk 3**), cognitieve stoornissen, veroorzaakt door neurodegeneratie, (**hoofdstuk 4**) en klachten samenhangend met inflammatie, verantwoordelijk voor de kenmerkende schubs (**hoofdstuk 5**).

METHODOLOGISCHE ASPECTEN

Enkele studies beschreven in dit proefschrift gaan over onderzoek waarbij patiënten zijn gevolgd gedurende enkele jaren (longitudinaal onderzoek). Dit soort onderzoek brengt bepaalde technische problemen met zich mee. Voordat de dikte van een bepaalde laag van het netvlies berekend kan worden, moeten OCT-scans eerst gesegmenteerd worden. Voor de pRNFL wordt dit door de computer automatisch en direct na het maken van de scan gedaan. Fabrikanten van OCT-apparaten verbeteren segmentatiealgoritmen continu, met het doel de diktemetingen accurater te maken. Echter, in een longitudinale setting zou dit kunnen leiden tot foutieve metingen, omdat de scans in de loop van tijd door verschillende segmentatiealgoritmen zijn gesegmenteerd. In **hoofdstuk 2.1** hebben we gekeken of dit het geval is door te onderzoeken of er een verschil in diktemetingen is bij twee opeenvolgende versies van één segmentatiealgoritme. Over de gehele onderzoekspopulatie vonden we een klein, maar significant verschil

in de pRNFL-dikte tussen de twee versies van het segmentatiealgoritme. Wanneer er werd gekeken naar individuele patiënten kon het verschil tussen de twee versies zelfs groot zijn. In het uiterste geval kon het verschil 6 µm zijn, wat veel hoger is dan het gemiddelde, jaarlijkse pRNFL-verlies bij een MS-patiënt (1-2 µm/jaar). We raden clinici en onderzoekers dan ook aan om er zeker van te zijn dat alle OCT-scans van een patiënt met dezelfde versie van het segmentatiealgoritme zijn gesegmenteerd voordat ze de resultaten interpreteren. Indien dit niet het geval is, dan kan het leiden tot een over- of onderschatting van de daadwerkelijke longitudinale atrofie.

OCT EN HET VISUELE SYSTEEM IN MS

De diagnose MS wordt gesteld op basis van de combinatie van doorgemaakte klachten, schubs waaronder een neuritis optica, en typische afwijkingen op een MRI-scan. Soms kan het echter moeilijk zijn om klinisch vast te stellen of iemand ooit een neuritis optica heeft doorgemaakt. Eerder onderzoek heeft aangetoond dat een doorgemaakte neuritis optica resulteert in atrofie van de binnenste twee lagen van het netvlies. Echter, de absolute dikte van deze twee lagen is geen goede maat voor het vaststellen van een doorgemaakte neuritis optica, omdat de variatie in dikte van het netvlies tussen personen (ook gezonde) groot is. Uit het onderzoek beschreven in **hoofdstuk 3.1** blijkt dat het *verschil* in dikte van het netvlies tussen de twee ogen van één patiënt wel met een hoge betrouwbaarheid het onderscheid kan maken tussen patiënten met en patiënten zonder een doorgemaakte neuritis optica. Wanneer het verschil in dikte van het netvlies tussen de twee ogen 5% of meer bedroeg, dan wees dit op een doorgemaakte neuritis optica. Het op deze manier kunnen opsporen van een doorgemaakte neuritis optica, zou kunnen leiden tot een eerdere diagnose van MS en daarmee een snellere behandeling. Of dit ook echt zo is, dient in toekomstige studies te worden onderzocht waarbij ons afkappunt van 5% als definitie van een doorgemaakte neuritis optica kan worden gebruikt. In **hoofdstuk 3.2** van dit proefschrift hebben we de relatie onderzocht tussen de dikte van de pRNFL en mGCIPL en de visuele functies (o.a. het zicht) en visusgerelateerde kwaliteit van leven (kwaliteit van leven op basis van problemen met het zien), gemeten middels een vragenlijst. Hieruit kwam naar voren dat atrofie van de binnenste twee lagen van het netvlies gepaard gaat met zowel lagere visuele functies als ook een lagere visusgerelateerde kwaliteit van leven. Deze associaties werden niet beïnvloed door het wel of niet hebben doorgemaakt van een neuritis optica. De resultaten laten zien dat OCT een geschikte en klinisch relevante uitkomstmaat is voor onderzoeken waarbij behandelingen van neuritis optica worden onderzocht.

OCT EN NEURODEGENERATIE IN MS

Ook patiënten die nooit een neuritis optica hebben doorgemaakt laten atrofie van de binnenste twee lagen van het netvlies zien, al is de mate van atrofie minder dan na een doorgemaakte neuritis optica. Er wordt verondersteld dat deze atrofie van het netvlies een weerspiegeling is van het algehele verlies van zenuwcellen in het centraal zenuwstelsel (neurodegeneratie). Het exacte mechanisme waarop neurodegeneratie leidt tot atrofie van het netvlies is nog niet helemaal duidelijk, maar de meest geaccepteerd verklaring is retrograde trans-synaptische degeneratie. Dit houdt in dat een beschadigde zenuwcel de beschadiging ‘doorgeeft’ aan een

andere zenuwcel waarmee deze verbonden is. Eerder onderzoek heeft laten zien dat de mate van atrofie van de binnenste twee lagen van het netvlies samenhangt met klinische maten van neurodegeneratie, waaronder hersenatrofie gemeten op MRI en lichamelijke invaliditeit. De relatie tussen atrofie van het netvlies en cognitieve stoornissen, ook een gevolg van neurodegeneratie, is minder goed onderzocht. In **hoofdstuk 4.1** hebben we deze laatste relatie bestudeerd. De resultaten tonen aan dat patiënten met cognitieve stoornissen beduidend meer atrofie laten zien van zowel de pRNFL als de mGCIPL. Andersom was het hebben van een dunnere pRNFL en mGCIPL geassocieerd met een hogere kans op het hebben van cognitieve stoornissen. Deze relatie tussen atrofie van het netvlies en cognitieve stoornissen werd echter alleen gevonden bij de patiënten die nog nooit een neuritis optica hadden doorgemaakt. De patiënten die wel een neuritis optica hadden doorgemaakt lieten dusdanig veel atrofie van het netvlies zien, dat de relatie werd gemaskeerd. Deze laatste bevinding maakt dat een eenmalige atrofiemeting minder bruikbaar is voor het vaststellen van neurodegeneratie bij die laatste groep patiënten.

Hoofdstuk 4.2 behandelt het vervolg op de studie beschreven in hoofdstuk 4.1, waarbij de patiënten twee en vier jaar na de initiële beoordeling opnieuw werden onderzocht. Bij deze studie werd gekeken naar *achteruitgang* in cognitief functioneren. We vonden dat *progressieve* (longitudinale) atrofie van de mGCIPL geassocieerd was met cognitieve achteruitgang. De dikte van de pRNFL en mGCIPL aan het begin van de studie was niet voorspellend voor cognitieve achteruitgang. Een andere, belangrijke uitkomst was dat de hoeveelheid *progressieve* atrofie niet verschilde tussen de ogen met en zonder een voorgeschiedenis van neuritis optica. Dit betekent dat progressieve atrofie van het netvlies ook bij patiënten die een neuritis optica hebben doorgemaakt als voorspellende factor gebruikt zou kunnen worden.

De resultaten van de twee bovenstaande studies onderstrepen dat atrofie van het netvlies bij MS-patiënten neurodegeneratie weerspiegelt en daarmee een maat is voor cognitieve en lichamelijke invaliditeit.

In **hoofdstuk 4.3** wordt een nieuw fenomeen beschreven dat op OCT-scans is ontdekt, genaamd peripapillaire hyperreflecterende eivormige massa-achtige structuren (PHOMS). De resultaten van het onderzoek tonen aan dat PHOMS significant vaker voorkomen bij MS-patiënten dan bij gezonde controles. Er is echter geen relatie tussen PHOMS en ziektekarakteristieken zoals leeftijd, ziekteduur, ziektetype, het gebruik van medicatie tegen MS of progressie van lichamelijke invaliditeit. De exacte aard van PHOMS is momenteel nog onduidelijk. Verder onderzoek naar dit fenomeen zou ons meer duidelijkheid kunnen verschaffen over de mechanismen die ten grondslag liggen aan neurodegeneratie in het centraal zenuwstelsel van MS-patiënten.

OCT EN INFLAMMATIE IN MS

Tot voor kort werd bij OCT-onderzoek bij MS-patiënten voornamelijk gekeken naar de relatie tussen neurodegeneratie en atrofie van de binnenste twee lagen van het netvlies. **Hoofdstuk**

5 beschrijft onderzoeken waarbij we hebben gekeken naar de relatie tussen OCT en inflammatie in MS. Zoals eerder benoemd, wordt inflammatie verantwoordelijk geacht voor de kenmerkende schubs die patiënten ervaren. In **hoofdstuk 5.1** hebben we de relatie onderzocht tussen inflammatie en een derde laag van het netvlies, genaamd de 'macular inner nucleair layer' (mINL). Hieruit komt naar voren dat een toename in dikte van de mINL samenhangt met het ontstaan van zowel een neuritis optica als schubs elders in het lichaam. De resultaten komen overeen met resultaten van andere onderzoeken bij MS-patiënten waarbij er een associatie werd gevonden tussen een verdikking van de mINL en tekenen van inflammatie (op MRI dan wel klinisch).

Eerdere onderzoeken hebben vastgesteld dat uveïtis vaker voorkomt bij MS-patiënten dan in de algemene populatie. Uveïtis is een ontsteking van de binnenkant van het oog en gaat gepaard met vertroebeling van het glasvocht. In **hoofdstuk 5.2** hebben we middels een nieuwe techniek, waarmee deze glasvochttroebeling automatisch kan worden gekwantificeerd, onderzocht of MS-patiënten meer vertroebeling laten zien als uiting van inflammatie. In ons cohort was er geen verschil in glasvochttroebeling tussen MS-patiënten en gezonde controles. Daarnaast was er geen relatie tussen de mate van glasvochttroebeling en tekenen van inflammatie zoals het ontstaan van nieuwe schubs, MRI-afwijkingen of mINL-dikte. Een kanttekening moet worden geplaatst bij deze resultaten aangezien het onderzoek is uitgevoerd onder patiënten met een langdurig beloop, waarbij de inflammatie vaak is uitgedoofd en er voornamelijk sprake is van neurodegeneratie.

CONCLUSIE

Alles bij elkaar genomen, zijn er sterke aanwijzingen dat schade aan het centraal zenuwstelsel van MS-patiënten wordt weerspiegeld in veranderingen in het netvlies. Dit maakt dat OCT een waardevol instrument is bij de klinische beoordeling van MS-patiënten, voor zowel visuele, cognitieve als lichamelijke klachten. De onderzoeken beschreven in dit proefschrift hebben bijgedragen aan de kennis over de toepasbaarheid van OCT in de klinische MS-praktijk. Op dit moment wordt OCT in enkele MS-centra in Nederland en wereldwijd reeds gebruikt voor het monitoren van het ziektebeloop. De hoop is dat met nieuw onderzoek het ook mogelijk wordt om middels OCT het ziektebeloop te voorspellen, zodat toekomstige schade kan worden voorkomen.

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BIOGRAPHY

Danko Čorić was born on 28 September 1987 in Zenica, Bosnia and Herzegovina (at that time part of Yugoslavia). In 1994, his parents, together with his sister and him, moved to the Netherlands due to the Bosnian war. After spending a year in a refugee center, they settled in Gouda. After finishing high school (St. Antoniuscollege, Gouda), he started medical school at Utrecht University. During the last year of his medical training, he completed a scientific internship at the department of neurology of the UMC Utrecht, where he investigated the risk factors associated with chronic idiopathic axonal polyneuropathy. Shortly after graduating from university in December 2012, he started working as a junior doctor at the department of neurology of the Maasstad Ziekenhuis in Rotterdam. At that time, he also moved to Rotterdam. In 2015, he started his PhD at the MS Center of the VU University Medical Center under the supervision of prof. dr. BMJ Uitdehaag, dr. A. Petzold and dr. LJ Balk. The research he performed there is described in this thesis. During his PhD he also spend some time abroad, working at City, University of London in London, UK. In October 2020, he started his residency program in psychiatry at GGZ Delfland in Delft.