A systematic review and meta–analysis of optical coherence tomography in multiple sclerosis

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Abstract

Optical coherence tomography (OCT) is a new method potentially applicable for the analysis of neurodegeneration in multiple sclerosis (MS) by capturing thinning of the retinal nerve fibre layer (RNFL). Metaanalyses of time domain OCT (TDOCT) data demonstrates RNFL thinning of 20 μm (95%CI 18-23, n=2063, p¡0.00001) following MS optic neuritis (MSON) and μm (95%CI 6-9, n=3154, p¡0.00001) in MS without MSON. The estimated RNFL thinning in patients with MS is above what is expected from normal ageing. The likely cause being retrograde trans-synaptic degeneration and progressive loss of retinal ganglion cells. The RNFL correlated with visual and neurological functioning as well as with paraclinical data. Promising developments in order to better understand the structurefunction relationship in MS pathophysiology include spectral/Fourierdomain OCT (SD/FDOCT) technology, polarisation sensitive OCT, fluorescence labelling, structural assessment of action potential propagation and segmentation algorithms allowing for quantitative assessment of different retinal layers.

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Keywords optical coherence tomography, retinal nerve fibre layer, axonal loss, neurodegeneration, multiple sclerosis

Contents

1 Introduction

Optical coherence tomography (OCT) is a non–invasive technique[1] which allows for an "optical biopsy" of accessible tissues such as the retina (Figure 1). Over the past decade OCT matured into an interesting, highly sensitive tool for imaging of neurodegeneration in multiple sclerosis (MS) research.[2,3] Because the retina is the only place where a tissue layer made up of axons can be imaged directly, quantification of the retinal nerve fibre layer (RNFL) has the potential to open a diagnostic window on monitoring neurodegeneration.

Here we present a systematic review of studies investigating OCT in patients with MS. Special care is taken to distinguish axonal damage caused by MS clinically evident optic neuritis (MSON) from more subtle retinal axonal damage in non–affected MS eyes. We will review the anatomical functional correlations, first focusing on the visual system and second, focusing on more global measures of disability in MS. The relationship of OCT data to established electrophysiological techniques and imaging modalities will also be discussed. Finally, we will provide a glimpse into future research areas of advanced OCT imaging that might influence assessment of axonal damage relevant to assessing a patient's MS activity and response to therapy.

2 Methods

Search strategy and selection criteria The review of the Dutch, English, French, German, Italian and Spanish literature was conducted on all studies using OCT in MS patients between the first description of the method by Huang[1] and May 2010, including manuscripts published ahead of print. We searched pubmed, EMBASE, medline, web of science and the Cochrane Register of Diagnostic Test Accuracy Studies using the search terms: multiple sclerosis, MS, optic neuritis, ON, optical coherence tomography, OCT, retinal nerve fibre layer, RNFL. From 99 studies identified, 62 were excluded because they were reviews, did not use the Stratus OCT, were single case reports, communications in response to an article, duplication of data already published on this cohort or data presentation were not detailed enough to allow inclusion into a meta–analysis. Studies that did not include a control cohort were only included if they compared the ON affected and not affected eye in MS patients. From the 37 included studies 35 presented data suitable for meta–analysis of RNFL thickness between groups.[4–38]

Statistical analysis The data analysis used the Cochrane Collaboration's Review Manager software package (RevMan5) following the guidance of the Diagnostic Test Accuracy (DTA) Working Group. The published RNFL thickness data was entered as a continuous variable. We used inverse variance, with random effects in the model. For effect measure we

chose the mean difference which allows to compare the RNFL thickness in μm between the groups of interest. Three group comparisons for the RNFL thickness were performed: (a) MSON eyes with controls eyes, (b) MS eyes without history of MSON and control eyes, (c) in the same MS patient the MSON eye with the non–affected eye. Regression analyses were performed using SAS software (version 9.1.3). A p-value of ≤ 0.05 was regarded as significant.

3 OCT in Multiple Sclerosis

Axonal loss in the retina With the invention of the ophthalmoscope (von Helmholtz, 1851), *in vivo* detection of atrophy of the optic disc became technically possible. For example, Gowers described sectoral RNFL loss in a woman suffering from syphilis. In his case the remaining nerve fibres were more visible because of swelling (plate II, figure 2 in reference[39]). A later case of sectoral RNFL loss in which sequential *in vivo* images permitted to evoke the concept of ascending axonal degeneration (now known as Wallerian degeneration) was facilitated by the rare presence of myelinated fibres in the retina.[40] Bachmann describes a 28 year old man in whom sudden loss of vision in the right eye on the 22nd of January 1920 was due to a central retinal artery occlusion. Within two months after the retinal vascular event, the RNFL loss became visible as degeneration of bundles of myelinated retinal axons (Figure 2 A), progressing over the following two months (Figure 2 B) and leading to complete optic atrophy and

loss of myelinated axons observed on fundus exam within one year (Figure 2 C).[40] There is good post–mortem evidence for RNFL thinning in MS.[41]

RNFL in MSON In multiple sclerosis, following optic neuritis (MSON), the degree of RNFL loss in thickness is in the range of 5–40 μm, averaging at 10–20 μm.[6] The finding was significant for all studies using time domain OCT (TD–OCT) technology based on the Stratus OCT (Zeiss, software version 3.0 & 4.0) that we identified in a systematic literature review. The summary data of 14 studies on MS patients with MSON,[4,10,11,16,18,19,21–27,30,31] containing a total of 2063 eyes tested with OCT are presented in Figure 3. From two of these studies the data published were not in a format allowing inclusion into the meta–analysis and more detailed information could not be obtained from the authors.[24,28] The results of the meta–analysis on the remaining 12 studies[4,10,11,16,18,21,22,25–27,30,31] are highly significant with an estimated average RNFL loss of -20.38 μm [-22.86, -17.91].

An important limitation of these studies is that optic nerve damage, independent of whether it occurs in the context of MS (MSON) or due to other causes (for a thorough clinical review see reference[42]) will cause some degree of RNFL loss. In an elegantly conducted small case series, Choi *et al.* present detailed OCT data on patients with several different kinds of optic neuropathy.[43] Any form of optic nerve damage was associated with marked thinning of the RNFL. Therefore the question arises as to whether the presence of MSON could have introduced a bias towards more damage and RNFL thinning into the studies presented in Figure 3.

In the absence of MSON, retrograde trans-synaptic retinal ganglion cell degeneration due to MS lesions within the posterior optic pathways could be a cause for RNFL loss. The existence of retrograde trans–synaptic retinal ganglion cell degeneration has been proven in patients with cerebrovascular accidents affecting the posterior visual pathways and cortex.[44,45]

In a combined MRI/OCT study Reich *et al.* showed that damage to the optic radiations in MS was associated with a reduced averaged global RNFL.[46] There is a need to investigate whether the recently recognised effect of post geniculate lesions[44,45] on the RNFL can be distinguished from RNFL loss caused by subclinical damage to the anterior visual pathways in MS as suggested by VEP studies.[47–51]

RNFL in MS without MSON We identified 16 studies which compared the RNFL thickness in patients with MS without evidence for ON to a control population,[4,10,11,14–16,19,21,22,25–28,30,31] of which 15 provided data in a format allowing inclusion into the meta–analysis.[4,10,11,14,16,21,22,25–28,30,31] The summary data on the 3154 eyes investigated is presented in Figure 4. The estimated RNFL loss (-7.08 μm) was less compared to MSON (-22.86 μm), but the 95% CI were markedly smaller [-8.65, -5.52]. This highlights the importance for carefully looking for evidence of MSON in order to minimise the risk that detection of the more subtle RNFL loss due to presumed retrograde trans–synaptic retinal ganglion cell degeneration may be masked by the pronounced loss caused by damage to the anterior visual pathways.

RNFL in MS comparing MSON eyes with the contralateral eye To address this question 27 studies compared the affected (MSON) eye to the clinically non–affected eye in MS patients.[4–6,8–11,13,16,17,21–25,27,29–38] The meta–analysis on the 4199 eyes investigated clearly illustrates the significant effect that presence of MSON has on RNFL (Figure 5). The estimate RNFL loss (-13.84 μm) is larger than what was seen for the comparison of MS without MSON to controls (-7.08 μm), but the confidence intervals were overlapping. An important prognostic finding of these studies is that after reaching a threshold of thinning to about 75 μm of RNFL thickness, the chances for recovery of visual function seem to be less.[6]

Conclusion Taken together the published data[4–27,29–38] suggests a relationship between RNFL thinning and MS pathology as illustrated in Figure 6. The retinal axons project through the optic nerve into the lateral geniculate nucleus (LGN). About 90% of retinal axons synapse in the LGN and travel with the optic radiations to the occipital cortex. The remaining 10% project into the pretectal region of the midbrain. Severe thinning of the RNFL layers follows MSON directly (Figure 6 B). These **acute** changes in MSON patients can be distinguished from more **chronic** changes caused by MS pathology of the optic pathways. An MS lesion affecting fibres in the optic radiation will cause Wallerian degeneration of some axons which reaches the LGN only after some time. Due to trans–synaptic degeneration retrograde axonal degeneration will eventually cause some degree of RNFL thinning (Figure 6 C). It is also likely that progressive loss of retinal

ganglion cells (RGC) occurs as a result of chronic changes in the optic nerves/anterior visual pathways themselves (Figure 6 D). To test this hypothesis we reviewed the literature for the time course of RNFL loss in MS patients with and without MSON.

4 Time course of RNFL loss in MS

As a rule of thumb, RNFL loss becomes readily detectable to OCT about 3 months after an acute optic neuritis (ON). Earlier reduction in RNFL thickness from axonal atrophy is clinically difficult to distinguish from a reduction due to resolution of axonal swelling, which is common in acute optic neuritis. Costello *et al.* presented longitudinal data on the RNFL thickness during the first 12 months following ON (see Figure 7). This data shows ongoing axonal loss in the affected eye for at least 12 months, but most thinning occurs by 6 months after injury.

An inverse correlation between disease duration and the averaged overall RNFL thickness was found by some R=-0.262, p=0.011,[19] R=-0.6, p=0.02,[25] p=0.03 (R–value not published),[10] but not by others.[15,16] Examining non–MSON eyes Henderson *et al.* found that the RNFL decreased by 0.12mum per year of disease (95%CI: -0.50, 0.25), but this failed statistical significance (p=0.513).[15] Investigating MSON eyes, Klistoner *et al.* did not find a correlation between RNFL thickness and disease duration (p=0.9, R–value not published).[16] The divergent results[10,15,16,19,25] may in part be explained by the variation in average disease duration and a bias in the population of

MS patients studied. A meta–regression analysis of the studies' raw data may help to shed light on the presumed association between RNFL loss and disease duration, but the most accurate information will come from longitudinal studies, where the RNFL thickness in individual patients can be studied over time. Talman *et al.* published longitudinal data from 593 eyes which were assessed at baseline and $\geq$ 6 months later.[38] Their statistical analysis was corrected for patients age and adjusted for within-patient and inter eye correlations. For MSON eyes the percentage decrease of RNFL thickness compared to baseline was 0.4% (0.4μm; 95%CI: 1.16, -0.35) for eyes with a 0.5–1 year follow–up period; 1.7% (1.6μm; 95% CI, 2.47, 0.70) for $>$1 to 2 years; 3.2% (2.9μm; 95% CI, 4.02, 1.86) for $>$2 to 3 years; and 6.7% (6.1μm; 95% CI, 7.73, 4.41) for $>$3 years of follow-up. In comparison the average RNFL thinning for disease–free control eyes was 0.5% (0.49μm; 95% CI, 1.36, -0.39) over a 3-year period.[38] Their pooled analysis (MSON eyes and non-MSON eyes) showed that each year of follow–up was associated with an , on averaged 2 μm increase of RNFL thinning (p$<$0.001, generalised estimating equation models).[38]

Conclusion One needs to be very careful drawing conclusions on the time course of RNFL loss in an individual eye from cross–sectional data. The data suggest that in MS without MSON the estimated yearly thinning of the overall RNFL ($\approx$ 2 μm[38]) is probably below the detection limit of TD-OCT systems. Theoretically, the newer Spectral or Fourier domain OCT (SD/FD-OCT) systems should be able to enable resolution at this

level,[52–55] but practically a resolution of 4–6 μ was found for the Heidelberg spectralis and Cirrus HD-OCT.[56] There was (yet) no quantifiable RNFL loss in patients with a recent onset (average of 4.3 months) clinically isolated syndrome (CIS) if compared to healthy controls.[57] The current duration of phase II trials in MS is frequently around 4–6 months and it is not likely that OCT will provide a reliable outcome over this time–scale. From the above data one would expect that MS patients without MSON will require follow–up for at least two years.

The longitudinal monitoring of the RNFL is technically challenging. Two methods have been validated clinically: topographic change analysis (TCA) and statistical image mapping (SIM).[58–61] There is no consensus yet on how to collect and analyse longitudinally collected OCT data in MS.

5 OCT and disability in MS

There are well characterised limitations to the clinical disability scales currently employed in MS research,[62] not least of which is that they fail to fully capture the range of disability seen in the disease, especially if they are not in the domain of patient mobility and motor function. Useful surrogate markers are challenging to validate.

The driving force of disability in MS, axonal loss, appears to be associated with changes in the RNFL which are statistically related to the changes observed in clinical disability progression.[4–27] (Figures 3–5). Because the RNFL is anatomically related to visual function, studies analysing

this relationship are reviewed first. There are good arguments that RNFL loss also reflects neurodegeneration on a more global level[2,3] stimulating additional review of the robustness of the relationship between RNFL loss and global clinical disability scales. In reviewing this literature, it is important to keep in perspective that almost all of the studies to date are performed on group analysis.

5.1 Visual function

Visual acuity Monocular visual acuity (VA) is commonly assessed using standard eye charts. The method was introduced by the Dutch ophthalmologist Hermann Snellen in 1862.[63] The current convention is to document the Snellen equivalent (20/10–20/200). There are methodological limitations of the Snellen charts and of the many published improvements this review will consider Early Treatment Diabetic Retinopathy Study (ETDRS) charts and low contrast acuity (Sloan charts) in a separate paragraph.

Loss of RNFL was associated with reduced Snellen VA in most[5,6,18,19,21,27,64–66] but not all[13] studies. A linear correlation between loss of Snellen VA and the RNFL was observed in three studies.[18,64,65] The strongest correlations ($R>0.6$) were found in studies including MSON patients.[18,64,65] Comparable results were found for use of standard Japanese decimal VA.[35]

Early Treatment Diabetic Retinopathy Study (ETDRS) charts The ETDRS charts have a number of advantages over the Snellen VA, one being that the use of logarithmic scaling (logMAR), permits adjustment of the VA

score to the viewing distance.[67] Essentially a logMAR score provides interval data which facilitate statistical analyses and is therefore recommended for use in clinical trials.[68] Trip *et al.* found a linear correlation between the interocular difference in the logMAR score and the RNFL thickness.[26] Henderson *et al* confirmed the correlation of the RNFL with the logMAR score (R=-0.54, $p<0.001$).[15] Costello *et al.* also observed such a correlation six months after MSON.[7] In a mixed cohort of untreated MS patients with or without MSON, Spain *et al.* reported a linear correlation (R=-0.53, $p<0.001$)[29] which is consistent with the results of Siepman *et al.* (R=-0.56, $p<0.01$).[37]

Low contrast letter acuity Balcer *et al.* first suggested integrating (binocular) low contrast letter acuity (Sloane charts) into a modified Multiple Sclerosis Functional Composite (MSFC) based on her observation that MS patients could be significantly ($p<0.0001$) better distinguished from controls at a 1.25% contrast level compared to ETDRS charts.[69] The authors also found Pelli–Robson charts to be of use, albeit at a lower level of significance (p=0.003). The results were confirmed in later studies.[38,70]

Fisher *et al.* found a one–line change of 1.25% low–contrast letter acuity for every 4μm of RNFL lost.[10] A strong correlation (R=0.63, p=0.02) between low contrast acuity and RNFL thickness in affected MSON eyes was described in two other studies,[11,27] a finding confirmed independently in patients with PPMS (R=-0.46, p=0.026).[15] A moderate correlation between the RNFL and 2.5% charts (R=0.39, $p<0.001$) and 1.25% charts

(R=0.31, p$<$0.001) was found in one study.[21] In line with these studies are the results of Spani *et al.* using 1.25% Sloan charts (R=-0.34, p=0.02).[29] Currently, there are no longitudinal studies showing good correlation between RNFL and VA over time.

Visual fields (VF) Multiple sclerosis can produce any type of VF defect. The most common VF defects in acute MSON are dense but transient scotoma which are central, altitudinal or centro–caecal.[42,71,72] Achromatic static perimetry is typically used for assessment of VF loss. The sensitivity of this technique is limited when supra–threshold screening strategies are used and improves with threshold estimation strategies.[73,74] The likelihood to underestimate VF loss depends on the number of stimuli tested, stimulus size, the threshold loss and the control of eye–movements.

Costello *et al.* used the central 30-2 full threshold strategy (Humphrey)[6] which is sensitive and gives a good overview. The authors found a relationship between the RNFL thickness and VF 3-6 months after ON. Below a threshold of 75 μm RNFL loss, a linear correlation was found with the VF mean deviation (MD) in decibels (dB) *. Importantly, there was no recovery of visual function in these patients. A RNFL thickness of less then 75 μm is considered to be a poor prognostic sign.[7,22,23] The results of Costello *et al.* are consistent with those by Trip *et al* who described a linear correlation between the interocular difference in the RNFL thickness and VF MD us-

*Authors note: the VF MD is one of four global indices provided by the Humphrey perimeter. The MD gives the average of differences from normal expected value for the patients age. The MD is useful for detecting diffuse VF loss as it is the case in MSON.

ing the same protocol.[26] A weak correlation between the averaged RNFL thickness and the MD VF was also confirmed by others.[15,19,37,64] Noval *et al.* described an association between RNFL loss and VF 1.5 and 3 months after MSON, but not any more after 6 months.[64] The authors conclude that OCT may detect RNFL damage at a level which is below the sensitivity of automated static perimetry which is consistent with other studies.[20]

Cheng *et al.* used a VF severity score and found a better correlation for the RNFL with the overall VF loss compared to quadrant VF loss.[5] The authors suggest this may be due to (a) diffuse instead of localised RNFL defects in MS, (b) a poor structure–to–function map, as reasons for the limited topographic correlations,[5] and suboptimal image registration on TD–OCT in some patients offers an alternative explanation. Trip *et al.* also failed to show an association between a temporal VF defect and loss of RNFL thickness in the corresponding RNFL sector.[26] They point out that the Humphrey 30-2 program tests far less points in the nasal and temporal sectors than in the superior and inferior sectors, potentially leading to greater noise due to reduced sampling.

5.2 Global clinical scores

EDSS Twelve studies examined the relationship between loss of RNFL and progression on the EDSS.[4,9,10,12,13,18,24,25,28,37,65,75] An inverse correlation between the RNFL and the EDSS was observed in 6 studies (R=-0.348,[24] R=-0.7,[25] R=-0.399,[75] R=-0.30,[37] R=-0.42 MS patients without

MSON[4] and partial R=-0.35 using the minimal RNFL thickness[12]). This data is consistent with analyses from two further studies describing a significant reduction of the RNFL with increasing EDSS percentiles.[10,13] Four studies did not find a relationship between the RNFL and the EDSS.[18,28,36,65] One of these studies included NMO and MS patients,[18] another presented detailed data on both variables but did not include a correlation analysis.[9]

The differences are at least in part explained by the heterogeneity of the groups investigated (see Figures 3–4). The strongest correlation between the EDSS and the RNFL was found for MS eyes which were not affected by MSON.[4,25] It is possible that the strong effect MSON has on RNFL thickness (Figure 3) masks more subtle changes caused by either asymptomatic axon damage or trans–synaptic axonal degeneration in non–affected MS eyes.

Therefore, future treatment trials using OCT as a secondary outcome measure for global disability will have to anticipate that predefined analyses on patients without MSON are likely to be of stronger statistical power than in patients with MSON and that alternative measures of disability scoring that better reflect generalised neurologic functioning, including cognition may need to be used which might better reflect diffuse axonal loss in the CNS.

The Multiple Sclerosis Severity Score (MSSS) The MSSS was developed to overcome the problem of changes in the EDSS with different disease durations when comparing groups. There was no correlation of the

RNFL with the MSSS in one study including patients with MSON.[65]

6 OCT and electrophysiology in MS

OCT allows to study of the *structural* properties of the retina thus complimenting the many electrophysiological techniques aimed at *functional* assessment. Research has been performed to find out possible associations between the two domains. Recalling the simplified sketch from Figure 6, one can formulate two key hypotheses to be tested: (1) is there a relationship between RNFL loss and VEP/PERG amplitude reduction (Figure 6 B)? (2) does VEP evidence for demyelination in the context of normal anterior visual pathways predict later development of RNFL loss with transynaptic retrograde degeneration (Figure 6 C)?

6.1 VEP

Following demyelination of the optic nerve the latency of visual evoked potentials (VEP) are typically prolonged. This finding may persist for many years and is regarded as a sensitive but not specific test. In a longitudinal study Brusa *et al.* were first to note a small decline of the VEP amplitude in the non–affected eye of MS patients with ON, suggesting that this may be due to axonal loss.[50] Advancing this concept, the same group demonstrated that reduction of RNFL thickness was indeed related to reduced VEP (whole–field VEP and central–field VEP),[26] a finding confirmed by others.[16,19,20,76] Klistorner *et al.* went on to show a high functional–

topographic correlation between multifocal VEP and the RNFL (inferior VF, R=0.84; superior VF, R=0.78; central VF, R=0.75; $p<0.000$ for all correlations).[77] Advances in multifocal VEP data analysis may render this technique more accessible.[78] Some authors[14,16,19,79] also found a correlation between the RNFL and VEP latencies.

The association between reduction in RNFL and VEP amplitudes lends further weight to the argument that demyelination related damage to the ON may cause either direct axon damage or may occur in the postgeniculate visual pathway, leading to retrograde axonal degeneration of the non–myelinated axons in the retina (Figure 6 B).

6.2 ERG

Using TD-OCT a correlation was found between the RNFL thickness and pattern ERG P50 latency as well as with the P50-P95 amplitude.[79] It was suggested that this may be due to loss of ganglion cells following damage to the optic nerve.[79] Others did not find a convincing relationship between the overall averaged RNFL and simple or multifocal ERG results,[14] possibly because multifocal ERG samples mainly the photoreceptor and bipolar cell activation and not ganglion cells, therefore a correlation may not necessarily be expected.

7 OCT and imaging in MS

It is beyond the scope of this review to adequately address the results of the body of literature on structural changes within the visual pathways assessed by brain imaging techniques and the relationship to OCT data. In brief, this relationship was investigated in a number of studies included in our systematic review.[11–13,24,25,80,81] These authors focused on optic nerve imaging[11,80,81] and full brain imaging techniques.[11–13,24,25,46] The published data suggest correlations of the RNFL with brain atrophy measures[12,13,25] but a consensus has yet to emerge which atrophy measure is the most relevant, with grey matter atrophy remaining a hot topic.[11] Other measures potentially to be considered are the normalised brain volume,[11,24] the T2 lesion volume,[11,24,25] the magnetisation transfer ratio,[11,81] the fractional anisotropy and diffusion tensor imaging.[11,82] Less promising seem to be the T1 lesion volume[11,13,25] and the mean parenchymal diffusivity.[11,46] For a more in–depth review see references.[?,3]

8 Future developments

OCT and the macula in MS Changes of macular volume, as well as inner and outer macular segments consistently show volume loss caused by loss of retinal ganglion cells (RGC).[12,15,19–23,26,32,37,83–85] All found the macular volume to be reduced in patients with MS if compared to controls. Loss of macular volume was correlated with loss of RNFL in 4 stud-

ies.[15,26,80,84] Of note retinal thickness in the macula is made up of many RGCs (cones in the macula have a 1:1 correlation with RGCs where as in the periphery many rods have a 1000:1 relationship with RGCs). Therefore the macula provides a model to test hypotheses on primary neuronal cell death followed by axonal loss. In a recent editorial,[86] Waxman asked three crucial questions: (1) what are the mechanisms involved in RGC apoptosis? (2) could this be following axonal pathology or dysfunction? (3) alternatively, could RGC apoptosis be an example of primary neuronal injury independent from axonal damage?

Polarisation–sensitive OCT (PS-OCT) Specific tissue properties can be further investigated by recording the polarisation state of back-scattered light.[87,88] Polarisation-sensitive OCT (PS-OCT) yields depth–resolved information on any light polarisation changing properties of the sample related to tissue birefringence.[87,89–91] Importantly, birefringence of the RNFL is related to the structure of dominant axonal filaments such as neurofilaments and microtubles.[92] The birefringence of the RNFL induces a quantifiable degree of phase retardation.[89–91,93]

The group of de Boer demonstrated that the birefringence of the RNFL is not constant, but varies by a factor of 3 around the optic nerve head, with higher values reported in the superior and inferior quadrants, and lower values in the nasal and temporal quadrant.[93] This distinguishes the RNFL from other retinal structures which are either polarisation–preserving (e.g. photoreceptor layer) or polarisation scrambling/depolarising (e.g. retinal

pigmented epithelium, RPE).[91] Because changes to the axonal cytoskeleton such as neurofilament compactness, phosphorylation and stoichiometry can precede axonal loss,[94,95] there may be a chance to detect early stages of axonal pathology in MS using PS-OCT. Experimentally it has already been shown that change in RNFL birefringence precedes RNFL loss.[96]

Fluorescence labelling Fluorescence labelling of a protein (annexin 5) which binds to a key component initiating apoptosis (phosphatidylserine) allowed for real–time *in vivo* monitoring of RGC apoptosis.[97] The detection of apoptosing retinal cells (DARC) technique provides a promising surrogate outcome for neuroprotective treatment strategies in glaucoma, dementia and potentially also MS.[98,99] Analogous, labelling of mitochondria may allow for *in vivo* testing of the "virtual hypoxia" hypothesis in MS.[100]

Retinal sector analysis This review did not include OCT data on sector analysis of the retina. It can be hypothesised that retinal axons of certain retinal sectors are more vulnerable then others in MS. Therefore, quantitative analysis of these sectors may allow for sensitive detection of axonal loss. However there is a large normal variation of the appearance of the optic disc influencing the RNFL thickness which ought to be controlled for.[101,102]

RNFL thickness & reflectivity maps The accurate localisation of focal and peripheral loss of retinal axons is challenging using individual circular RNFL scans at the optic nerve head. One possible approach is the development of RNFL thickness maps.[90] Because the loss of retinal axons in MS is more diffuse than the arcuate bundle loss seen in glaucoma it can be speculated that an integrative approach combining PS-OCT data with RNFL thickness maps may have the potential to predict the topography of RNFL loss in MS.

Retinal layer segmentation algorithms With the introduction of SD/FD–OCT retinal layer image quality potentially allows for segmentation and quantification of individual layers. There is histological evidence that MS not only affects the RNFL but also cellular layers.[41] Therefore new segmentation algorithms for quantitative analyses of individual retinal layers may enable us to better investigate progression of neurodegeneration in MS.

Doppler OCT and vascular changes in MS There is some evidence that vascular co-morbidity is a poor prognostic sign in MS.[103] Changes of the retinal vasculature such as perivasculitis are recognised in MS. Perivasculitis leads to extravascular hyaline deposits, hence the descriptive name vascular sheathing. It is likely that these changes lead to a more rigid retinal vasculature and thus a quicker pulse propagation from the posterior (choriodal) to the anterior (retinal vasculature) circulation.[17]

This could be investigate by combining SD-OCT with Doppler velocimetry (SD-ODT). SD-ODT is non-invasive and allows for accurate topographic localisation of retinal blood vessels.

OCM and action potentials The influence that action potential propagation has on light properties has been recognised by Frank[104] who cited his work together with Kornakova[105] in 1947. The field flourished over the next 30 years (reviewed in[106]). Using optical coherence microscopy (OCM) the structural assessment of an action potential became reality.[107,108] At present only *in vitro* monitoring of action potential propagation is possible. Functional imaging of the human retina *in vivo* would be highly desirable to investigate whether axonal dysfunction may precede RGC and/or RNFL loss.

9 Conclusion

There is much excitement about OCT in MS research and it may be permitted to cite one of the first neurologists who made extensive use of the ophthalmoscope, Hughlings Jackson (1835-1911): “It is not too much to say that, without an extensive knowledge of ophthalmology, a methodological investigation of diseases of the nervous system is not merely difficult, but impossible.”[109]

In conclusion, OCT is a relatively new and promising technique for potential monitoring neuroprotective treatment trials in MS. First, there is a

clear pathological correlate (axonal loss). Second, the analytical reproducibility is excellent. Third, the sensitivity to change is twice as high as the normal physiological changes during ageing and over a magnitude above the averaged changes seen after ON. Fourth, it is of high clinical relevance, correlating with clinical measures (loss of visual function) thus capturing a fundamental feature associated with disability progression. Fifth, there is data from brain imaging and electrophysiological studies which suggests that the integration of OCT into MS research may allow for a more accurate view of structure–function relationships in understanding the pathophysiology of this enigmatic disease. Finally, it is predictive of a clinical outcome (poor visual recovery). It is proposed to investigate the role OCT for future MS research within a concentrated, resource saving approach using an international workforce (OCTiMS).

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JdB: has no funding from industry. Research funding and funding for equipment from government and non governmental foundations: VICI career award. Title: Minimally invasive optical diagnostics in medicine. 09NIG 03 (FOM). Title: Minimally invasive optical coherence tomography for diagnosis and staging of early lung cancer lesions. R21-RR023139

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CP, RK, AG, PV, SS and PC are sitting on the Novartis steering committee for a multi-center observational study: ”A 3-year, open-label, multi-center, multi-cohort, parallel-group study to validate optical coherence tomography in patients with multiple sclerosis” and receive honoraria.

Author's contributions AP: conceived the idea for this review, performed the literature search, systematic review and meta-analysis. Wrote the first draft of the manuscript. JdB: revised the manuscript, performed an independent literature research and provided Figures 8 and 9A. SS: revised the manuscript. PV: revised the manuscript. RK: revised the manuscript and performed an independent literature research. AG: revised the manuscript.

PC: revised the manuscript. CP: revised the manuscript.

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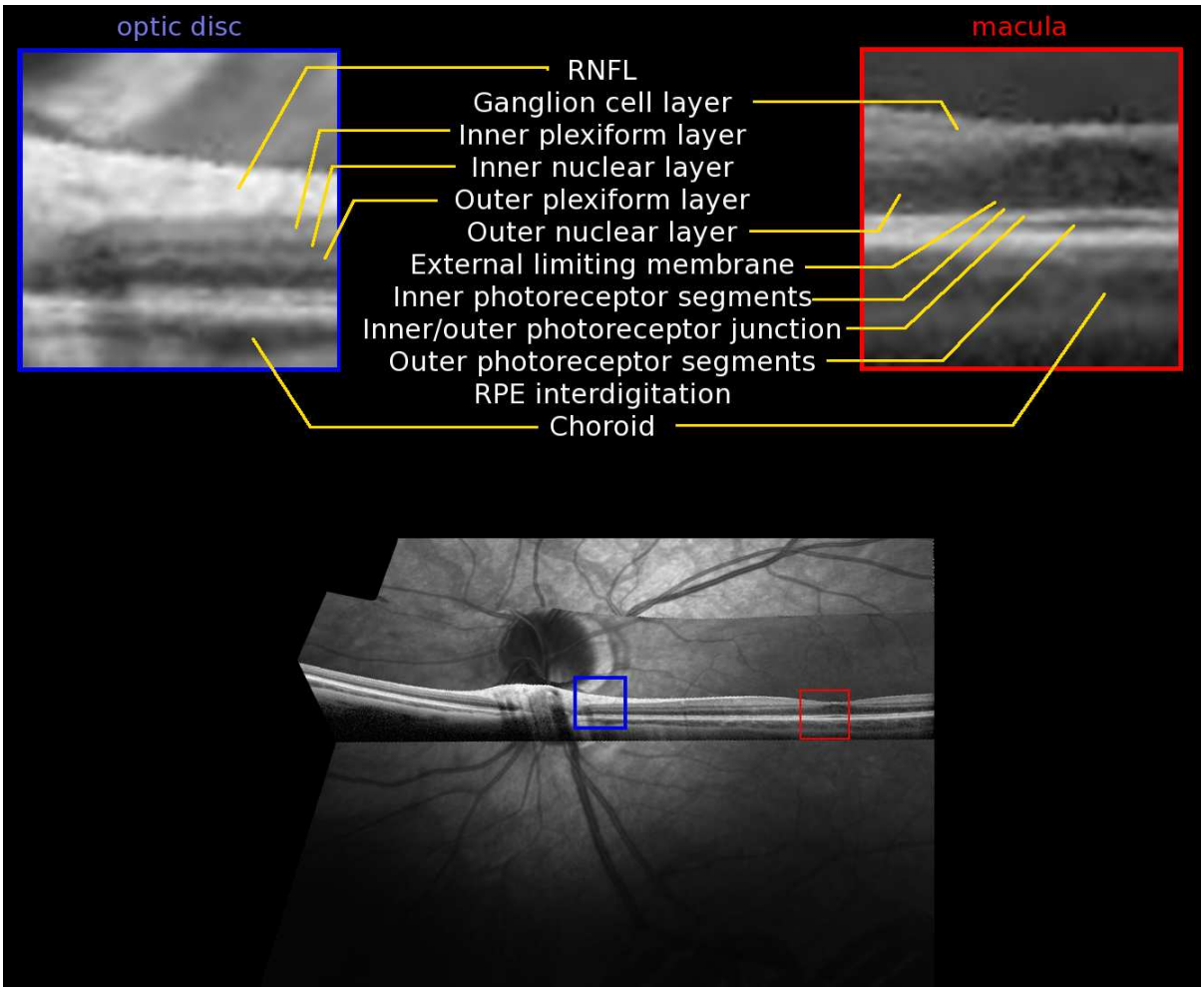


Figure 1: *SD/FD–OCT image of a normal eye showing the different retinal layers. The inlay shows an enlarged part of the optic disc (blue frame) and macula (red frame).*

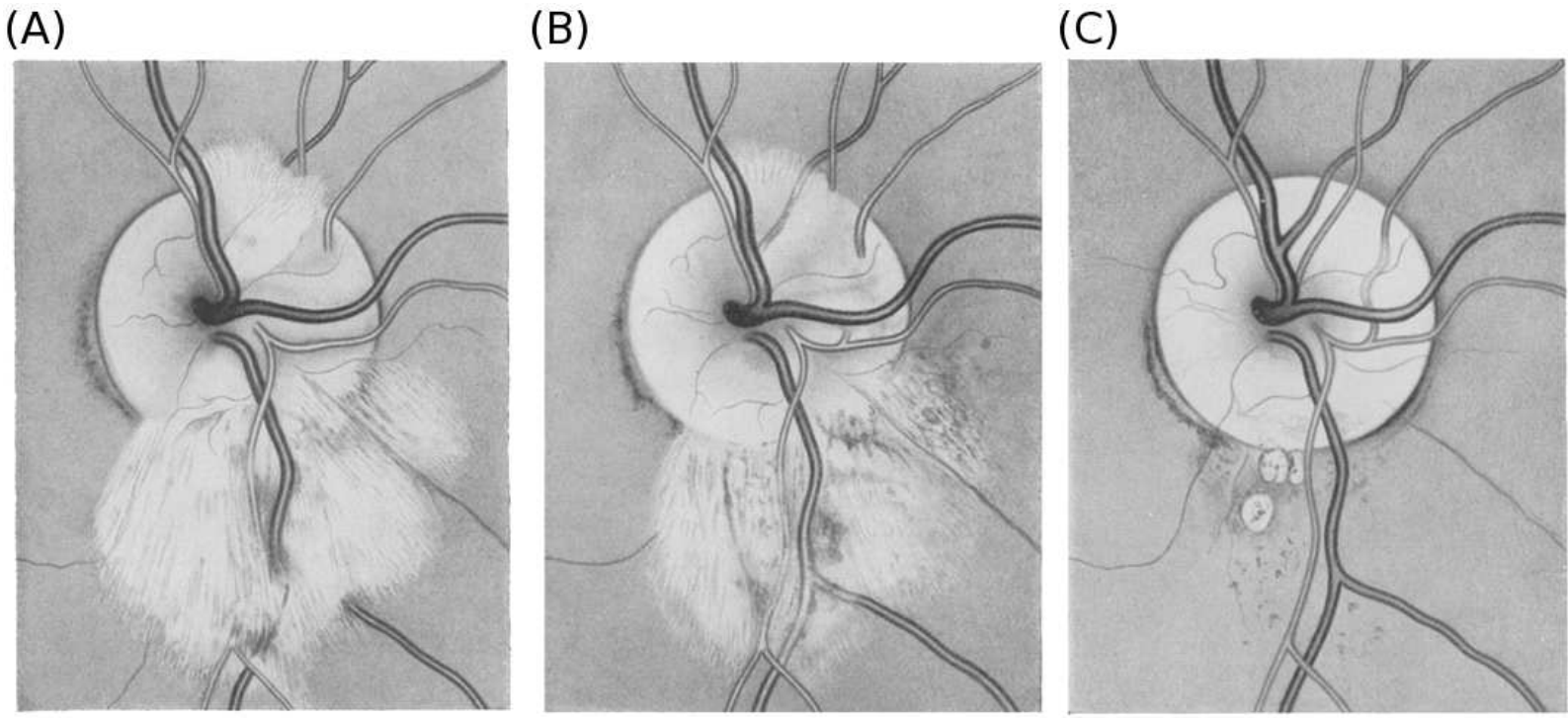
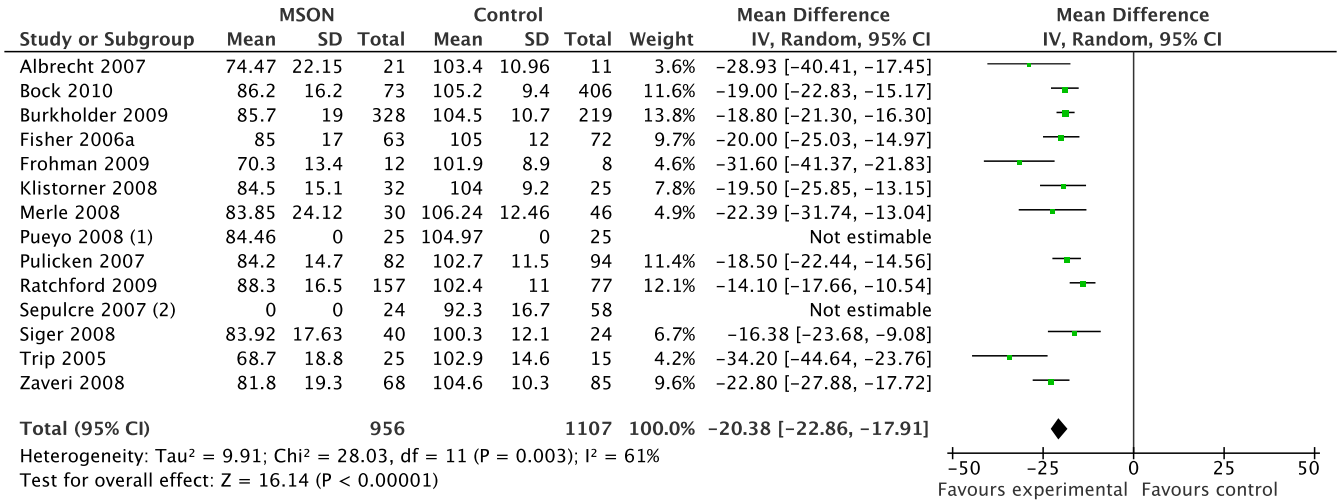
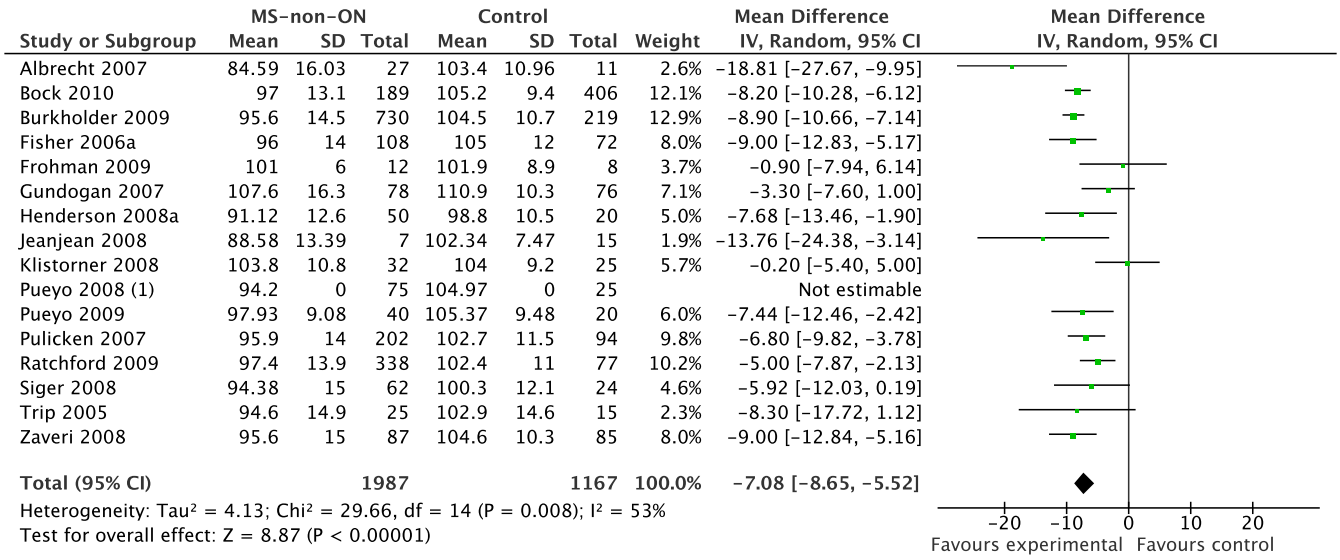


Figure 2: *These hand sketched images illustrates probably for the first time the development of axonal degeneration in the retina. At the time, this observation was only apparent thanks to two anatomical oddities coinciding. Firstly the presence of myelinated axons within the retina (a developmental occurrence), seen here as a white bundle on the top and larger white bundle on the bottom of the optic disc. Secondly, occurrence of a central retinal artery occlusion in the same patient which caused retinal axon degeneration over time. (A) A few dark gaps are visible between the inferior bundle of myelinated axons (sketch taken 2 months after sudden loss of vision in the right eye), indicating the beginning of loss of axons and their myelination (B) axonal loss becomes more marked after 4 months and (C) complete optic atrophy is the end result one year after a presumed embolus of the central retinal artery in the 28 year old boy with a mitral valve insufficiency. Bachmann already speculated in 1921 that the mechanism leading to this fundus appearance may be due to ascending (Wallerian) axonal degeneration. Note that the very occasion presence of myelinated retinal axons as seen in this sketch will influence RNFL data acquired by OCT, since intraretinal myelination results in a thicker, more highly reflective RNFL in those areas. MS patients in whom myelinated retinal axons are observed can appear as normal or above normal outliers in treatment trials using OCT as an outcome measure, especially if the myelinated retinal axons are undamaged initially. Reprinted with permission from.*[40]



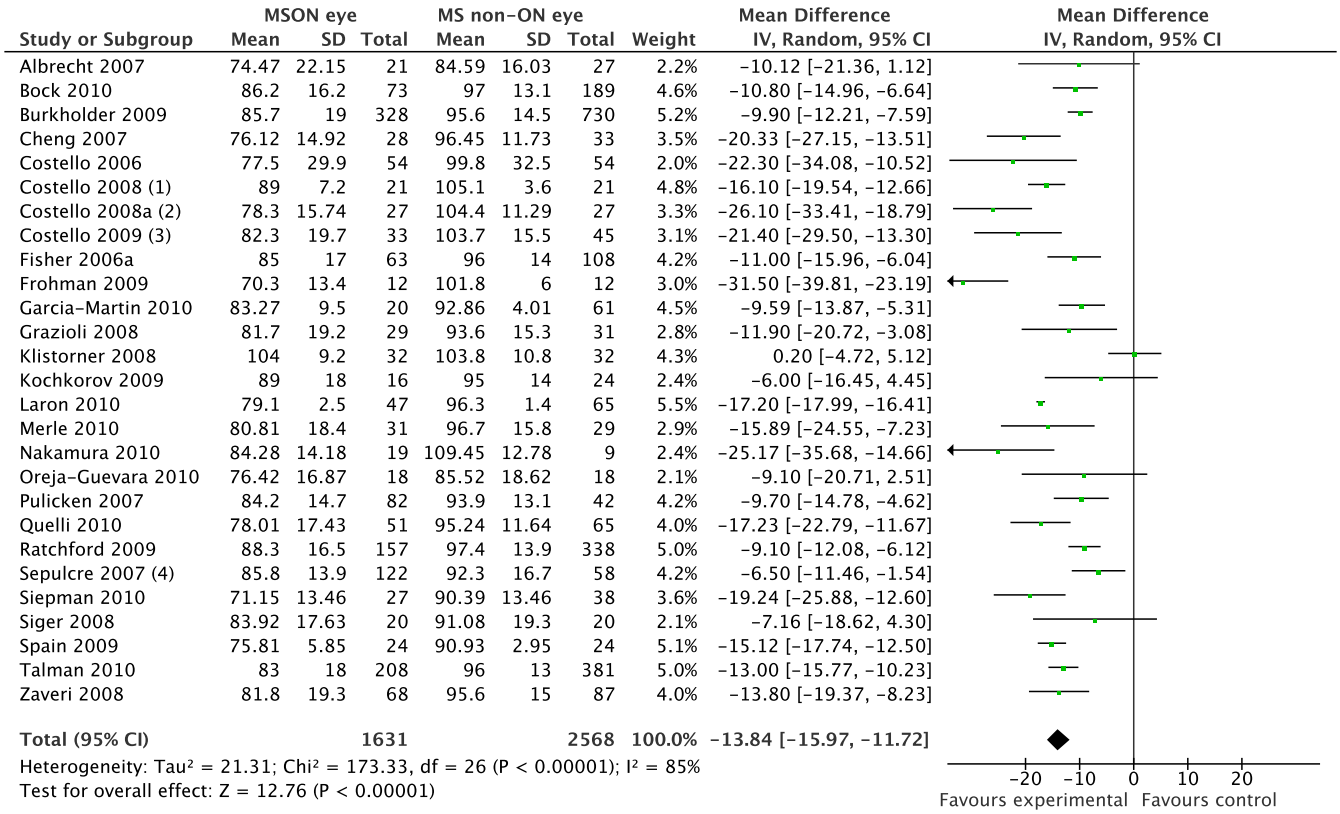
(1) Standard deviation not available from author
(2) Data (mean+/-SD) of ON eyes not published and not available from author.

Figure 3: *Meta–analysis of OCT studies in MS patients who did suffer from MSON. The overall averaged RNFL (mean±SD) and number of eyes (not subjects!) investigated is shown for patients and normal subjects. To the right is the data expressed as micron difference in RNFL thickness of eyes that had optic neuritis compared to normal eyes; length of horizontal bar is the 95% confidence interval for each study.*



(1) Standard deviation not available from author.

Figure 4: *Meta-analysis of OCT studies in MS patients who did not suffer from ON, by history. The overall averaged RNFL (mean±SD) and number of eyes investigated is shown, similar to Figure 3 Note that the bar graph to the right, which summarises the difference in RNFL in the asymptomatic eyes compared to normal eyes, shows there is loss of RNFL even in eyes supposedly not suffering from previous optic neuritis.*



(1) Data on one year after ON. The authors also present 2-year data which is not included into this analysis.
(2) The one year (13-18 months followup) is taken. Data on standard deviation kindly provided by Dr Fiona Costello.
(3) The one year data on RRMS is presented.
(4) This study included 24 ON eyes and 98 eyes without ON. More details not available from authors.

Figure 5: *Meta-analysis of OCT studies comparing MSON and non–affected eyes in MS patients. The overall averaged RNFL (mean±SD) and number of eyes investigated is shown. The difference between the MSON and MS-non-ON eyes shown in this figure is less than the difference between MSON eyes and normal eyes Figure 3 (please note that the scale of the x–axis differs between the two Figures).*

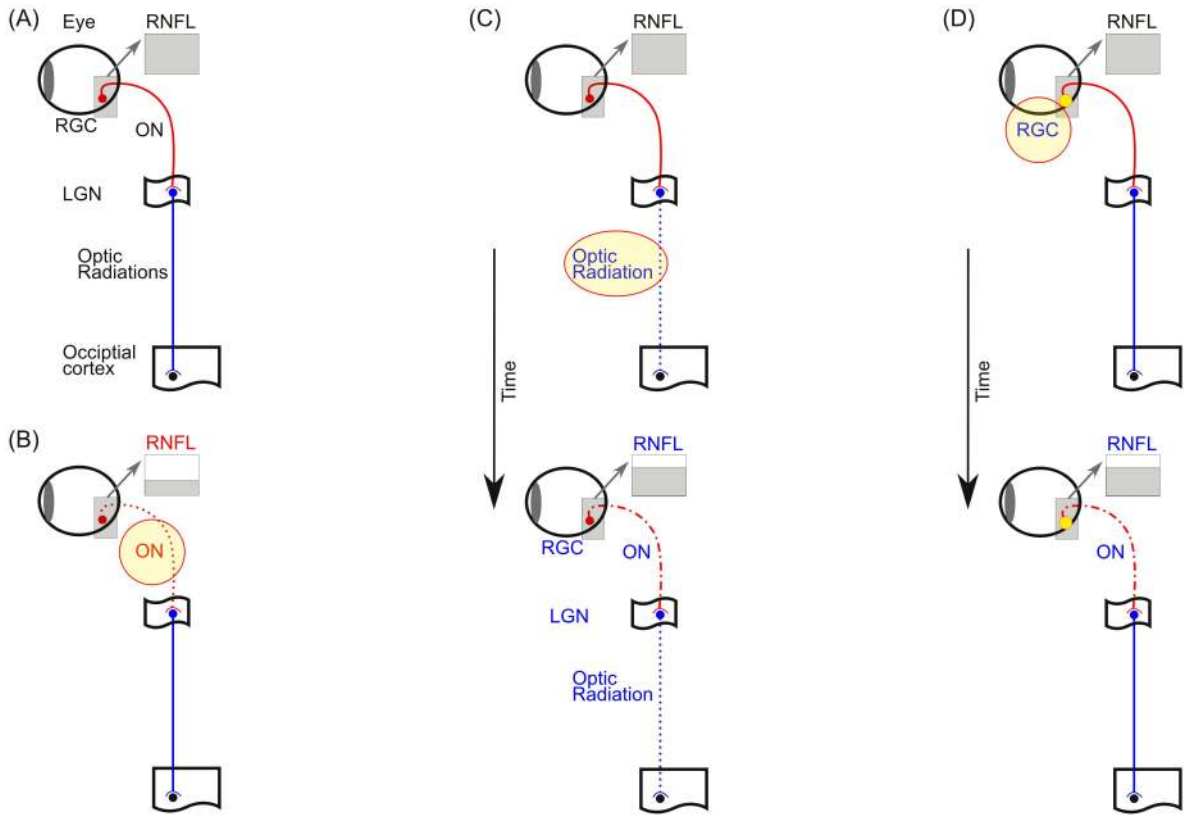


Figure 6: ***A model of the presumed relationship between RNFL thickness and MS pathology.*** *(A) A simplified sketch of the normal human visual pathways. The retinal ganglion cells (RGC) send their unmyelinated axons into the eye were they form the retinal nerve fibre layer (RNFL, grey inlay), travel to the optic disc and leave the orbit. Once the axons passed the sclera they become myelinated and form the optic nerve (ON). After passing through the chiasma were the temporal fibres cross (not shown) they are called the optic tract. The optic tract wraps around the midbrain and enters the lateral geniculate nucleus (LGN) where all axons must synapse. After the LGN the axons fan out through the deep white matter (optic radiations) to reach the occipital cortex. (B) MSON (red) directly causes* ***acute*** *axonal loss in the ON (red dotted line) leading to marked thinning of the RNFL (small grey box). (C) MS lesions within the optic radiations (blue dotted line) do not immediately result in RNFL thinning. This is thought to be a* ***chronic*** *consequence of trans–synaptic axonal loss through the LGN. With time (black arrow) trans–synaptic axonal degeneration causes a relative smaller degree of axonal loss in the ON (red dashed–dotted line) with a quantifiable degree of RNFL loss (grey box). (D) Progressive loss of RGC (yellow dot) is a likely result of* ***chronic*** *changes in the anterior visual pathways themselves and causes a small degree of RNFL loss (grey box).*

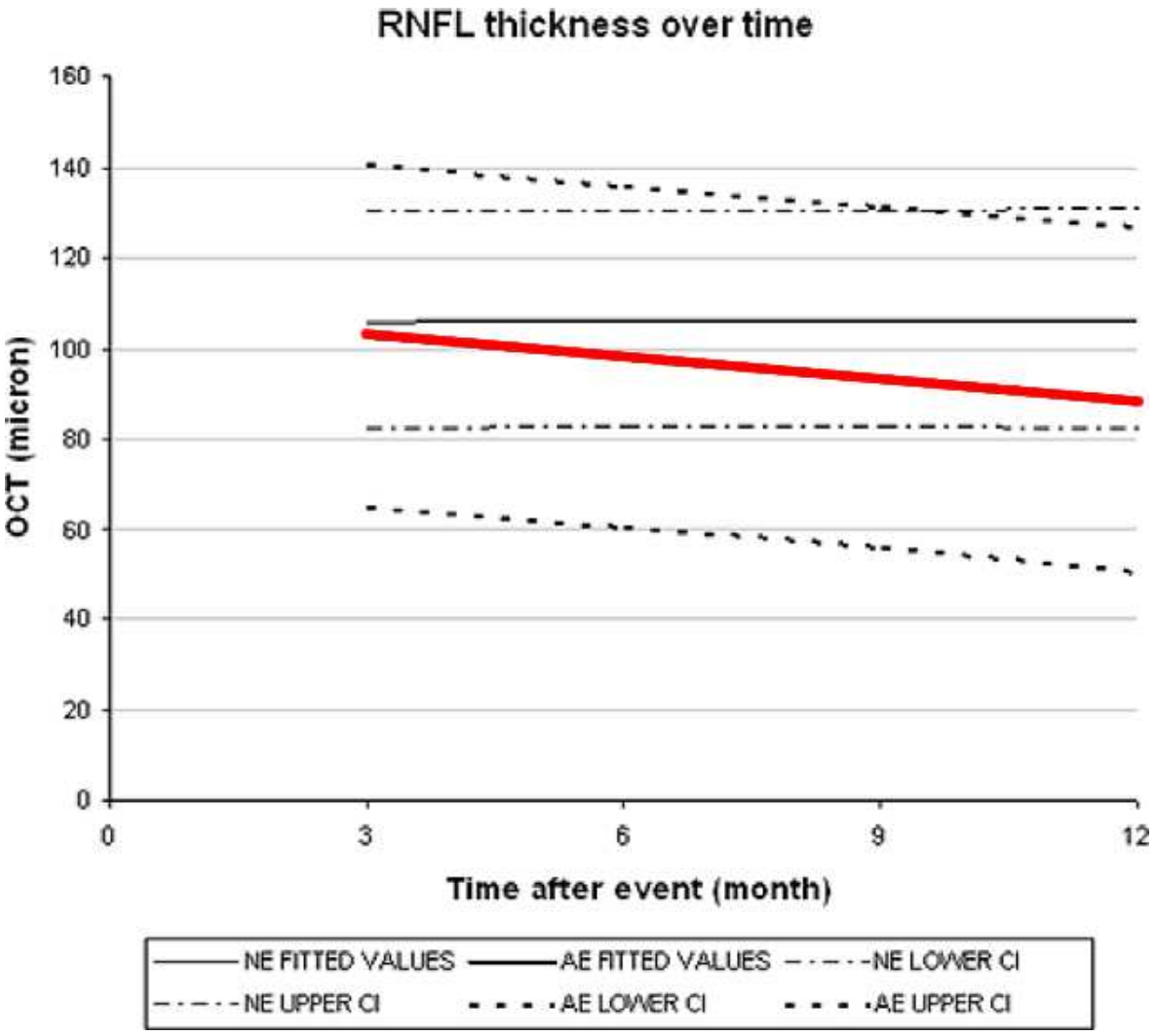


Figure 7: *Longitudinal profile of OCT measurements in affected (ON) and non–affected eyes from patients with MS. Image modified from reference*[7] *with permission.*