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DOI:
10.1016/S1474-4422(17)30278-8

Document Version
Accepted author manuscript

Citation for published version (APA):
ERN-EYE IMSVISUAL (2017). Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. *The Lancet Neurology*, *16*(10), 797-812. https://doi.org/10.1016/S1474-4422(17)30278-8

Published in:
The Lancet Neurology

Retinal layer segmentation in multiple sclerosis: a systematic review and meta–analysis

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July 3, 2017

Abstract

Background Structural retinal imaging biomarkers are important for early recognition and monitoring of inflammation and neurodegeneration in multiple sclerosis (MS). With introduction of spectral domain optical coherence tomography (SD–OCT) supervised automated segmentation of individual retinal layers became possible. We aimed to investigate which retinal layers show atrophy associated with neurodegeneration in MS when measured using SD-OCT.

Methods In this systematic review and meta–analysis we searched for articles in Pubmed, Web of Science and Google Scholar between

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November 22nd 1991 and April 19th, 2016 for OCT, MS, demyelination and optic neuritis. Data were taken from cross–sectional cohorts as well as from one follow–up point, at least 3 months after onset, from longitudinal studies. Data on eyes were classified into healthy controls, MS with associated optic neuritis (MSON) and MS without optic neuritis (MSNON). Individual layer segmentation performance was rated by random effects meta–analysis for MSNON versus control eyes, MSON versus control eyes and MSNON versus MSON eyes.

Findings Of 25497 record identified, 110 articles were eligible and 40 reported data (of in total 5776 eyes of patients with MS and 1697 eyes of healthy controls), which met published OCT quality control criteria and were suitable for meta–analyses.

The meta–analyses of SD-OCT data suggests thinning of the peripapillary retinal nerve fibre layer (RNFL) in MS associated with optic neuritis (MSON [N=1030 eyes], mean difference with healthy control eyes [N=1333] -20.10 μm (95%CI -22.76 to -17.44, p$<$0.00001) and -7.41 μm (95%CI -8.98 to -5.83, p$<$0.00001) in MSNON [N=2463 eyes] if compared to controls [N=1279 eyes]. Longitudinally, the peripapillary RNFL atrophy rate ranged from -0.36 to -1.49 μm/year.

Retinal layer segmentation of the macula revealed RNFL thinning of -6.18 μm (95%CI -4.28 to -8.07, p$<$0.00001) in MSON and -2.15 μm (95%CI -3.15 to -1.15, p$<$0.0001) and MSNON compared to controls. Atrophy of the macular ganglion cell layer and inner plexiform

layer (GCIPL) was -16.42 μm (95%CI -13.60 to -19.23, p$<$0.00001) for MSON and -6.31 μm (95%CI -4.87 to -7.75, p$<$0.00001) for MSNON compared to controls. A small degree of INL thickening has been related to inflammatory disease activity in MSON (0.77 μm, 95%CI 0.25 to 1.28, p=0.003). Atrophy was not observed for any of the retinal layers beyond the inner nuclear layer (INL). There was no statistical difference in outer nuclear layer and outer plexiform (ONPL) thickness between either MSNON or MSON with controls. There was a small degree of ONPL thickening comparing MSON and MSNON eyes (1.21, 95%CI 0.24 to 2.19, p=0.01). Relevant sources of bias were excluded by Funnel plots.

Interpretation The most robust primary outcomes for neurodegeneration in MSNON and MSON were the peripapillary RNFL and macular GCIPL. Inflammatory disease activity may be captured by the INL. Because of the consistency, robustness and large effect size, we recommend the inclusion of the pRNFL and macular GCIPL in clinical practice for diagnosis, monitoring of progression and research.

Funding This study was not funded.

Keywords spectral–domain optical coherence tomography, retinal layer segmentation, optic neuritis, multiple sclerosis.

Introduction

Optical coherence tomography (OCT) is a high resolution imaging technique suitable for sophisticated post–processing[1,2]. Since our last meta–analysis[3] time domain (TD-) retinal OCT has been overtaken by spectral domain (SD-) OCT in clinical practise[4]. The much higher resolution of SD–OCT now permits for individual retinal layer thickness analyses[5–8]. This has added an additional ten retinal layers to the well investigated retinal nerve fibre layer (RNFL)[9]. Five of these layers have been analysed systematically in patients with MS. These are the ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL) and the outer nuclear layer (ONL). The present meta–analysis investigated what additional information can be derived by retinal layer segmentation in multiple sclerosis (MS) and MS associated optic neuritis (MSON).

Methods

Search strategy and selection criteria This study was a systematic review and meta-analysis of individual retinal layer thickness in MS. The review of the Dutch, English, French, German, Italian and Spanish literature was conducted by AP and L Balk on all studies (cross-sectional and longitudinal) using OCT in MS patients since discovery of the method November 22nd 1991 by Huang[1] and April 19th, 2016, including manuscripts published ahead of print. We searched PubMed, Web of Science and Google Scholar using a hierarchical search strategy. First we searched for OCT including the brand and device names of the major commercial suppliers. Next we refined this search by using the search terms: multiple sclerosis, demyelination, optic neuritis and the abbreviations MS, CIS, RRMS, SPMS, PPMS, ON and MSON. Articles were reviewed for use of SD-OCT. A diagnosis of MS and multiple sclerosis associated optic neu-

ritis (MSON) were defined as per consensus[10–13]. Articles were excluded if they did not contain patients with MS; included fewer than ten subjects; did not use SD-OCT; did not separate MSON from non MSON eyes; were communications in response to an article; were duplication of data already published from the same cohort; reported data in a format other than mean (SD) or mean (SEM; study authors were contacted and asked to supply this information). Articles which did not contain a group of control patients were excluded if they did not contain data permitting to compare MSON eyes with the fellow eye. Conflicts on inclusion of data were resolved by consensus (AP, LB).

Data analysis Data were independently extracted by two authors (AP, LB). Extracted data consisted of mean thickness and standard deviations of individual retinal layers (RNFL, ganglion cell layer [GCL], inner plexiform layer [IPL], a combination of GCL and IPL, inner nuclear layer [INL],

outer nuclear layer [ONL], outer plexiform layer [OPL] or a combination of ONL and OPL) of eyes of patients with MS (with and without history of MSON) and healthy control subjects. Because of the anatomical structure of the retina (see supplementary text) data were reported for the RNFL at the optic disc and macula, but for all other layers only at the macula. To solve conflicts of inclusion for the meta-analysis authors were approached per mail regarding inclusion criteria, timing of events and presentation of data (mean, standard deviation, numbers). Key papers excluded from the meta-anlysis because of unsuitable or duplicate data were still referenced in the systematic review. No grey literature sources were assessed and only summary estimates were used. The primary outcome measure was thickness (in μm) of pRNFL, GLC, IPL, GCIPL, INL and ONPL, in eyes with MSON and without MSON (MSNON) and in healthy control eyes. Results were reported as mean difference (in μm, with 95%CI) between the

MSON, MSNON and control groups for all retinal layers. Variability within studies (sampling error) and between studies was assessed with the I^2 estimate of heterogeneity. Retinal OCT data for different SD-OCT devices were analysed together. Data were taken from cross-sectional studies and from one single time point from longitudinal studies. The baseline OCT values were taken from longitudinal studies which did not include acute optic neuritis. Because the time lag between onset of MSON and ensuing retinal layer atrophy, follow-up data was taken from these studies from one single time point which had to be at least three months after onset of MSON[14]. No subgroup analyses according to disease course were performed which would have led to loss of power and because the new, relevant, classification into 'active' and 'stable' disease by Lublin et al.[15] has not yet been applied systematically. Data on individual retinal layer thickness were entered for each group of eyes as mean thickness in μm, with standard deviation in

order to compare the predefined groups (MSON eyes, MSNON eyes and eyes of healthy control subjects). Categorization of the groups was done on eye level, instead of patient level. For OCT research specific quality assessment we used the Advised Protocol for OCT Study Terminology and Elements (APOSTEL) recommendations[9]. The APOSTEL recommendations were based on validated OCT quality control criteria[16,17]. P values of 0.05 or less were considered significant. Publication bias was assessed with funnel plots. To account for publication bias, the results of the funnel plots were reported as Supplementary Data.

The present data analyses on SD-OCT was in design identical to our previous meta-analysis on time domain OCT[3]. This will enable better comparison of the TD-OCT meta–analysis with present SD-OCT meta–analysis data. We used Cochrane Collaboration's Review Manager software package (Review Manager (RevMan) [Computer program]. Version

5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.) following the guidance of the Diagnostic Test Accuracy (DTA) Working Group. Retinal layer thickness data were entered as a continuous variable. We used inverse variance, with random effects (DerSimonian and Laird random-effects). The choice of random effects instead of a fixed effects analysis was made because of the level of heterogeneity between studies reported previously[3].

Another reason for random effects analyses is related to the different OCT devices and segmentation algorithms used in the studies. On an individual patient level they are not directly comparable[18]. On a group level the degree of atrophy can still be extracted, but study heterogeneity will increase. We have therefore colour coded and labelled data derived by different OCT manufactures.

The results of the meta–analysis were summarised for related retinal

layers. For each layer subgroup analyses were presented first for the comparison of patients with MS and MSON and control subjects. Next, the comparison of patients with MS who did not experience an episode of MSON with control subjects. Finally, the comparison of eyes with and without MSON in patients with MS. The reader is advised that interpretation of the quantitative statistical data cannot be extrapolated to individual patients for small retinal layer thickness changes because the axial resolution of current SD-OCT devices used in clinical routine is about 3-7 μm.

Role of the funding source There was no funding source for this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 summarises the selection process of the 110 articles identified which did use SD-OCT in MS (for full list of references please refer to supplementary text). Of these articles 40 presented data suitable (five after contacting the authors) for meta–analysis of retinal layer thickness between groups[6,14,19–56]. The study characteristics are presented in Table 1.

The following paragraphs summarise the meta–analysis for SD-OCT of the **pRNFL and also the macular RNFL (mRNFL)**. All OCT data refer to number of eyes.

The degree of atrophy of the pRNFL following MSON was highly significant and averaged at -20.10 μm (95%CI -17.44 to -22.76, p$<$0.00001, Figure 2 A). Findings were significant for each of the 19 studies included[6,20–22,24,25,29,30,33,35,36,39,42] Data were based on 2363 eyes.

Consistent with this finding, there was also significant atrophy of the mRNFL averaging at -6.18 μm (95%CI -4.28 to -8.07, p$<$0.00001, Figure 2 A). Data were based on 506 eyes from seven studies[6,20,26,32,34,48,54].

In MSNON atrophy of the pRNFL averaged at -7.41 μm (95%CI -5.83 to -8.98, p$<$0.00001) compared to controls (Figure 2 B). Data were based on 3742 eyes from 20 studies[6,20–22,24,25,27–30,33,35,37,39,42,44,50,54,55,57].

Six studies reported data on the mRNFL[6,20,26,32,54,57]. The atrophy averaged at -2.15 μm (95%CI -1.15 to -3.15, p$<$0.0001, Figure 2 B). Data were based on 1030 eyes.

The atrophy of the pRNFL in eyes from MSNON patients compared to those with MSON showed a mean difference of -11.25 μm (95%CI -9.50 to -13.00, p$<$0.00001, Figure 2 C). Data were from 3972 eyes from 22 studies[14,19–21,23–25,29–31,33,35,36,39,40,42,46,47,49,50,54,56].

Four studies reported data on the mRNFL[20,26,31,32]. The atrophy aver-

aged at -3.68 μm (95%CI -1.27 to -6.10, p=0.003, Figure 2 C). Data were based on 615 eyes.

Effect sizes for the pRNFL and mRNFL were summarised in 3. Funnel blots did not reveal a publication bias (see Supplementary data).

The meta-analysis for the GCL and IPL shows that in patients with MSON there was significant atrophy of the GCL and IPL averaging at 16.42 μm (95%CI -13.60 to -19.23, p$<$0.00001, Figure 4A). Data were calculated from 1673 eyes from 17 studies[6,20,22,24,26,30,32–35,43,48,50,52–55]. Most studies reported the combined GCIPL thickness[6,22,24,26,30,32–35,43,50,52–55] and only two (Balk et al, Schneider et al) published the GCL thickness[20,48].

In patients with MSNON the mean difference compared with the controls in atrophy of the GCL and IPL was -6.31 μm (95%CI -4.87 to -7.75, p$<$0.00001, Figure 4B). Data were from 2367 eyes from 18 studies[6,20,22,24,26,30,32,33,35,37,44,50–55] Seventeen studies measured the GCIPL[6,22,24,26,30,32,33,35,37,44,50–55,57] and

only one the GCL[20].

Atrophy of the GCP and IPL was more marked in MSON eyes compared to MSNON, mean difference -8.81 (95%CI -7.12 to -10.50, p$<$0.00001, Figure 4C). Data were calculated from 2319 eyes from 21 studies[6,14,19,20,24,26,30–33,35,40,41,46,49,5

Eighteen studies measured the GCIPL (also reported as GCIP[9])[6,19,24,26,30,32,33,35,40,41,46,49,50,52–

and three Balk et al, Costello et al and Hadhoum et al) the GCL alone[14,20,31].

Effect sizes for the GCIPL are summarised in 3. Funnel blots did not reveal a publication bias (see Supplementary data).

The meta-analysis for the INL does not provide evidence for atrophy of the INL, but thickness increased modestly following MSON. The mean difference between the MSON and control groups showed thickening of the INL after MSON 0.77 μm (95%CI 0.25 to 1.28, p=0.003, Figure 5A). Data were from 885 eyes published in eight studies[6,20,26,32,34,48,52,54].

The INL remained essentially unchanged in MSNON eyes (Figure 5B,

p=0.18). Compared to control subjects the 95%CI (-0.17 to 0.89 μm) of the INL in the patient cohort did cross the zero line in the Forest plot. Eight studies contributed to this analysis of data from 1182 eyes[6,20,26,32,37,38,52,54].

A thickened INL was observed in MSON eyes compared to MSNON eyes. The average thickening was small (mean difference 0.65 μm, 95%CI 0.23 to 1.08, p=0.003, Figure 5C). Data were available from 1075 eyes from seven studies[19,20,26,31,32,46,52,54]. Effect sizes for the INL are summarised in 3. Funnel blots did not reveal a publication bias (see Supplementary data).

The meta-analysis for the ONPL shows that following MSON the average ONPL was marginally thickened in eyes with MSON and control eyes (Figure 5D, p=0.23). Data were based on 645 eyes from four studies[20,48,53,54].

In MSNON eyes, the ONPL was minimally thinner compared to con-

trol eyes (Figure 5E, p=0.14). Data were based on 954 eyes from five studies[20,37,53,54,57].

The ONL appeared to be mildly thickened in MSON eyes compared to MSNON eyes (Figure 5F). The average increase of ONPL thickness was 1.21 μm (95%CI 0.24 to 2.19, p=0.01). Data were based on 1071 eyes from six studies[19,20,31,46,53,54].

Effect sizes for the ONPL are summarised in 3. Funnel blots did not reveal a publication bias (see Supplementary data).

Discussion

In this meta-analysis, the data suggest that MSNON and MSON eyes are associated with atrophy of the retinal ganglion cells (GCL and GCIPL) and their axons (pRNFL and mRNFL). Importantly, the effect sizes shown for the present SD-OCT based meta–analysis of the pRNFL very closely

matched the effect sizes from an earlier TD–OCT based meta–analysis[3]. This emphasises the robustness and accuracy of the pRNFL as a measure for neurodegeneration in MS and MSON spanning two generations of OCT device technology. Although the meta-analyses in this review is the first providing a valuable summary of available data on individual retinal layer thickness patients with MS, it should be noted that meta-analyses are based on solely observational studies, which is not without limitations[58,59].

It was not possible to accurately resolve individual layers of the macular with TD-OCT[3,60]. This study shows that using SD-OCT, the mRNFL, GCL/GCIPL, INL and ONL/ONPL can now be reliably quantified with data suitable for meta-analyses. These new quantitative layer segmentation data extend on earlier pRNFL data by demonstrating that inner retinal layer atrophy is severe after MSON, but still considerable and significant in patients with MS who never experienced MSON compared to controls. Inter-

estingly, on a group level different segmentation algorithms deliver comparable data. This is consistent with an earlier head-to-head comparison of OCT devices in patients with MS[61].

In human vision the first-, second-, and third-order neurons and their axons are hard–wired into the human brain and transmit analogue and digital signals[62]. This hard-wired single pathway enables the retinotopic map of the human visual cortex[63]. Anatomically the GCL, mRNFL and pRNFL represent the first unit within this hard–wired pathway. Axonotmesis at any point in this hard–wired pathway is understood to give rise to retrograde trans–synaptic axonal degeneration which will inexorably cause inner retinal layer atrophy[64]. Trans–synaptic degeneration affects the dorsal LGN, but stops at the INL (detailed discussion in supplementary text).

Six studies reported longitudinal data[41,46,65–68]. Talman using TD-OCT reported an annual atrophy rate of -1.4 μm/year in 381 patients with MS,

which was closely matched by the SD-OCT data (-1.49 μm/year, n=96) from Narayanan[41,67]. Later studies found the annual pRNFL atrophy rate to be about 66% less marked averaging at -0.36 μm/year (n=107)[46], -0.5 μm/year (n=45)[66] and -0.53 μm/year (n=168)[65]. One study (n=58) found no significant changes over a two year period[68].

The differences in annual atrophy rates may partly be explained by differences of the demographic data. The highest annual atrophy rate was found in patients without MSON and a shorter disease duration[65]. A plateau effect was observed in patients with a longer disease duration ($>$ 20 years)[65]. Likewise presence of MSON resulted in a higher annual atrophy rate in eyes with MSON (-0.91 μm/year) compared to eyes without MSON (-0.53 μm/year)[66]. But this was opposite to what Narayanan had reported with a lower annual atrophy rate in eyes with MSON (-1.27 μm/year) compared to eyes without MSON (-1.49 μm/year)[41].

A conservative estimate from these data is that or a 1 μm loss every 1-2 years with an OCT device accuracy threshold of about 2–3 μm, a trial of 2–3 years with active patients would be powered for probing a neuroprotection against pRNFL atrophy. During the early disease course a shorter trial duration may be sufficient[65]. Good mechanisms to be target by trials with the pRNFL as an outcome measure are inflammatory disease activity in MS[57,69,70] as well as non–demyelinating mechanisms such as for example mitochondrial dysfunction[71,72]. Finally, SD–OCT segmentation has been used as an outcome marker in a recent remyelination trial[73].

A limitation of pRNFL data not directly evident from the meta-analyses is caused by disc oedema at presentation[74]. The elegant longitudinal study buy Kupersmith *et al.* clearly demonstrated superiority of the GCIPL layer compared to the pRNFL for detection of early atrophy following MSON. This notwithstanding, the averaged atrophy of the pRNFL following MSON

was 20.38 μm (95% CI 17.91 to 22.86) for TD-OCT data and 20.10 μm (95%CI 17.44 to 22.76) for SD-OCT data. In MS without MSON averaged atrophy of the pRNFL was 7.08 μm (95%CI 5.52 to 8.65) for TD-OCT data and 7.41 μm (95%CI 5.83 to 8.98) for SD-OCT data. Finally, comparison of MSON and non MSON eyes showed averaged pRNFL atrophy of 13.84 μm (95%CI 11.72 to 15.97) for TD-OCT and 11.25 μm (95%CI 9.50 to 13.00) for SD-OCT data. The almost identical findings for TD- and SD-OCT data highlight that the pRNFL is well suited for use as a outcome measure in clinical trials. There is new evidence that achievement of no evident disease activity (NEDA) with disease modifying treatment is related with less marked atrophy of the pRNFL longitudinally[57].

Consistent with the data from the RNFL there is a grading of atrophy of the GCL and IPL, which is most severe in MSON, followed by eyes of MS patients without MSON and control subjects. Because of the poor image

contrast between the GCL and the IPL most studies reported a combined measurement of these two layers.

An important advantage of the GCIPL compared to the pRNFL is that atrophy becomes detectable much earlier[74,75]. Already one month after MSON thinning of the GCIPL becomes quantifiable compared to baseline values, whilst for the pRNFL the advice is to wait at least three months. Reassuringly, this finding is corroborated by a different meta-analysis which also included neuromyelitis optica and which was published whilst present manuscript was under review[76].

In addition, the retinal ganglion cell layer complex is the thickest in the macula. Therefore, this layer has a large dynamic range and it appears that because most of the MS related damage includes the macula, the GCIPL is a good biomarker for neurodegeneration in the visual pathway in MS. In cases with severe atrophy of the pRNFL following MSON a flooring

effect may prevent observation of further atrophy around the optic disc, but the GCIPL will still be useful.

There was no atrophy observed for the INL. In contrast thickening of this layer was significant more substantial following MSON compared to MS without MSON. A relationship between INL thickening as a sign of inflammatory activity has also been reported[57,69]. Importantly, longitudinal data demonstrated that INL microcysts were mostly ($>$80%) transient (dynamic)[77,78]. A transient increase of INL thickness may be a sign of retinal inflammation or failure of maintaining the retinal fluid homeostasis[79], consistent with the original description of MMO in MS[80]. There are now several independent lines of evidence suggesting existence of a retinal glymphatic system with a prominent role for the INL[79,81,82]. Segmentation of the INL will be relevant for studies on the effect and treatment of inflammatory disease activity in MS. Future developments in this field will include

OCT angiography[79,81,82].

Taken together the meta-analyses suggest that there are no significant changes of the ONPL in either MSON eyes or eyes of patients without MSON compared to controls. There was however, a small degree of ONPL thickening in MSON eyes compared to non MSON eyes which is caused by a very mild degree of thickening in the former and thinning in the latter. This is a consistent observation from the literature on MSON and other forms of acute optic neuritis (neuromyelitis optica, anti-MOG), typically during the acute phase[11]. This is now confirmed by new prospective evidence for ONL thickening in anti-MOG ON[83]. An increased MRI DIR signal has also been associated with ONPL thickening[31]. It has been hypothesised that ONPL thickening might be caused by traction, inflammation and oedema[19,84]. The need for rigorous OCT quality control[16,85] here cannot be overemphasised because the outer retinal layers are par-

ticularly vulnerable to an easily overlooked artefact caused by placement of the measurement beam[86,87]. We anticipate that recognition of outer retinal layer volume changes will become more relevant for the differential diagnosis of MSON from other causes of optic neuritis[63,83,88].

A limitation to current date studies is the difficulty obtaining retinal tissue for detailed histological investigations[89]. A potential advantage is the availability of electrophysiological techniques[49,90]. Clinically it is well recognised that conduction block can be caused by any structural or inflammatory lesion affecting the optic pathways. Typically these lesions are nowadays revealed by MRI brain imaging. Therefore the application of MRI based diagnostic criteria for MS[10] to the cohorts subject to present meta–analysis render this type of study contamination most unlikely. The potential to combine now OCT with pattern and multifocal electroretinogram, visual evoked potentials and MRI provides a powerful tool for the com-

bined assessment of structure and function in cohorts of homogeneous pathology[4,11].

Will all segmented retinal layers be needed for clinical practise and trials? Probably not. For practical reasons a reasonable minimalistic approach will suffice with the pRNFL if taken at an appropriately chosen time point. At least three months after MSON. For clinical trials and longitudinal studies on neurodegeneration one would recommend as a minimum the pRNFL and mGCIPL[91]. Those studies focusing on inflammation as well are advised to consider the INL as well. The mRNFL is, given effect size and error bar distribution (Figure 3) the least sensitive measure. The mRNFL may however be regarded as a “backup” in those patients were imaging of the optic disc proofs technically too difficult.

In summary, SD–OCT based layer segmentation has unravelled the progression of neurodegeneration on a structural level. Atrophy affects

axons and neurons of the hard–wired visual pathway, namely the pRNFL, mRNFL and GCIPL. A new physiological barrier to retrograde trans–synaptic axonal degeneration has been confirmed, namely the INL. On basis of this, transient INL volume changes may be indicative of inflammatory disease activity and response to disease modifying treatment in MS.

Contributors

AP: conceived the idea for this review, performed the literature search, systematic review and meta-analysis. Wrote the first draft of the manuscript. LJ Balk: contributed to the literature research, contributed to the statistical analyses and revised the manuscript. LJ Balcer : revised the manuscript. OO: revised the manuscript. PAC: revised the manuscript. FC: revised the manuscript. TCF: revised the manuscript. EMF: revised the manuscript. EHML: revised the manuscript. AG: revised the manuscript. RK: revised the manuscript. SS: revised the manuscript. PVE: revised the manuscript. PVI: revised the manuscript. FP: revised the manuscript.

Declaration of interest

AG: reports grants and other from Inception Biosciences, other from Medimmune, grants from National MS Society, grants from NIH, other from

Mylan/Sandoz/Dr. Reddy/ Amneal/Momenta/Synthon, other from JAMA Neurology, outside the submitted work.

AP reports that the VUmc MS Centre Amsterdam participated in the OCTIMS trial and the centre has received research support for OCT projects from the Dutch MS Society. The research of AP was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

PV (Villoslada): has received honorarium from Heidelberg Engineering in 2014, has received unrestricted research grants from Novartis (including OCTIMS study), Biogen, Genzyme and Roche and has participated in advisory boards for Novartis, Roche, Genzyme and Biogen in 2016. PV hold stocks in the folloeing spin-offs Bionure Inc, Spire Bioventures, Mintlabs

and Health Engineering.

TF: reports personal fees from Acorda, personal fees from Novartis, personal fees from Genzyme

EF: has received speaker fees from Novartis, Acorda, Genzyme, and TEVA.

SS: reports grants from University of Zurich, Clinical Research Priority Program (CRPP), grants from Swiss Multiple Sclerosis Society, during the conduct of the study; personal fees from Bayer Healthcare, personal fees from Biogen, personal fees from Merck, grants and personal fees from Novartis, grants and personal fees from Sanofi-Genzyme, personal fees from TEVA, personal fees from Roche, outside the submitted work; .

PV (Vermersch): Honoraria and consulting fees from Biogen, Sanofi-Genzyme, Bayer, Novartis, Teva, Merck-Serono, Roche and Almirall. Research supports from Biogen, Bayer, Novartis, Sanofi-Genzyme and Merck-

Serono.

EHML (Martinez-Lapiscina) receives funding from the Instituto de Salud Carlos III, Spain and Fondo Europeo de Desarrollo Regional (FEDER) (JR16/00006), Grant for MS Innovation and Marató TV3 Charitable Foundation. She is a researcher in the OCTIMS study, an observational study (that involves no specific drugs) to validate SD-OCT as a biomarker for multiple sclerosis, sponsored by Novartis. EHMLP has received speaking honoraria from Biogen and Genzyme and travel reimbursement from Genzyme, Roche for international and national meetings over the last 3 years. She has participated in a scientific board from Genzyme in 2015. She is a member of the working committee of International Multiple Sclerosis Visual System (IMSVISUAL) Consortium.

OO: has received grants from Novartis, grants and personal fees from Biogen, Genzyme–Sanofi, Merck–Serono, Novartis and Teva Pharmaceu-

ticals Industries.

RK reports receipt of grants from the US Department of Defense (DOD) and Veterans Affairs Office of Research and Development (VA-ORD), and the Chronic Effects of Neurotrauma Consortium (CENC); DOD/VA; Center for the Prevention and Treatment of Visual Loss, C9251-C, Veterans Administration Rehabilitation Research Development (RRD), VA-ORD; I01 RX000889-01A1 Veterans Administration Rehabilitation Research and Development (RRD), VA-ORD; 1IO1 RX002101 Veterans Administration, VA-ORD (RRD); 1R01EY023279-01, National Eye Institute (NEI) W81XWH-16-1-0071 Department of Defense, DOD CDMRP USAMRAA; and W81XWH-16-1-0211 Department of Defense, DOD CDMRP USAMRA. The University of Iowa Neuro-ophthalmology Division also participated in the Novartis sponsored OCTiMS Study as one of the research sites and RK served on the OCTiMS Steering Committee.

PAC (Calabresi): has received grants from Biogen-IDEC, Teva, Novartis, Annexon, and Medimmune. He has received consulting fees from; Biogen-IDEC and Vertex.

FP has received research support and personal compensation for activities with Alexion, Chugai, Biogen, Bayer, MerckSerono, Teva, Genzyme, Novartis, MedImmune. FP is sitting on the steering committee of the MedImmune N-Momentum study and receives honoraria. FP receives funding from Deutsche Forschungsgemeinschaft, Bundesministerium für Bildung und Forschung, and Guthy Jackson Charitable Foundation.

FC: Has received consulting fees from Clene, Merck- Serono and PRIME. She is participating as a principal investigator in the Novartis funded OCTiMS study.

LB (Balcer): reports personal fees from Biogen.

LJB (Balk): The VUMC MS Centre Amsterdam received financial re-

search support for OCT projects from TEVA; the VUmc MS Centre Amsterdam participated in the OCTIMS trial.

FP, AP, RK, AG, PV (Villoslada), PV (Vermersch), 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.

Acknowledgement

We would like to thanks the following authors for providing details on their published data needed for the meta–analysis: Dr Benjamin Knier, Professor Thomas Korn, Dr Raed Behbehani, Dr Elena Garcia–Martin.

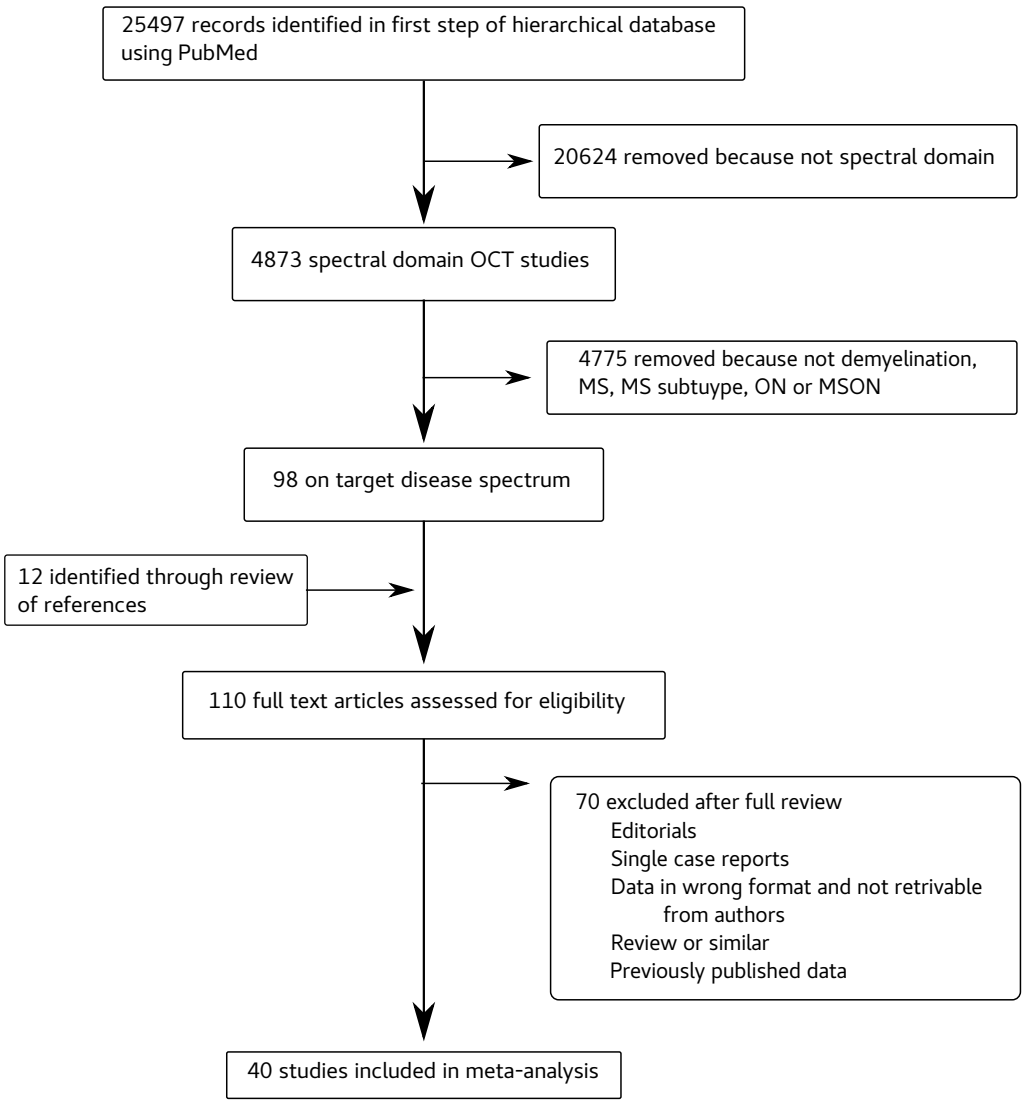


Figure 1: *Study selection.*

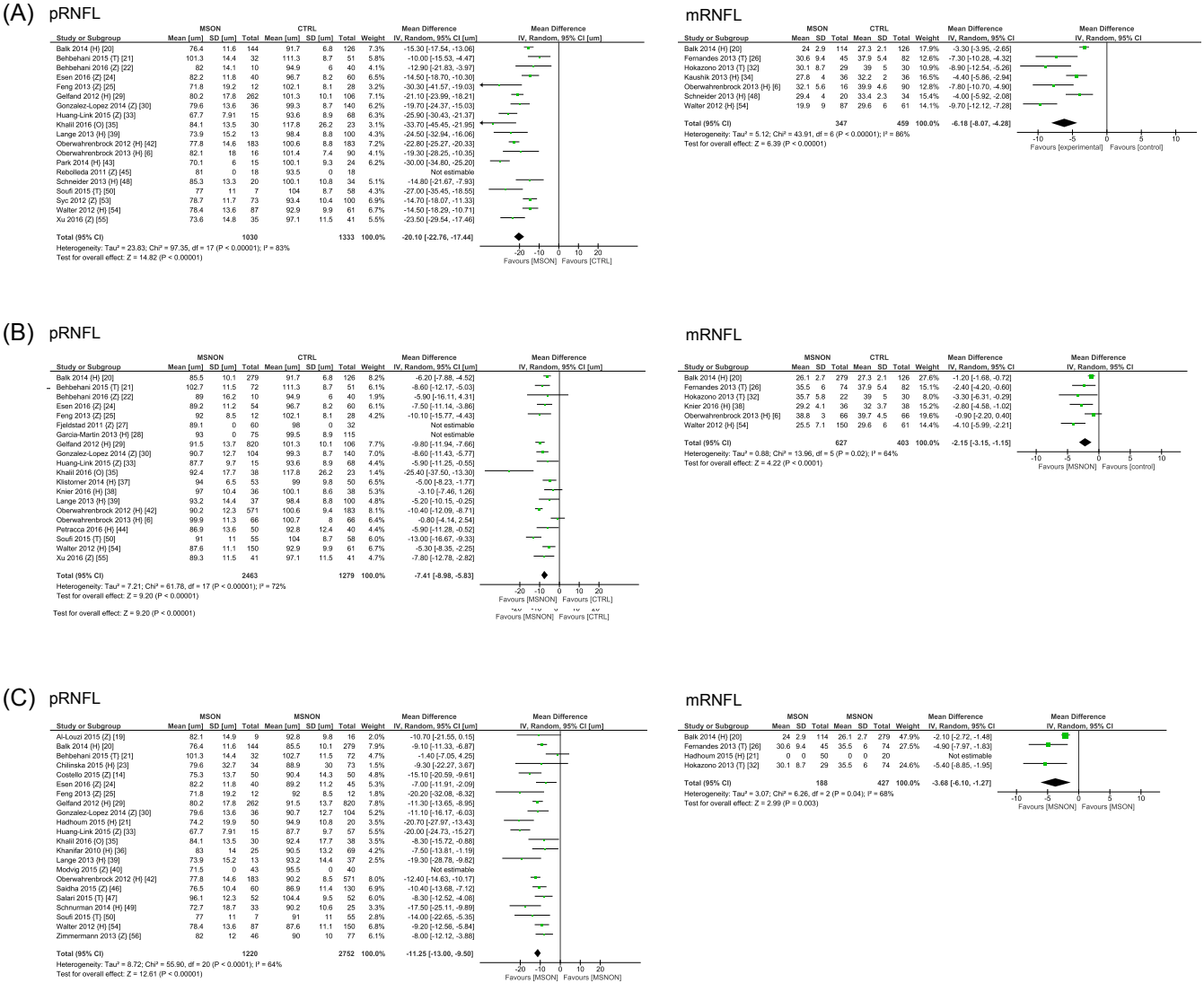


Figure 2: *Meta–analysis of peripapillary (pRNFL) and macular RNFL (mRNFL) SD–OCT data in MS patients who **(A)** did suffer from MSON, **(B)** never suffered from MSON and **(C)** comparison of MSON and MSNON eyes. The overall averaged RNFL (mean±SD) and number of eyes included is shown for patients and normal subjects. The micron difference in RNFL thicknesses is shown to the right with the length of de horizontal bar indicating the 95% confidence interval. The four SD–OCT devices used were indicated as H (Spectralis, Heidelberg Engineering), Z (Cirrus, Zeiss), O (RTVue, Optovue) and T (3D OCT-2000, Topcon). In the graph "favours" indicates greater atrophy in the group named in brackets. For corresponding Funnel plots see Supplementary data.*

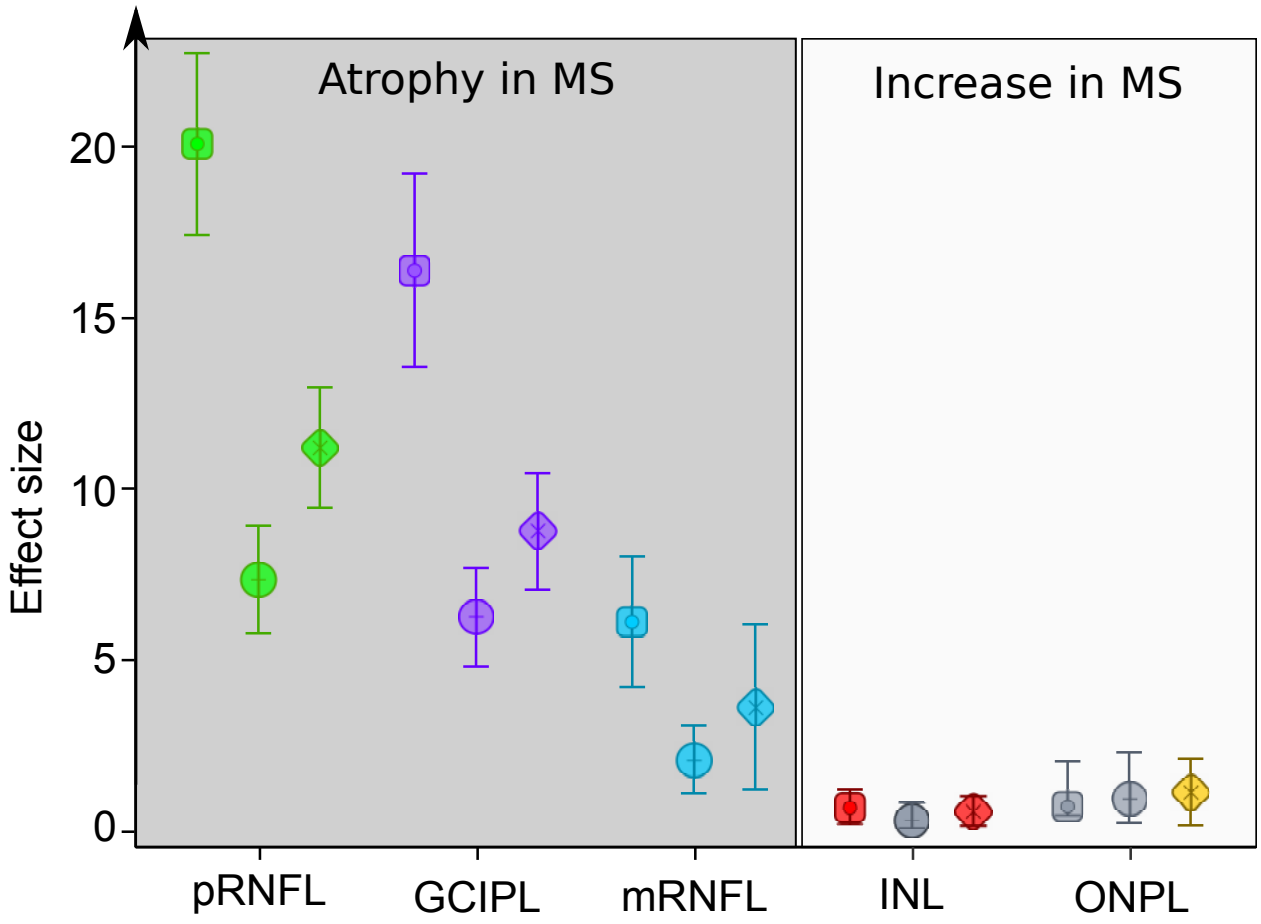
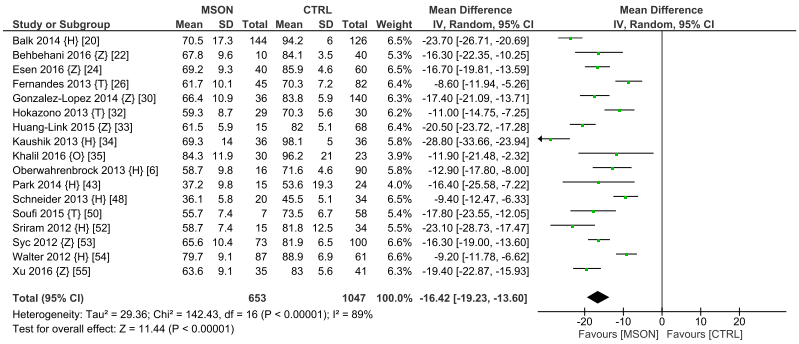


Figure 3: *SD-OCT layer segmentation performance rating in patients with MSON versus controls (squares), MSNON versus controls (circles) and MSNON versus MSON (diamonds). Head-to-head OCT layer segmentation performance based on average effect sizes. Segmented layers shown in green (pRNFL), purple (GCIPL) and blue (mRNFL) are significant with good effect sizes. The effect size was small for the INL and only in presence of MSON (red). The effect size was minimal for the ONPL comparing MSON with MSNON (brown). Effect sizes shown in grey were non-significant. The patient to control effect size were all shown as positives to allow for a clear comparison between individual layers. The bars indicate the 95% CI. The grey shaded areas indicate layers with atrophy (thinning) or increase (thickening).*

(A)



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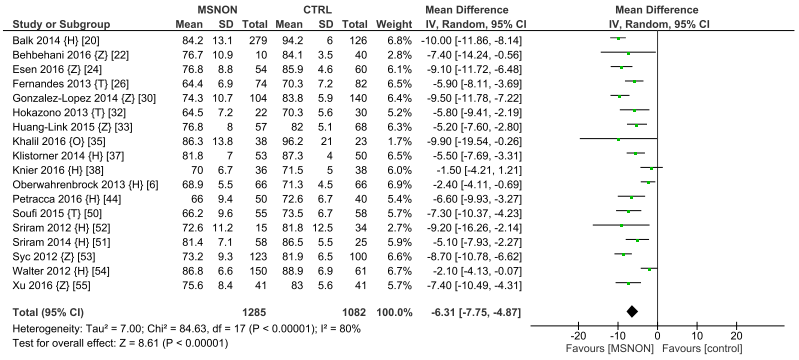


(C)

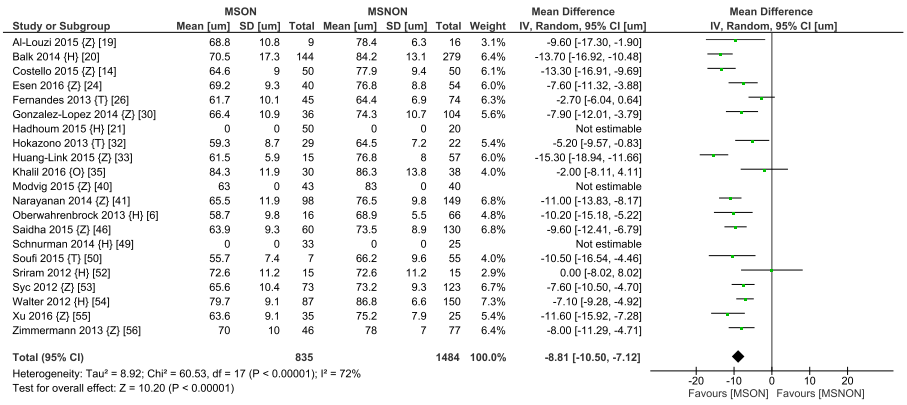
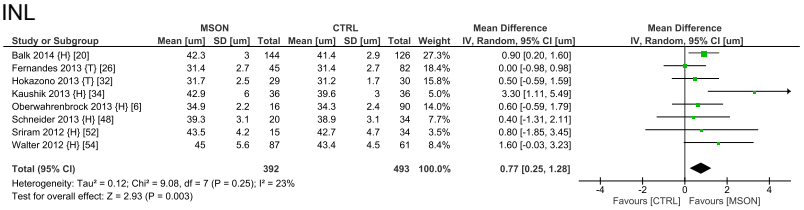


Figure 4: *Meta–analysis of macular GCL and IPL SD–OCT data in MS patients who **(A)** did suffer from MSON, **(B)** never suffered from MSON and **(C)** comparison of MSON and MSNON eyes. For corresponding Funnel plots see Supplementary data.*

(A) INL



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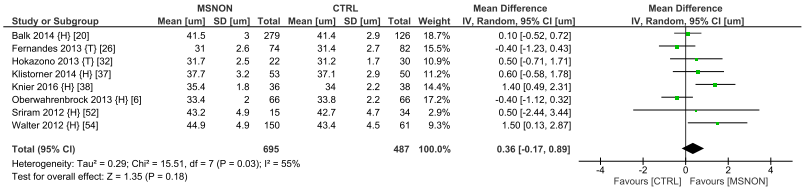
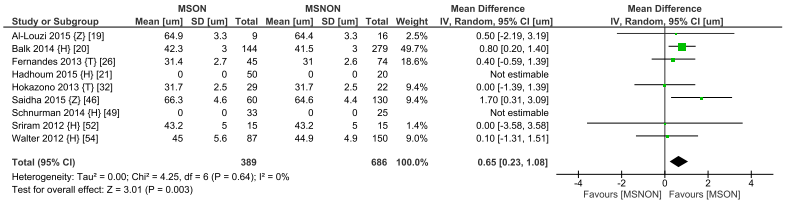
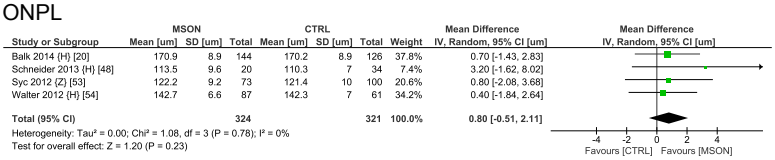


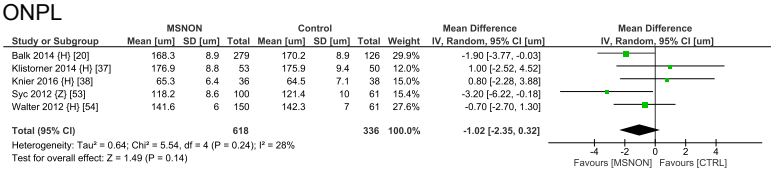
(C) INL



(D) ONPL



(E) ONPL



(F) ONPL

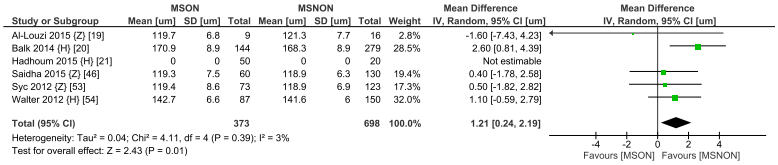


Figure 5: *Meta–analysis of macular INL SD–OCT data in MS patients who **(A)** did suffer from MSON, **(B)** never suffered from MSON and **(C)** comparison of MSON and MSNON eyes. Meta–analysis of macular OPL and ONL (ONPL) SD–OCT data in MS patients who **(D)** did suffer from MSON, **(E)** never suffered from MSON and **(F)** comparison of MSON and MSNON eyes. For corresponding Funnel plots see Supplementary data.*

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