

Supplementary materials

Supplementary Table 1. Diagnostic performance of the inter-eye differences of the mGCIPL at three time points. This represents the usefulness as a cross sectional measure for DIS.

mGCIPL	Group	Time	AUC	95% CI	P-value ^a	Sensitivity ^b	Specificity ^b
IEPD	HC - MS	BL	0.81	0.70 – 0.92	<u>reference</u>	58.2%	95.5%
IEAD	HC - MS	BL	0.80	0.68 – 0.92	0.281	54.6%	95.5%
IEPD	HC - MS	Y2	0.78	0.67 – 0.92	0.377	59.4%	94.7%
IEAD	HC - MS	Y2	0.78	0.66 – 0.90	0.354	56.6%	93.7%
IEPD	HC - MS	Y4	0.80	0.68 – 0.91	0.526	56.7%	88.9%
IEAD	HC - MS	Y4	0.77	0.65 – 0.89	0.115	53.9%	88.9%
IEPD	HC - BMSON	BL	0.89	0.76 – 1.00	<u>reference</u>	79.0%	95.5%
IEAD	HC - BMSON	BL	0.88	0.75 – 1.00	0.567	73.7%	95.5%
IEPD	HC - BMSON	Y2	0.93	0.81 – 1.00	0.270	85.7%	84.2%
IEAD	HC - BMSON	Y2	0.90	0.78 – 1.00	0.740	66.7%	94.7%
IEPD	HC - BMSON	Y4	0.89	0.77 – 1.00	0.966	85.0%	88.9%
IEAD	HC - BMSON	Y4	0.86	0.71 – 1.00	0.474	75.0%	88.9%
IEPD	HC - UMSON	BL	0.88	0.77 – 0.98	<u>reference</u>	84.2%	95.5%
IEAD	HC - UMSON	BL	0.85	0.73 – 0.97	0.105	79.0%	95.5%
IEPD	HC - UMSON	Y2	0.87	0.77 – 0.98	0.799	83.3%	84.2%
IEAD	HC - UMSON	Y2	0.86	0.74 – 0.98	0.395	77.8%	94.7%
IEPD	HC - UMSON	Y4	0.90	0.80 – 1.00	0.315	85.3%	88.9%
IEAD	HC - UMSON	Y4	0.88	0.77 – 0.99	0.965	79.4%	88.9%
IEPD	HC - NMSON	BL	0.73	0.59 – 0.88	<u>reference</u>	41.5%	95.5%
IEAD	HC - NMSON	BL	0.72	0.57 – 0.87	0.605	30.2%	95.5%
IEPD	HC - NMSON	Y2	0.66	0.51 – 0.81	0.205	49.0%	84.2%
IEAD	HC - NMSON	Y2	0.67	0.52 – 0.83	0.316	36.7%	94.7%
IEPD	HC - NMSON	Y4	0.68	0.53 – 0.84	0.213	42.0%	88.9%
IEAD	HC - NMSON	Y4	0.66	0.50 – 0.81	0.076	28.0%	88.9%

mGCIPL: macular ganglion-cell inner plexiform layer; IEPD: intereye percentage difference; IEAD: intereye absolute difference; MS: multiple sclerosis; BMSON: bilateral MS associated optic neuritis; UMSON: unilateral MS associated optic neuritis; nMSON: no MS associated optic neuritis; AUC: area under the curve; CI: confidence interval. BL: baseline; Y2: Year 2; Y4: Year 4

mGCIPL data was available for the following number of healthy controls / unilateral MSON / bilateral MSON / non-MSON: baseline 22/38/19/53, Year 2 19/36/21/49, Year 4 18/34/20/50

^a: P-value refers to the comparison of the ROC curve to the reference ROC curve (baseline IEPD)

^b: sensitivity and specificity are based on a 5% cut-off for the IEPD and a 4 µm cut-off for the IEAD

Supplementary Table 2. The diagnostic performance of atrophy rates of the mGICPL over time for MS patients compared to healthy controls

Time	AUC	95% CI	P-value
BL->y2	0.57	0.43 – 0.70	<u>reference</u>
y2->y4	0.58	0.45 – 0.72	0.895
BL->y4	0.49	0.36 – 0.62	0.487

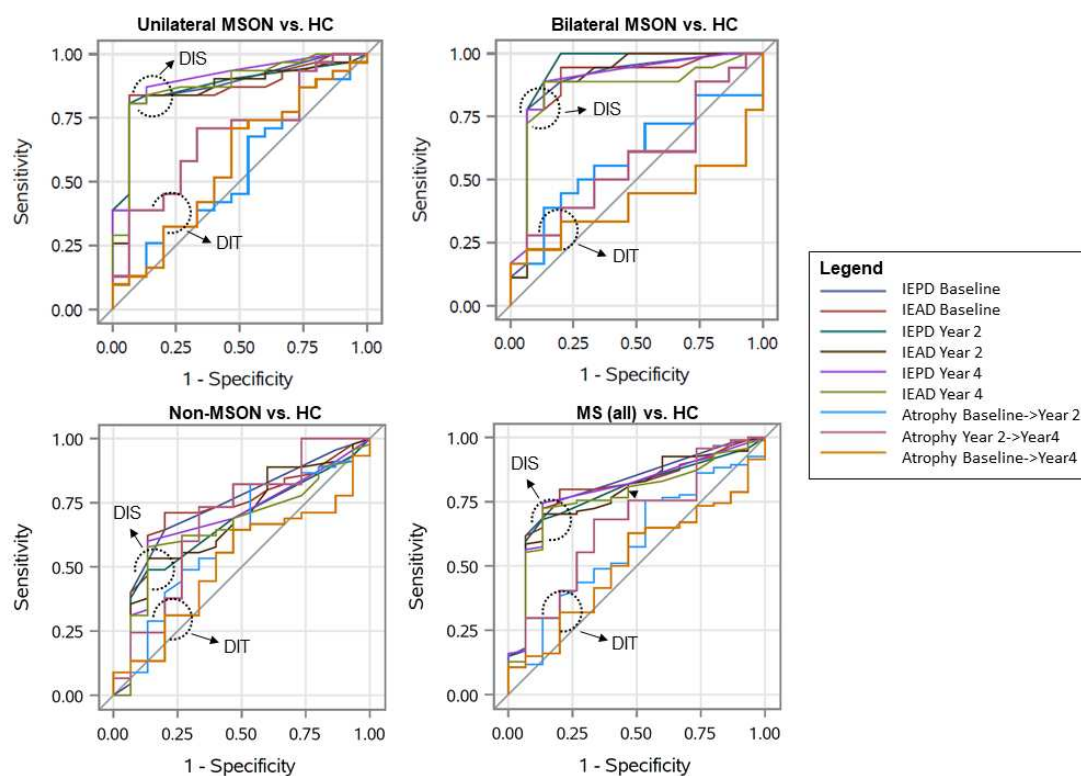
AUC: area under the curve; CI: confidence interval. BL: baseline; Y2: Year 2; Y4: Year 4
mGICPL data was available for the following number of MS patients/healthy controls: BL->Y2: 119/22, y2->y4: 120/21, BL->y4: 122/23

Supplementary Table 3. Sensitivity and specificity of previously reported optimized thresholds for the mGCIPL IEPD and IEAD

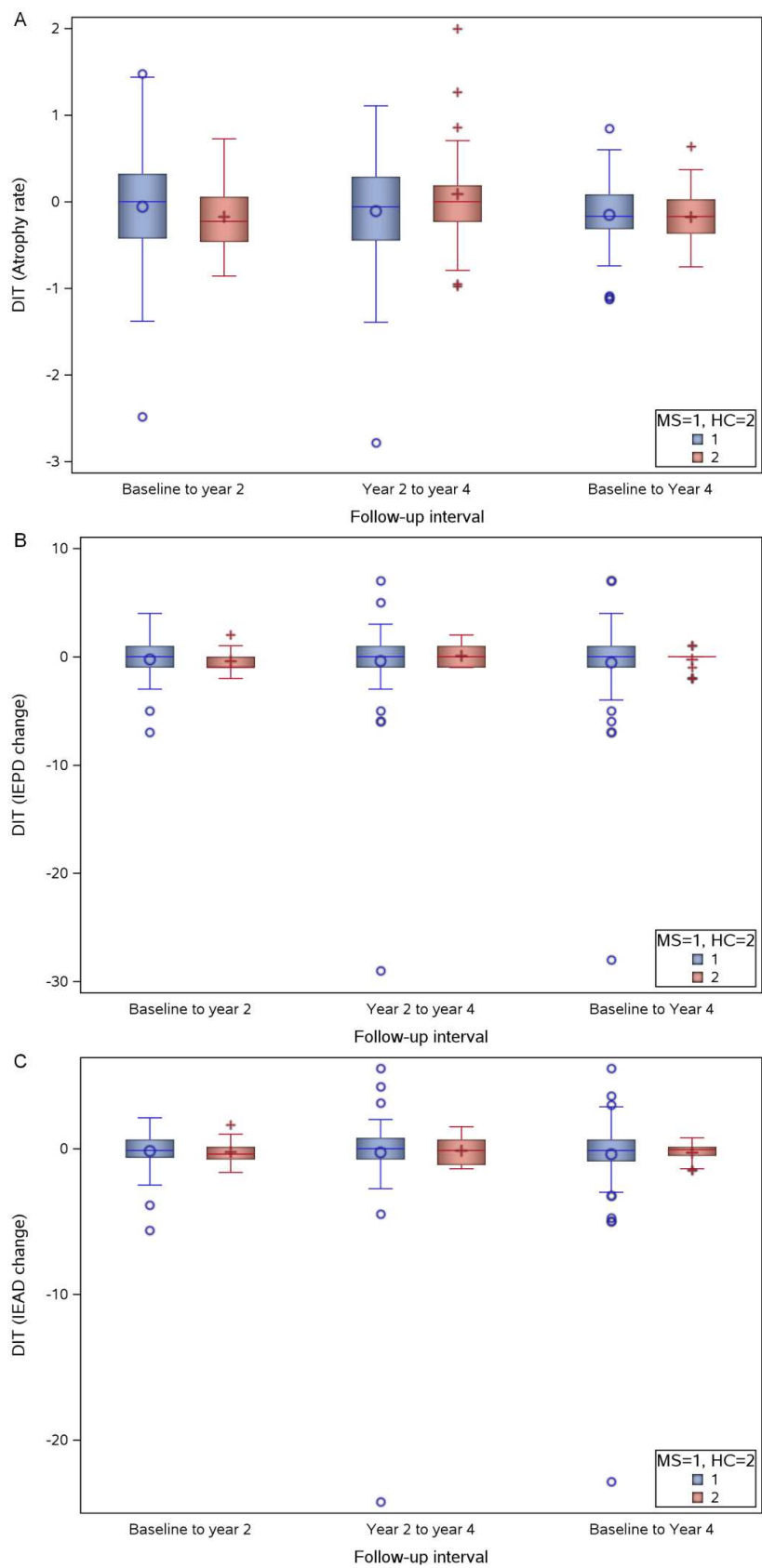
OCT measure	Optimized cut-off	Reference	Group comparison	Specificity	Sensitivity
IEPD mGCIPL	5 %	Coric et al. 2017	Symptomatic bilateral MSON vs. healthy controls	97 %	86 %
IEPD mGCIPL	6 %	Coric et al. 2017	Symptomatic unilateral MSON vs. healthy controls	97 %	70 %
IEAD mGCIPL	4.0 μm	Nolan-Kenney 2019	Symptomatic unilateral MSON vs. non-MSON	77 %	68 %
IEAD mGCIPL	3.5 μm	Behbehani 2020	Unilateral optic neuritis vs. healthy controls	98%	100 %
IEAD mGCIPL	2.83 μm	Davion 2020	Symptomatic unilateral or bilateral MSON vs. non-MSON ^a	67.4 %	67.3 %
IEAD mGCIPL	2.83 μm	Outteryck 2020	Symptomatic ON in CIS patients, with vs. without an optic nerve lesion on 3D-DIR MRI	83.3 %	88.2 %
IEPD mGCIPL	8 %	Outteryck 2020	Symptomatic ON in CIS patients, with vs. without an optic nerve lesion on 3D-DIR MRI	95.6 %	73.5%
IEAD mGCIPL	1.42 μm	Outteryck 2020	CIS patients with vs. without an asymptomatic optic nerve lesion on 3D-DIR MRI	72.6 %	89.3 %
IEPD mGCIPL	2 %	Outteryck 2020	CIS patients with vs. without an asymptomatic optic nerve lesion on 3D-DIR MRI	69.4 %	89.3 %

OCT: optical coherence tomography; IEPD: inter-eye percentage difference; IEAD: inter-eye absolute difference; mGCIPL: macular ganglion cell inner plexiform layer; MSON: MS associated optic neuritis. DIR: double inversion recovery.

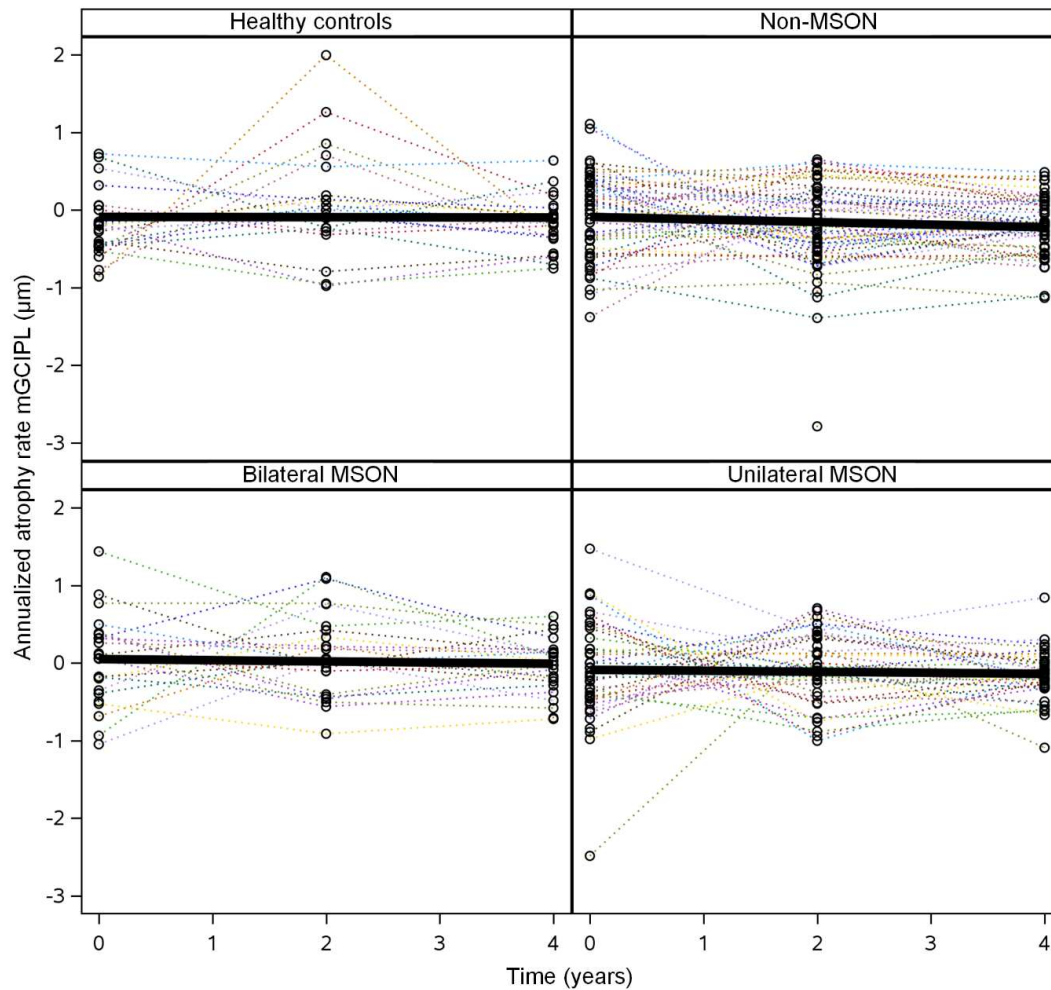
^aOnly patients with relapsing-remitting MS treated with natalizumab were included



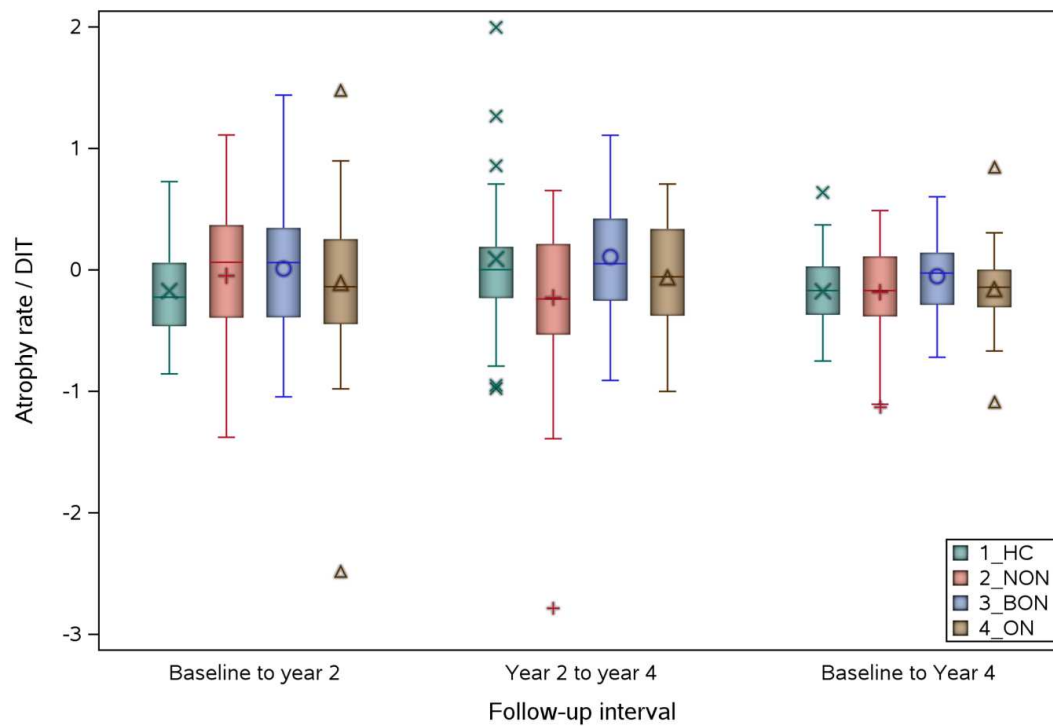
Supplementary Figure 1. Receiver Operator Characteristics (ROC) curves showing the diagnostic accuracy for differentiation between the MS (sub)group and healthy controls. Inter Eye Percentage Difference (IEPD) and Inter Eye Absolute Difference (IEAD) lines represent usefulness for dissemination in space (DIS) and the atrophy rate for dissemination in time (DIT). (A) patients with unilateral MS associated optic neuritis (MSON) versus healthy controls; (B) patients with bilateral MS associated optic neuritis (MSON); C) MS patients (all) versus healthy controls.



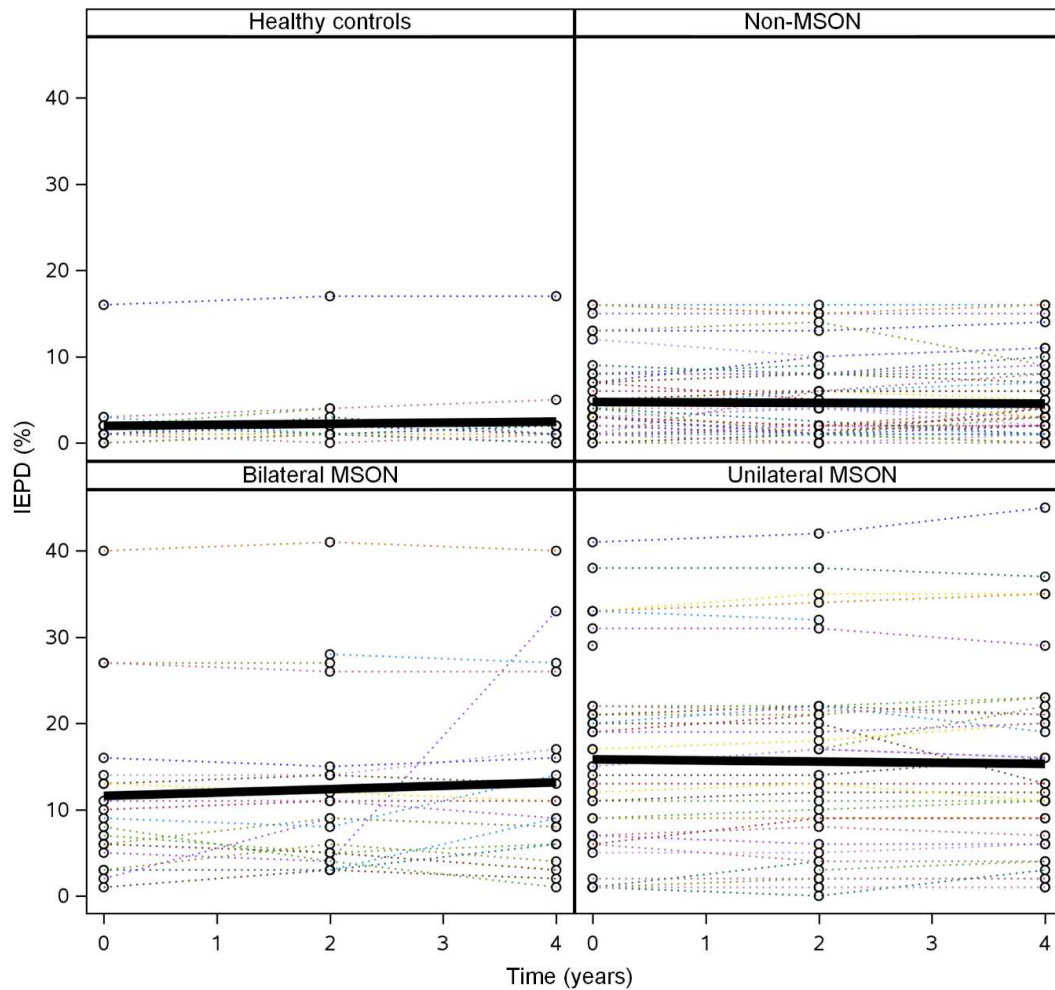
Supplementary Figure 2. Box-and-Whisker plots showing (A) the annualized atrophy rate of the macular ganglion cell inner plexiform layer (mGCIPL), (B) the change of the IEPD over time and (C) the change of the IEAD over time. HC: healthy controls (red); MS: patients with MS (blue). The median (bold horizontal line), 25-75 percentiles (box), 5-95 percentiles (whiskers), mean (symbol in the box) and outliers (symbols outside the box) are shown.



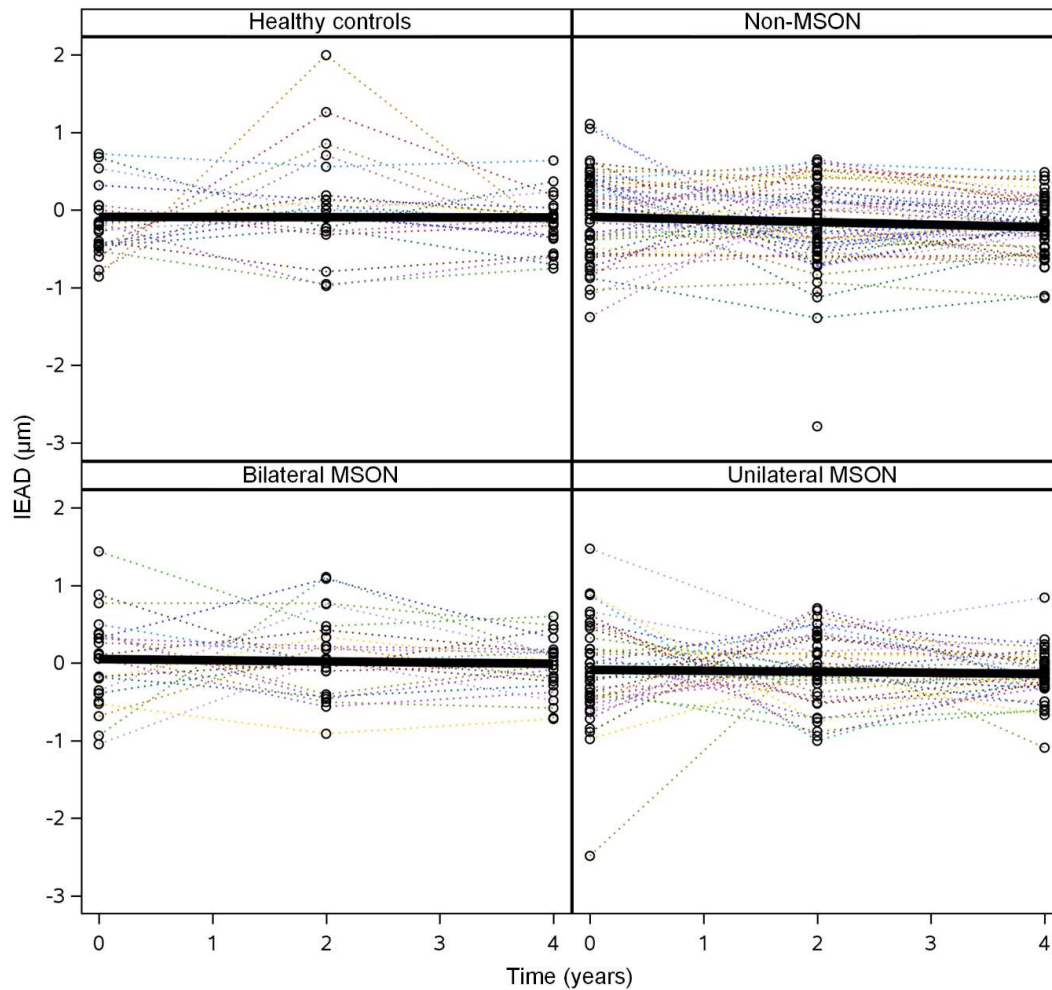
Supplementary Figure 3. Line graphs showing the annualized atrophy rate of the macular ganglion cell inner plexiform layer (mGC IPL) at year 0 (baseline), year 2 and year 4 of the healthy controls and MSON subgroups. The coloured dotted lines represent the individual subjects, and the solid black line represents the mean change. MSON: MS associated optic neuritis



Supplementary Figure 4. Box-and-Whisker plots showing the annualized atrophy rate of the macular ganglion cell inner plexiform layer (mGC IPL) of the healthy controls and MSON subgroups from baseline to year 2, year 2 to year 4 and baseline to year 4. HC: healthy controls (green); NON: no MS associated optic neuritis (red); BON: bilateral MS associated optic neuritis (blue); ON: unilateral MS associated optic neuritis (yellow). The median (bold horizontal line), 25-75 percentiles (box), 5-95 percentiles (whiskers), mean (symbol inside the box) and outliers (symbols outside the box) are shown.



Supplementary Figure 5. Line graphs showing the inter-eye percentage difference (IEPD) of the macular ganglion cell inner plexiform layer (mGCIPL) at year 0 (baseline), year 2 and year 4 of the healthy controls and MSON subgroups. The coloured dotted lines represent the individual subjects, and the solid black line represents the mean change. MSON: MS associated optic neuritis.



Supplementary Figure 6. Line graphs showing the inter-eye absolute difference (IEAD) of the macular ganglion cell inner plexiform layer (mGCIPL) at year 0 (baseline), year 2 and year 4 of the healthy controls and MSON subgroups. The coloured dotted lines represent the individual subjects, and the solid black line represents the mean change. MSON: MS associated optic neuritis.