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.

<table>
<thead>
<tr>
<th>mGCIPL</th>
<th>Group</th>
<th>Time</th>
<th>AUC</th>
<th>95% CI</th>
<th>P-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEPD</td>
<td>HC - MS</td>
<td>BL</td>
<td>0.81</td>
<td>0.70 – 0.92</td>
<td>reference</td>
<td>58.2%</td>
<td>95.5%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - MS</td>
<td>Y2</td>
<td>0.78</td>
<td>0.67 – 0.92</td>
<td>0.377</td>
<td>59.4%</td>
<td>94.7%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - MS</td>
<td>Y4</td>
<td>0.80</td>
<td>0.68 – 0.91</td>
<td>0.526</td>
<td>56.7%</td>
<td>88.9%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - MS</td>
<td>Y4</td>
<td>0.77</td>
<td>0.65 – 0.89</td>
<td>0.115</td>
<td>53.9%</td>
<td>88.9%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - BMSON</td>
<td>BL</td>
<td>0.89</td>
<td>0.76 – 1.00</td>
<td>reference</td>
<td>79.0%</td>
<td>95.5%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - BMSON</td>
<td>Y2</td>
<td>0.93</td>
<td>0.81 – 1.00</td>
<td>0.270</td>
<td>85.7%</td>
<td>84.2%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - BMSON</td>
<td>Y4</td>
<td>0.89</td>
<td>0.77 – 1.00</td>
<td>0.966</td>
<td>85.0%</td>
<td>88.9%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - BMSON</td>
<td>Y4</td>
<td>0.86</td>
<td>0.71 – 1.00</td>
<td>0.474</td>
<td>75.0%</td>
<td>88.9%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - UMSON</td>
<td>BL</td>
<td>0.88</td>
<td>0.77 – 0.98</td>
<td>reference</td>
<td>84.2%</td>
<td>95.5%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - UMSON</td>
<td>Y2</td>
<td>0.87</td>
<td>0.77 – 0.98</td>
<td>0.799</td>
<td>83.3%</td>
<td>84.2%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - UMSON</td>
<td>Y4</td>
<td>0.90</td>
<td>0.80 – 1.00</td>
<td>0.315</td>
<td>85.3%</td>
<td>88.9%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - UMSON</td>
<td>Y4</td>
<td>0.88</td>
<td>0.77 – 0.99</td>
<td>0.965</td>
<td>79.4%</td>
<td>88.9%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - NMSON</td>
<td>BL</td>
<td>0.73</td>
<td>0.59 – 0.88</td>
<td>reference</td>
<td>41.5%</td>
<td>95.5%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - NMSON</td>
<td>Y2</td>
<td>0.66</td>
<td>0.51 – 0.81</td>
<td>0.205</td>
<td>49.0%</td>
<td>84.2%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - NMSON</td>
<td>Y4</td>
<td>0.67</td>
<td>0.52 – 0.83</td>
<td>0.316</td>
<td>36.7%</td>
<td>94.7%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - NMSON</td>
<td>Y4</td>
<td>0.68</td>
<td>0.53 – 0.84</td>
<td>0.213</td>
<td>42.0%</td>
<td>88.9%</td>
</tr>
<tr>
<td>IEPD</td>
<td>HC - NMSON</td>
<td>Y4</td>
<td>0.66</td>
<td>0.50 – 0.81</td>
<td>0.076</td>
<td>28.0%</td>
<td>88.9%</td>
</tr>
</tbody>
</table>

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; UMSON: 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

<table>
<thead>
<tr>
<th>Time</th>
<th>AUC</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL-&gt;y2</td>
<td>0.57</td>
<td>0.43 – 0.70</td>
<td>reference</td>
</tr>
<tr>
<td>y2-&gt;y4</td>
<td>0.58</td>
<td>0.45 – 0.72</td>
<td>0.895</td>
</tr>
<tr>
<td>BL-&gt;y4</td>
<td>0.49</td>
<td>0.36 – 0.62</td>
<td>0.487</td>
</tr>
</tbody>
</table>

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 MS patients/healthy controls: BL->Y2: 119/22, y2->y4: 120/21, BL->y4: 122/23
<table>
<thead>
<tr>
<th>OCT measure</th>
<th>Optimized cut-off</th>
<th>Reference</th>
<th>Group comparison</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEPD mGCIPL</td>
<td>5 %</td>
<td>Coric et al. 2017</td>
<td>Symptomatic bilateral MSON vs. healthy controls</td>
<td>97 %</td>
<td>86 %</td>
</tr>
<tr>
<td>IEPD mGCIPL</td>
<td>6 %</td>
<td>Coric et al. 2017</td>
<td>Symptomatic unilateral MSON vs. healthy controls</td>
<td>97 %</td>
<td>70 %</td>
</tr>
<tr>
<td>IEAD mGCIPL</td>
<td>4.0 μm</td>
<td>Nolan-Kenney 2019</td>
<td>Symptomatic unilateral MSON vs. non-MSON</td>
<td>77 %</td>
<td>68 %</td>
</tr>
<tr>
<td>IEAD mGCIPL</td>
<td>3.5 μm</td>
<td>Behbehani 2020</td>
<td>Unilateral optic neuritis vs. healthy controls</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td>IEAD mGCIPL</td>
<td>2.83 μm</td>
<td>Davion 2020</td>
<td>Symptomatic unilateral or bilateral MSON vs. non-MSON&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.4 %</td>
<td>67.3 %</td>
</tr>
<tr>
<td>IEAD mGCIPL</td>
<td>2.83 μm</td>
<td>Outteryck 2020</td>
<td>Symptomatic ON in CIS patients, with vs. without an optic nerve lesion on 3D-DIR MRI</td>
<td>83.3 %</td>
<td>88.2 %</td>
</tr>
<tr>
<td>IEPD mGCIPL</td>
<td>8 %</td>
<td>Outteryck 2020</td>
<td>Symptomatic ON in CIS patients, with vs. without an optic nerve lesion on 3D-DIR MRI</td>
<td>95.6 %</td>
<td>73.5%</td>
</tr>
<tr>
<td>IEAD mGCIPL</td>
<td>1.42 μm</td>
<td>Outteryck 2020</td>
<td>CIS patients with vs. without an asymptomatic optic nerve lesion on 3D-DIR MRI</td>
<td>72.6 %</td>
<td>89.3 %</td>
</tr>
<tr>
<td>IEPD mGCIPL</td>
<td>2 %</td>
<td>Outteryck 2020</td>
<td>CIS patients with vs. without an asymptomatic optic nerve lesion on 3D-DIR MRI</td>
<td>69.4 %</td>
<td>89.3 %</td>
</tr>
</tbody>
</table>

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.

<sup>a</sup>Only patients with relapsing-remitting MS treated with natalizumab were included.

Supplementary Table 3. Sensitivity and specificity of previously reported optimized thresholds for the mGCIPL IEPD and IEAD.
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 (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 4. Box-and-Whisker plots showing the annualized atrophy rate of the macular ganglion cell inner plexiform layer (mGCIPL) 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.