| | RRMS | OCT HC | p-value |
|---|---|---|---|
| G-pRNFL | 99.77 ± 10.57 | 98.11 ± 10.45 | 0.531 |
| ***PMB-pRNFL*** | **52.24 ± 8.90** | **55.05 ± 6.71** | **0.047** |
| T-pRNFL | 67.78 ± 12.39 | 71.20 ± 9.58 | 0.092 |
| TS-pRNFL | 136.64 ± 19.39 | 135.57 ± 15.87 | 0.686 |
| TI-pRNFL | 144.90 ± 22.22 | 145.81 ± 21.06 | 0.778 |
| N-pRNFL | 75.58 ± 14.76 | 72.68 ± 14.47 | 0.332 |
| NS-pRNFL | 110.75 ± 21.14 | 105.92 ± 19.77 | 0.221 |
| NI-pRNFL | 118.96 ± 23.74 | 110.26 ± 25.52 | 0.082 |
| RNFL TV ($mm^3$) | 0.89 ± 0.10 | 0.87 ± 0.07 | 0.322 |
| RNFL IR NASAL (µm) | 20.42 ± 3.94 | 20.27 ± 2.01 | 0.761 |
| RNFL OR NASAL (µm) | 47.52 ± 7.27 | 47.45 ± 4.88 | 0.898 |
| RNFL IR SUPERIOR (µm) | 24.25 ± 4.09 | 23.99 ± 2.32 | 0.632 |
| RNFL OR SUPERIOR (µm) | 36.59 ± 5.40 | 35.53 ± 4.05 | 0.226 |
| RNFL IR TEMPORAL (µm) | 16.56 ± 3.39 | 16.04 ± 1.26 | 0.218 |
| ***RNFL OR TEMPORAL*** (µm) | **17.62 ± 2.62** | **16.91 ± 1.19** | **0.034** |
| RNFL IR INFERIOR (µm) | 24.73 ± 3.97 | 25.17 ± 2.50 | 0.435 |
| RNFL OR INFERIOR (µm) | 38.70 ± 5.95 | 37.94 ± 4.16 | 0.394 |
| GCL TV ($mm^3$) | 1.12 ± 0.10 | 1.12 ± 0.09 | 0.975 |
| GCL IR NASAL (µm) | 52.31 ± 5.55 | 53.53 ± 4.83 | 0.190 |
| GCL OR NASAL (µm) | 39.48 ± 4.17 | 39.44 ± 3.80 | 0.972 |
| GCL IR SUPERIOR (µm) | 53.11 ± 4.74 | 53.83 ± 3.98 | 0.384 |
| GCL OR SUPERIOR (µm) | 35.21 ± 3.41 | 35.09 ± 3.33 | 0.858 |
| GCL IR TEMPORAL (µm) | 49.72 ± 5.06 | 49.92 ± 4.85 | 0.806 |
| GCL OR TEMPORAL (µm) | 38.58 ± 4.46 | 38.44 ± 3.42 | 0.826 |
| GCL IR INFERIOR (µm) | 53.04 ± 4.51 | 53.62 ± 4.14 | 0.476 |
| GCL OR INFERIOR (µm) | 34.61 ± 3.24 | 33.92 ± 3.12 | 0.267 |
| IPL TV ($mm^3$) | 0.93 ± 0.07 | 0.92 ± 0.07 | 0.406 |
| IPL IR NASAL (µm) | 43.18 ± 3.93 | 43.46 ± 3.52 | 0.695 |
| IPL OR NASAL (µm) | 31.52 ± 3.32 | 31.18 ± 3.02 | 0.576 |
| IPL IR SUPERIOR (µm) | 43.92 ± 3.61 | 44.32 ± 3.37 | 0.559 |
| IPL OR SUPERIOR (µm) | 28.11 ± 2.71 | 27.86 ± 2.72 | 0.611 |
| IPL IR TEMPORAL (µm) | 42.09 ± 3.86 | 41.77 ± 3.33 | 0.623 |
| IPL OR TEMPORAL (µm) | 34.21 ± 3.09 | 33.45 ± 2.83 | 0.157 |
| IPL IR INFERIOR (µm) | 44.08 ± 3.55 | 43.78 ± 3.33 | 0.622 |
| IPL OR INFERIOR (µm) | 27.41 ± 2.64 | 26.87 ± 2.52 | 0.271 |
| INL TV ($mm^3$) | 0.97 ± 0.06 | 0.95 ± 0.06 | 0.055 |
| INL IR NASAL (µm) | 39.95 ± 4.87 | 39.05 ± 4.41 | 0.234 |
| INL OR NASAL (µm) | 33.82 ± 2.77 | 32.97 ± 2.52 | 0.074 |
| INL IR SUPERIOR (µm) | 39.36 ± 3.88 | 37.90 ± 3.42 | **0.026** |
| INL OR SUPERIOR (µm) | 33.71 ± 2.35 | 33.17 ± 2.21 | 0.199 |
| INL IR TEMPORAL (µm) | 38.20 ± 4.15 | 37.32 ± 3.79 | 0.203 |
| INL OR TEMPORAL (µm) | 33.14 ± 2.68 | 32.68 ± 2.64 | 0.317 |
| INL IR INFERIOR (µm) | 38.49 ± 3.54 | 37.77 ± 3.67 | 0.235 |
| INL OR INFERIOR (µm) | 33.36 ± 2.34 | 32.69 ± 2.19 | 0.107 |
| OPL TV ($mm^3$) | 0.82 ± 0.07 | 0.81 ± 0.07 | 0.482 |
| OPL IR NASAL (µm) | 33.97 ± 9.96 | 32.91 ± 8.41 | 0.496 |
| OPL OR NASAL (µm) | 29.27 ± 4.03 | 28.28 ± 3.90 | 0.150 |
| OPL IR SUPERIOR (µm) | 33.13 ± 5.23 | 32.77 ± 4.99 | 0.678 |
| OPL OR SUPERIOR (µm) | 26.88 ± 2.13 | 26.19 ± 2.06 | 0.070 |
| OPL IR TEMPORAL (µm) | 33.60 ± 6.92 | 35.77 ± 9.94 | 0.091 |
| OPL OR TEMPORAL (µm) | 28.29 ± 2.78 | 27.96 ± 2.98 | 0.455 |
| OPL IR INFERIOR (µm) | 31.45 ± 4.27 | 32.69 ± 6.51 | 0.099 |
| OPL OR INFERIOR (µm) | 26.64 ± 2.01 | 26.62 ± 2.65 | 0.962 |
| ONL TV ($mm^3$) | 1.76 ± 0.19 | 1.77 ± 0.18 | 0.711 |

| | | | |
|---|---|---|---|
| ONL IR NASAL (µm) | 72.87 ± 13.38 | 74.54 ± 12.47 | 0.460 |
| ONL OR NASAL (µm) | 58.57 ± 8.50 | 59.29 ± 8.11 | 0.627 |
| ONL IR SUPERIOR (µm) | 70.55 ± 8.53 | 71.97 ± 8.25 | 0.357 |
| ONL OR SUPERIOR (µm) | 62.34 ± 6.67 | 63.51 ± 6.02 | 0.342 |
| ONL IR TEMPORAL (µm) | 70.18 ± 10.43 | 69.08 ± 12.30 | 0.592 |
| ONL OR TEMPORAL (µm) | 58.04 ± 6.89 | 58.88 ± 6.38 | 0.483 |
| ONL IR INFERIOR (µm) | 70.36 ± 8.18 | 69.12 ± 9.41 | 0.436 |
| ONL OR INFERIOR (µm) | 55.08 ± 6.65 | 54.44 ± 6.05 | 0.628 |
| RPE TV ($mm^3$) | 0.41 ± 0.04 | 0.41 ± 0.03 | 0.952 |
| RPE IR NASAL (µm) | 16.92 ± 1.79 | 16.60 ± 1.51 | 0.284 |
| RPE OR NASAL (µm) | 14.59 ± 1.65 | 14.76 ± 1.60 | 0.562 |
| RPE IR SUPERIOR (µm) | 16.05 ± 1.62 | 16.00 ± 1.44 | 0.847 |
| RPE OR SUPERIOR (µm) | 14.61 ± 1.64 | 14.15 ± 1.56 | 0.120 |
| RPE IR TEMPORAL (µm) | 15.78 ± 1.65 | 15.59 ± 1.61 | 0.502 |
| RPE OR TEMPORAL (µm) | 13.41 ± 1.47 | 13.50 ± 1.59 | 0.748 |
| RPE IR INFERIOR (µm) | 15.55 ± 1.46 | 15.60 ± 1.29 | 0.817 |
| RPE OR INFERIOR (µm) | 13.86 ± 1.47 | 13.90 ± 1.45 | 0.896 |

**eTable 1. Peripapillary RNFL thicknesses and macular layer volumes in patients and HC.** All values are shown as mean ± SD. Abbreviations: G global, PMB Papillo-macular Bundle, T temporal, TS Temporal Superior, TI Temporal Inferior, N Nasal, NS Nasal Superior, NI Nasal Inferior. RNFL Retinal Nerve Fibre Layer, GCL Ganglion Cell Layer, IPL Inner Plexiform Layer, INL Inner Nuclear Layer, OPL Outer Plexiform Layer, ONL Outer Nuclear Layer, RPE Retinal Pigmented Layer. TV Total Volume, N Nasal, S Superior, T temporal, I Inferior.

| OCT-values | HRF location | p-value | $\beta$-value |
|---|---|---|---|
| **RNFL-TV** | GCIP HRF | 0.715 | -0.097 |
| | INL HRF | 0.180 | -0.240 |
| | IRL HRF | *0.076* | -0.264 |
| **RNFL N-IR** | GCIP HRF | 0.780 | -0.002 |
| | INL HRF | 0.880 | 0.000 |
| | IRL HRF | 0.630 | -0.001 |
| **RNFL N-OR** | GCIP HRF | 0.552 | 0.002 |
| | INL HRF | 0.341 | -0.002 |
| | IRL HRF | 0.546 | -0.001 |
| **RNFL S-IR,** | GCIP HRF | 0.829 | 0.001 |
| | INL HRF | 0.784 | 0.001 |
| | IRL HRF | 0.805 | -0.001 |
| **RNFL S-OR** | GCIP HRF | 0.584 | -0.003 |
| | INL HRF | 0.102 | -0.005 |
| | IRL HRF | *0.067* | -0.005 |
| **RNFL T-IR** | GCIP HRF | 0.408 | -0.006 |
| | INL HRF | 0.458 | 0.003 |
| | IRL HRF | 0.764 | -0.001 |
| **RNFL T-OR** | GCIP HRF | 0.213 | -0.012 |
| | INL HRF | 0.176 | 0.006 |
| | IRL HRF | 0.941 | -0.000 |
| **RNFL I-IR** | GCIP HRF | 0.446 | -0.005 |
| | INL HRF | 0.343 | -0.003 |
| | IRL HRF | *0.066* | -0.005 |
| **RNFL I-OR** | GCIP HRF | 0.658 | -0.002 |
| | INL HRF | **0.006** | -0.009 |
| | IRL HRF | **0.007** | -0.007 |
| **GCIP TV** | GCIP HRF | 0.560 | -0.093 |
| | INL HRF | 0.887 | -0.017 |
| | IRL HRF | 0.716 | -0.036 |
| **GCIP N-IR** | GCIP HRF | 0.661 | 0.001 |
| | INL HRF | 0.488 | 0.001 |
| | IRL HRF | 0.445 | 0.001 |
| **GCIP N-OR** | GCIP HRF | 0.417 | -0.003 |
| | INL HRF | 0.775 | -0.001 |
| | IRL HRF | 0.503 | -0.001 |
| **GCIP S-IR** | GCIP HRF | 0.678 | 0.001 |
| | INL HRF | 0.600 | 0.001 |
| | IRL HRF | 0.415 | 0.002 |
| **GCIP S-OR** | GCIP HRF | 0.178 | -0.006 |
| | INL HRF | 0.145 | -0.005 |
| | IRL HRF | *0.050* | -0.005 |
| **GCIP T-IR** | GCIP HRF | 0.991 | 0.000 |
| | INL HRF | 0.663 | 0.001 |
| | IRL HRF | 0.581 | 0.001 |

| | | | |
|---|---|---|---|
| **GCIP T-OR** | GCIP HRF | 0.950 | -0.000 |
| | INL HRF | 0.377 | 0.002 |
| | IRL HRF | 0.286 | 0.002 |
| **GCIP I-IR** | GCIP HRF | 0.839 | 0.001 |
| | INL HRF | 0.350 | 0.002 |
| | IRL HRF | 0.407 | 0.002 |
| **GCIP I-OR** | GCIP HRF | 0.737 | -0.002 |
| | INL HRF | 0.881 | -0.000 |
| | IRL HRF | 0.940 | -0.000 |
| **IPL TV** | GCIP HRF | 0.519 | -0.230 |
| | INL HRF | 0.684 | 0.110 |
| | IRL HRF | 0.938 | 0.017 |
| **IPL N-IR** | GCIP HRF | 0.908 | 0.001 |
| | INL HRF | 0.409 | 0.004 |
| | IRL HRF | 0.530 | 0.002 |
| **IPL N-OR** | GCIP HRF | 0.272 | -0.008 |
| | INL HRF | 0.786 | 0.001 |
| | IRL HRF | 0.664 | -0.002 |
| **IPL S-IR** | GCIP HRF | 0.895 | 0.001 |
| | INL HRF | 0.556 | 0.003 |
| | IRL HRF | 0.524 | 0.002 |
| **IPL S-OR** | GCIP HRF | 0.134 | -0.014 |
| | INL HRF | *0.052* | -0.013 |
| | IRL HRF | **0.020** | -0.013 |
| **IPL T-IR** | GCIP HRF | 0.772 | 0.002 |
| | INL HRF | 0.215 | 0.005 |
| | IRL HRF | 0.168 | 0.005 |
| **IPL T-OR** | GCIP HRF | 0.687 | -0.003 |
| | INL HRF | 0.132 | 0.009 |
| | IRL HRF | 0.213 | 0.006 |
| **IPL I-IR** | GCIP HRF | 0.793 | 0.002 |
| | INL HRF | 0.356 | 0.004 |
| | IRL HRF | 0.385 | 0.003 |
| **IPL I-OR** | GCIP HRF | 0.527 | -0.006 |
| | INL HRF | 0.833 | -0.001 |
| | IRL HRF | 0.714 | -0.002 |
| **INL TV** | GCIP HRF | 0.107 | 0.686 |
| | INL HRF | **<0.001** | 1.211 |
| | IRL HRF | **<0.001** | 1.036 |
| **INL N-IR** | GCIP HRF | 0.365 | 0.004 |
| | INL HRF | **0.010** | 0.007 |
| | IRL HRF | **0.009** | 0.006 |
| **INL N-OR** | GCIP HRF | 0.547 | 0.005 |
| | INL HRF | **0.014** | 0.013 |
| | IRL HRF | *0.077* | 0.008 |
| **INL S-IR** | GCIP HRF | 0.148 | 0.009 |

| | | | |
|---|---|---|---|
| | INL HRF | **<0.001** | 0.016 |
| | IRL HRF | **<0.001** | 0.014 |
| **INL S-OR** | GCIP HRF | 0.279 | 0.011 |
| | INL HRF | **0.011** | 0.018 |
| | IRL HRF | **0.015** | 0.014 |
| **INL T-IR** | GCIP HRF | **0.049** | 0.012 |
| | INL HRF | **0.001** | 0.012 |
| | IRL HRF | **<0.001** | 0.013 |
| **INL T-OR** | GCIP HRF | 0.445 | 0.007 |
| | INL HRF | 0.947 | 0.000 |
| | IRL HRF | 0.600 | 0.003 |
| **INL I-IR** | GCIP HRF | *0.054* | 0.012 |
| | INL HRF | **0.019** | 0.010 |
| | IRL HRF | **0.001** | 0.011 |
| **INL I-OR** | GCIP HRF | 0.123 | 0.016 |
| | INL HRF | **0.008** | 0.019 |
| | IRL HRF | **0.001** | 0.019 |
| **OPL TV** | GCIP HRF | 0.385 | -0.318 |
| | INL HRF | 0.924 | -0.022 |
| | IRL HRF | 0.682 | -0.080 |
| **OPL N-IR** | GCIP HRF | 0.528 | -0.002 |
| | INL HRF | 0.549 | 0.001 |
| | IRL HRF | 0.953 | 0.000 |

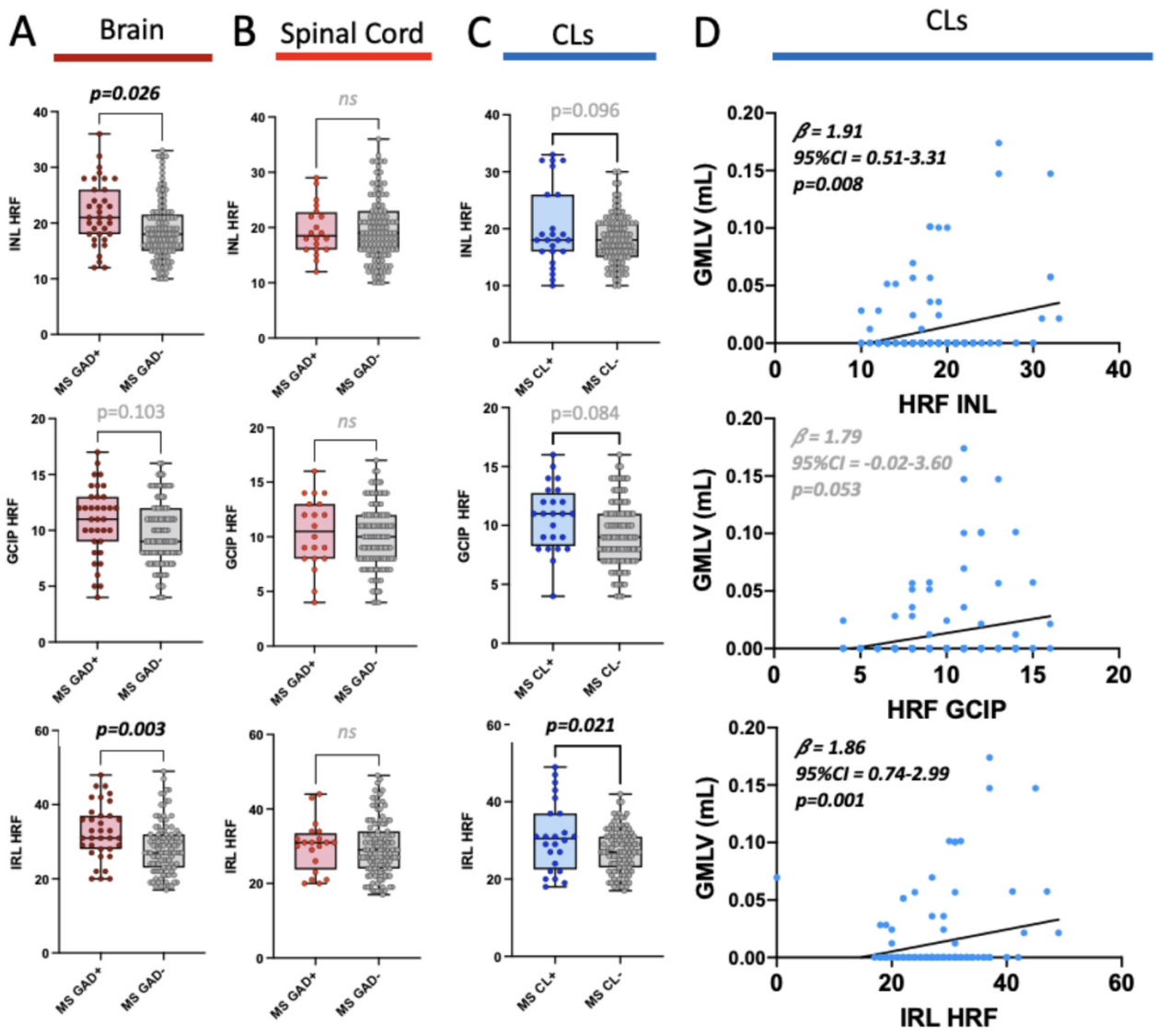
| | | | |
|---|---|---|---|
| **OPL N-OR** | GCIP HRF | 0.388 | -0.005 |
| | INL HRF | 0.514 | 0.002 |
| | IRL HRF | 0.759 | 0.001 |
| **OPL S-IR** | GCIP HRF | 0.203 | -0.006 |
| | INL HRF | **0.019** | -0.006 |
| | IRL HRF | **0.018** | -0.005 |
| **OPL S-OR** | GCIP HRF | 0.652 | 0.005 |
| | INL HRF | 0.869 | 0.001 |
| | IRL HRF | 0.325 | 0.006 |
| **OPL T-IR** | GCIP HRF | 0.725 | -0.001 |
| | INL HRF | 0.300 | -0.002 |
| | IRL HRF | 0.279 | -0.001 |
| **OPL T-OR** | GCIP HRF | 0.668 | 0.003 |
| | INL HRF | 0.348 | -0.004 |
| | IRL HRF | 0.854 | -0.001 |
| **OPL I-IR** | GCIP HRF | 0.557 | -0.003 |
| | INL HRF | **0.040** | 0.006 |
| | IRL HRF | 0.314 | 0.002 |
| **OPL I-OR** | GCIP HRF | 0.417 | -0.009 |
| | INL HRF | 0.341 | 0.007 |
| | IRL HRF | 0.842 | 0.001 |
| **ONL TV** | GCIP HRF | 0.957 | -0.008 |
| | INL HRF | 0.824 | 0.024 |

| | | | |
|---|---|---|---|
| | IRL HRF | 0.777 | 0.025 |
| **ONL N-IR** | GCIP HRF | 0.564 | 0.001 |
| | INL HRF | 0.338 | -0.001 |
| | IRL HRF | 0.842 | -0.000 |
| **ONL N-OR** | GCIP HRF | 0.981 | -0.000 |
| | INL HRF | 0.625 | -0.001 |
| | IRL HRF | 0.759 | -0.001 |
| **ONL S-IR** | GCIP HRF | 0.389 | 0.002 |
| | INL HRF | 0.215 | 0.002 |
| | IRL HRF | 0.165 | 0.002 |
| **ONL S-OR** | GCIP HRF | 0.668 | -0.002 |
| | INL HRF | 0.983 | 0.000 |
| | IRL HRF | 0.748 | -0.001 |
| **ONL T-IR** | GCIP HRF | 0.952 | 0.000 |
| | INL HRF | 0.202 | 0.002 |
| | IRL HRF | 0.283 | 0.001 |
| **ONL T-OR** | GCIP HRF | 0.689 | -0.001 |
| | INL HRF | 0.401 | 0.002 |
| | IRL HRF | 0.556 | 0.001 |
| **ONL I-IR** | GCIP HRF | 0.821 | 0.001 |
| | INL HRF | 0.667 | -0.001 |
| | IRL HRF | 0.964 | 0.000 |
| **ONL I-OR** | GCIP HRF | 0.742 | -0.001 |

| | | | |
|---|---|---|---|
| | INL HRF | 0.979 | 0.000 |
| | IRL HRF | 0.919 | 0.000 |
| **RPE TV** | GCIP HRF | 0.970 | 0.025 |
| | INL HRF | 0.896 | -0.062 |
| | IRL HRF | 0.980 | -0.010 |
| **RPE N-IR** | GCIP HRF | 0.338 | 0.013 |
| | INL HRF | 0.343 | 0.008 |
| | IRL HRF | 0.114 | 0.011 |
| **RPE N-OR** | GCIP HRF | 0.575 | -0.008 |
| | INL HRF | 0.603 | 0.005 |
| | IRL HRF | 0.808 | -0.002 |
| **RPE S-IR** | GCIP HRF | 0.883 | -0.002 |
| | INL HRF | 0.719 | 0.003 |
| | IRL HRF | 0.937 | 0.001 |
| **RPE S-OR** | GCIP HRF | 0.648 | 0.007 |
| | INL HRF | **0.044** | 0.020 |
| | IRL HRF | *0.097* | 0.013 |
| **RPE T-IR** | GCIP HRF | 0.601 | -0.007 |
| | INL HRF | 0.129 | -0.014 |
| | IRL HRF | *0.073* | -0.013 |
| **RPE T-OR** | GCIP HRF | 0.704 | 0.007 |
| | INL HRF | 0.652 | -0.005 |
| | IRL HRF | 0.867 | -0.002 |

| | | | |
|---|---|---|---|
| **RPE I-IR** | GCIP HRF | 0.730 | -0.006 |
| | INL HRF | **0.009** | -0.027 |
| | IRL HRF | *0.057* | -0.017 |
| **RPE I-OR** | GCIP HRF | 0.990 | -0.000 |
| | INL HRF | 0.132 | -0.015 |
| | IRL HRF | 0.457 | -0.006 |
| Thalaic Volue | GCIP HRF | *0.051* | -0.067 |
| | INL HRF | 0.991 | 0.000 |
| | IRL HRF | 0.284 | -0.024 |
| ***LGN Volue*** | ***GCIP HRF*** | ***0.003*** | ***-0.002*** |
| | INL HRF | 0.773 | -0.000 |
| | IRL HRF | *0.059* | -0.001 |
| Precentral Thickness | GCIP HRF | 0.904 | 0.030 |
| | INL HRF | 0.582 | 0.109 |
| | IRL HRF | 0.567 | 0.090 |
| Pericalcarin Thickness | GCIP HRF | 0.247 | -0.297 |
| | INL HRF | 0.178 | -0.276 |
| | IRL HRF | *0.081* | -0.285 |
| Optic radiation White atter Lesion Volue | GCIP HRF | 0.755 | 0.015 |
| | INL HRF | 0.211 | 0.046 |
| | IRL HRF | 0.231 | 0.035 |

**eTable 2. Correlations between HRF count in the inner retina and macular thickness and the volumes.** Ten layers were considered; in addition, each layer was also divided in 2 rings (Inner Ring, IR; Outer Ring, OR) and 4 sectors (N, Nasal; S, Superior; T, Temporal; I, Inferior). RNFL Retinal Nerve Fibre Layer, GCL Ganglion Cell Layer, IPL Inner Plexiform Layer, INL Inner Nuclear Layer, OPL Outer Plexiform Layer, ONL Outer Nuclear Layer, RPE Retinal Pigmented Layer. TV Total Volume.




**eFigure 1. HRF associated with brain inflammatory parameters.** Both IRL and INL HRF count are increased in presence of brain (A), but not spinal cord (B) gadolinium enhancing lesion. C) Patients with cortical lesions had significantly higher IRL HRF count. While INL and IRL HRF count associated with Grey Matter Lesion Volume (GMLV), no association with White Matter Lesion Volume (WMLV) was observed. Abbreviations: CLs: cortical lesions; WMLs: White Matter Lesions.

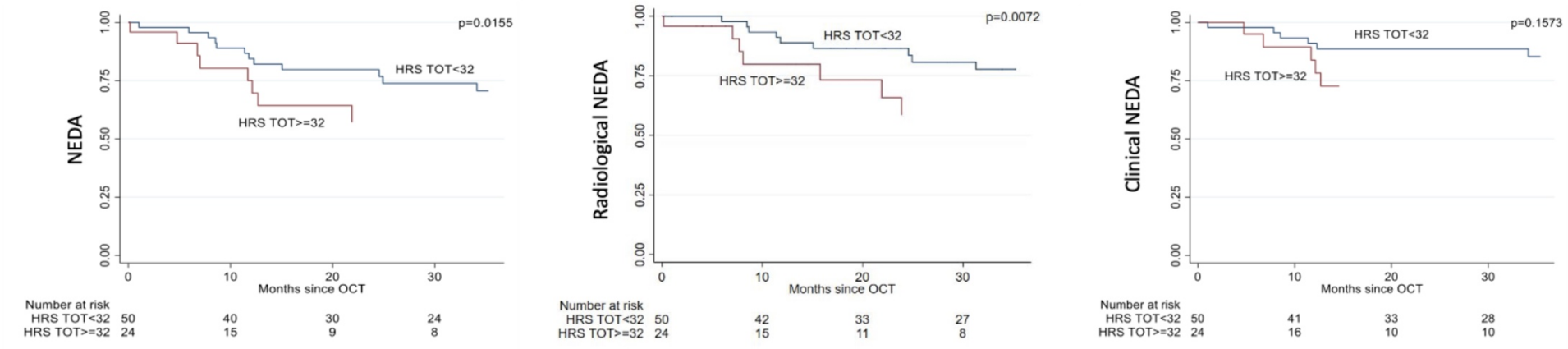



**Figure 2. NEDA condition can be predicted applying specific IR HRF count cut-off.** Kaplan–Meier curves and Log-rank tests according to total HRF. A cut-off value of 32 was found using time-dependent ROC curves with the Liu approach. In line with this cut-off, both radiological NEDA and NEDA significantly differed.

**eTable 3. Clinical and radiological parameters observed in the followed-up cohort.**

| | | |
|---|---|---|
| **First-line DT confirmed** | 58 patients | *10 IFN*<br>*14 Glatiramer acetate*<br>*26 Dimethyl fumarate*<br>*8 Teriflunomide* |
| **Second-line DT** | 13 patients | *10 Natalizumab*<br>*2 Alemtuzumab*<br>*1 Ocrelizumab* |
| **DT not determined *** | 8 patients | |
| **Clinical relapses**<br>• Total<br>• Non ocular relapses | <br>• 19 (16 patients)<br>• 12 (11 patients) | 3 patients had 2 clinical relapses during the follow-up.<br>1 patient had 2 non-ocular relapses. |
| **New T2/FLAIR lesions** | 24 patients | 12 patients: 1 new lesion<br>6 patients: 2 new lesions<br>3 patients: 3 new lesions<br>1 patient: 4 new lesions<br>2 patients: 5 new lesions |
| **New T1 Gd+ lesions** | 11 patients | 9 patients: 1 gad+ lesion<br>2 patients: 2 gad+ lesions. |

* these patients were lost at follow-up within 3 months.