Diagnostic Accuracy of Spectralis SD OCT Automated Macular Layers Segmentation to Discriminate Normal from Early Glaucomatous Eyes

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Purpose: To evaluate the accuracy of the macular retinal layer segmentation software of the Spectralis spectral-domain (SD) optical coherence tomography (OCT) device (Heidelberg Engineering, Inc., Heidelberg, Germany) to discriminate between healthy and early glaucoma (EG) eyes.

Design: Prospective, cross-sectional study.

Participants: Forty EG eyes and 40 healthy controls were included.

Methods: All participants were examined using the standard posterior pole and the peripapillary retinal nerve fiber layer (pRNFL) protocols of the Spectralis OCT device. Using an Early Treatment Diabetic Retinopathy Study circle at the macular level, the automated retinal segmentation software was applied to determine thicknesses of the following parameters: total retinal thickness, inner retinal layer (IRL), macular retinal nerve fiber layer (mRNFL), macular ganglion cell layer (mGCL), macular inner nuclear layer (mINL), macular outer plexiform layer (mOPL), macular outer nuclear layer (mONL), photoreceptors (PR), and retinal pigmentary epithelium (RPE). The ganglion cell complex (GCC) was determined by adding the mRNFL, mGCL, and mIPL parameters and the ganglion cell layer–inner plexiform layer (mGCC-IPL) was determined by combining the mGCL and mIPL parameters. Thickness of each layer was compared between the groups, and the layer and sector with the best area under the receiver operating characteristic curve (AUC) were identified.

Main Outcome Measures: Comparison of pRNFL, IRL, mRNFL, mGCL, mIPL, mGCC, mGCC-IPL, mINL, mOPL, mONL, PR, and RPE parameters and total retinal thicknesses between groups for the different areas and their corresponding AUCs.

Results: Peripapillary RNFL was significantly thinner in the EG group globally and in all 6 sectors assessed (P < 0.0005). For the macular variables, retinal thickness was significantly reduced in the EG group for total retinal thickness, mIRL, mRNFL, mGCL, and mIPL. The 2 best isolated parameters to discriminate between the 2 groups were pRNFL (AUC, 0.956) and mRNFL (AUC, 0.906). When mRNFL, mGCL, and mIPL measurements were combined (mGCC and mGCC plus mIPL), then its diagnostic performance improved (AUC, 0.940 and 0.952, respectively).

Conclusions: Macular RNFL, mGCC-IPL, and mGCC measurements showed a high diagnostic capability to discriminate between healthy and EG participants. However, macular intraretinal measurements still have not overcome standard pRNFL parameters. Ophthalmology 2017;124:1218-1228 © 2017 by the American Academy of Ophthalmology

Supplemental material available at www.aaojournal.org.
(GCL) is thicker than the RNFL, with an RGC body diameter approximately 10 to 20 times larger compared with their axons. In addition, the central retina has less variability in cell density compared with peripheral retina or optic nerve head parameters or even peripapillary RNFL (pRNFL) thickness. Thus, quantifying RGC loss in the macula has been shown to allow early detection of glaucomatous damage, in some cases even 5 years before functional damage can be detected. In this regard, in vivo thickness measurement of the GCC and GCL plus inner plexiform layer (IPL; GCL-IPL) with spectral-domain (SD) optical coherence tomography (OCT) devices have shown very good discriminative diagnostic ability, especially in early glaucoma (EG), but also in moderate to severe glaucoma. The new segmentation software designed for the Spectralis SD OCT (Heidelberg Engineering, Inc., Heidelberg, Germany) enables the independent quantification of all the retinal layers in the macula, including separate measurements of the 3 layers most affected by glaucoma: the mRNFL, mGCL, and mIPL. This differentiation also may contribute to improving our knowledge of the pathogenesis early in the disease in terms of which layer is affected first (cell body vs. axons).

The purpose of this study was to assess the diagnostic accuracy of this new macular retinal layer segmentation software to discriminate between healthy participants and participants with EG. This ability also was compared with that shown by the more conventional analysis of pRNFL thickness.

**Methods**

**Study Design**

This prospective, cross-sectional, multicenter, observational study complied with the tenets of the Declaration of Helsinki and was approved by the Ethical Committee of Hospital de l’Esperança-Parc de Salut Mar. Informed consent was obtained from all the participants. One eye of each participant was included in this study according to the eligibility criteria described below. If both eyes met the eligibility criteria, one eye was selected randomly.

**Participants**

The study was undertaken from February 2015 through May 2015. Participants included in this study were recruited consecutively at the Departments of Glaucoma of Hospital de l’Esperança-Parc de Salut Mar, Institut Catalá de la Retina, and Institut de la Màcula in Barcelona, Spain. All participants underwent a complete ophthalmic examination that included best-corrected visual acuity, pachymetry, slit-lamp biomicroscopy of the anterior and posterior segments, Goldmann applanation tonometry, peripapillary and macular imaging using Spectralis SD OCT (Heidelberg Eye Explorer version 1.9.13.0, Spectralis Viewing Module 6.5.2.0; Heidelberg Engineering). Swedish interactive threshold algorithm standard strategy, program 24-2 of the Humphrey Field Analyzer (Carl Zeiss Meditec, Jena, Germany), was used for VF testing of each eye. Reliability criteria were fixation losses of 20% or less, false-positive results of 15% or less, and false-negative results of 33% or less.

Inclusion criteria were as follows: patients older than 18 years of age, best-corrected visual acuity better than 20/40, refractive error of less than 5 spherical dioptries and 2 dioptries of cylinder, and open angle on gonioscopy. Exclusion criteria were previous intraocular or laser surgery except uncomplicated cataract surgery 6 months before examination, history or evidence of retinal or macular pathologic features (including drusen), systemic diseases or neurologic disorders that could produce VF or optic disc defects, and failure to obtain reliable standard automated perimetry results.

**Test Methods**

**Reference Standard.** The target condition (EG) was defined using both structural and functional evidence as suggested by Foster et al and as usually performed clinically. Glaucomatous VF defects were defined according to the criteria of Anderson in which at least 1 of the following had to be present: having a cluster of 3 or more nonedge points with $P < 0.05$ and at least 1 point with $P < 0.01$ in the pattern deviation probability plot, or pattern standard deviation of less than 5%, or glaucoma hemifield test results outside normal limits. Both EG and healthy participants underwent VF examination at least twice before the study was initiated.

Early glaucoma patients had to have elevated basal intraocular pressure (>21 mmHg), glaucomatous optic disc abnormalities evaluated by 2 glaucoma specialists (M.P. and A.G.; defined as thinning of the neuroretinal rim, notches, peripapillary hemorrhages, or RNFL defects), and glaucomatous VF defects with a mean deviation (MD) of more than −6 dB. Healthy controls had a normal optic nerve head appearance, intraocular pressure of 21 mmHg or less, and normal VF results.

**Index Test: Spectral-Domain Optical Coherence Tomography Imaging.** All participants were examined using the standard posterior pole and pRNFL protocols of the Spectralis OCT. Images had to have a quality index of at least 20 to be included in the study. Images with artifacts were excluded.

Images were acquired using the automated eye alignment eye-tracking software (TruTrack; Heidelberg Engineering) to obtain perifoveal volumetric retinal scans comprising 61 single lines of 15 frames (30° × 25° volume scan centered at the fovea). Peripapillary RNFL thickness (from internal limiting membrane to the inner aspect of the retinal pigment epithelium [RPE]) also was measured in all participants in a standard fashion using the instrument’s RNFL protocol (circular 3.5-mm diameter, 768 A-scans) and was segmented automatically using the Spectralis software. Correction for fovea–disc orientation was provided automatically by the software with the Fovea-Disc Alignment system. In all cases, foveal fixation and segmentation were checked to be correct.

Using these specific protocols, images were obtained by 3 experienced operators in 3 different centers and were sent to the main center using the Spectralis raw data sharing system, where all the images were reviewed exhaustively by a glaucoma specialist (M.P.) who was masked to clinical information and then assessed quality, alignment, and artifacts and performed macular segmentation. Layer-by-layer segmentation was executed automatically in this instrument using the new software for the Spectralis OCT (Fig 1), and it was checked to be adequate in the 61 B-scans of each imaged eye using the criteria of Ishikawa et al as a reference. In detail, we excluded eyes with incorrigible segmentation failures, which were defined as obvious disruption of the detected border, border wandering (detected border jumping to and from different anatomic structures), or both within more than 5% consecutively (i.e., an uninterrupted error) or 20% cumulatively (i.e., adding up all errors amounted to 20%
We then selected the retinal thickness map analysis to evaluate numeric averages of the different layers for each of 9 areas as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) circle (Fig 2). This consists of 3 rings of 1-mm (inner), 3-mm (intermediate), and 6-mm (outer) diameter centered at the fovea. The intermediate and outer rings were then divided into 4 zones: superior, nasal, inferior, and temporal. The average of all measurements within the inner 1-mm circle was defined as central foveal thickness. The following macular measurements were measured in each of the 9 macular areas defined by the ETDRS circle described above: (1) total retinal thickness, (2) inner retinal layers (IRLs; comprising the inner limiting membrane and the external limiting membrane), (3) mRNFL, (4) mGCL, (5) mIPL, macular inner nuclear layer, (6) macular outer plexiform layer, (7) macular outer nuclear layer, (8) photoreceptors, and (9) RPE. Global macular volume for each layer (in cubed millimeters) also was recorded. The measurements obtained for each layer in these 9 zones were recorded and used in the analysis. Mean pRNFL in each of the 6 studied zones was determined: superotemporal, superonasal, nasal, inferonasal, temporal, and inferotemporal.

**Statistical Analyses**

Baseline descriptive characteristics (age, gender, eye, B-scan quality index, and VF MD) were compared between healthy and EG groups using an unpaired Student t test for quantitative variables (after checking normality of the distribution) and the Fisher exact test for categorical variables. Then, mean thickness in different layers was compared between the groups in each sector of the ETDRS circle. The layers compared were the standard pRNFL, retina, mRNFL, macular IRL (mIRL), mGCL, mIPL, macular inner nuclear layer, macular outer plexiform layer, macular outer nuclear layer, photoreceptors, and RPE. The comparison of RPE thickness between groups was used as a negative control (a comparison where no difference is expected).
The diagnostic accuracy of the single best parameter in each layer (in terms of statistical significance and effect measure) to differentiate between healthy and diseased eyes was compared by means of the area under the receiver operating characteristics (ROC) curve (AUC). The curves show the values at different levels of sensitivity (ability to detect truly diseased patients) on the y-axis and 1-specificity (false-positive rate) on the x-axis for each parameter. The AUC summarizes the global value of the parameter, where values closer to 1 represent higher diagnostic discriminant ability. The sensitivity at 80% specificity, sensitivity at 95% specificity, positive and negative likelihood ratio, positive predictive value, negative predictive value, and best cutoff point at 95% specificity also were calculated. Also, the ROC curves and corresponding AUCs for the best macular sector in inner layers were compared using a chi-square test for difference between AUCs, including a de novo computed macular ganglion cell complex (mGCC; combining pRNFL, mGCL, and mIPL) and the mGCL plus mIPL (mGCL-mIPL). Agreement between papillar and macular parameters was evaluated with a Venn diagram showing cases of glaucoma (defined as abnormal with 95% specificity based on the best cutoff point) identified by the best pRNFL and the best macular parameters.

Finally, the relationship between functional (as measured through MD loss on the VF in decibels) and structural loss (mean thickness for pRNFL and volume for all other layers) in all patients was determined using Spearman’s ρ correlation coefficient. A locally weighted scatterplot smoother curve was fit to aid interpretation of the relationship between these variables. Briefly, a locally weighted scatterplot smoother is a nonparametric regression method in which a low-degree polynomial is fitted only to a subset of points near each x-value, with lower weights given to the points progressively further away from the particular x-value. The resulting curve fits the data better than the straight line obtained with conventional linear regression.

Missing data or intermediate index test or reference standard results were excluded from the analysis (see Fig 3 for flowchart of participants). All potentially eligible patients from the 3 centers involved who were willing to participate were recruited consecutively during the course of the study (February–May 2015), therefore, no formal sample size calculations were performed.

The method of Bonferroni was used to correct for multiple comparisons of mean layer thickness between healthy and EG eyes. Because there were 107 planned comparisons, statistical significance was considered if the P value was less than 0.0005 (approximately 0.05/107). For all other analyses, a P value of less than 0.05 was considered statistically significant. All analyses were 2-tailed. Stata IC version 13.1 (StataCorp, College Station, TX) was used to analyze the results.

Table 1. Comparison of Baseline Features between the Early Glaucoma and Healthy Control Groups

<table>
<thead>
<tr>
<th>Features</th>
<th>Early Glaucoma (n = 40)</th>
<th>Healthy Control (n = 40)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>66.3 (7.7)</td>
<td>65.3 (8.4)</td>
<td>0.55</td>
</tr>
<tr>
<td>Female gender</td>
<td>50.0</td>
<td>57.5</td>
<td>0.65</td>
</tr>
<tr>
<td>Right eye</td>
<td>50.0</td>
<td>52.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Scan quality (peripapillary)</td>
<td>25.3 (3.3)</td>
<td>25.3 (3.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Scan quality (macular)</td>
<td>25.1 (3.5)</td>
<td>25.0 (3.3)</td>
<td>0.95</td>
</tr>
<tr>
<td>Mean deviation (dB)</td>
<td>−2.26 (1.82)</td>
<td>−0.04 (1.41)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) for quantitative variables and percentages for categorical variables.
There were no differences in age, gender, or image quality between groups. As expected, MD was significantly worse in the EG group for the following layers and sectors (P < 0.0005). The EG group was significantly thinner in the EG group globally and in all 6 sectors assessed (P < 0.0005). For the macular parameters, retinal thickness was reduced significantly (P < 0.0005); the best parameter (sector) of each macular layer: mRNFL, mGCL, and the newly calculated mGCC (AUC, 0.940) for the temporal-inferior pRNFL was larger than the AUC for outer-inferior mIRL (P < 0.0005), but all other paired comparisons between these 4 parameters were not statistically significant (P ≥ 0.10). Figure 6 shows ROC curves and corresponding AUCs for the best parameter (sector) of each macular layer: mRNFL, mGCL, mIPL, and the newly calculated mGCC (AUC, 0.940) and mGCL-mIPL (AUC, 0.952). The between-group differences were statistically significant (P = 0.02). On paired comparisons, the AUC for temporal-inferior pRNFL was larger than the AUC for outer-inferior mIRL (P = 0.02) and the inner-temporal mGCL (P = 0.005), but all other paired comparisons between these 4 parameters were not statistically significant (P ≥ 0.10).

### Table 2. Diagnostic Performance in Decreasing Order of the Most Relevant Parameters on Each Layer on Bivariate Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Area under the Receiver Operating Characteristic Curve (95% Confidence Interval)</th>
<th>Sensitivity at 80% Specificity</th>
<th>Sensitivity at 95% Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>LR +</th>
<th>LR −</th>
<th>Best Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNFL T/Inf</td>
<td>0.956 (0.912—0.999)</td>
<td>92.5</td>
<td>87.5</td>
<td>92.1</td>
<td>88.1</td>
<td>17.5</td>
<td>0.13</td>
<td>123</td>
</tr>
<tr>
<td>mGCL-mIPL O/T</td>
<td>0.952 (0.911—0.992)</td>
<td>90.0</td>
<td>67.5</td>
<td>89.2</td>
<td>83.7</td>
<td>13.5</td>
<td>0.34</td>
<td>56</td>
</tr>
<tr>
<td>mGCC O/T</td>
<td>0.940 (0.893—0.986)</td>
<td>90.0</td>
<td>65.0</td>
<td>89.2</td>
<td>83.7</td>
<td>13.0</td>
<td>0.37</td>
<td>76</td>
</tr>
<tr>
<td>mRNFL O/Inf</td>
<td>0.906 (0.836—0.976)</td>
<td>87.5</td>
<td>72.5</td>
<td>89.7</td>
<td>87.8</td>
<td>14.5</td>
<td>0.29</td>
<td>32</td>
</tr>
<tr>
<td>mIRL O/Inf</td>
<td>0.876 (0.800—0.952)</td>
<td>80.0</td>
<td>67.5</td>
<td>82.1</td>
<td>80.5</td>
<td>13.5</td>
<td>0.34</td>
<td>192</td>
</tr>
<tr>
<td>Retina O/Inf</td>
<td>0.862 (0.782—0.942)</td>
<td>80.0</td>
<td>60.0</td>
<td>82.1</td>
<td>80.5</td>
<td>12.0</td>
<td>0.42</td>
<td>266</td>
</tr>
<tr>
<td>mGCL Inn/T</td>
<td>0.858 (0.777—0.938)</td>
<td>75.0</td>
<td>55.0</td>
<td>82.7</td>
<td>75.6</td>
<td>14.3</td>
<td>0.47</td>
<td>37</td>
</tr>
<tr>
<td>mIPL Inn/T</td>
<td>0.854 (0.775—0.934)</td>
<td>70.0</td>
<td>45.0</td>
<td>77.8</td>
<td>72.7</td>
<td>12.2</td>
<td>0.57</td>
<td>34</td>
</tr>
</tbody>
</table>

**Results**

**Participants**

One hundred eyes meeting inclusion criteria were evaluated for this study. Three eyes were excluded in the healthy group and 17 in the EG group (see Fig 3 for the flow of participants by group). Considering the 20 excluded eyes overall (3 healthy eyes and 17 EG eyes), 9 eyes were excluded for low-quality signal strength (macular only in 4, peripapillary only in 3, and 2 in both macula and peripapillary images), 5 eyes were excluded because of artifacts (i.e., incomplete images; 1 in the peripapillary images and 4 in the macular scans), and 6 were excluded because of incorrigible macular segmentation. Analysis finally was performed on 40 glaucomatous eyes of 40 participants and 40 healthy eyes of 40 normal participants. Of the final 80 eyes included, segmentation was modified manually in 7 (2 in the normal group and 5 in the glaucoma group). The demographic and clinical characteristics of each group are summarized in Table 1. There were no differences in age, gender, or image quality between groups. As expected, MD was significantly worse in patients with the target condition (MD, −2.26±1.82 dB) than in normal participants (MD, −0.04±1.41 dB). All the ophthalmic examinations, perimetry tests, and OCT analyses were performed within 1 week of the participant’s date of enrollment into the study.

**Test Results**

The comparison of the mean thickness of each retinal layer in each sector of the ETDRS circle (and volume for the global macula, 6 mm) between groups is shown in Figure 4 (for complete data, please see Tables S1 and S2, available at www.aaojournal.org). Peripapillary RNFL was significantly thinner in the EG group globally and in all 6 sectors assessed (P < 0.0005). For the macular parameters, retinal thickness was reduced significantly in the EG group for the following layers and sectors (P < 0.0005): total retinal thickness (inner temporal, outer superior, and outer inferior), mIRL (inner inferior and temporal and all the outer sectors), mRNFL (inner inferior, outer superior, outer nasal, and outer inferior), mGCL (all the sectors studied except the central area), and mIPL (inner superior, inner inferior, inner temporal, outer inferior, and outer temporal). Volume was significantly lower for the EG group in the IRL, mRNFL, mGCL, and mIPL (P < 0.0005) when compared with healthy controls. The rest of parameters and layers did not show statistically significant differences.

Table 2 shows the values for AUC; sensitivity at 80% specificity; and sensitivity, positive likelihood ratio (LR+) and negative likelihood ratio (LR−), positive predictive value, negative predictive value, and best cutoff values at 95% specificity for the single best parameter in each layer for which at least 1 statistically significant result was obtained (Table S1, available at www.aaojournal.org). The 3 best parameters were the temporal-inferior sector of pRNFL (AUC, 0.956), the outer-inferior sector of mRNFL (AUC, 0.906), and the outer-inferior sector of mIPL (AUC, 0.876). The mGCL and mIPL parameters showed an AUC of 0.858 and 0.854 for the inner-temporal and inner-inferior sectors, respectively. The corresponding ROC curves for the 3 parameters with the largest AUCs (temporal-inferior pRNFL, outer-inferior mRNFL, and outer-inferior mIPL) and mGCL (inner temporal) are shown in Figure 5. There were statistically significant differences between these 4 parameters (P = 0.0024). The AUC for temporal-inferior pRNFL was larger than the AUC for outer-inferior mIRL (P = 0.02) and the inner-temporal mGCL (P = 0.005), but all other paired comparisons between these 4 parameters were not statistically significant (P ≥ 0.10). Figure 6 shows ROC curves and corresponding AUCs for the best parameter (sector) of each macular layer: mRNFL, mGCL, mIPL, and the newly calculated mGCC (AUC, 0.940) and mGCL-mIPL (AUC, 0.952). The between-group differences were statistically significant (P = 0.02). On paired comparisons, the data represent mean (standard deviation) thickness in micrometers except for volume (Vol), which is measured in cubed millimeters. Differences between groups may not be exactly the subtraction of one group from the other because of rounding. Statistically significant results after Bonferroni correction (P < 0.0005) are marked with an asterisk (*) and shaded in the corresponding sector. All the graphics are shown as right-eye orientation. IRL = macular inner retinal layer; mGCL = macular ganglion cell layer; mINL = macular inner nuclear layer; mIPL = macular inner plexiform layer; mONL = macular outer nuclear layer; mOPL = macular outer plexiform layer; mRNFL = macular retinal nerve fiber layer; PR = photoreceptor; RPE = retinal pigment epithelium.
the AUC for mGCC was statistically larger than the AUC for mGCL (\(P = 0.03\)) and mIPL (\(P = 0.01\)), whereas the mGCL-mIPL AUC was larger than the AUC for mGCL (\(P = 0.01\)) and mIPL (\(P = 0.004\)). All other paired comparisons were not statistically significant (\(P \geq 0.17\)).

Using the best cutoff point to compare the agreement between pRNFL versus mRNFL and pRNFL versus mGCL-mIPL to diagnose glaucoma (Fig 7), most glaucomatous patients were found to show abnormal results with 95% specificity with both macular and peripapillary parameters (28/40 with mRNFL and 26/40 with mGCL-mIPL). However, pRNFL alone was able to identify 9 and 7 eyes that would have been missed by mGCL-mIPL and mRNFL, respectively. Inversely, only 1 eye was found to be glaucomatous with macular and not peripapillary parameters.

The correlation between functional and structural damage in each layer in all eyes is shown in Table 3. This association was significant for pRNFL, total retinal volume, IRL, mRNFL, mGCL, and IPL (\(P < 0.0005\)). Figure 8 shows the scatterplots of the statistically significant relationships with the locally weighted scatterplot smoother, suggesting that the relationship between each layer thickness or volume and VF MD seems linear for most layers in the range evaluated (mild functional loss).

**Discussion**

Recently, there has been an increasing interest on the importance of evaluating the macula in glaucoma diagnosis and management.\(^{18}\) Glaucomatous damage to the macula is frequent, can occur early in the disease, and can be underestimated with standard VF tests.\(^{19}\) Moreover, GCL thickness measurements show less variability than conventional RNFL and optic disc parameters.\(^{8}\) Since the performance of some initial studies with time-domain OCT,\(^{16,20}\) the advent of SD OCT technology has permitted a more rapid acquisition of retinal images at a higher axial-image resolution, allowing the discrimination and measurement of individual retinal layers.\(^{21}\) Currently, there are different OCT instruments available, and
Table 3. Spearman’s ρ Correlation Coefficient between Mean Deviation in the Visual Field and Each Retinal Layer Volume or Thickness in All Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spearman’s ρ</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNFL</td>
<td>0.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Retina</td>
<td>0.43</td>
<td>0.0001</td>
</tr>
<tr>
<td>mRNFL</td>
<td>0.42</td>
<td>0.0001</td>
</tr>
<tr>
<td>mIRL</td>
<td>0.47</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mGCL</td>
<td>0.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mPL</td>
<td>0.43</td>
<td>0.0001</td>
</tr>
<tr>
<td>mNL</td>
<td>0.03</td>
<td>0.82</td>
</tr>
<tr>
<td>mOPL</td>
<td>-0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>mONL</td>
<td>0.12</td>
<td>0.28</td>
</tr>
<tr>
<td>Photoreceptors</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>RPE</td>
<td>-0.10</td>
<td>0.38</td>
</tr>
</tbody>
</table>

mGCL = macular ganglion cell layer; mNL = macular inner nuclear layer; mPL = macular inner plexiform layer; mIRL = macular inner retinal layer; mONL = macular outer nuclear layer; mOPL = macular outer plexiform layer; mRNFL = macular retinal nerve fiber layer; pRNFL = peripapillary retinal nerve fiber layer; RPE = retinal pigment epithelium.

For pRNFL, the measure used is mean thickness (in micrometers). For all other layers, total volume (cubed millimeters) is used.

depending on the model, different retinal segmentation algorithms are provided. Thus, the Cirrus HD-OCT (Carl Zeiss Meditec) evaluates the GCL together with the IPL (GCL-IPL), and the RTVue OCT (Optovue, Inc., Fremont, CA) examines the entire GCC (RNFL, GCL, and IPL). The new software of the Spectralis OCT is able to measure each of the 3 layers of the complex individually as well as the rest of retinal layers. Most of the OCT studies evaluating internal retinal layers in the macula have shown a diagnostic accuracy similar to pRNFL thickness. However, this type of isolated ganglion cell analysis improves glaucomatous diagnostic capacity remains unclear.

In this study, we found that using this new macular segmentation software of the Spectralis OCT, pRNFL still performed better than the other retinal macular layers assessed separately (AUC, 0.956). The parameter with the second best AUC was mRNFL outer inferior (AUC, 0.906), which was lower than pRNFL, but the differences were not statistically significant. Isolated mGCL showed less diagnostic ability (AUC, 0.858; P = 0.005). Even in cases of disagreement between macular and peripapillary parameters using the best cutoff point to identify glaucoma (abnormal with 95% specificity; Fig 7), pRNFL measurements were able to identify 9 eyes that would have been missed by mGCC-IPL versus only 1 patient who would have been diagnosed by macular parameters only. Similarly, earlier studies comparing time-domain and SD OCT macular and peripapillary measurements have shown comparable discriminating power for glaucoma detection, and 2 recent systematic reviews have concluded that pRNFL parameters are still preferable to macular parameters for diagnosing manifest glaucoma, but that the differences are small. In contrast, a previous study with a preliminary version of this segmentation found that mRNFL was the best discriminating parameter even compared with pRNFL (AUC, 0.742 and 0.595, respectively). These differences, including their lower AUC values, may be because in this study, controls were compared with glaucoma suspects instead of eyes with established disease. More recently, a similar study using the same new segmentation software found that mRNFL (AUC, 0.915) and mGCL (AUC, 0.914) had the best AUCs and that they were not statistically different from pRNFL (AUC, 0.878), suggesting that the ability of mRNFL and mGCL to discriminate glaucoma was high and comparable with that of pRNFL. Several reasons may explain these small differences. First, their population was Korean, with a higher prevalence of normal-tension glaucoma with glaucomatous damage closer to the fovea and higher chances of detecting it with a macular scan. Second, approximately 35% of the glaucomatous eyes included had moderate to severe disease, and therefore their mean MD was greater than in our study (mean MD, −5.47±6.79 dB vs. −2.26±1.82 dB). Third, pRNFL AUC was calculated using global pRNFL thickness only, which may underestimate regional papillary damage (favoring macular parameters), especially considering that most of the glaucomatous eyes included showed mild disease (65%).

We wanted to study if there were differences in glaucomatous diagnostic performance if GCC layers are measured separately or as a whole. In this regard, we calculated 2 de novo parameters adding layers accordingly (GCL-IPL and GCC). In our study, this improved macular diagnostic accuracy, and mGCL-IPL (outer temporal) and mGCC (outer temporal) showed very good discriminative results (AUC, 0.952 and 0.940, respectively), comparable with those obtained by pRNFL and better than isolated mGCL and mIPL (P < 0.05). Although not strictly comparable, these results are similar to those of previous studies performed by Mwanza et al using HD-Cirrus in which minimum GCL-IPL and inferotemporal GCL-IPL had an AUC of 0.959 and 0.956, respectively, and were not different to that of the best pRNFL and optic nerve head parameters. An explanation for this could be that segmenting thinner layers may be less precise than considering layers together, or that the separation from cell bodies and dendrites may be somehow unnatural. In this regard, the same study evaluating SD Spectralis segmentation software and discussed previously also found that the diagnostic ability of mGCC was the highest (AUC, 0.925), but was not significantly different from that of mGCL (AUC, 0.914), mGCL-mIPL (AUC, 0.895), or pRNFL (AUC, 0.878). Further studies to compare these measurements are needed to investigate these hypotheses.

The regional distribution of the RNFL defects observed was consistent with the data available in the literature. Superior and inferior zones of pRNFL showed the best diagnostic performance. At the level of the macula, the fiber layer is thicker in areas closer to the disc, and that is where the highest differences were found (Fig 4). For the rest of the ganglion cell parameters, differences were found overall, except for the fovea, in which the density of ganglion cells is very low.

Considering our results, we hypothesize that the first detectable changes at early stages of glaucoma may occur at
the level of the RNFL containing the ganglion cell axons, whereas modifications in the layer containing the ganglion cell bodies (GCL) and dendrites (IPL) may take place at a later stage. This reasoning is in agreement with previous histologic findings that indicate that most cell body death happens after axon degeneration. Nevertheless, and given the cross-sectional nature of our study, longitudinal studies are needed to confirm the timing of these events. Additionally, the behavior of RNFL and GCL thickness in OCT could be different in other optic neuropathies. Early mGCL thinning has been found to precede pRNFL damage in multiple sclerosis with and also without prior clinical optic neuritis and at early stages (2 weeks after onset) of ischemic optic neuropathy.

Finally, in these EG eyes (mean MD, −2.28 dB), the association of VF MD with global measurements was statistically significant for pRNFL thickness, total retinal volume, IRL volume, mGCL volume, and mIPL volume. Global mGCL volume showed a correlation to MD ($r = 0.51; P < 0.0001$) of a strength similar to that demonstrated between MD and pRNFL thickness ($r = 0.53; P < 0.0001$). These results are similar to those reported in other studies.

This study has several limitations. First, although ultimately we did not use the OCT results (index test) directly to
diagnose the target condition, we did use optic disc appearance (which is not independent from RNFL) to classify the cases, and this may have restricted the entry of participants into either the healthy or the EG group (incorporation bias), which may have overestimated diagnostic accuracy. However, the most widely accepted definition of glaucoma is based both on structural and functional criteria, and this is what is carried out clinically to ensure that the condition is present. Moreover, we considered that it was particularly important to keep structural criteria in this study to be able to include very early glaucoma cases in whom visual defects are scarce and frequently not typical. Second, our study included only eyes diagnosed with glaucoma based on abnormal perimetric results and a MD of not more than −6 dB, without inclusion of patients showing structural damage alone. Third, macular parameters may not be obtainable adequately in a non-negligible proportion of eyes (16%). Fourth, the lack of a normative database for the Spectralis software does not allow us to calculate how many patients were in or out of the normal range. If that information was available, it would be very interesting to know how many patients had normal pRNFL parameters while mRNFL or mGCL already were affected or vice versa. However, we tried to provide similar information about this agreement using a Venn diagram showing the number of cases defined as abnormal by the best peripapillary and macular parameters.

In conclusion, our findings indicate that with the new Spectralis retinal segmentation software, pRNFL thickness has still not been overcome as a surrogate marker in glaucoma assessment. However, isolated mRNFL showed a high ability to discriminate between healthy and EG participants. When mRNFL, mGCL, and mIPL measurements were added and assessed together (mGCC and mGCL-mIPL), then its diagnostic accuracy improved to being equivalent of that of pRNFL. A significant structural–functional association was found between MD of the VF and mean volumes of the GCC layers.

References


Footnotes and Financial Disclosures

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Abbreviations and Acronyms:
+ = positive; − = negative; AUC = area under the receiver operating characteristic curve; EG = early glaucoma; ETDRS = Early Treatment Diagnostic Retinopathy Study; GCC = ganglion cell complex; GCL = ganglion cell layer; GCL-IPL = ganglion cell layer plus inner plexiform layer; Inn = inner; Inf = inferior; IPL = inner plexiform layer; IRL = inner retinal layer; LR = likelihood ratio; MD = mean deviation; mGCC = macular ganglion cell complex; mGCL = macular ganglion cell layer; mGCL-mIPL = macular ganglion cell layer plus macular inner plexiform layer; mINL = macular inner nuclear layer; mIPL = macular inner plexiform layer; mIRL = macular inner retinal layer; mONL = macular outer nuclear layer; mOPL = macular outer plexiform layer; mRNFL = macular retinal nerve fiber layer; O = outer; OCT = optical coherence tomography; PR = photoreceptors; pRNFL = peripapillary retinal nerve fiber layer; RGC = retinal ganglion cell; RNFL = retinal nerve fiber layer; ROC = receiver operating characteristic; RPE = retinal pigmentary epithelium; SD = spectral-domain; Sup = superior; T = temporal; VF = visual field.

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