Automatic Segmentation of Posterior Pole Retinal Layers In Patients with Early Stage Glaucoma Using Spectral Domain Optical Coherence Tomography

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Abstract

Purpose: To measure Ganglion Cell Layer (GCL) and Retinal Nerve Fiber Layer (RNFL) thickness of the retinal posterior pole in patients with early stage primary open-angle glaucoma (POAG) using the new automatic segmentation technology of spectral domain optical coherence tomograph (SD-OCT).

Methods: 37 clinical records of patients with early glaucoma (grade 1 to 2 according to the Glaucoma Staging System 2) and 40 age and sex-matched controls were considered in this case-control observational retrospective study. Automated segmentation of GCL and RNFL was performed in one randomly selected eye from the electronic OCT records of each participant using the new Spectralis SD-OCT segmentation technology (Heidelberg Engineering, Inc., Heidelberg, Germany). Thickness of different retinal layers was obtained from each Posterior Pole volumetric scan. Measurements of the peripapillary RNFL thickness (pRNFLT) were also obtained and then compared with those of posterior pole RNFL thickness (ppRNFLT).

Results: Both GCL and RNFL were significantly thinner at the retinal posterior pole in the POAG group as compared to the control group (p<0.0001). Furthermore, pRNFLT was significantly thinner in the glaucoma group as compared to the control group (p<0.0001). Measurements of pRNFLT were significantly correlated with those of the ppRNFLT (Pearson’s coefficient r=0.863).

Conclusions: The new Spectralis SD-OCT automatic segmentation tool may be useful in evaluating structural damage in patients with early glaucoma, by providing complementary measurements to the clinical assessment of glaucoma that could be used in conjunction with other relevant parameters in the diagnosis and the evaluation of the progression of the disease.

Keywords: Glaucoma; SD-OCT; Ganglion cell layer; Retinal nerve fiber layer; Retinal posterior pole

Introduction

Glaucoma is an optic neuropathy that can lead to progressive and irreversible vision loss due to ganglion cell death. It clinically manifests as characteristic optic nerve head (ONH) and retinal nerve fiber layer (RNFL) alterations with correlating visual field changes [1] and represents the second cause of partially preventable blindness globally [2]. Optical coherence tomography, a well-accepted tool for the fiber

large volumes of data is of great value for clinicians and scientists to quickly investigate retinal and optic nerve head alterations.

Evaluation of intra-retinal layer thickness plays an important role in the diagnosis and monitoring of various ocular diseases.

Several computer-automated algorithms for intra-retinal layer segmentation have been proposed to overcome the limits of most of the commercial systems, in terms of capacity to measure the thickness of only a few retinal layers. These algorithms applied to the high resolution SD-OCT instruments allow quantitative evaluation of the thickness of all intra-retinal layers [11].

Segmentation is a critical step towards reliable quantification of total retinal, RNFL and GCC thickness that actually represent helpful parameters in elucidating either the presence or progression of glaucoma. However, fully automated segmentation of retinal OCT scans is challenging due to intrinsic speckle noise, the possible presence of blood vessels and other artifacts (e.g. motion, reduced illumination).

The aim of this case-control study was to evaluate the Ganglion Cell Layer (GCL) and Retinal Nerve Fiber Layer (RNFL) thickness at the retinal Posterior Pole in patients with early stage primary open angle glaucoma (POAG) and to compare the obtained results with those of
healthy subjects, using the new "Spectralis" spectral domain optical coherence tomography (SD-OCT) segmentation technology (Spectralis device, software version 6.0).

Materials and Methods

The study was approved by the internal review board of the University Hospital of Tor Vergata, Rome and the research followed the tenets of the Declaration of Helsinki.

The medical records of 37 glaucoma patients from the glaucoma clinic of the University Hospital of Tor Vergata were included in this study.

All subjects considered in this study had extensive ophthalmologic examinations available in their clinical records, including best-corrected visual acuity (BCVA), Goldmann applanation tonometry, central corneal thickness (CCT) by ultrasound pachymetry measurements, gonioscopy, slit-lamp biomicroscopy with dilated fundus examination, Standard Automated Perimetry (SAP) with SITA-Standard program 24-2 of a Humphrey Field Analyzer (model 750, Zeiss Humphrey Systems, Dublin, CA, USA).

Diagnosis of glaucoma was based on the occurrence of typical glaucomatous optic disc changes with corresponding visual field defects. Optic disc changes consisted in cup-to-disc ratio (CDR) greater than 0.5 in either eye and/or CDR asymmetry greater than or equal to 0.2 and/or presence of focal thinning of the rim in either eye.

A glaucomatous visual field defect was defined as a glaucoma hemifield test outside of the range of normal limits, pattern standard deviation (PSD) with a P value less than 5%, or a cluster of three points or more in the pattern deviation plot in a single hemifield (superior or inferior) with a P value of less than 5%, one of which having a P value of less than 1%.

All of the patients were classified as having early stage glaucoma (1 and 2) according to the Glaucoma Staging System 2 criteria [12].

All patients affected by glaucoma were treated in accordance with the EGS guidelines [13].

Those with the presence of any retinal pathology or optic nerve disease other than glaucoma and spherical or cylindrical refractive errors higher than 3 and 2 diopters (D) respectively identified in medical records were excluded.

Medical records of 40 age and sex-matched healthy subjects formed the control group. Inclusion criteria were as follows: spherical or cylindrical refractive errors lower than 3 and 2 D, respectively; normal intraocular pressure (<21 mmHg); normal CCT, normal appearance of the optic disc; normal visual field; no significant ocular disease found by routine ophthalmologic examination; and no family history of glaucoma or systemic diseases with possible ocular involvement.

Measurements of Posterior Pole RNFL and GCL thickness and peripapillary RNFL thickness were performed using SD-OCT imaging (Spectralis; Heidelberg Engineering, Heidelberg, Germany).

Both eyes of each included patient were considered, but only one eye was randomly chosen for statistical analysis.

Images were acquired using image alignment eye-tracking software (TruTrack; Heidelberg Engineering GmbH) as this improves scan reproducibility [14].
retinal nerve fiber layer, along a 6° radius circle scan centered on the optic nerve head.

All scans using the Posterior Pole and RNFLt protocols were acquired after pupil dilation with 0.5% tropicamide and 10% phenylephrine (Visumidriatic Fenilefrina, Visufarma).

Only high-quality scans, defined as scans with signal quality >25, where the quality score range is 0 (poor quality) to 40 (excellent quality), without discontinuity or misalignment, poor illumination, involuntary saccadic eye movements or blinking artifacts on careful visual inspection were used for analysis. The same experienced operator applied manual correction to the OCT automatic segmentation when necessary; however these cases were excluded from the study.

For maximum reproducibility, an internal fixation target was used [15]. In particular, to acquire the posterior pole and the peripapillary RNFL scans, the patient was asked to fixate on a central target and a nasal target, respectively.

Repeatability and reproducibility of the retinal layers thickness measurements obtained from the Spectralis posterior pole scans using the new segmentation algorithm were evaluated from the OCT electronical records of 8 healthy subjects who had undergone the Posterior Pole scan three times on the same day in the same eye. In particular, repeatability and reproducibility were calculated for each of the 64 volumetric units constituting the "posterior pole" scan map of each segmented layer.

Statistical analysis

All data were initially entered into an EXCEL database (Microsoft, Redmond, Washington – United States) and the analysis was performed using the SPSS vers. 20.0 (SPSS, Chicago, Illinois, USA) and the NCSS and PASS vers.11.0 for power analysis.

Values in the text and tables are presented as the mean ± standard deviation (SD). Preliminary analyses were performed to ensure that there was no violation of the assumptions of normality and linearity.

Descriptive statistics consisted of the mean ± standard deviation values for parameters with Gaussian distributions (after confirmation with histograms and the Kolgomorov-Smirnov test). The level of statistical power to detect a difference between the two groups was 99% with an alpha=0.5 for N=37 subjects.

The data were analyzed using a one-way analysis of variance (ANOVA) and a Bonferroni test for multiple comparisons.

The coefficient of variation (CV %) was used to determine repeatability and reproducibility of SD-OCT measurements of all the analyzed retinal layers, for each of the 64 volumetric units constituting the "posterior pole" scan map.

Pearson's test was used to identify statistical correlations between pRNFLt and ppRNFLt. A p value of <0.05 was considered statistically significant.

Results

A total of 37 eyes from 37 patients (22 males and 15 females, mean age 59.4 ± 7.6 years, range 49 to 70 years) with early stage glaucoma (18 stage 1 and 19 stage 2 POAG patients) and 40 eyes of 40 healthy individuals (23 males and 17 females, mean age 58.3 ± 9.7 years, range 49 to 71) were included in the study. 3 POAG patients were excluded from the study, as manual adjustments of B-scan retinal segmentation were necessary.

Mean refractive error was -1.70 ± 2.31 diopters, ranging from -2.50D to +1.00D.

Both intraocular pressure (mean value 15.3 ± 1.2 mmHg) and central corneal thickness (mean value 557 ± 8 micron) were in the normal range.

Furthermore, the above parameters did not differ significantly between groups (p=0.543, and 0.413, respectively).

Thickness measurements of the different retinal layers provided by the new segmentation algorithm of the Spectralis SD-OCT “Posterior Pole” scans showed significant thinning of the total retinal, RNFL and GCL thickness in patients with early glaucoma when compared to controls, as demonstrated by the ANOVA test (p<0.0001). These results are presented in Table 1; graphs in Figures 2 and 3 show the statistically significant differences in the thickness of GCL and RNFL between the two groups. Figure 4 shows a comparison between a Posterior Pole GCL thickness map of a glaucoma patient and of a healthy subject, as obtained using the inbuilt Spectralis mapping software.
ANOVA test

<table>
<thead>
<tr>
<th>Group</th>
<th>N*</th>
<th>Retina**</th>
<th>GCL**</th>
<th>RNFL**</th>
<th>P§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)#</td>
<td></td>
</tr>
<tr>
<td>Glaucoma</td>
<td>37</td>
<td>274.8 ± 15.0</td>
<td>27.6 ± 4.4</td>
<td>34.0 ± 7.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>294.13 ± 9.0</td>
<td>32.6 ± 9.0</td>
<td>45.0 ± 5.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*N: number of sample subjects **Retina: total retinal thickness; RNFL: retinal nerve fiber layer; GCL: ganglion cell layer #mean ± SD: mean value ± Standard deviation §p: p value

Table 1: Spectralis SD-OCT retinal posterior pole thickness values of Retina, Ganglion Cell Layer and Retinal Nerve Fiber Layer in patients with glaucoma compared to controls.

Figure 4: Comparison between Ganglion Cell Layer thickness map of a glaucoma patient (left) and of a healthy subject (right) as obtained using the inbuilt Spectralis mapping software.

Peripapillary RNFL thickness values differed significantly globally and in all sectors (p<0.001) between the two groups.

Pearson’s correlation coefficient analysis showed that Posterior Pole RNFL thickness (ppRNFLt) correlated strongly with pRNFLt values (r=0.863) in glaucoma patients.

Signal strength did not differ between the glaucoma group and the healthy group (p=0.762).

The within-subject median CV% values and the between-subject median CV% values of all repeated measurements were found to be <5% for Total Retinal Thickness (Retina), GCL and Posterior Pole RNFL values, as shown in Tables 2 and 3, respectively.

Table 4 shows the between-subject median CV% values of each of the 64 retinal volumetric (total retinal thickness) units constituting the Spectralis Posterior Pole map. The values reproduce the topographical arrangement of the Spectralis Posterior Pole map with the exclusion of fovea-to-disc inclination; specifically T stands for temporal, N nasal, S superior and I inferior. Values in bold characters indicate very high reproducibility of the SD-OCT repeated measurements, while values in black indicate high reproducibility.
<table>
<thead>
<tr>
<th>Layer</th>
<th>CV%*</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>0.00</td>
<td>2.29</td>
<td></td>
</tr>
<tr>
<td>Subject 2</td>
<td>0.25</td>
<td>0.00</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Subject 3</td>
<td>0.77</td>
<td>0.18</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Subject 4</td>
<td>0.75</td>
<td>0.17</td>
<td>2.73</td>
<td></td>
</tr>
<tr>
<td>Subject 5</td>
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<td>0.17</td>
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<td>Subject 6</td>
<td>0.52</td>
<td>0.00</td>
<td>0.93</td>
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<td>Subject 7</td>
<td>0.54</td>
<td>0.17</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Subject 8</td>
<td>0.73</td>
<td>0.17</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>RNFL**</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<td>0.00</td>
<td>10.19</td>
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<td>18.55</td>
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<tr>
<td>Subject 4</td>
<td>4.55</td>
<td>0.93</td>
<td>14.04</td>
<td></td>
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<tr>
<td>Subject 5</td>
<td>4.61</td>
<td>0.00</td>
<td>0.78</td>
<td></td>
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<tr>
<td>Subject 6</td>
<td>3.03</td>
<td>0.00</td>
<td>12.49</td>
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<td>3.85</td>
<td>0.00</td>
<td>13.07</td>
<td></td>
</tr>
<tr>
<td>GCL**</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 1</td>
<td>2.63</td>
<td>0.00</td>
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<td>2.67</td>
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<td>7.81</td>
<td></td>
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<tr>
<td>Subject 4</td>
<td>3.74</td>
<td>0.00</td>
<td>16.47</td>
<td></td>
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<tr>
<td>Subject 5</td>
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<td>0.00</td>
<td>19.68</td>
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<tr>
<td>Subject 8</td>
<td>3.23</td>
<td>0.00</td>
<td>16.90</td>
<td></td>
</tr>
</tbody>
</table>

*CV% : Coefficient of Variation; **Retina: total retinal thickness; RNFL: retinal nerve fiber layer; GCL: ganglion cell layer

Table 2: Within-subject Coefficient of Variation % for the different retinal layers: median, minimum and maximum values of Posterior Pole total retinal (Retina), Retinal Nerve Fiber Layer (RNFL) and Ganglion Cell Layer (GCL) thickness.

**Table 3:** Between-subject Coefficient of Variation % for the different retinal layer thicknesses: median, minimum and maximum values.

<table>
<thead>
<tr>
<th>N</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.98</td>
<td>0.48</td>
<td>0.57</td>
</tr>
<tr>
<td>0.67</td>
<td>0.75</td>
<td>0.50</td>
</tr>
<tr>
<td>0.92</td>
<td>0.56</td>
<td>0.75</td>
</tr>
<tr>
<td>0.68</td>
<td>0.66</td>
<td>0.50</td>
</tr>
<tr>
<td>0.48</td>
<td>0.63</td>
<td>0.37</td>
</tr>
<tr>
<td>0.73</td>
<td>0.45</td>
<td>0.76</td>
</tr>
<tr>
<td>0.32</td>
<td>0.62</td>
<td>0.80</td>
</tr>
<tr>
<td>0.76</td>
<td>0.00</td>
<td>0.62</td>
</tr>
<tr>
<td>0.82</td>
<td>0.64</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Bold characters indicate a very high repeatability of the SD-OCT repeated measurements, while values in black indicate a high repeatability. T: Temporal; N: Nasal; S: Superior; I: Inferior

**Table 4:** Between-subject Coefficient of Variation %: median values of Posterior Pole total retinal thickness (Retina).

Table: Retina: total retinal thickness; RNFL: retinal nerve fiber layer; GCL: ganglion cell layer

**Discussion**

The new segmentation algorithm of the Spectralis SD-OCT allowed us to demonstrate a significant reduction of Total Retina, GCL and RNFL thickness in the whole retinal posterior pole in a group of patients with early glaucoma when compared to controls (p<0.000, Figures 1 and 2).

There are several reasons to perform an accurate study of the central retina in these patients: one of which being that more than 50% of the ganglion cells are located in this region and the GCL is more than one cell-layer thick at this level. Moreover, it is well known that in addition to the changes that occur in the optic nerve head and RNFL, the macular region also manifests significant changes in the eyes of patients suffering from POAG [16,17].

There are several reasons to perform an accurate study of the central retina in these patients: one of which being that more than 50% of the ganglion cells are located in this region and the GCL is more than one cell-layer thick at this level. Moreover, it is well known that in addition to the changes that occur in the optic nerve head and RNFL, the macular region also manifests significant changes in the eyes of patients suffering from POAG [16,17].

As such, the macular area and posterior pole may represent anatomic sites of particular significance in the identification of morphological changes in POAG patients. Compared to OCT evaluation limited to the macular area, whole retinal Posterior Pole analysis may better represent the structural correlates of glaucomatous functional damage by SAP visual fields performed by ophthalmologists in normal clinical practice.

Alterations of the central retina have been previously described using time-domain OCT in eyes affected by glaucoma, in addition to peripapillary RNFL thinning.

However, pRNFLt may also be altered in high myopia, tilted disc, papilledema and pseudopapilledema. On the other hand, pRNFLt may also be altered by ocular diseases other than glaucoma (for example: epiretinal membranes, macular edema).

Therefore, clear differentiation of the ganglion cell layer and the retinal nerve fiber layer at the posterior pole as separate entities, together with the measurement of thickness, is of particular interest in providing a more reliable structural evaluation of eyes of POAG patients.

Previous studies using Stratus OCT have reported slight inferiority of macular thickness measurements when compared to peripapillary RNFL measurements in detecting glaucomatous change [3,4].

One possible reason may be that the limited resolution of Stratus OCT only permits measurement of the entire retinal thickness, whereas the changes in glaucoma preferentially occur at the inner retinal layers.

However SD-OCT is capable of measuring individual layers of the retina and as such is more likely to capture these changes in the macular area, and therefore analysis of the macular ganglion cell complex using this technique has been proposed as a surrogate method for the evaluation of glaucomatous damage [18-23]. Recent studies have demonstrated that thickness measurements of the innermost layers of the retina, namely the retinal nerve fiber layer, ganglion cell layer and inner plexiform layer using a SD-OCT ganglion cell complex analysis algorithm, were as effective as RNFL measurements in detecting structural changes in glaucoma and in monitoring the progression of the disease [19-26].

A significant decrease of peripapillary RNFL thickness was similarly found in our early POAG patients using the Spectralis RNFL scanning protocol as compared to the control group (p<0.001). However, in the present study, in addition to pRNFLt we also measured RNFL thickness of the whole retinal posterior pole in these patients. Of note, our results demonstrated in glaucoma patients a significant correlation...
between ppRNFLt values, as obtained by automated segmentation of the Posterior Pole scan, with pRNFLt (r=0.863) as obtained by the inbuilt scanning protocol. Only recently has the repeatability of pRNFLt values with Fourier-domain OCT been reported in the findings of the Advanced Imaging for Glaucoma Study: Design, Baseline Characteristics, and Inter-site Comparison [27].

Awareness of the level of repeatability and reproducibility of such measurements is very important in terms of clinical application: a previous study reported an excellent repeatability and reproducibility of thickness measurements of each of the individual retinal layers at the centre of the fovea in a young, healthy cohort, using the newly available Spectralis segmentation software [28].

In our paper, this segmentation software was applied to the whole retinal posterior pole (and not only to the macular area) in eyes of patients with early glaucoma, and repeatability of the whole Posterior Pole volumetric scan was evaluated for each segmented retinal layer.

Using this new method a greater proportion of the retina possibly affected by glaucomatous structural damage was successfully measured layer by layer.

In this first attempt, we focused in particular on the analysis of the RNFL and GCL, the two specific layers most affected by glaucoma.

For the above mentioned reasons, ascertainment of the repeatability and reproducibility of the isolate ganglion cell layer thickness measurement is an important feature of the SD-OCT automatic segmentation, in order to separate real changes from the intrinsic variability of this method.

Our results showed differing CV% values corresponding with distinct retinal layers in healthy subjects. CV% values of within- and between-subject repeated total retinal thickness measurements were highly precise, as illustrated in Tables 2 and 3. However, CV% values of repeated Posterior Pole GCL and RNFL measurements appeared only slightly less consistent, although still within a range of good repeatability and reproducibility.

This may be due to an intrinsically wider variability of measurement of the Spectralis automatic segmentation algorithm when accurately identifying single layers of the total retina.

It has to be pointed out that in a few cases (8% of total screened POAG patients) the automated segmentation algorithm failed to correctly identify different retinal layers and therefore performance of manual re-segmentation was necessary. In particular, imperfect segmentation occurred at the level of the RNFL in 3 eyes of the POAG patients. The possible consequences of this manual correction on the inter-observer variability are evident, and as such those cases of manual correction were excluded from the study.

Despite the fact that use of automated algorithms has undoubtedly enhanced the quantitative evaluation of different retinal layers using advanced SD-OCT imaging techniques, a careful inspection by an experienced observer of all the segmentations obtained using the new Spectralis software is still mandatory.

Glaucoma is a disease that causes progressive loss of vision related to retinal ganglion cell death. It is conceivable that the degree of visual function loss would be proportional to the rate of ganglion cell loss. Harwerth and Quigley found a quantitative relationship between ganglion cell density and visual sensitivity in POAG [29].

In this initial study, we did not perform any structure-function correlations between perimetric indices, such as mean deviation (MD) and pattern standard deviation (PSD) and SD-OCT results on account of the small number of patients. For the same reason, differential evaluation of layers other than RNFL and GCL was not performed, since the analysis was exclusively focused on retinal layers most affected by glaucoma in this preliminary phase of the study. Further assessment of any difference in all retinal layers will undoubtedly be of future interest, together with investigation of structure-function correlations.

Further work is needed to estimate the reliability of such measurements in eyes with more advanced stages of glaucoma in which the “floor effect”, poor fixation and disruptions in retinal morphology might increase measurement variability [30]. Furthermore, it has been demonstrated that reductions in both RNFL and ganglion cell complex thickness can predict the development of glaucomatous VF loss in suspected and pre-perimetric glaucoma patients [31]. We feel that the significant rate of thinning found in eyes from patients in our study with early stage glaucoma, using the new automatic segmentation algorithm, could also be a useful tool in patients suffering from glaucoma at different stages.

However, to the best of our knowledge this is the first study in which the new algorithm of the Spectralis SD-OCT has been used to perform automatic segmentation of the posterior pole in patients with early glaucoma. This seems to be a promising tool useful, in conjunction with all the other relevant clinical parameters, in the detection and monitoring of structural damage from glaucoma.

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