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A Combined Index of Structure and Function for Staging Glaucomatous Damage

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Abstract

Purpose—To present and evaluate a new combined index of structure and function (CSFI) for staging and detecting glaucomatous damage.

Methods—Observational study including 333 glaucomatous eyes (295 with perimetric glaucoma and 38 with preperimetric glaucoma) and 330 eyes of healthy subjects. All eyes were tested with standard automated perimetry (SAP) and spectral domain optical coherence tomography (SDOCT) within 6 months. Estimates of the number of retinal ganglion cells (RGC) were obtained from SAP and SDOCT and a weighted averaging scheme was used to obtain a final estimate of the number of RGCs for each eye. The CSFI was calculated as the percent loss of RGCs obtained by subtracting estimated from expected RGC numbers. The performance of the CSFI for discriminating glaucoma from normal eyes and the different stages of disease was evaluated by receiver operating characteristic (ROC) curves

Results—The mean CSFI, representing the mean estimated percent loss of RGCs, was 41% and 17% in the perimetric and pre-perimetric groups, respectively ($P<0.001$). They were both significantly higher than the mean CSFI in the normal group ($P<0.001$). The CSFI had larger ROC curve areas than isolated indexes of structure and function for detecting perimetric and preperimetric glaucoma and differentiating among early, moderate and advanced stages of visual field loss.

Conclusion—An index combining structure and function performed better than isolated structural and functional measures for detection of perimetric and preperimetric glaucoma as well as for discriminating different stages of the disease.

INTRODUCTION

Glaucoma is an optic neuropathy characterized by progressive neuroretinal rim thinning, excavation and loss of the retinal nerve fiber layer.¹ These structural changes are usually accompanied by functional losses, which may ultimately result in a significant decrease in vision-related quality of life. Staging the severity of glaucomatous damage is an essential component in guiding management decisions and providing prognostic information. Patients with severe damage may be at an increased risk for developing functional impairment and, therefore, may require more aggressive treatment than those with mild or moderate damage.

Additionally, staging systems may be used to monitor disease progression over time and also to evaluate treatment efficacy.

The most common test used to stage glaucoma severity is clinical standard automated perimetry (SAP). Visual field defects on SAP have been shown to be associated with retinal ganglion cell (RGC) loss both in experimental and clinical glaucoma.² Additionally, SAP defects are related to measures of functional impairment in the disease and, therefore, may be used to gauge the impact of the disease on quality of vision. However, experimental studies have shown that as many as 40–50% of RGCs may need to be lost before the decrease in threshold sensitivity exceeds normal variability and reaches statistical significance.^{2–4} In fact, qualitative and quantitative analyses of the optic nerve and retinal nerve fiber layer (RNFL) have shown that significant structural changes are present in many patients before detectable changes in SAP.^{5–13}

Although many different staging schemes using SAP have been proposed, it is clear that a classification system that only considers SAP abnormalities may result in gross underestimation of the amount of damage in early disease. On the other hand, the utility of structural measurements in moderate and advanced stages of the disease has been questioned.^{14–18} There is evidence that RNFL and optic disc assessment by imaging technologies may not provide adequate sensitivity to follow patients who present with severe glaucomatous damage. In this situation, SAP losses are still the best method to quantify the impact of the disease and monitor its progression.

The apparent disagreement between structural and functional measurements of the disease seem to be largely derived from the different algorithms and measurement scales as well as the different variability characteristics of the tests commonly used to assess structural and functional losses. In fact, Harwerth and colleagues² demonstrated that structural and functional tests are in agreement as long as one uses appropriate measurement scales for neural and sensitivity losses and considers factors such as the effect of aging and eccentricity on estimates of neural losses. In a series of investigations, they demonstrated that estimates of RGC losses obtained from clinical perimetry agreed closely with estimates of RGC losses obtained from RNFL assessment by optical coherence tomography.² The results of their model provided a common domain for expressing results of structural and functional tests, i.e., the estimates of RGC losses, opening the possibility of combining these different tests to improve the reliability and accuracy of estimates of the amount of neural losses and develop a combined staging system for glaucoma severity.

In the current study, we propose a new index to estimate glaucoma severity based on a combination of functional measurements obtained by SAP and structural assessment by spectral domain optical coherence tomography (SDOCT). We show that the index performed well in discriminating diseased from non-diseased patients and provided a better estimate of the stage of glaucoma severity compared to the isolated use of functional or structural measures.

METHODS

This was an observational study. Participants from this study were included in two prospective longitudinal studies designed to evaluate optic nerve structure and visual function in glaucoma (the African Descent and Glaucoma Evaluation Study [ADAGES] and the Diagnostic Innovations in Glaucoma Study [DIGS]). The 3-site ADAGES collaboration includes the Hamilton Glaucoma Center at the Department of Ophthalmology, University of California-San Diego (UCSD) (data coordinating center), the New York Eye and Ear Infirmary and the Department of Ophthalmology, University of Alabama, Birmingham

(UAB). Although the DIGS includes only patients recruited at UCSD, the protocols of the two studies are identical. The institutional review boards at all 3 sites approved the study methodology, which adhered to the tenets of the Declaration of Helsinki and to the Health Insurance Portability and Accountability Act. Methodological details have been described previously.¹⁹

At each visit during follow-up, subjects underwent a comprehensive ophthalmologic examination including review of medical history, best-corrected visual acuity, slit-lamp biomicroscopy, intraocular pressure (IOP) measurement, gonioscopy, dilated fundoscopic examination, stereoscopic optic disc photography, and automated perimetry using Swedish Interactive Threshold Algorithm (SITA Standard 24-2). Only subjects with open angles on gonioscopy were included. Subjects were excluded if they presented with a best-corrected visual acuity less than 20/40, spherical refraction outside ± 5.0 diopters and/or cylinder correction outside 3.0 diopters, or any other ocular or systemic disease that could affect the optic nerve or the visual field.

The study included 333 eyes of 246 glaucoma patients diagnosed based on evidence of presence of repeatable glaucomatous visual field defects or documented history of progressive glaucomatous optic neuropathy. From the 333 eyes, 295 had evidence of glaucomatous visual field defects based on repeatable abnormal visual field test results defined as a pattern standard deviation (PSD) outside of the 95% normal confidence limits, or a Glaucoma Hemifield Test result outside normal limits. An additional group of 38 eyes had evidence of progressive glaucomatous change in the appearance of the optic disc as assessed by masked grading of simultaneous stereoscopic optic disc photographs (TRC-SS; Topcon Instrument Corp of America, Paramus, New Jersey, USA), despite absence of statistically significant visual field losses. The evidence of progressive glaucomatous damage had to be present before the imaging test date and the details of the methodology employed to grade optic disc photographs at the UCSD Optic Disc Reading Center have been provided elsewhere.^{20, 21} This latter group was used to assess the ability of the proposed staging system to quantify damage in patients with confirmed preperimetric glaucoma.

The control group consisted of 330 eyes from 171 healthy participants. These subjects were recruited from the general population and were required to have a normal ophthalmologic examination and IOP below 22mmHg in both eyes, but results of visual field tests were not used as inclusion or exclusion criteria.

Visual Field Testing

All patients underwent SAP testing using SITA-standard 24-2 strategy less than 6 months apart from imaging. All visual fields were evaluated by the UCSD Visual Field Assessment Center (VisFACT).²² Visual fields with more than 33% fixation losses or false-negative errors, or more than 15% false-positive errors were excluded. The only exception was the inclusion of visual fields with false-negative errors of more than 33% when the field showed advanced disease (MD lower than -12 dB). Visual fields exhibiting a learning effect (i.e., initial tests showing consistent improvement on visual field indexes) were also excluded. Visual fields were further reviewed for the following artifacts: lid and rim artifacts, fatigue effects, inappropriate fixation, evidence that the visual field results were due to a disease other than glaucoma (such as homonymous hemianopia), and inattention. The VisFACT requested repeats of unreliable visual field test results, and these were obtained whenever possible.

Spectral-Domain OCT

The Cirrus HDOCT (software version 5.2, Carl Zeiss Meditec Inc., Dublin) was used to acquire RNFL measurements in the study. It uses a superluminescent diode scan with a center wavelength of 840 nm and an acquisition rate of 27 000 A-scans per second at an axial resolution of 5 μm . The protocol used for RNFL thickness evaluation was the optic disc cube. This protocol is based on a 3-dimensional scan of a $6 \times 6 \text{ mm}^2$ area centered on the optic disc where information from a $1024 \text{ (depth)} \times 200 \times 200$ -point parallelepiped is collected. Then, a 3.46-mm diameter circular scan (10.87mm length) is automatically placed around the optic disc, and the information about parapapillary RNFL thickness is obtained. Because information from the whole region is obtained, it is possible to modify the position of the scan after the exam is taken. To be included, all images were reviewed for non-centered scans and had to have signal strength >6 , the absence of movement artifacts, and good centering around the optic disc.

Combined Structure and Function Index

The development of the combined index of structure and function to measure disease severity was based on previous work by Harwerth and colleagues² on the development and validation of a model linking structure and function in glaucoma. Based on experimental studies in monkeys, the authors first derived an empirical model relating sensitivity measurements in SAP to histological RGC counts as a function of retinal eccentricities. The experimental results were then translated to clinical perimetry in humans. The following formulas were proposed to estimate the number of RGC somas in an area of the retina corresponding to a specific SAP test field location at eccentricity ec with sensitivity s in dB:

$$\begin{aligned} m &= [0.054 * (ec * 1.32)] + 0.9 \\ b &= [-1.5 * (ec * 1.32)] - 14.8 \\ gc &= \{[(s - 1) - b] / m\} + 4.7 \\ SAPrgc &= \sum 10^{(gc * 0.1)} \end{aligned}$$

In the above formulas, m and b represent the slope and intercept, respectively, of the linear function relating ganglion cell quantity (gc) in dB to the visual field sensitivity (s) in dB at a given eccentricity. By applying the above formulas, one can obtain a SAP-derived estimate of the total number of RGCs ($SAPrgc$) by adding the estimates from all locations in the visual field. The structural part of the model consisted in estimating the number of RGC axons from RNFL thickness measurements obtained by optical coherence tomography. The model took into account the effect of aging in the axonal density and the effect of disease severity on the relationship between the neuronal and non-neuronal components of the RNFL thickness estimates obtained by OCT. To derive the total number of RGC axons from the global RNFL thickness measurement obtained by OCT ($OCTrgc$), one can apply the following formulas:

$$\begin{aligned} d &= (-0.007 * \text{age}) + 1.4 \\ c &= (-0.26 * \text{MD}) + 0.12 \\ OCTrgc &= 10^{\{[\log([\text{average RNFL thickness} * 10.87 * d]) * 10] - c\}} \end{aligned}$$

In the above formulas, d corresponds to the axonal density (axons/ μm^2) and c is a correction factor for the severity of disease to take into account remodeling of the RNFL axonal and non-axonal composition. The above calculations allow one to estimate the number of RGCs from two sources, one functional and one structural, and a strong relationship was demonstrated between the two estimates in external validation cohorts. However, although

Harwerth et al proposed a model linking structure and function, no attempt was made to develop an index combining structural and functional estimates that could be clinically used to stage glaucoma severity. We propose the following calculations to develop such an index. In order to derive a combined index, we simply averaged the estimates of RGC numbers obtained from SAP and OCT, but weighting according to severity of disease. As clinical perimetry and imaging tests accuracies have been proposed to be inversely related to disease severity, we propose a weighted scale combining the estimates of RGC numbers from both tests:

$$wrgc = (1 + MD/30) * OCTrgc + (-MD/30) * SAPrgc$$

The weights were chosen to reflect the inverse relationship with disease severity of SAP and OCT estimates, along the scale of MD values ranging from 0 to -30dB. After estimates of *wrgc* were obtained, a linear regression model was run to relate *wrgc* estimates to age and optic disc area in the normal control population. The purpose was to develop a model to predict expected RGC numbers according to age and optic disc area. In order to avoid model overfitting, the regression parameters were obtained using only half of the normal eyes (development sample). After the expected number of RGCs was calculated for each eye, an estimate of the percent RGC loss for each eye was obtained by subtracting measured from estimated RGC numbers. The percent estimate of RGC loss should reflect an estimate of glaucomatous damage obtained by combining data from structural and functional measurements (CSFI, combined structure-function index), as calculated below:

$$CSFI = [(expected\ RGC\ number - wrgc) / (expected\ RGC\ number)] * 100$$

Statistical Analysis

The performance of the CSFI for discriminating glaucoma from normal eyes and the different stages of disease was compared to those of other indexes previously used to stage disease severity such as MD and the Visual Field Index (VFI), as well as to the SDOCT parameter average RNFL thickness. Receiver operating characteristic (ROC) curves were built, and the area under the ROC curves (AUC) was used to summarize the diagnostic accuracy for each parameter. Perimetric and preperimetric glaucomatous eyes were compared to normal eyes in the validation sample, i.e., excluding the eyes previously used to obtain the regression parameters described above. An AUC equal to 1 represents perfect discrimination, whereas an AUC of 0.5 represents chance discrimination. AUCs and 95% confidence intervals were obtained for each parameter after adjusting for age. A bootstrap resampling procedure ($n = 1000$ resamples) was used to derive confidence intervals. Age adjustment was performed using a ROC regression model, as previously described. The model is able to adjust for the differences in variables between control and cases by fitting a linear regression of the marker distribution on the adjustment variables among controls. Standardized residuals based on this fitted linear model are used in place of the marker values for cases and controls. To account for the potential correlation between eyes, the cluster of data for the study subject was considered as the unit of resampling when calculating standard errors. This procedure has been previously used to adjust for the presence of multiple correlated measurements from the same unit.²³

All statistical analyses were performed with commercially available software (Stata version 12; StataCorp, College Station, TX). The alpha level (type I error) was set at 0.05.

RESULTS

From the 333 glaucomatous eyes, 295 (89%) had perimetric glaucoma and 38 (11%) had preperimetric glaucoma. The eyes were compared to 165 eyes from 85 healthy subjects included in the validation sample. The mean ages of perimetric glaucoma and preperimetric glaucoma participants were 69 ± 11 and 66 ± 10 , respectively. They were both significantly higher than that of control subjects (60 ± 11 ; $P < 0.01$ for both comparisons). Age differences were adjusted for in the ROC analyses.

Table 1 shows estimates of the different parameters obtained in the study. There was a strong correlation between RGC estimates obtained from SAP and OCT data in the eyes included in the study ($r = 0.89$; $P < 0.001$) (Figure 1). Figure 2 shows histograms of calculated weighted estimates of RGC numbers combining structural and functional tests (*wrgc*), according to the diagnostic categories. The mean estimated number of RGCs in the group with perimetric glaucoma was 524,545 compared to 748,731 in the preperimetric group and 973,120 in normal eyes. The results of the linear regression model relating estimated RGC numbers to age and optic disc area in the normal eyes from the development sample are presented on Table 2. There was a significant relationship between RGC number and age, with an estimated loss of 9,249 RGCs per year older in normal subjects (Figure 3). Also, each 0.1mm^2 larger optic disc area corresponded to an increase in 11,607 RGCs.

The mean CSFI, representing the mean estimated percent loss of RGCs, was 41% and 17% in the perimetric and pre-perimetric groups, respectively ($P < 0.001$). They were also both significantly higher than the mean CSFI in the normal group ($P < 0.001$) (Table 1). Figure 4 shows a boxplot graph of the CSFI values according to diagnostic category. Table 3 shows the areas under the ROC curves for the parameters investigated in the study. The CSFI had an ROC curve area of 0.94 to discriminate glaucomatous from normal eyes. The performance of the CSFI was superior to that of SDOCT parameter average RNFL thickness ($\text{AUC} = 0.92$; $P = 0.008$) and the global visual field indexes MD ($\text{AUC} = 0.88$; $P < 0.001$) and VFI ($\text{AUC} = 0.89$; $P < 0.001$). Analyses were also performed by subgroups of perimetric and preperimetric glaucoma. For detection of perimetric glaucoma, the CSFI also performed significantly better than average RNFL thickness and MD ($P < 0.001$ for both comparisons), but not significantly different from the VFI ($P = 0.16$) (Table 4). For detecting preperimetric glaucoma, the CSFI had an ROC curve area of 0.85, which was superior to that of the VFI ($\text{AUC} = 0.51$; $P < 0.001$) and MD ($\text{AUC} = 0.63$; $P < 0.001$). The ability to detect preperimetric glaucoma with the CSFI was similar to that of the SDOCT parameter average RNFL thickness ($\text{AUC} = 0.88$; $P = 0.32$). Figure 5 shows ROC curves for the different parameters for detection of perimetric and preperimetric glaucoma.

We also evaluated the ability of the CSFI in discriminating eyes with different stages of glaucomatous visual field loss as determined by the Hodapp-Anderson-Parrish (HAP) classification system. According to the HAP, from the 295 eyes with glaucomatous visual field loss, 189 had early damage, 49 had moderate and 57 had advanced. Table 4 shows the values of the parameters calculated in the study for these different severity groups. The AUC for the CSFI for separating early from moderate visual field loss was $0.94 (\pm 0.02)$, compared to only $0.77 (\pm 0.02)$ for the SDOCT average RNFL thickness ($P < 0.001$). For separating moderate from advanced glaucomatous field loss, the AUC of the CSFI was $0.96 (\pm 0.02)$, which was again significantly better than that for average RNFL thickness ($\text{AUC} = 0.70 \pm 0.05$; $P < 0.001$). The CSFI also performed better than average RNFL thickness to discriminate eyes with preperimetric glaucoma from those with early visual field loss (0.73 ± 0.04 vs. 0.60 ± 0.04 , respectively; $P < 0.001$). Figure 6a shows the relationship between MD and CSFI whereas Figure 6b shows the relationship between MD and average

thickness. It can be seen that the CSFI agrees more closely with MD than the parameter average RNFL thickness in moderate and advanced stages of the disease.

Figure 7 illustrates a case of preperimetric glaucoma included in the study. The eye had clear evidence of documented progressive optic disc change on stereophotographs before the imaging test date but still presented with visual fields that were statistically within normal limits. Results of the SDOCT exam show pronounced RNFL thinning, with average thickness of 68 μ m. The CSFI for the eye was 39%, indicating a loss of 39% of the estimated number of RGCs compared to the age expected number. Figure 8 shows two eyes with advanced glaucoma, one with MD of -15.12dB and another with MD of -23.61. Despite the important differences in visual field damage between the two cases, SDOCT results were similar in the two eyes with the same value of average thickness of 50 μ m. The CSFI clearly distinguished between the eyes with values of 74% for the former and 85% for the latter.

DISCUSSION

In the current study, we propose a new index combining information on structural and functional damage in glaucoma which can be used to stage and provide diagnostic information on the disease. The index performed significantly better than isolated measures of structure and function for diagnosing pre-perimetric and perimetric glaucoma. In addition, the index also performed better in discriminating different stages of the disease, suggesting that it might also be helpful for staging and monitoring patients over time.

Several staging systems for glaucoma have been proposed in the literature.^{24–29} Most of them have been based solely on information extracted from visual fields. Visual field-based staging systems assume that all patients with statistically normal fields should be grouped at a single stage and, therefore, they do not differentiate whether the patient is actually a healthy subject, has suspicious findings for the disease or evidence of glaucomatous neuropathy despite absence of detectable field losses. Experimental and clinical research, however, has shown that a substantial number of RGCs may need to be lost before detectable changes are observed in the visual field.² Evidence of structural damage to the optic disc and RNFL has been demonstrated in patients with statistically normal visual fields using different imaging technologies and conventional stereophotographs.^{5, 8, 20, 21} More importantly, these structural changes have been shown to carry prognostic information, being strongly associated with risk of development of future functional losses in the disease.⁵ In our study, patients with preperimetric glaucoma had an estimated mean number of RGCs of 748,731 which was approximately 23% lower than the mean number of 973,720 cells measured in the healthy eyes included in the validation sample. Differences in the number of cells could be partially explained by age differences in the two groups. Therefore, we calculated the CSFI which corresponds to a percent estimate of loss compared to the age-expected number of RGCs. Patients with pre-perimetric glaucoma had a mean CSFI of 17% which was still significantly higher than that of healthy subjects. The diagnosis of preperimetric glaucoma in our study was based on documented evidence of progressive optic disc change in stereophotographs. Due to the wide variability of the optic nerve appearance, a single optic disc examination is frequently not diagnostic in the early stages of glaucoma.^{5, 21} In the absence of visual field loss, a diagnosis of certainty of glaucoma can only be given by demonstrating a previous history of progressive glaucomatous changes to the optic nerve. We demonstrated that the CSFI performed well in differentiating eyes with preperimetric glaucoma from healthy subjects, with an ROC curve area of 0.85, similar to what can be obtained from analysis using SDOCT average thickness.

Staging systems based on optic disc appearance or quantitative assessment of the optic disc and RNFL have also been proposed.^{27, 30} These classification systems are limited by the

decreasing performance of imaging instruments to discriminate among the different stages of disease with increasing severity of damage. Sihota et al³¹ reported an area under the ROC curve of only 0.705 for discriminating early to moderate visual field losses with the OCT parameter average thickness. A weak performance was also reported in separating moderate from advanced cases with an ROC curve area of only 0.737. These values are very similar to those found in our study for the SDOCT parameter average thickness, with corresponding areas under the ROC curve of 0.77 and 0.70, respectively. Longitudinal studies have also shown an inverse relationship between disease severity and ability to detect change with imaging devices.^{15, 17, 32} These findings collectively suggest that the use of a structure-only staging system is likely to be inadequate once the patient has been diagnosed with visual field loss. In contrast, the use of a combined index of structure and function allowed excellent separation between the different stages of the disease. The CSFI had areas under the ROC curve of 0.94 to separate early from moderate loss and 0.96 for discriminating moderate from advanced loss. Although these results may seem obvious as the CSFI actually incorporates visual function information used to define severity or classifying the groups, they need to be seen in the context of the overall performance of the CSFI. The CSFI performed well not only to differentiate the different stages of glaucomatous visual field loss but also in detecting preperimetric glaucoma. Therefore, using a single index combining structure and function, we were able to detect the earliest stages of damage while retaining the ability to differentiate among the different stages of the disease in more advanced cases, a task that was poorly performed when visual field data or OCT data were used in isolation.

It is important to note that some overlap in CSFI values was seen among the different studied groups as shown on Figure 4. However, this is a limitation inherent to any parameter assessing biologic variables and could also be related to the variability of the tests used to obtain estimates of RGC numbers. Both SAP and OCT have test-retest variability and this will obviously translate into CSFI variability. This should not have affected the comparisons performed in our study, however, it indicates the need for clinicians to obtain multiple tests to improve reproducibility, as currently performed in clinical practice.

We based our estimates of SAP and OCT-derived RGC numbers on previously published work by Harwerth and colleagues.² Using normal monkeys and monkeys with laser-induced experimental glaucoma, they showed that SAP sensitivity values can provide good estimates of the amount of histologically-measured RGC counts in the retina. These estimates agreed closely with those obtained from OCT RNFL thickness data. They showed a strong linear relationship between the number of RGC somas and axons obtained from functional and structural measures, respectively, when retinal eccentricity and appropriate measurement scales for neural and sensitivity losses were used. The linear relationship suggests that the lack of sensitivity of SAP for detection of early glaucomatous damage is most likely not the result of true structural changes occurring in the absence of functional losses, but is rather related to the logarithmic scale used for SAP sensitivity measurements, as well as the magnitude of change required to reach statistically significant levels of abnormality.^{6, 33} The logarithmic scale compresses the range of losses in early stages of the disease while expanding the range in later stages. These findings could suggest that a simple linearization of SAP data could improve detection of early damage. However, this is usually not the case. In fact, the ROC curve for detecting preperimetric glaucoma using estimates of RGC number from SAP (SAPrgc) in our study was still only 0.69, much inferior to that of RGC estimates from OCT data (0.88). As SAP data is originally acquired using staircase procedures based on a logarithmic scale (dB), SAP is not good at estimating small amounts of ganglion cell losses at early stages of the disease. In contrast, by expanding the range of the scale at later stages, SAP might be more sensitive to small changes in the number of RGCs which do not seem to produce detectable changes in RNFL thickness. Despite these

observations, the ability to express results of functional and structural tests in the same domain opens the possibility of combining the information from the two tests to increase the precision of RGC estimates, as performed in our study. By combining the estimates, one increases the precision of the final estimate of neuronal losses to better stage glaucomatous damage. However, instead of simply averaging the two estimates, we used a weighting scheme based on MD values. This was done in order to take into consideration differences in performance of SAP and imaging tests at different stages of the disease for the reasons described above.

Our study has limitations. We used empirically-derived formulas to estimate the number of RGCs from SAP and OCT data. Although estimates obtained from these formulas have been validated in multiple external cohorts, the original formula for estimating RGCs from OCT data was based on an older version of the technology, time-domain OCT. In our study, we used the same previously derived formulas, but data were obtained by SDOCT and it is possible that modifications would be necessary to compensate for the change in technologies. However, the agreement between SAP and OCT data found in our study was similar to that reported by Harwerth et al², suggesting that major modifications are probably not necessary. Another potential limitation of our study is that we used only global measures of visual function and structural damage. A sectorial analysis may provide a better representation of localized damage and improved detection of glaucoma. However, the use of sectorial information may be difficult to interpret in the context of a staging system. Additionally, sectorial information will be more variable and not necessarily better for monitoring changes over time. Further studies should evaluate whether a combination of sectorial structure and function data could improve detection and staging of glaucomatous damage. Another limitation of our study is that the presence of media opacities could potentially affect SAP-derived estimates of RGCs and, therefore, calculations of the CSFI. This is a potential limitation of most visual field-based staging systems, as they usually base their classifications at least in part on values of the MD index. However, by combining functional and structural measurements, our approach potentially reduces the effect of media opacities by relatively decreasing the influence of SAP-derived data on the final estimates of neuronal losses. Nevertheless, clinicians should be aware of the effect of media opacities when evaluating functional changes and quality of imaging test results in glaucoma patients.

The CSFI has several desirable properties for use as a staging index. It discriminates well among the different stages of the disease and has a very intuitive interpretation as the overall percent loss of neuronal tissue. In addition, it is provided on a continuous scale avoiding the artificial categorization of the disease continuum. However, it should be emphasized that an ideal staging system for glaucoma would be highly predictive of the degree of disability from the disease. Although SAP measurements have been related to measures of quality of vision in patients with glaucoma, such relationship is usually weak. Recent studies have proposed different methods to evaluate the degree of functional impairment caused by the disease and future studies should be performed attempting to correlate proposed staging systems to results of these tests or develop staging systems based on results of tests directly measuring functional impairment in glaucoma.^{34, 35} The methods described in our study to estimate RGC counts from a combination of structure and function could also be used to provide a useful parameter for longitudinal monitoring of glaucomatous changes. We are conducting additional studies to investigate this possibility.

In conclusion, an index combining structure and function performed better than isolated structural and functional measures for detection of perimetric and preperimetric glaucoma as well as for discriminating different stages of the disease. Further studies should evaluate the ability of the proposed index to monitor glaucomatous changes over time.

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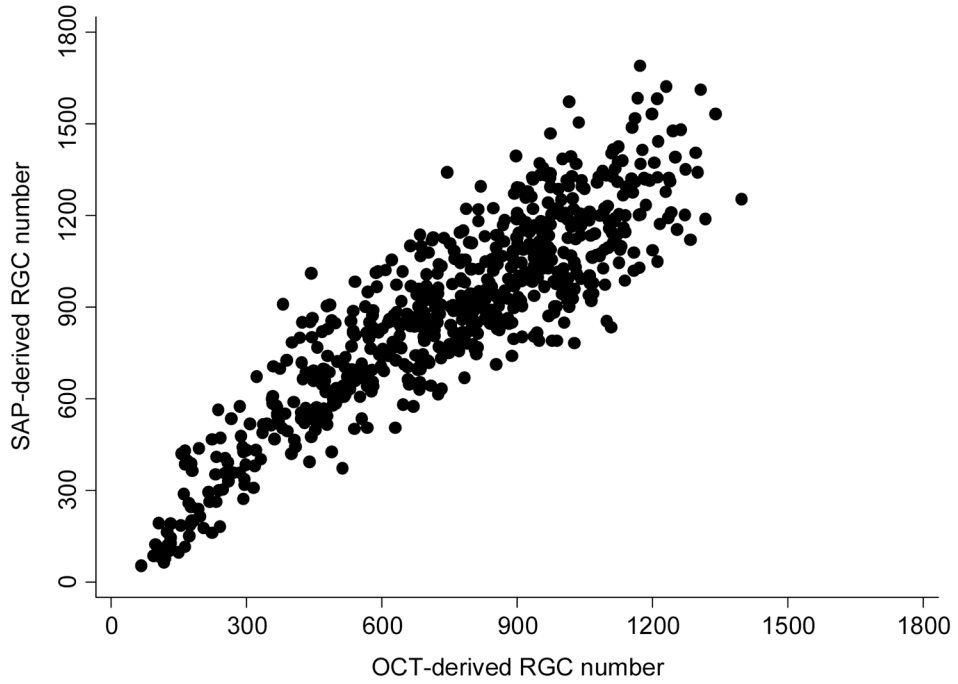


Figure 1. Scatterplot illustrating the relationship between the number of retinal ganglion cells (RGC) derived from standard automated perimetry (SAP) sensitivity data and the number of RGCs estimated from analysis of the retinal nerve fiber layer by optical coherence tomography (OCT).

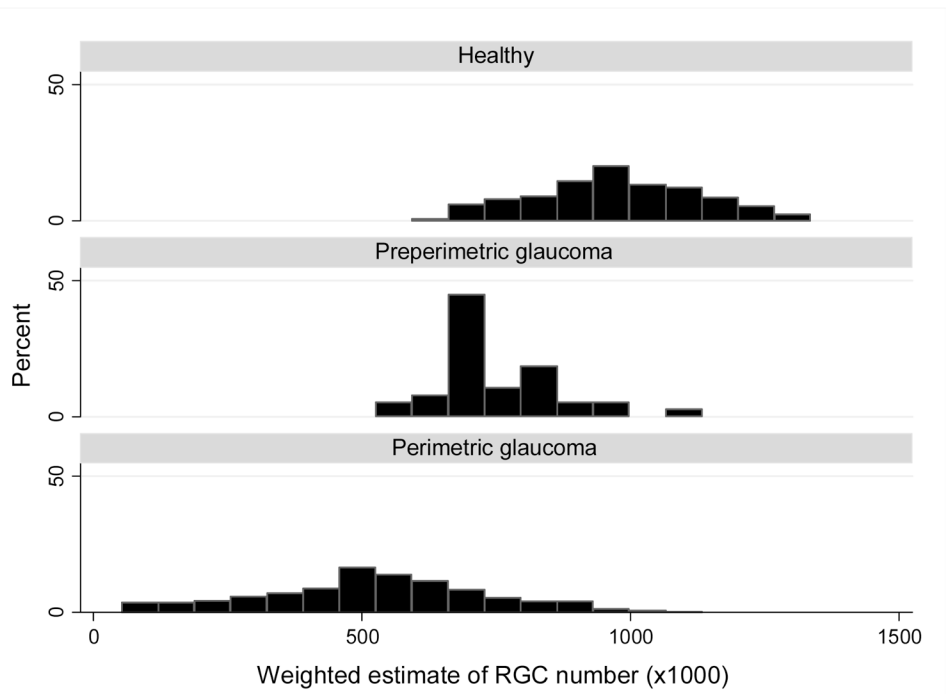


Figure 2. Histograms illustrating the distribution of the number of estimated retinal ganglion cells according to the different diagnostic categories.

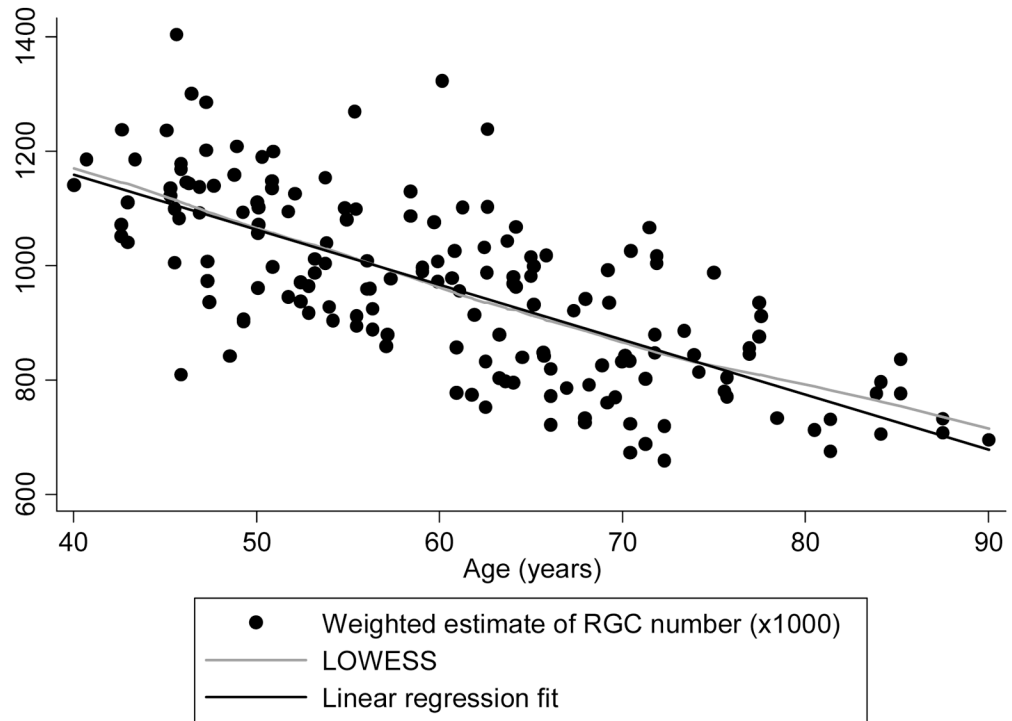


Figure 3. Relationship between the weighted estimate of number of retinal ganglion cells (RGC) and age. A locally weighted scatterplot smoothing (lowess) shows that a linear regression fits the data well.

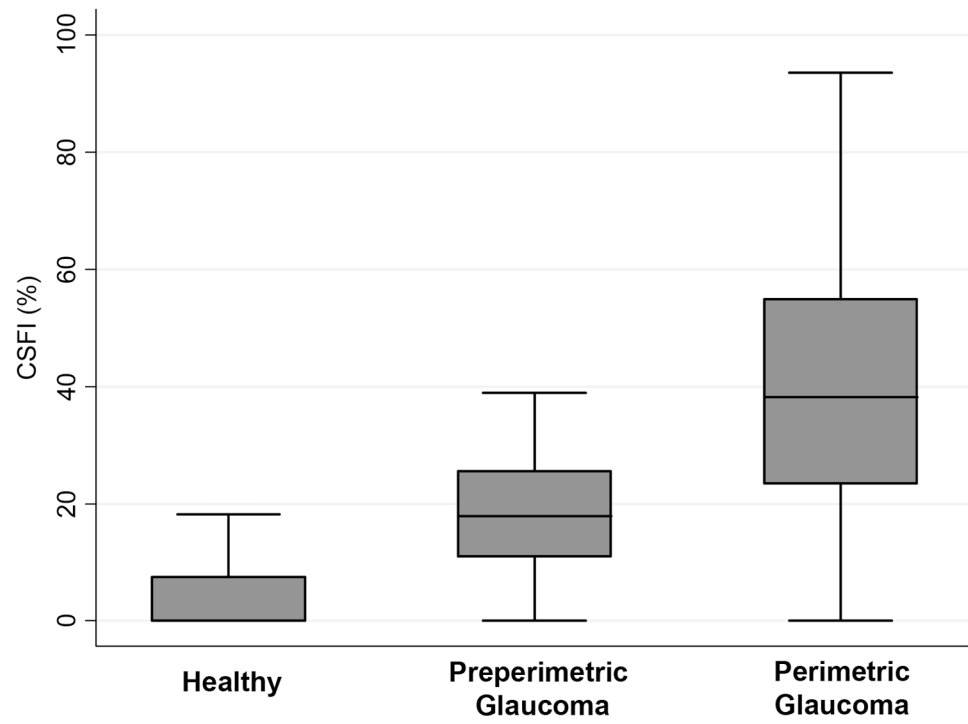


Figure 4. Boxplots illustrating the distribution of the values of the combined index of structure and function (CSFI) according to the different diagnostic categories.

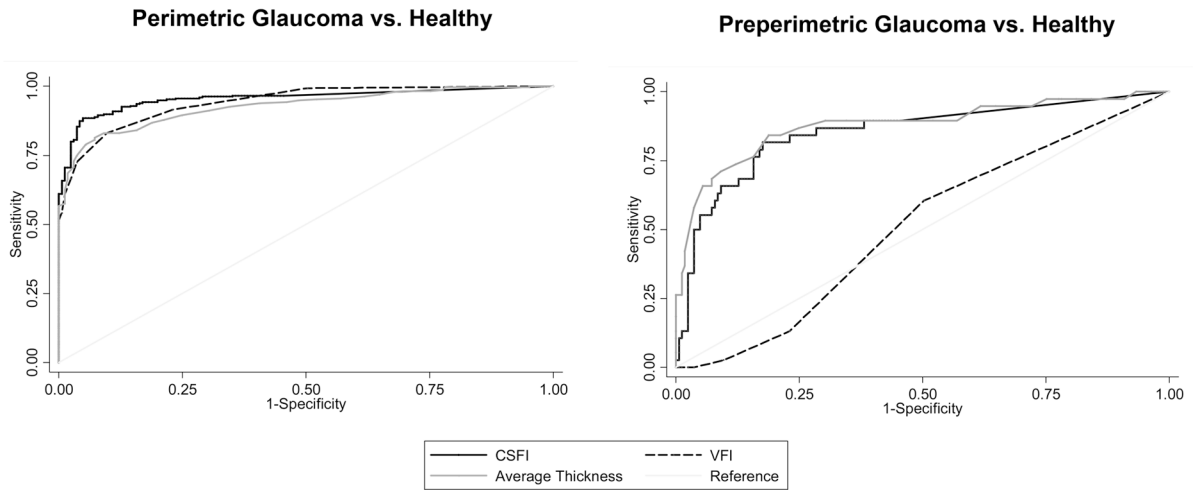


Figure 5. Receiver operating characteristic (ROC) curves for discriminating between perimetric glaucoma and healthy eyes (left) and between preperimetric glaucoma and healthy eyes (right). ROC curves are shown for the parameters CSFI (combined index of structure and function), average retinal nerve fiber layer (RNFL) thickness and VFI (Visual Field Index).

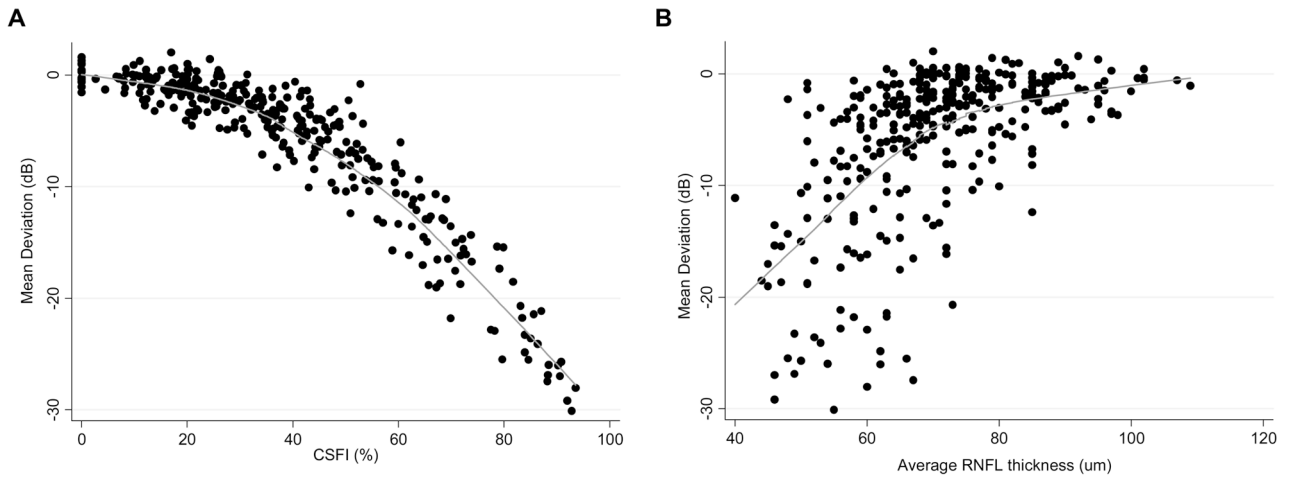


Figure 6.

A. Scatterplot illustrating the relationship between mean deviation (MD) and the CSFI (combined index of structure and function) with superimposed locally weighted scatterplot smoothing (lowess). B. Scatterplot illustrating the relationship between MD and average retinal nerve fiber layer (RNFL) thickness with superimposed lowess. There is much more scatter around the lowess curve for the average thickness compared to the CSFI.

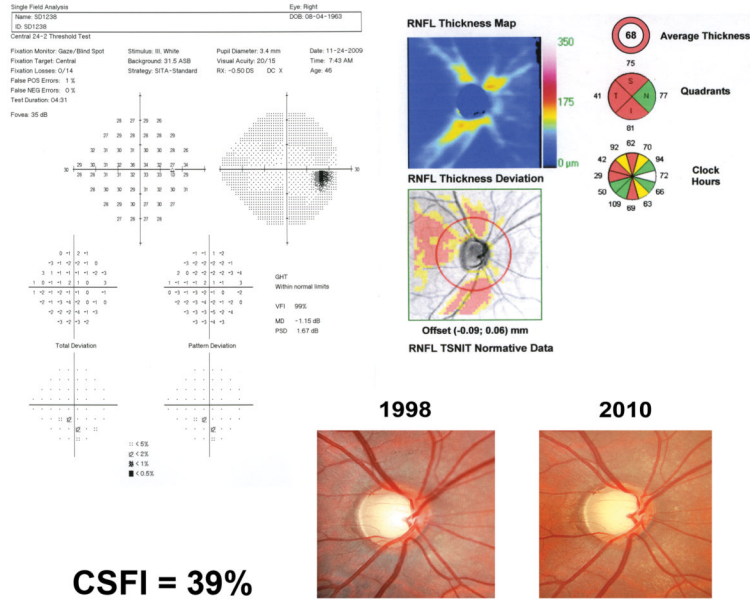


Figure 7. Eye with preperimetric glaucoma included in the study. The eye had evidence of progressive optic disc change on stereophotographs (superior and inferior rim thinning), but still presented with visual fields that were statistically within normal limits. Results of the optical coherence tomography (OCT) exam show pronounced retinal nerve fiber layer thinning with average thickness of 68μm, compatible with the changes seen on optic disc photographs. The combined index of structure and function (CSFI) was 39%, indicating a loss of 39% of retinal ganglion cells compared to the age expected number.

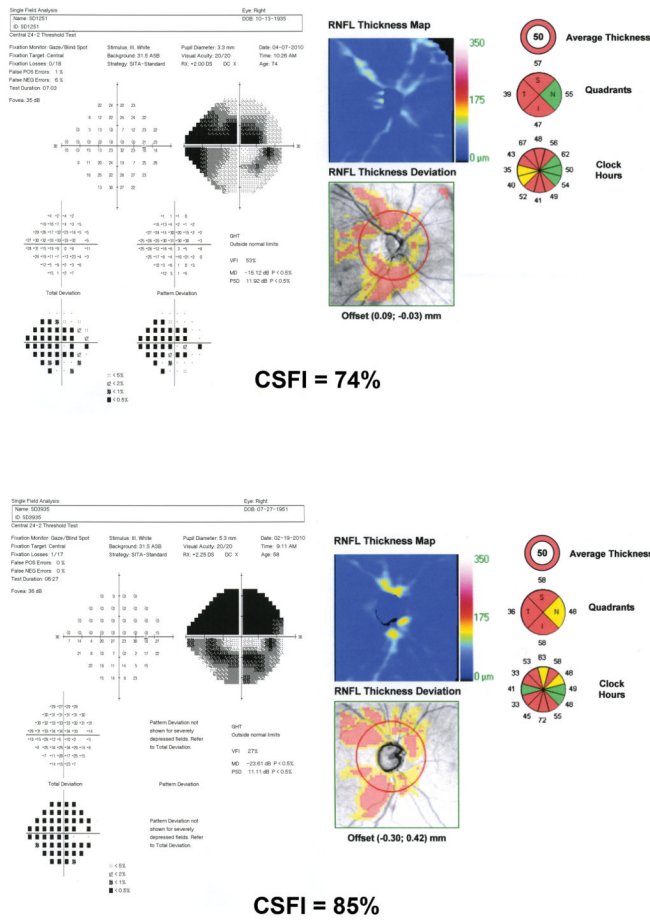


Figure 8. Two eyes with advanced glaucoma, the superior one shows mean deviation (MD) of -15.12 dB and the inferior one, MD of -23.61 . Despite the important differences in visual field damage between the two cases, the optical coherence tomography results were similar in the two eyes with the same value of average retinal nerve fiber layer (RNFL) thickness of $50\mu\text{m}$. The combined index of structure and function (CSFI) shows markedly different results between the eyes, with values of 74% for the former and 85% for the latter.

Table 1

Mean values of the different parameters calculated in the study in perimetric glaucoma, preperimetric glaucoma and healthy eyes.*

	Perimetric glaucoma (n = 295)	Preperimetric glaucoma (n = 38)	Healthy (n = 165)	P (Perimetric glaucoma vs. healthy)	P (Preperimetric glaucoma vs. healthy)
MD \bar{x} , dB	-4.01 (-1.79, -9.40)	-0.32 (-1.33, 0.47)	0.17 (-0.85, 1.05)	<0.001	0.015
PSD \bar{x} , dB	4.80 (2.59, 9.77)	1.52 (1.41, 1.76)	1.60 (1.34, 1.85)	<0.001	0.407
VFI \bar{x} , %	92 (77, 97)	99 (99,100)	99 (99, 100)	<0.001	0.825
Average thickness , μ m	69 (13)	78 (10)	94 (9)	<0.001	<0.001
SAPrgc , x1000 cells	660 (277)	944 (148)	1075 (208)	<0.001	<0.001
OCTrgc , x1000 cells	502 (221)	749 (107)	977 (156)	<0.001	<0.001
wrgc , x1000 cells	525 (210)	749 (105)	973 (154)	<0.001	<0.001
CSFI , %	41 (22)	17 (10)	4 (7)	<0.001	<0.001

* Values are given as mean (standard deviation), unless otherwise indicated

\bar{x} Median (first quartile, third quartile)

MD – mean deviation; PSD – pattern standard deviation; VFI – visual field index; SAPrgc – Number of retinal ganglion cells estimated from SAP sensitivity values; OCTrgc – number of retinal ganglion cells estimated from optical coherence tomography data; wrgc – weighted estimated of the number of retinal ganglion cells; CSFI – combined index of structure and function.

Table 2

Results of the linear regression model evaluating the association between the weighted number of retinal ganglion cells and age and optic disc area in healthy eyes. *

Parameter	Coefficient	95% CI	P
Age, per year older	-9249	-10613 to -7885)	<0.001
Optic Disc Area, per 0.1mm ² larger	11607	6077 to 17138	<0.001
Constant	1301098	1163399 to 1438796	<0.001

*Data from the 165 healthy eyes included in the development sample.

Table 3

Areas under the receiver operating characteristic (ROC) curves and standard errors for the parameters evaluated in the study.

	Glaucoma vs. Healthy	Perimetric glaucoma vs. Healthy	Preperimetric glaucoma vs. Healthy
MD	0.88 (0.01)	0.92 (0.01)	0.63 (0.05)
PSD	0.88 (0.01)	0.94 (0.01)	0.46 (0.05)
VFI	0.89 (0.01)	0.94 (0.01)	0.51 (0.04)
Average thickness	0.92 (0.01)	0.93 (0.01)	0.88 (0.04)
<i>wrgc</i>	0.95 (0.01)	0.96 (0.01)	0.88 (0.03)
CSFI	0.94 (0.01)	0.96 (0.01)	0.85 (0.04)

MD – mean deviation; PSD – pattern standard deviation; VFI – visual field index; *wrgc* – weighted estimated of the number of retinal ganglion cells; CSFI – combined index of structure and function.

Table 4

Values of the parameters obtained in the study for the different stages of glaucoma severity based on the Hodapp-Anderson-Parrish classification.

	Early glaucoma (n = 189)	Moderate glaucoma (n = 49)	Advanced glaucoma (n = 57)
MD[†] , dB	-2.3 (-3.7, -1.0)	-8.2 (-9.7, -7.0)	-17.4 (-23.3, -14.7)
PSD[†] , dB	3.0 (2.1, 4.6)	9.9 (7.2, 11.6)	11.6 (9.4, 13.6)
VFI[†] , %	96 (93, 98)	80 (75, 84)	51 (32, 58)
Average thickness , μm	74 (12)	65 (10)	57 (9)
SAPrgc , x1000 cells	812 (180)	540 (157)	260 (138)
OCTrgc , x1000 cells	628 (156)	376 (82)	193 (70)
wrgc , x1000 cells	641 (147)	422 (82)	227 (100)
CSFI , %	28 (13)	52 (8)	75 (11)

MD – mean deviation; PSD – pattern standard deviation; VFI – visual field index; SAPrgc – Number of retinal ganglion cells estimated from SAP sensitivity values; OCTrgc – number of retinal ganglion cells estimated from optical coherence tomography data; wrgc – weighted estimated of the number of retinal ganglion cells; CSFI – combined index of structure and function.