The Structure and Function Relationship in Glaucoma: Implications for Detection of Progression and Measurement of Rates of Change

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Purpose. To evaluate the relationship between change in estimated retinal ganglion cell (RGC) counts and change in measures of functional and structural damage in glaucoma, from cross-sectional data.

Methods. The study included 397 eyes of 397 patients with glaucoma, suspects, and healthy individuals. All eyes underwent testing with standard automated perimetry (SAP) and spectral-domain optical coherence tomography (SD-OCT). Estimates of retinal ganglion cell (RGC) counts were obtained from SAP and SD-OCT using a previously derived algorithm. Smoothing spline curves were fitted to investigate the relationship between functional/structural parameters and RGC counts. The first derivatives (i.e., slopes) of these curves were obtained to investigate the relationship between changes in these measures.

Results. A nonlinear relationship was observed between SAP mean deviation (MD) and RGC counts. The same amount of RGC loss corresponded to largely different amounts of MD change depending on the stage of the disease. For SDOCT average retinal nerve fiber layer (RNFL) thickness, a linear relationship was seen with RGC counts throughout most of the spectrum of disease, but reaching a plateau in advanced glaucoma. Changes in RGC counts for eyes with early damage corresponded to small changes in MD, but to relatively larger changes in RNFL thickness. For eyes with advanced disease, changes in RGC counts produced relatively larger changes in MD but only small or no changes in average RNFL thickness.

Conclusions. The analysis and interpretation of rates of SAP and SD-OCT change, as indicators of the velocity of neural damage in glaucoma, should take into account the severity of the disease. (*Invest Ophthalmol Vis Sci.* 2012;53:6939–6946) DOI: 10.1167/iovs.12-10345

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Glaucoma is a neurodegenerative disease caused by progressive retinal ganglion cell (RGC) loss associated with characteristic structural changes in the optic nerve and retinal nerve fiber layer (RNFL). The neural insult can result in functional losses and decrease in vision-related quality of life. Detection of progression and estimation of rates of disease deterioration are essential in order to evaluate risk of functional impairment and establish treatment strategies.[1,2]

Standard automated perimetry (SAP) is the most commonly used method for assessing rates of visual function loss in glaucoma and estimating risk of impairment from the disease. Rates of change using SAP have traditionally been measured using linear regression over time with parameters such as mean deviation (MD) and expressed in units of decibels/year (dB/y).[3–9] A recent review by Chauhan et al.[10] has suggested that rates of MD change in dB/y are "pragmatic estimates of visual disability patients can suffer at given rates of progression and baseline damage." According to the authors, a patient with early visual field loss (MD = –4 dB) and a rapid rate of progression (–2 dB/y), could be expected to develop total disability (–30 dB) in 13 years. Using the same reasoning, the authors concluded that a rate of change slower than –0.5 dB/y would in general be considered slow and unlikely to lead to blindness in the patient's lifetime. Such reasoning is fundamentally based on the assumption that rates of MD change over time are linear. However, there is very little evidence in the literature to support this assumption. Using data from experimental glaucoma in rhesus monkeys, Harwerth et al.[11,12] showed that perimetric sensitivity has a linear relationship with histological RGC counts in a log-log scale—that is, when expressed in decibels, SAP sensitivity thresholds are linearly related to RGC counts also expressed in decibels. Analysis of the results published by Harwerth et al. indicate that in order for the rate of visual field loss to be linear in dB/y, the rate of RGC loss would also need to be linear in dB/y, which would imply an exponential *decrease* in RGC losses as glaucoma progresses. Recent work by Hood et al.[13] shows a curvilinear relationship between functional and structural measures and analysis of their work also implies that in order for rates of visual field loss to be linear on a decibel scale, rates of structural losses would have to decrease over time. These findings are also corroborated by other studies of structure and function in glaucoma.[14,15]

Although direct RGC counting in vivo is not yet possible in humans, the use of empirical formulas derived from clinical structural and functional tests provide estimates of the number of RGCs, which have been shown to correlate well with histologic counts in experimental glaucoma models.[12,16,17] In recent studies, we proposed a method for estimating the amount of RGC loss from a combination of retinal nerve fiber layer (RNFL) assessment with optical coherence tomography and SAP.[16,17] The estimates of RGC counts performed

significantly better than isolated structural and functional parameters for staging the disease and monitoring glaucomatous progression.

In the current study, we evaluated the relationship between change in estimated RGC counts and change in measures of functional and structural damage in the disease. The relationship between changes in different variables was obtained by calculating derivatives from curves fitted to cross-sectional data obtained from glaucoma, suspect, and healthy eyes.

Methods

This was an observational study. Participants from this study were included in a prospective longitudinal study designed to evaluate optic nerve structure and visual function in glaucoma (DIGS: Diagnostic Innovations in Glaucoma Study) conducted at the Hamilton Glaucoma Center, University of California, San Diego. Participants in the DIGS were longitudinally evaluated according to a pre-established protocol that included regular follow-up visits in which patients underwent clinical examination and several other imaging and functional tests. All the data were entered in a computer database. All participants from the DIGS study who met the inclusion criteria described below were enrolled in the current study. Informed consent was obtained from all participants. The University of California, San Diego Human Subjects Committee approved all protocols and the methods described adhered to the tenets of the Declaration of Helsinki.

Subjects underwent a comprehensive ophthalmologic examination including review of medical history, best-corrected visual acuity (BCVA), 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 BCVA less than 20/40, spherical refraction outside ±5.0 diopters (D) and/or cylinder correction outside 3.0 D, or any other ocular or systemic disease that could affect the optic nerve or the visual field.

The study included 397 eyes of 397 patients. From the 397 eyes, 122 (31%) had glaucomatous visual field defects; 80 (20%) eyes had evidence of glaucomatous optic neuropathy (GON) but without visual field abnormalities; 98 (25%) eyes had ocular hypertension; and 97 (24%) were healthy eyes. Glaucomatous visual field losses were based on presence of repeatable abnormal SAP with pattern standard deviation (PSD) outside of the 95% normal confidence limits or a Glaucoma Hemifield Test result outside normal limits. Evidence of GON was based on masked grading of simultaneous stereoscopic optic disc stereophotographs. Eyes were classified as having GON if they had neuroretinal rim thinning, excavation, or RNFL defects.

Visual Field Testing

All patients underwent SAP testing using SITA-standard 24-2 strategy less than 30 days apart from imaging. All visual fields were evaluated by the UCSD Visual Field Assessment Center (VisFACT).[18] 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

OCT equipment (Cirrus HD-OCT software version 5.2; Carl Zeiss Meditec, Inc., Dublin, Ireland) 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 6×6 mm^2 area centered on the optic disc where information from a 1024 (depth) × 200 × 200-point parallelepiped is collected. Then, a 3.46-mm diameter circular scan (10,870 μm 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 noncentered scans and had to have signal strength greater than or equal to 7, the absence of movement artifacts, and good centering around the optic disc.

Estimates of RGC Counts

Estimates of RGC counts were obtained based on previous work by Harwerth and colleagues[12,16,17] 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 used 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:

$$m = [0.054(\text{ec} \times 1.32)] + 0.9$$

$$b = [-1.5(\text{ec} \times 1.32)] - 14.8$$

$$gc = \{[(s - 1) - b]/m\} + 4.7$$

$$\text{SA}Prgc = \sum 10^{(gc \times 0.1)}$$

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(ies) in dB at a given eccentricity. To account for the total number of ganglion cells in an area of the retina, the cell density derived from each perimetry measurement was considered to be uniform over an area of retina corresponding to an area of 6×6 degrees of visual space that separates test locations in SAP. By applying the above formulas, a SAP-derived estimate of the total number of RGCs (SA*Prgc*) was obtained 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 (OCT*rgc*), we applied the following formulas:

$$d = (-0.007 \times \text{age}) + 1.4$$

$$c = (-0.26 \times \text{MD}) + 0.12$$

$$a = \text{average RNFL thickness} \times 10,870 \times d$$

$$\text{OCT}rgc = 10^{\left[\left(\log(a) \times 10 - c\right) 0.1\right]}$$

In the above formulas, d corresponds to the axonal density (axons/μm^2), c is a correction factor for the severity of disease to take into account remodeling of the RNFL axonal and non-axonal composition. The variable a corresponds to the number of axons passing toward the optic disc at the point of the OCT circumference. These calculations provide estimates of the number of RGCs from two sources, one functional and one structural. The final estimate of RGC count was obtained by simply averaging the functional and structural estimates:

$$\text{Estimated RGC count} = (\text{OCT}rgc + \text{SA}Prgc)/2$$

Statistical Analysis

The primary purpose of the analysis was to quantify the relationship between change in structural and functional parameters and change in estimated RGC counts as assessed from cross-sectional data. For this purpose, cubic smoothing spline curves were fitted to investigate the relationship of the functional/structural parameters and RGC counts and first derivatives (i.e., slopes) of these curves were obtained to investigate the relationship between changes in these measures.

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

Results

Table 1 shows demographic and clinical characteristics of the eyes included in the study. Average estimated RGC counts in eyes with glaucomatous visual field loss, GON with normal visual fields, and ocular hypertensive eyes were significantly different than that of healthy eyes, after adjustment for age differences between groups ($P < 0.03$ for all comparisons). Figure 1 shows boxplots of the distribution of estimated RGC counts in each one of the groups.

Figure 2A shows the relationship between MD and estimated RGC counts. A curve was fit to the data using smoothing splines. The fit had an adjusted R^2 value of 0.94. Figure 2B shows the first derivatives obtained from the curve shown on Figure 2A and plotted against estimated RGC counts. The derivatives correspond to the slopes of the curve shown on Figure 2A at different RGC count values. They indicate the amount of change in MD per 10,000 RGCs. For example, for an eye with 600,000 RGCs, an additional loss of 10,000 RGCs would correspond to a change of approximately 0.2 dB in MD. As Figure 2B shows, the amount of change in MD corresponding to a change in RGC count varies with the RGC counts (i.e., with disease severity). Alternatively, the same amount of RGC loss will correspond to largely different amounts of MD change according to the stage of the disease. Table 2 shows the amount of MD change that would correspond to different amounts of RGC losses at different stages of the disease. For example, for an eye with an estimated RGC count of 1,020,000 and MD of 0.4 dB, which corresponded to the median value in healthy eyes, a loss of 10,000 RGCs would correspond to a very small loss of 0.04 dB in MD. In this situation, even a very large loss of RGCs of 100,000 cells would still correspond to only 0.33 dB change in MD. In contrast, for an eye with severe glaucomatous damage with an estimated RGC count of 281,000 cells and MD of −15dB, a loss of 10,000 RGCs would correspond to 0.47 dB. A loss of 100,000 RGCs would correspond to a loss of 5.78 dB.

Figure 3A shows the relationship between average RNFL thickness and estimated RGC counts. A curve was also fit to the data using smoothing splines (adjusted $R^2 = 0.74$); but in contrast to the plot of MD versus RGC counts, it shows a linear relationship between average thickness and RGC counts throughout most of the spectrum of disease. However, at low RGC count values, the curve levels off and reaches a plateau with average RNFL thickness values rarely falling below 50 μm and with no values below 40 μm. Figure 3B shows the first derivatives obtained from the curve shown on Figure 3A and plotted against estimated RGC counts. The derivatives correspond to the slopes of the curve shown on Figure 3A at different RGC count values. They indicate the amount of change in average RNFL thickness per 10,000 RGCs. For values of estimated RGC count greater than 500,000 cells, corresponding to average RNFL thickness of approximately 65 μm, the amount of change in average RNFL thickness per 10,000 RGCs remains approximately constant at 0.5 μm. However, for values of RGC count lower than 500,000 cells, the values of the derivatives fall quickly, reaching values close to 0 for RGC counts lower than 200,000 cells, which correspond to an average RNFL thickness of approximately 55 μm. Table 3 shows the amount of SD-OCT average RNFL thickness change that would correspond to different amounts of RGC losses at different stages of the disease. For example, for an eye with an estimated RGC count of 1,020,000 and average thickness of 91 μm, a loss of 10,000 RGCs would correspond to a loss of 0.5 μm in RNFL thickness. A loss of RGCs of 100,000 cells would correspond to approximately 5 μm. For an eye with severe glaucomatous damage with an estimated RGC count of 281,000 cells and an average thickness of 57 μm, a loss of 10,000 RGCs would correspond to 0.3 μm, whereas a loss of 100,000 RGCs would correspond to only 1.5 μm.

Figure 4 shows a combined plot illustrating the relationship between MD, average RNFL thickness, and estimated RGC counts. As the plot shows, changes in RGC counts for eyes with early damage will correspond to small changes in MD, but to relatively larger changes in SD-OCT average RNFL thickness. For eyes with advanced disease, the opposite occurs with changes in RGC counts producing relatively larger changes in MD, but only small or no changes in average RNFL thickness.

Discussion

In the present study, empirical formulas were used to estimate RGC counts in order to better understand the implications of the structure and function relationship for estimating progression and measuring rates of change in glaucoma. We showed that interpretation of rates of change measured by SAP or SD-OCT, as indicators of the amount of neural damage, depends on the stage of disease. These findings have significant implications for the use of these tests to monitor glaucoma patients and individuals suspected of having the disease in clinical practice.

Analysis of the relationship between MD and RGC counts as shown in Figure 2 indicated that, at early stages of the disease, significant losses of RGCs would correspond to relatively small changes in MD This finding agrees with the large amount of evidence indicating that progressive optic disc or RNFL changes can frequently be seen before the appearance of statistically significant defects on SAP.[13,19–26] In fact, experimental studies have shown that as many as 25% to 50% of RGCs may need to be lost before the decrease in SAP threshold sensitivity values exceed normal variability and reaches statistical significance.[11,12,27] Motivated by the Fechner law,[28] scaling of perimetric stimulus intensities has been incorporated into standard perimetric testing, where the stimulus intensities are scaled by a logarithmic transformation to decibel units of attenuation for both the intensity staircase procedure for threshold measurements as well as for the report of the final threshold intensity. Several investigators have suggested that such scaling may introduce an artifactual relationship between structural and functional measurements in glauco-

Table 1. Clinical and Demographic Characteristics of the Eyes Included in the Study according to the Diagnostic Category

	Healthy, $n = 97$	OHT, $n = 98$	GON with Normal Visual Field, $n = 80$	Glaucomatous Visual Field Loss, $n = 122$
Age, y	60 ± 12	65 ± 11	68 ± 10	69 ± 11
Race, %				
Caucasian	71%	87%	86%	52%
African-American	29%	13%	14%	48%
Sex, %				
Female	30%	33%	41%	52%
MD*	0.4 (−0.4, 1.15)	0.4 (−0.3, 1.1)	−0.1 (−1.0, 0.6)	−4.9 (−11.0, −1.9)
PSD*	1.6 (1.3, 1.8)	1.5 (1.3, 1.7)	1.7 (1.5, 2.0)	6.5 (2.7, 10.8)
Average RNFL thickness, μm	91.5 ± 8.8	87.4 ± 8.1	82.3 ± 12.2	67.6 ± 12.0
RGC count, cells	1,035,573 ± 177,678	984,023 ± 151,411	888,098 ± 176,488	557,800 ± 245,350

Values represent mean ± standard deviation, unless otherwise indicated.
* Median (interquartile range).

ma.[13–15,20] The logarithmic scale would accentuate sensitivity changes in the visual field at low decibel values and minimize changes at high decibel levels, as shown in Figure 2. However, although this has served to increase awareness for early diagnosis of the disease based on optic nerve and retinal nerve fiber layer examinations, the issue of potential underestimation of rates of change with SAP has been largely ignored.

The vast majority of studies evaluating rates of change in glaucoma have used linear estimation of rates of change using indexes such as SAP MD, expressed in dB/y. However, the exponential relationship between visual field sensitivity in dB and structural measurements as shown in our study and in several previous clinical and experimental investigations,[12,13,15,29–32] suggests that a linear rate of change in decibels would only be possible if the rate of RGC loss in glaucoma decreases exponentially over time. That is, the amount of RGCs lost per unit of time would be a constant proportion of the remaining RGCs. This implies that the probability of an RGC dying would be constant and memoryless over time. Although this hypothesis cannot be completely ruled out, it seems rather unlikely based on experimental and clinical evidence.[33] In fact, longitudinal studies with imaging devices have suggested linear rates of structural loss over time that would be incompatible with linear rates of visual field loss in decibels per unit of time.[26,34–37]

A few previous studies have attempted to evaluate the issue of linearity of visual field losses over time. Bengtsson and colleagues[38] evaluated whether linear regression of the initial visual field index (VFI) values on a series of visual fields was a reliable estimator of future values for this index. Although the authors claimed a good predictive ability, analysis of the data reveals that a large number of patients (30%) had a difference between predicted and observed VFI values greater than 10%, which can be considered quite substantial. Further, the presence of treatment effects during follow-up would certainly confound the results, potentially leading to a decrease in rates of change that could otherwise be exponentially increasing over time. In another study, McNaught et al.[39] compared different curve-fitting models on visual field data obtained from five patients followed untreated over time for an average of 5.7 years. Fifteen visual field data points were available during follow-up. The authors obtained R^2 values of 0.61 and 0.53 for exponential and linear models, respectively, when all data were used to fit the models. Subsequently, they fit the models to the

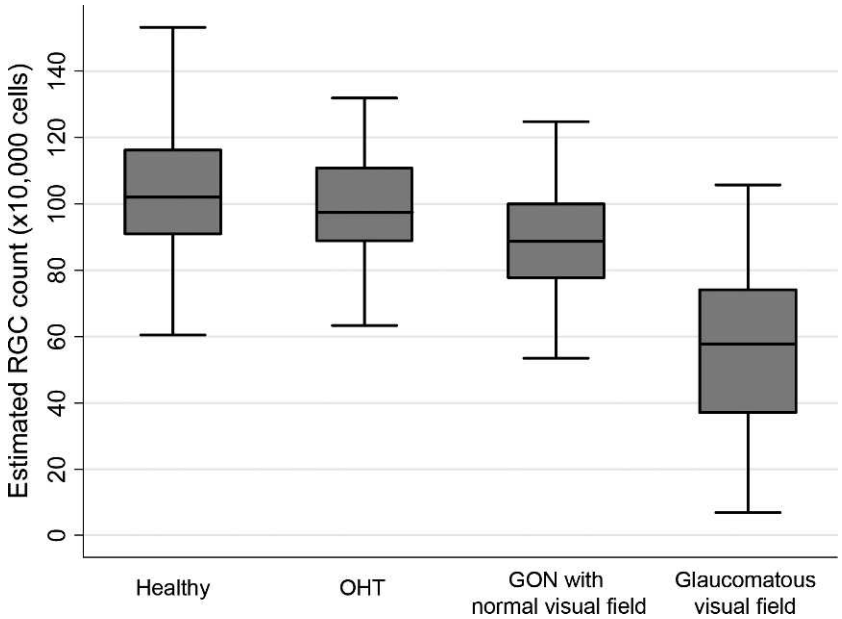

Figure 1. Boxplot graph illustrating the distribution of estimates of RGC counts in the four diagnostic categories: healthy eyes, eyes with ocular hypertension (OHT), eyes with GON but normal visual fields, and eyes with glaucomatous visual fields.

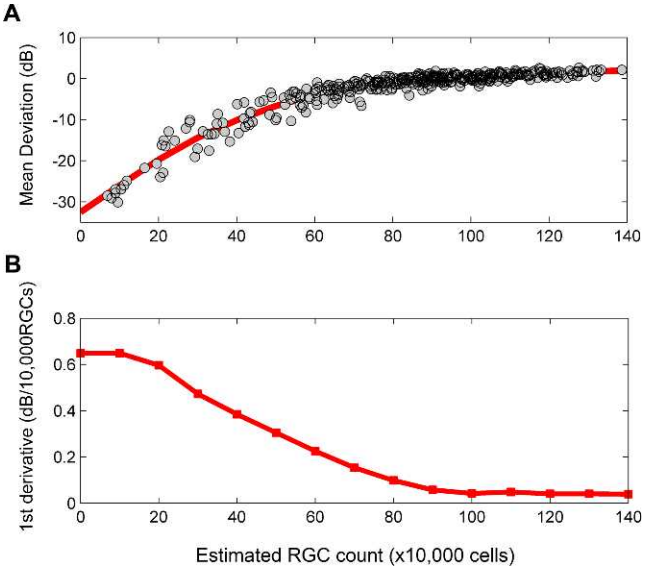

Figure 2. Analysis of the relationship between visual field parameters and estimated RGC counts. (**A**) Relationship between MD and estimated RGC counts. (**B**) First derivatives of the *curve* shown on Figure 2A plotted against estimated RGC counts. The derivatives indicate the amount of change in MD per 10,000 RGCs at different levels of RGC counts.

first five data points and evaluated the ability of the different models in predicting the subsequent 10 data points. As the linear model resulted in lower predictive error than the other models, the authors concluded that the linear model would be the most adequate for visual field data expressed in decibels. However, it would certainly be difficult to fit any reliable model other than perhaps a linear one with only five data points. The findings of McNaught et al.[39] may also be the result of a restricted window of observation on the natural course of the disease. When only a relatively short period of time is evaluated, it is likely that a linear fit will provide a good model for the data, as when a curve is approximated by several contiguous short lines, such as in piecewise regression. It is important to emphasize, however, that linear rates of visual field loss in dB/y may still provide clinically relevant information with regard to the presence of change in visual function over time, especially in moderate to advanced damage and over relatively short periods of follow-up.

The nonlinear relationship between MD and RGC counts indicates that face value interpretations of rates of MD loss over time can be misleading. A recent review of Chauhan and colleagues[10] has suggested that rates of MD change slower than −0.5 dB/y would be unlikely to lead to visual disability whereas rates faster than −2 dB/y should be considered as indicative of fast progression. As Figure 2 and Table 2 demonstrate, a change of −0.5 dB in MD in early stages of the disease (with initial MD close to 0 dB) would correspond to a loss of approximately 100,000 RGCs. Such loss would actually be greater than the loss of approximately 35,000 cells that would be associated with a 2-dB change in MD for an eye with severe damage and MD of −15 dB (Table 2). As another example, consider an eye with initial RGC count of 1,000,000 that is showing a linear rate of loss of 100,000 RGCs per year. This eye would lose all RGCs in 10 years. However, analysis of rates of MD change during the first year of the disease would indicate a rate of only approximately −0.3 dB/y (Table 2). Sole reliance on linear rates of MD change in this situation could potentially lead to severe underestimation of the risk of functional impairment. In fact, by the time a rate of loss of 100,000 RGCs/year corresponds to −2 dB/y, the eye would have close to 650,000 RGCs, or a loss of 35% of neural tissue.

Table 2. Change in SAP MD Index Corresponding to Different Amounts of Change in Estimated RGC Counts at Different Stages of the Disease

Stage of Disease		Change in MD, dB, for a Change of:		
MD, dB	Estimated RGC Count	10,000 RGCs	35,000 RGCs	100,000 RGCs
0.4*	1,020,000	0.04	0.11	0.33
−2	710,000	0.15	0.56	1.79
−5	560,000	0.25	0.94	2.98
−10	403,000	0.39	1.34	3.99
−15	281,000	0.47	1.78	5.78
−20	193,000	0.64	2.35	7.02
−25	121,000	0.71	2.53	7.25

* Average MD of the healthy eyes included in the study.

Although it should be recognized that additional losses of visual function in patients with severe damage would carry a higher risk of producing disability than in those with normal visual fields or early visual field loss, the underestimation of rates of neural damage by SAP in early stages of the disease could potentially lead to underestimation of the risk of functional impairment from the disease. Even if one recognizes the nonlinear relationship between MD and RGC counts as shown in Figure 2 and interprets MD values accordingly, small rates of change in dB/y will be more difficult to detect due to the variability of measurements and, therefore, sole reliance on SAP for measurement of rates of change will still have the potential for underestimating neural losses. It could be argued that treatment could be started later once the eye has shown clear evidence of significant rates of change on SAP. However, it is important to emphasize that if treatment is initiated late in the course of the disease, a much slower rate of change will have to be achieved in order to prevent development of functional impairment than what would be necessary if treatment had been started earlier. Although it is generally possible to slow down the rate of disease progression and keep patients close to stability even if they have moderate or advanced damage,[40] this usually requires more aggressive interventions compared with what would be necessary if treatment had been started at an earlier stage.

The relationship between SDOCT average RNFL thickness and RGC counts as shown on Figure 3 was fundamentally different than that for SAP. The relationship was linear throughout most of the spectrum of damage, with a 0.5-μm change in average RNFL thickness corresponding to a 10,000 change in estimated RGC counts. In a recent work, patients with progressive glaucoma had a mean rate of estimated RGC losses of −33,369 cells/year.[16] Such rate would correspond to a rate of approximately −1.7 μm/year of loss in average RNFL thickness, a number that is similar to rates of structural change found in other studies.[41] The linear relationship between average thickness and RGC counts indicate that imaging instruments could be used to gauge information on rates of neural losses in early disease, when rates of SAP change can be misleading. Our observations are in agreement with several previous studies showing significant rates of structural damage in eyes with early glaucoma in the absence of apparent visual field deterioration.[23–26,34,35,42–49] For RGC counts below 500,000 RGCs, there was a decrease in the first derivatives of the relationship between average thickness and RGC counts (Fig. 3B). This would indicate that equivalent amounts of RGC loss would correspond to progressive smaller changes on RNFL

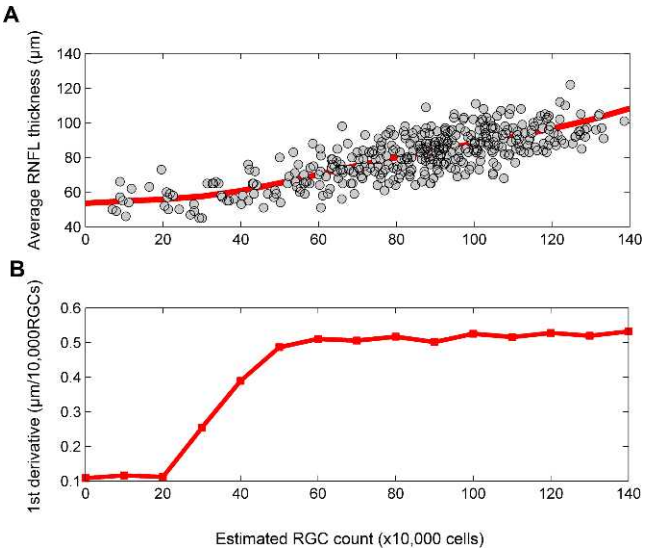

Figure 3. Analysis of the relationship between structural parameters and estimated RGC counts. (**A**) Relationship between SD-OCT, average RNFL thickness, and RGC counts. (**B**) First derivatives of the *curve* shown on Figure 3A plotted against estimated RGC counts. The derivatives indicate the amount of change in average RNFL thickness per 10,000 RGCs at different levels of RGC counts.

thickness as measured by SD-OCT. An eye with 500,000 RGCs would have a predicted average thickness of 65 µm and an MD of −6.7 dB. At this point, assessment of rates of change with SD-OCT would get progressively less helpful. For estimated RGC counts lower than 200,000 cells, corresponding to an average RNFL thickness of 55 µm and MD of −19.5 dB, changes in RGC counts would be largely undetected by SD-OCT with derivatives close to zero. The value of 55 µm seem to approximately correspond to a floor of the instrument and average RNFL thickness measurements rarely fall significantly below this level. The presence of such floor has been shown by several previous investigations and seems to be related to the presence of non-neural or glial tissue, as well as to the dynamic range of the instrument.[12,13,50] The relationship between average RNFL thickness and RGC counts shown on Figure 3 suggests that SD-OCT would be most useful in relatively early stages of damage. This agrees very well with several previous observations about the diagnostic accuracy of OCT in glaucoma. Sihota et al.[51] found that the OCT had poor ability in discriminating eyes with early from moderate glaucoma with a receiver operating characteristic (ROC) curve area of 0.705. For discriminating eyes with moderate to severe damage, the ROC curve area was 0.737 and for severe versus blind glaucoma, it was only 0.635.

The differences between SAP and SD-OCT in their relationships with estimated RGC counts are represented in Figure 4. Analysis of Figure 4 helps us understand the differences between these two tests in their abilities to detect damage at different stages of the disease, as discussed above. In addition, it helps explain, at least in part, disagreements that are commonly seen between these tests when they are used to assess progression in the disease. Analysis of Figure 4 also helps explain some other interesting clinical observations. It has been shown, for example, that eyes with more severe visual field losses at baseline are at higher risk of developing further progressive visual field losses.[52] Such observation may be simply the result that in eyes with more advanced damage, a smaller number of RGC losses would be necessary to produce relatively larger changes in MD compared with eyes with early damage (Table 2). Interestingly, in longitudinal studies with imaging instruments, the relationship between severity of

Table 3. Change in OCT Average RNFL Thickness Measurements Corresponding to Different Amounts of Change in Estimated RGC Counts at Different Stages of the Disease

Stage of Disease		Change in Average RNFL Thickness, µm, for a Change of:		
MD, dB	Estimated RGC Count	10,000 RGCs	35,000 RGCs	100,000 RGCs
0.4*	1,020,000	0.5	1.9	5.4
−2	710,000	0.5	1.7	5.0
−5	560,000	0.5	1.5	4.5
−10	403,000	0.4	1.0	2.6
−15	281,000	0.3	0.6	1.5
−20	193,000	0.05	0.1	0.4
−25	121,000	0.03	0.1	0.3

* Average MD of the healthy eyes included in the study.

disease at baseline and progressive RNFL or rim area damage has been shown to be the opposite of that described for visual fields.[25,34,35,53] That is, eyes with larger baseline values and less severe disease were those with faster rates of structural change. This again could be explained by the relative decrease in the ability of imaging devices to detect structural changes in more advanced glaucoma.

It should be noted that the predictions reported in our study for the relationships between structural and functional tests versus estimated RGC counts refer to averages of large samples of eyes. Due to the variability of measurements and number of RGCs, the actual values for individual eyes can be quite variable. It is also important to emphasize that our conclusions would remain unchanged if the analysis of the relationship between MD and RGC counts was performed using RGC counts estimated from OCT data only (see Supplementary Material and Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10345/-/DCSupplemental). Also, although SAP MD is an age-adjusted index, whereas average RNFL thickness and RGC counts are not, similar results were obtained when we used mean sensitivity values for the whole visual field

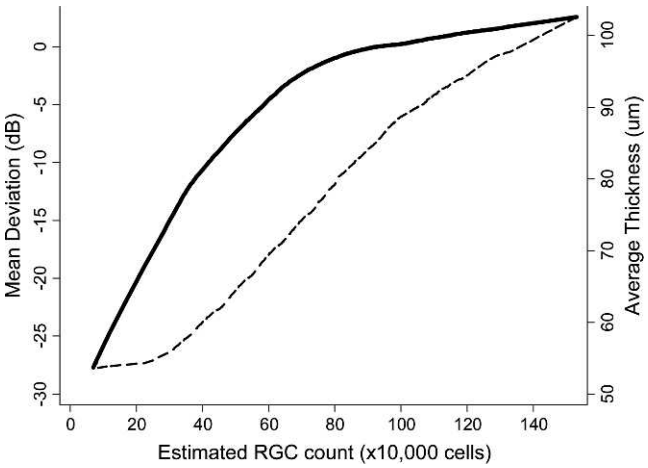

Figure 4. Relationship between MD, average RNFL thickness measurements, and estimated RGC counts. At early stages of damage (high RGC counts), changes in estimated RGC counts correspond to relatively smaller changes in MD (*continuous line*) and relatively larger changes in average RNFL thickness (*dashed line*). At advanced stages of damage (low RGC counts), changes in estimated RGC counts correspond to relatively large changes in MD, but only small changes in average RNFL thickness.

instead of MD (see Supplementary Material and Supplementary Fig. S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10345/-/DCSupplemental). This suggests that our conclusions were not dependent on the specific test used to estimate RGC counts.

Our study has limitations. We used empirically derived formulas to estimate the number of RGCs from SAP and OCT data and our estimates of RGC counts were not based on direct histologic RGC counts in humans. It could be argued that our observations are just the result of the empirical formulas used to obtain RGC counts. However, several pieces of evidence give support to our method. The empirical formulas derived by Harwerth and colleagues[12] have been validated on histological studies in monkeys that have a visual system almost indistinguishable to that of humans. The relationship between predicted RGC counts and histologically measured RGC numbers had an R^2 of 0.9, indicating an almost perfect predictive value. Therefore, if the empirical formulas closely predict the histological counts, there is little reason to believe that our findings are just an artifact from the calculations. The validity of our analyses is also supported by their ability to explain results of several clinical investigations related to detection of progression with functional and structural tests, as discussed above. Another limitation of our study is that estimates of RGC counts relied on the 24-2 test of SAP, which potentially leads to undersampling of the macular region. The additional use of SAP tests with higher concentration of points in the macular area could potentially improve the estimates of RGC counts.[54–56]

As two sources of RGC count estimates were available, one structural and one functional, we simply averaged the two estimates to obtain the final RGC count for the purposes of this study. However, in previous investigations, we have shown that a weighted (by disease severity) combination of estimates of RGC counts obtained by SAP and OCT actually performs better for detection of glaucoma and assessment of progression than simply averaging the two estimates.[16,17] This agrees with the observations presented on the current study about the different performances of these tests according to the severity of disease. It should be noted that a simple transformation of SAP sensitivity data into RGC counts does not improve detection of early disease with this instrument. In a previous work, we showed that SAP RGC counts have an area under the ROC curve of only 0.69 to detect preperimetric glaucomatous damage based on progressive optic disc changes.[17] Such observation is most likely the result of the fact that SAP data is originally acquired in decibels. This reinforces the need for a weighted estimate of the final RGC counts if one wants to maximize the performance for detection of early disease and progressive damage. In the current study, we did not use the weighting system to calculate final estimates of RGC counts in order to avoid potential biases when studying the relationships among SAP, OCT, and RGC counts.

In conclusion, our findings suggest that the analysis and interpretation of rates of SAP and OCT change over time in glaucoma should depend on the stage of the disease. The limitations of these tests suggest a strong need for approaches combining structural and functional data for detection of progression and estimation of rates of change in the disease.

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