Predicting Progression in Glaucoma Suspects With Longitudinal Estimates of Retinal Ganglion Cell Counts

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Purpose. We evaluated the ability of baseline and longitudinal estimates of retinal ganglion cell (RGC) counts in predicting progression in eyes suspected of having glaucoma.

Methods. The study included 288 glaucoma suspect eyes of 288 patients followed for an average of 3.8 ± 1.0 years. Participants had normal standard automated perimetry (SAP) at baseline. Retinal nerve fiber layer thickness assessment was performed with optical coherence tomography (OCT). Progression was defined as development of repeatable abnormal SAP or glaucomatous progressive optic disc changes. Estimates of RGC counts were obtained by combining data from SAP and OCT according to a previously described method. Joint longitudinal survival models were used to evaluate the ability of baseline and rates of change in estimated RGC counts for predicting progression over time, adjusting for confounding variables.

Results. A total of 48 eyes (17%) showed progression during follow-up. The mean rate of change in estimated RGC counts was −18,987 cells/y in progressors versus −8,808 cells/y for nonprogressors ($P < 0.001$). Baseline RGC counts and slopes of RGC loss were significantly predictive of progression, with HRs of 1.56 per 100,000 cells lower (95% confidence interval [CI], 1.18–2.08; $P = 0.002$) and 2.68 per 10,000 cells/y faster loss (95% CI, 1.22–5.90; $P = 0.014$), respectively. The longitudinal model including estimates of RGC counts performed significantly better than models including only structural or functional indexes separately.

Conclusions. Baseline and longitudinal estimates of RGC counts may be helpful in predicting progression and performed significantly better than conventional approaches for risk stratification of glaucoma suspects.

Keywords: glaucoma, visual field, optical coherence tomography, optic nerve head, intraocular pressure

Glaucoma is an optic neuropathy characterized by progressive neuroretinal rim thinning, excavation, and loss of the retinal nerve fiber layer (RNFL).[1] These structural changes usually are accompanied by functional loss, which may result in visual disabilities, such as decreased reading speed, ability to walk, and ability to drive, and eventually blindness.[2,3] Among patients with suspected glaucoma or with risk factors for the disease, only a proportion will have clear signs of damage during follow-up.[4] Therefore, stratification of patients according to the risk of disease may provide better allocation of resources, allowing more frequent monitoring and earlier intervention in those at higher risk, while avoiding unnecessary interventions and treatment side effects in those deemed at low risk.

Studies have shown that certain risk factors, such as older age, high IOP, thinner central cornea, and disk hemorrhages, are predictive of the development of primary open-angle glaucoma among those suspected of having the disease.[4,5] In addition, certain visual field parameters and structural characteristics of the optic nerve can help predict those eyes with a higher chance of clear signs of glaucomatous damage developing during follow-up.[4–6] Although different structural and functional parameters have been shown to have predictive ability, few attempts have been made to apply a combination of these parameters to improve prediction of damage.[7,8] Due to the different characteristics of structural and functional tests, it is possible that a combined approach could perform better than the isolated use of structural or functional tests.

A combined structure and function approach to estimate retinal ganglion cell (RGC) counts has been described by Medeiros et al.,[9,10] with the purpose of merging results of structural and functional tests into a single index that could be used for diagnosis, staging, and detecting glaucomatous progression. The index uses estimates of RGC counts obtained by previously derived empirical formulas. The estimates of RGC counts are obtained from two sources: one structural, RNFL thickness assessment with optical coherence tomography (OCT); and one functional, standard automated perimetry (SAP). These estimates then are combined using a weighted average to provide a single estimate of the RGC count for a particular eye. For each eye, an index (combined structure and function index [CSFI]), representing the percent estimate of

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RGC loss compared to the age-expected number of RGCs, also has been proposed.

The use of a combined structure and function measure to estimate RGC losses has been shown to perform better than isolated structural and functional parameters for diagnosing and staging glaucomatous damage, as well as for detecting progressive disease.[9,10] However, its performance for predicting progressive glaucomatous damage has not yet been evaluated to our knowledge. Therefore, the purpose of our study was to evaluate the ability of baseline and longitudinal estimates of RGC counts in predicting progressive glaucomatous damage in subjects suspected of having the disease followed over time. We also evaluated whether the proposed method improves risk stratification of patients with suspected glaucoma compared to isolated structural or functional measures.

Methods

This was an observational cohort study. The study participants were selected from 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 included the Hamilton Glaucoma Center at the Department of Ophthalmology, University of California, San Diego (La Jolla, CA; data coordinating center); the New York Eye and Ear Infirmary (New York, NY); and the Department of Ophthalmology, University of Alabama (Birmingham, AL). Although the DIGS includes only patients recruited at the University of California, San Diego, the protocols of the two studies are identical. Methodologic details have been described previously.[11]

All patients from the DIGS and ADAGES who met the inclusion criteria described below were enrolled in our study. Informed consent was obtained from all participants. This prospectively designed study received institutional review board approval at all involved sites. The methodology adhered to the tenets of the Declaration of Helsinki, and to the Health Insurance Portability and Accountability Act.

At each visit during follow-up, subjects underwent a comprehensive ophthalmologic examination, including review of medical history, best-corrected visual acuity, slit-lamp biomicroscopy, IOP measurement with Goldmann applanation tonometry, gonioscopy, dilated funduscopic examination, stereoscopic optic disc photography, SAP, and OCT testing. Central corneal thickness (CCT) was calculated as the average of three measurements obtained during the same visit using an ultrasound pachymeter (Pachette GDH 500; DGH Technology, Inc., Philadelphia, PA). Optic disc area was measured using a confocal scanning laser ophthalmoscope (HRT-II; Heidelberg Engineering GmbH, Heidelberg, Germany). Only subjects with open angles on gonioscopy were included. Subjects were excluded if they had a best-corrected visual acuity of less than 20/40, spherical refraction outside ±5.0 diopters (D), cylinder correction outside 3.0 D, or a combination thereof; or any other ocular or systemic disease that could affect the optic nerve or visual field. One eye of each patient was selected randomly for analysis.

This study included eyes suspected of having glaucoma at the baseline visit. This was based on the presence of suspicious appearance of the optic disc (neuroretinal rim thinning, excavation, or suspicious RNFL defects) or elevated IOP (>21 mm Hg), but normal SAP tests at baseline. Normal visual fields were defined based on mean deviation (MD) and pattern standard deviation (PSD) within 95% confidence limits (95% CI), and a Glaucoma Hemifield Test (GHT) within normal limits. All visual fields were evaluated by the UCSD Visual Field Assessment Center (VisFACT).[12] Visual fields with more than 33% fixation losses or false-negative errors, or more than 15% false-positive errors were excluded.

Each patient was required to have a minimum of 4 OCT examinations per eye during a minimum of two years of follow-up. During follow-up, patients were treated at the discretion of the attending ophthalmologist.

Optical Coherence Tomography

Subjects underwent ocular imaging with dilated pupils using the time-domain Stratus OCT (Carl-Zeiss Meditec, Inc., Dublin, CA). The fast RNFL algorithm was used to obtain RNFL thickness measurements with the OCT. Three images were acquired from each subject, with each image consisting of 256 A-scans along a 3.4-mm diameter circular ring around the optic disc. The average parapapillary RNFL thickness (360° measure) was calculated automatically by the software built into the Stratus OCT (Carl-Zeiss Meditec, Inc.) and was used in the study. Quality assessment of OCT scans was evaluated by Imaging Data Evaluation and Assessment (IDEA) Center experienced examiners masked to the subject's results of the other tests. Good-quality scans had to have focused images from the ocular fundus, signal strength of more than 7, and presence of a centered circular ring around the optic disc. RNFL scans also were evaluated as to the adequacy of the algorithm for detection of the RNFL. Only scans without overt algorithm failure in detecting the retinal borders were included in the study.

Estimates of Retinal Ganglion Cell Counts

The method for obtaining estimates of RGC counts has been described in detail by Medeiros et al.[9] Briefly, the method uses information from structural and functional tests to derive a final estimate of RGC count for a particular eye. The empirical formulas for estimating RGC counts for each test separately were developed previously from experimental studies in monkeys,[13] and subsequently validated on clinical and histologic studies in humans.[14]

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 decibels:

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

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

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

$$SAPrgc = \sum 10^{\wedge}(gc \times 0.1)$$

Where m and b represent the slope and intercept, respectively, of the linear function relating ganglion cell quantity (*gc*) in decibels to the visual field sensitivity (*s*) in decibels at a given eccentricity (*ec*). By applying the formulas, one can obtain a SAP-derived estimate of the total number of RGCs (SAP*rgc*) by adding the estimates from all the 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 OCT. To derive the total number of RGC axons from the global RNFL thickness measurement obtained by OCT (OCT*rgc*), one can apply the following

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formulas:

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

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

$$OCTrgc = 10\text{^}\{(\log[average\ RNFL\ thickness \times 10870 \times d] \times 10 - c) \times 0.1\}$$

Where d corresponds to the axonal density (axons per micrometers squared) and c is a correction factor for the severity of disease to consider remodeling of the RNFL axonal and nonaxonal composition. The model considered the effect of aging in the axonal density and the effect of disease severity on the relationship between the neuronal and nonneuronal components of the RNFL thickness estimates obtained by OCT. The above calculations allow one to estimate the number of RGCs from 2 sources, 1 functional and 1 structural, and a strong relationship was demonstrated between the 2 estimates in external validation cohorts. 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 SAP and OCT test accuracies have been proposed to be inversely related to disease severity,[15] we used the following weighted scale combining the estimates of RGC numbers from both tests to obtain a final estimate:

$$Estimated\ RGC\ count = \left(1 + \frac{MD}{30}\right) \times OCTrgc + \left(-\frac{MD}{30}\right) \times SAPrgc$$

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 −30 dB.

The expected RGC count for each eye was calculated based on a previous study that described a linear regression model that relates RGC estimates to age and optic disc area in a normal control population.[9] The model predicts expected RGC counts according to age in years and optic disc area in mm^2. 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 counts. The percent estimate of RGC loss by the CSFI should reflect an estimate of glaucomatous damage obtained by combining data from the structural and functional measurements, as calculated below:

$$CSFI = \left[\frac{expected\ RGC\ count - estimated\ RGC\ count}{expected\ RGC\ count}\right] \times 100$$

Follow-Up and Definition of Study Endpoints

The study endpoints were defined as the development of repeatable abnormal visual field defects and/or progressive optic disc changes during follow-up. Development of a visual field defect was defined as the presence of 3 consecutive abnormal SAP tests during follow-up. An abnormal visual field was defined as a PSD with $P < 0.05$ and/or a GHT with outside normal limits result.

Progressive optic disc damage was evaluated by masked assessment of optic disc stereophotographs obtained during follow-up. Simultaneous stereoscopic optic disc photographs (TRC-SS; Topcon Instrument Corp of America, Paramus, NJ) were reviewed with a stereoscopic viewer (Pentax Stereo Viewer II; Asahi Optical Co., Tokyo, Japan). Each grader was masked to the temporal sequence of the photographs. Definition of change was based on focal or diffuse thinning of the neuroretinal rim, increased excavation, and the appearance or enlargement of RNFL defects. Discrepancies between the two graders were resolved either by consensus or by adjudication of a third experienced grader. Only photographs of adequate quality were included.

Eyes that experienced an endpoint during the study follow-up were denominated as "progressors," whereas eyes that did not experience the study endpoint were denominated as "nonprogressors." For progressors, follow-up time was defined as the time between the OCT baseline visit and the date of the first abnormal visual field result or the first optic disc stereophotograph showing deterioration (the study endpoint). For nonprogressors in visual field and optic disc evaluations, follow-up time was defined as the time between the OCT baseline visit and date of last available follow-up. OCT exams were acquired within 3 months of corresponding visual fields and optic disc photographs.

To evaluate whether baseline and longitudinal measurements were predictive of the study endpoints, only tests acquired before the event date were analyzed in the study. Eyes that did not experience the study endpoint were considered censored at the last follow-up visit. All tests up to the last available follow-up date were analyzed for these eyes.

Statistical Analysis

The primary purpose of the study was to determine whether baseline and longitudinal estimates of RGC counts and CSFI values were predictive of progression in glaucoma suspects. A joint longitudinal survival model was used to investigate the relationship between longitudinal measurements and risk of progression. These models are ideally suited to study the association between changes in a longitudinal marker and the risk for an event, and have been described in detail previously.[16,17] In brief, they are composed of a longitudinal submodel and a survival submodel, which are tied together by sharing random effects. The longitudinal submodel was composed of a linear mixed model with the following formulation:

$$\begin{cases} y_i(t) = m_i(t) + \varepsilon_i(t) \\ m_i(t) = X_i\beta + Z_i b_i \\ b_i \sim N(0, D), \varepsilon_i(t) \sim N(0, \sigma^2) \end{cases}$$

The model specifically accounts for measurement error of the marker by postulating that the observed level of the outcome $y_i(t)$, corresponding to the estimated RGC count measurements, equals the unobserved true value $m_i(t)$ plus a random error term, $\varepsilon_i(t)$. The mixed model assumes random slopes and random intercepts, allowing different rates of change and intercept values for each eye.

To quantify the strength of the association between the longitudinal marker and the risk for the event (development of visual field loss and/or progressive optic disc damage), a survival submodel was used with the form:

$$h_i(t) = h_0(t)\exp(\gamma_1^T w_i + \gamma_2^T v_i + \alpha_1 m_i[t_0] + \alpha_2 m_i'[t]),$$

where $m_i' = \frac{d}{dt} m_i(t)$.

In the survival submodel, $h_i(t)$ determines the hazard function at time t, h_0 denotes the baseline hazard function specified by a Weibull distribution, w_i is a vector of baseline covariates with corresponding vector of coefficients (γ_1), v_i is a vector of time-dependent covariates with corresponding vector of coefficients (γ_2). This model was estimated jointly with the longitudinal submodel and allowed an evaluation of the

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Table 1. Baseline Demographic and Clinical Characteristics of Study Patients

Variables	Progressors, $n = 48$	Nonprogressors, $n = 240$	P
Age at baseline, y	60.4 ± 10.8	57.0 ± 11.2	0.056
Sex, % female	60%	63%	0.703
Race			
Caucasian, %	69%	63%	0.442
African American, %	31%	37%	
Spherical equivalent, D	−0.50 ± 1.83	−0.63 ± 1.82	0.655
IOP, mm Hg	19.0 ± 4.2	18.3 ± 3.6	0.215
CCT, μm	560 ± 39	549 ± 40	0.081
SAP MD, dB	−0.84 ± 1.03	0.19 ± 1.11	<0.001
SAP PSD, dB	1.75 ± 0.39	1.52 ± 0.31	<0.001
Average RNFL thickness, μm	85.5 ± 10.9	95.9 ± 11.3	<0.001
Estimated RGC count	848,827 ± 158,081	1,026,569 ± 167,928	<0.001
CSFI, %	17.1% ± 13.2%	2.3% ± 13.3%	<0.001

relationship between the true marker values $m_i(t)$ and the risk for the event. We were interested mainly whether the slopes of change in the marker (i.e., estimated RGC counts or CSFI values) were associated with risk of progression. Therefore, m_i' measured the first derivative (slope) of the marker profile and the coefficient α_2 measured how strongly associated was the value of the slope of the true longitudinal marker at time t with the risk for an event at the same time point, adjusting for the intercept value and values of other covariates. The interpretation of α is straightforward as in regular survival models, with $exp(\alpha)$ corresponding to the hazard ratio (HR) for a one unit change in the slope of the marker. We initially obtained a survival model including only the longitudinal marker information (intercepts and slopes), and subsequently we built a multivariable model adjusting for the baseline covariates age and CCT, and for the time-dependent covariate IOP. These variables have been reported to be associated significantly with the risk of development of glaucomatous visual field loss or optic disc deterioration among patients with ocular hypertension or suspected glaucoma.[4,5]

To assess and compare the importance of variables in determining the outcome, we used an R^2 index proposed by Royston.[18] The modified R^2 index is equivalent to the coefficient of determination of a linear model and measures the amount of variation in the outcome (survival time) explained by the predictor, or, in other words, the strength of the relationship between the predictor and the outcome in a survival model. The modified R^2 index has been proposed as the best way to assess prognostic information of survival models.[19] CIs for the modified R^2 indices and the P values for comparison of between models were obtained by bootstrapping, with 1000 replications.

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

Results

We studied 288 eyes of 288 patients with suspected glaucoma at baseline. Table 1 shows demographic and clinical characteristics of the patients included in the study. A total of 48 eyes (17%) showed progression during follow-up as assessed by visual fields or optic disc photographs. Of these 48 eyes, 23 (48%) progressed by visual fields only, 11 (23%) progressed by optic disc stereophotographs only, and 14 (29%) progressed by both tests. Mean follow-up time until the first endpoint for progressors was 4.1 ± 1.0 years. Mean follow-up time for nonprogressors was 3.7 ± 1.0 years.

The mean (± SD) estimated RGC count at baseline was 848,827 ± 167,928 cells for progressors versus 1,026,569 ± 158,081 cells for nonprogressors ($P < 0.001$). Corresponding numbers for CSFI were 17.1% ± 13.2% and 2.3% ± 13.3%, respectively ($P < 0.001$). Table 2 shows values of mean rates of change over time in the progressor and nonprogressor groups for the parameters evaluated in the study. The mean rate of change in estimated RGC counts was −18,987 cells/y in progressors versus −8,808 cells/y for nonprogressors ($P < 0.001$). Figure 1 shows raw measurements of the estimated RGC counts over time for progressors and nonprogressors, whereas Figure 2 shows the distribution of rates of change in estimated RGC counts in the two groups. Rates of change in CSFI also were significantly faster for progressors compared to nonprogressors (1.04%/y vs. −0.04%/y, respectively, $P < 0.001$).

Table 3 reports HRs for the risk of development of study endpoints for the different variables evaluated. Baseline estimated RGC counts were significantly predictive of progression, with a HR of 1.41 for each 100,000 cells lower (95% CI, 1.11–1.79; $P = 0.005$). Slopes of change in RGC counts also were significantly predictive. Each 10,000 cells/y faster rate of RGC loss corresponded to a 2.4 times higher risk of progression (HR, 2.42; 95% CI, 1.15–5.13; $P = 0.020$), after

Table 2. Mean (±SD) Rates of Change in Progressors and Nonprogressors for the Different Parameters Evaluated in the Study

Parameter	Progressors, $n = 48$	Nonprogressors, $n = 240$	P
Estimated RGC count, cells/y	−18,987 ± 6,239	−8808 ± 6233	<0.001
CSFI, %/y	1.04 ± 0.70	−0.04 ± 0.69	<0.001
Average RNFL thickness, μm/y	−0.92 ± 0.58	−0.41 ± 0.47	<0.001
SAP MD, dB/y	−0.07 ± 0.09	0.04 ± 0.07	0.002
SAP PSD, dB/y	0.11 ± 0.07	0.01 ± 0.03	<0.001

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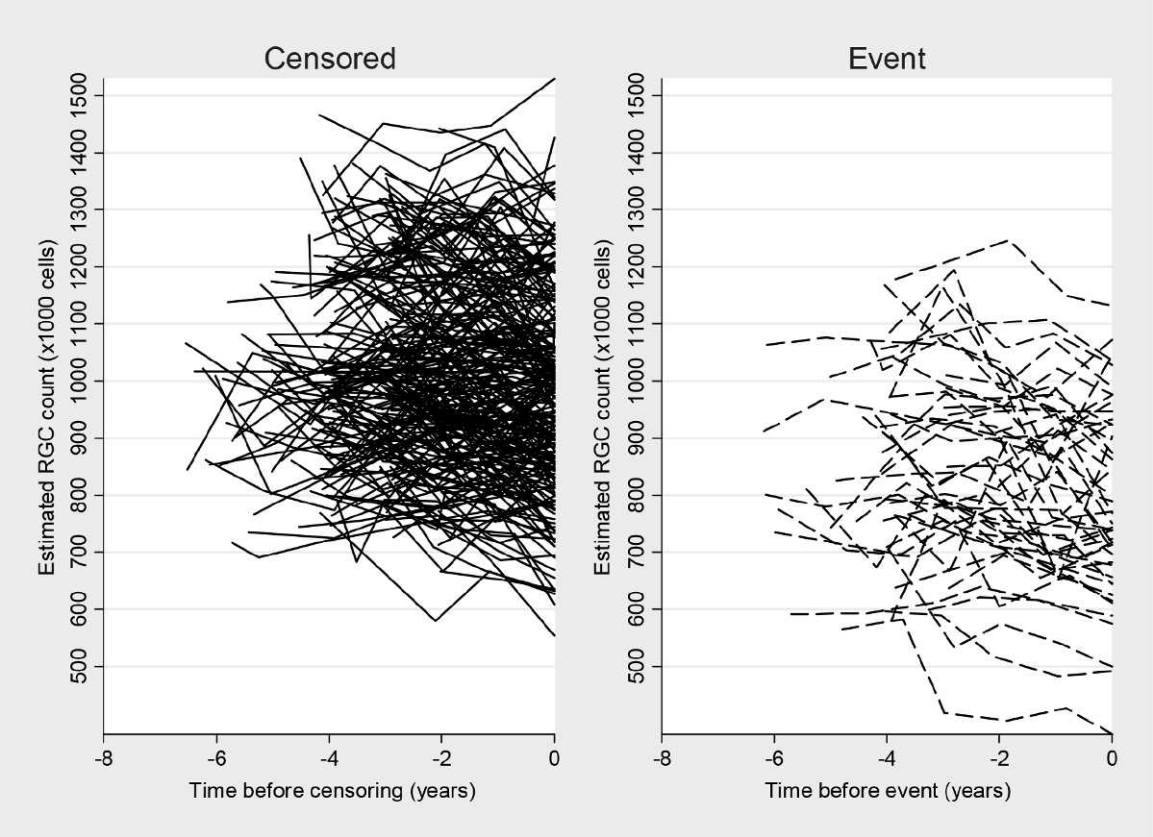

Figure 1. Raw values of estimated RGC counts over time for progressors and nonprogressors.

adjustment for baseline estimated RGC counts. In the multivariable model adjusting also for baseline age, CCT, and time-dependent follow-up IOP measurements, baseline RGC counts and slopes of estimated RGC loss still were significantly predictive of progression, with HRs of 1.56 per 100,000 cells lower (95% CI, 1.18–2.08; $P = 0.002$) and 2.68 per 10,000 cells/y faster loss (95% CI, 1.22–5.90; $P = 0.014$), respectively. Baseline and slopes of CSFI change also were significantly predictive of progression in the adjusted multivariable model, with HRs of 1.52 per 10% higher CSFI at baseline (95% CI, 1.15–2.03; $P = 0.005$) and 2.14 per 1%/y faster rate of CSFI change (95% CI, 1.14–4.02; $P = 0.018$), respectively.

The R^2 index was used to evaluate and compare the predictive abilities of the different models (Table 4). We compared the predictive abilities of the baseline models, that is, models that included information only from the baseline visit, to those of the longitudinal models, that is, models that included information from baseline as well as follow-up visits. For estimated RGC counts, the longitudinal model performed significantly better than the baseline model with R^2 of 82% vs. 31%, respectively ($P < 0.05$). Similar difference was seen for the CSFI models, with R^2 values of 76% vs. 36% for the longitudinal and baseline models, respectively ($P < 0.05$). Longitudinal models for estimated RGC counts and CSFI were significantly better than the equivalent models, including OCT RNFL thickness or visual field parameters separately ($P < 0.05$ for all comparisons, Table 4).

From the results of the joint model it also was possible to obtain individual survival probabilities for specific eyes based on estimated RGC counts obtained during follow-up. Figure 3 shows predicted survival probabilities for two eyes, one that showed a relatively fast decline in estimated RGC counts during follow-up (right panel) and another that showed relatively stable measurements over time (left panel). A comparison of the predicted survival probabilities shows that the eye with faster slope of change had much lower predicted probabilities of survival. This eye, in fact, showed development of visual field loss and optic disc progression during follow-up, whereas the eye with stable estimates of RGC counts did not show any changes on visual fields or optic disc photographs. Figure 4 shows how survival probabilities can be updated continuously during follow-up as more information becomes available. Optic disc changes as seen in stereophotographs and visual field results for the same eye also are shown. The predicted survival probabilities were relatively high when only baseline measurements were considered. As more information became available and a clear trend of loss in estimated RGC number was observed, the model estimated much lower probabilities of survival. The results were in agreement with changes observed on optic disc photographs and the eye also developed a visual field defect during follow-up.

Discussion

In our study, we showed that baseline and longitudinal estimates of RGC counts obtained from a combination of structural and functional tests were predictive of future development of visual field loss or optic disc deterioration in patients suspected of having glaucoma. Eyes with lower estimated RGC counts at baseline, and especially those with faster rates of change over time, had a greater risk of evidence of progression developing during the follow-up period. In addition, the combined structure and function measures had superior ability compared to functional and structural evalua-

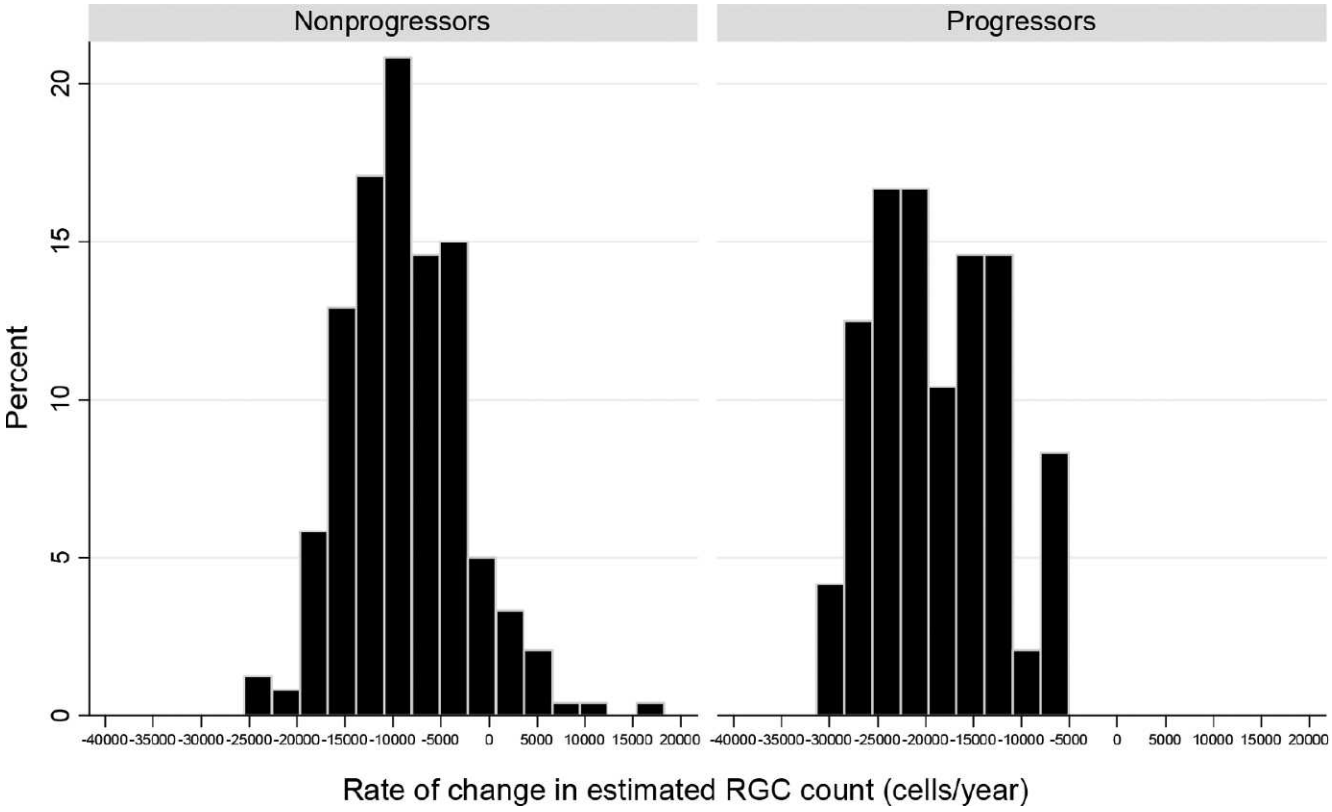

Figure 2. Distribution of rates of change in estimated RGC counts in progressors versus nonprogressors.

tions used separately in predicting which patients had glaucomatous progression during follow-up.

Glaucoma suspect eyes that had evidence of visual field loss or optic disc deterioration had significantly lower estimates of RGC counts and higher CSFI values at baseline compared to those eyes that did not have signs of progression. However, although the baseline models were significantly predictive of the outcome, a substantial gain in prediction was obtained by analyzing the longitudinal data during follow-up. The mean rate of change in estimated RGC counts for eyes with progression by SAP and/or optic disc photographs was −18,987 cells/y, which was over 2 times faster than in eyes that did not show progression by these methods (−8,808 cells/y). Importantly, the mean rate of change in estimated RGC counts for nonprogressors was similar to previously reported rates of age-related RGC losses in normal eyes.[20] However, there was a wide range of rates of change in estimated RGC counts as shown in Figure 2, and some eyes in the nonprogressor group

Table 3. HRs for Prediction of Progression Obtained From the Joint Longitudinal Survival Models

	Univariable		Multivariable*	
	Hazard Ratio	***P***	**Hazard Ratio**	***P***
Estimated RGC count				
Intercept, per 100,000 cells lower	1.41 (1.11–1.79)	0.005	1.56 (1.18–2.08)	0.002
Slope, per 10,000 cells/y faster decrease	2.42 (1.15–5.13)	0.020	2.68 (1.22–5.90)	0.014
CSFI				
Intercept, per 10% higher	1.55 (1.17–2.05)	0.002	1.52 (1.15–2.03)	0.005
Slope, per 1%/y faster increase	2.14 (1.14–4.00)	0.017	2.14 (1.14–4.02)	0.018
Average RNFL thickness				
Intercept, per 10 μm lower	1.59 (1.17–2.03)	0.003	1.55 (1.11–2.16)	0.011
Slope, per 1 μm/y faster decrease	1.97 (1.05–3.71)	0.035	2.09 (1.03–4.22)	0.040
SAP MD				
Intercept, per 0.1 dB lower	1.08 (1.03–1.12)	0.001	1.07 (1.03–1.12)	0.001
Slope, per 0.1 dB/y faster decrease	2.25 (1.24–4.07)	0.007	2.20 (1.23–3.92)	0.008
SAP PSD				
Intercept, per 0.1 dB higher	1.10 (1.03–1.18)	0.005	1.01 (0.97–1.05)	0.593
Slope, per 0.1 dB/y faster increase	1.87 (1.51–2.31)	<0.001	1.80 (1.29–2.51)	0.001

* Multivariable models adjust for baseline age, CCT, and IOP measurements obtained during follow-up.

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Table 4. Predictive Abilities for the Different Baseline and Longitudinal Models as Measured by the R^2

	R^2 (95% CI) Baseline Model	R^2 (95% CI) Longitudinal Model
Estimated RGC count	31% (14%–50%)	82% (72%–91%)
CSFI	36% (18%–59%)	76% (65%–87%)
Average RNFL thickness	24% (10%–47%)	51% (37%–67%)
SAP MD	19% (4%–38%)	45% (30%–66%)
SAP PSD	12% (4%–36%)	62% (52%–83%)

had relatively fast rates of change. It is possible that these eyes might have suffered signs of visual field loss or progressive disc damage on photographs if the follow-up time had been longer.

The use of a joint longitudinal survival model allowed us to quantify the ability of rates of change in the different parameters in predicting the risk of progression taking into account the censored aspect of the data, while also adjusting for the effect of confounding variables. For estimated RGC counts, each 10,000 cells/y faster rate of RGC loss corresponded to approximately 2.7 times higher risk of progression over time in a multivariable model adjusting for age, CCT, and IOP values acquired during follow-up. The predictive abilities of the longitudinal models were significantly better than those of models including only baseline information, as shown in Table 4. The longitudinal model including baseline and rates of change in estimated RGC counts had an R^2 of 82%, that is, this model was able to explain 82% of the variation in the outcome defined by SAP and stereophoto disc progression. Similar results were found when data were analyzed in terms of the CSFI. The longitudinal models using combined structure and function information also performed better than the longitudinal models using structural or functional tests separately. Interestingly, although 77% of the eyes with progression had evidence of visual loss during follow-up, the longitudinal models including rates of change in MD or PSD had R^2 values of only 45% and 62%, respectively. This likely is explained by the fact that progression to glaucoma also was defined by the presence of progressive optic disc damage during follow-up.[21]

In addition, the variability of visual field measurements over time may have weakened the predictive performance of longitudinal models including only visual field data. The longitudinal model including only OCT average RNFL thickness data also performed worse than the longitudinal model combing structure and function, with an R^2 of only 51%. This result suggests that a combined analysis of structural and functional data seemed to provide additional information that can help predict those glaucoma suspects more likely to develop clear signs of disease.

The joint longitudinal survival model presented in our study also allowed estimation of individual survival probabilities over time. Using this model, the risk of progression can be updated as information on predictive factors is made continuously available over time. Such an approach offers significant advantages over currently available predictive models or risk calculators designed to estimate risk of glaucoma development, which use only baseline information.[22,23] In fact, a recent study by Song et al. suggested that risk estimates obtained in ocular hypertensive patients over time using an available risk calculator can vary by a 10-fold magnitude due to variability of clinical measurements over time.[24] As Figure 4 illustrates, the joint modeling approach allows probabilities of progression to be updated continuously as more information becomes available, resulting in more effective use of clinical information. Similarly, the two eyes shown in Figure 3 had similar baseline measurements, but their risks of progression were very different when longitudinal information was incorporated into the model. We recently have demonstrated this approach in another study showing that longitudinal rim area measurements obtained by confocal scanning laser ophthalmoscopy were predictive of development of functional losses in glaucoma. In that study, the longitudinal model incorporating rim area measurements had a predictive ability with R^2 of 62%.[25] Compared to the results of the current study, the findings indicated that a superior predictive performance can be obtained by combining longitudinal structural and functional information. It should be noted that the use of a joint longitudinal survival model allowed us to evaluate the "true" association between longitudinal changes in the proposed marker and the outcome by taking into account measurement error. However, it is important to emphasize that the accuracy

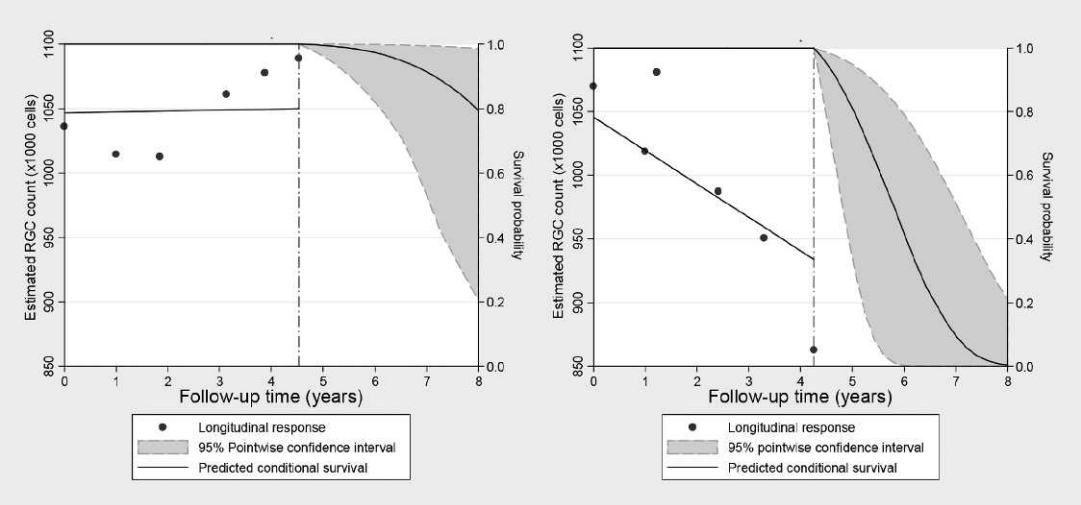

Figure 3. Predicted survival probabilities for two eyes, one that showed a relatively fast rate of change in estimated retinal ganglion cell counts during follow-up (*right*) and another that showed stable measurements over time (*left*). A comparison of the predicted survival probabilities shows that the eye with fast progression had much lower predicted probabilities of survival, that is, retaining a normal visual field or showing stable optic disc assessment on stereophotographs. This eye, in fact, showed development of visual field loss and progressive optic disc change during follow-up, whereas the eye with stable measurements did not show any change on the standard tests.

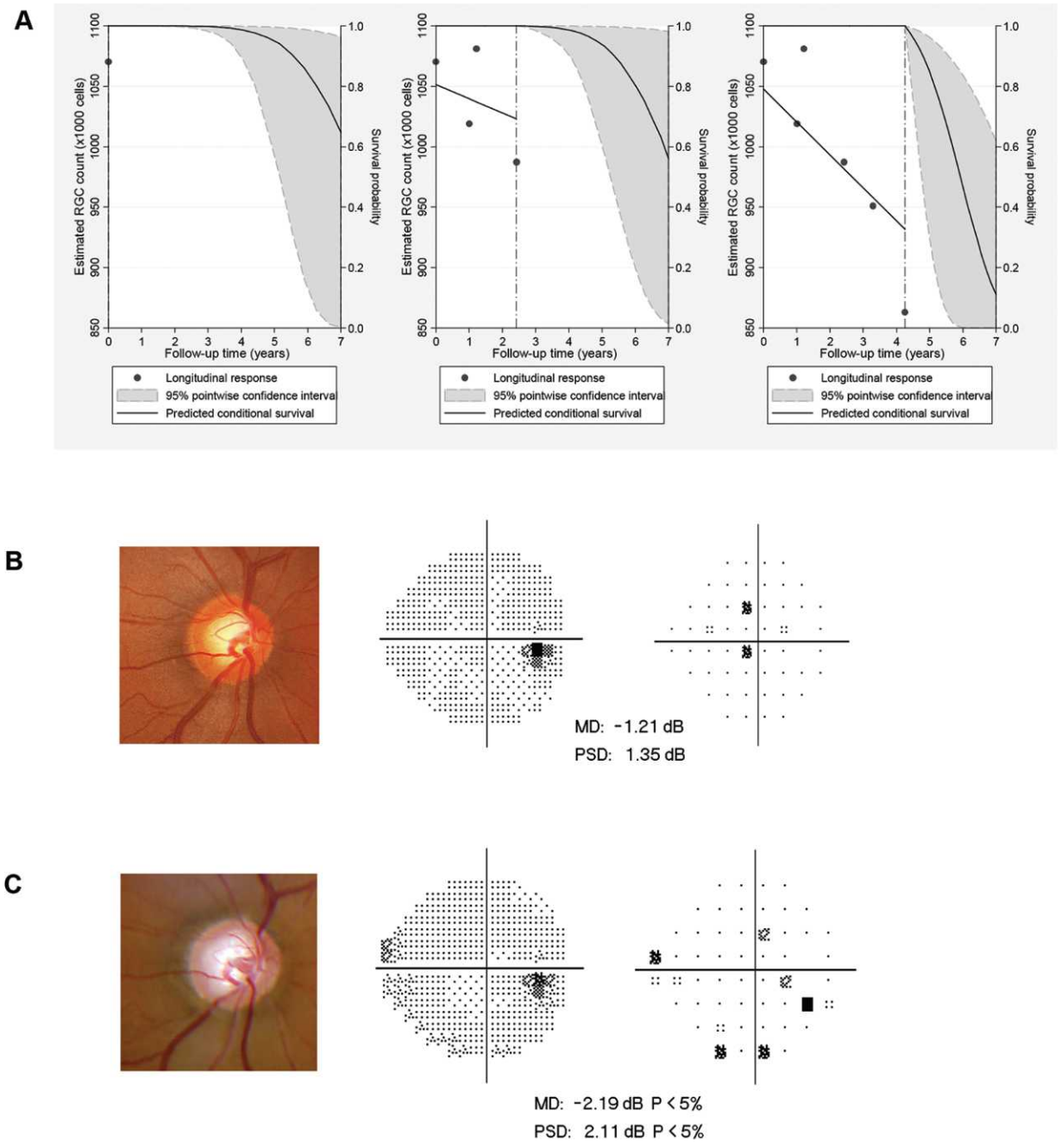

Figure 4. Example of how survival probabilities can be updated as more information on predictive factors becomes available during follow-up. (**A**) *Left*: shows survival probabilities after considering only the baseline data. The model estimated that the probability of retaining a normal visual field and stable optic disc over time was relatively high. As more information became available (*middle* and *right*), the survival probabilities were updated. The estimated survival probabilities became much lower as the result of progressive losses in estimated RGC number over time. (**B**) Baseline optic disc photograph and visual field (*grayscale* and *pattern deviation plot*) results from the same eye shown in (**A**). (**C**) Optic disc photograph from the same eye showing progressive neuroretinal rim thinning during follow-up. The visual field shows evidence of abnormality as indicated by the pattern standard deviation (PSD) outside normal limits.

and precision of the predictions for individual eyes will depend largely on the variability and number of follow-up measurements available over time. Small number of measurements or large variability will result in large CIs, indicating considerable uncertainty in the predictions.

Several other methods to combine structure and function in glaucoma have been described in the literature.[6–8,26–29] A previous study by Boland and Quigley described a different method to combine structure and function in a single index, based on the calculation of probability of abnormality for each point in the visual field and for each sector of the optic disc using confocal scanning laser ophthalmoscopy.[30] The method showed a better performance than the structural parameters evaluated by confocal scanning laser ophthalmoscopy, but had a performance only similar to visual field global indexes.[30] To the best of our knowledge, the predictive ability for

glaucomatous damage of the index described by Boland and Quigley has not yet been evaluated. Further longitudinal studies should compare the abilities of different proposed methods of combining structure and function for diagnosing and predicting glaucomatous progression.

Interestingly, the proportion of glaucoma suspect eyes with progression only by visual fields was larger than the proportion of patients with progression only by optic disc stereophotographs in our study. This result differs from the Ocular Hypertension Treatment Study (OHTS), which found a larger proportion of conversion by optic disc assessment than by visual field.[31] The reason for this difference probably is related to the inclusion criteria of the studies. In the OHTS, subjects were included only if they had normal optic disc appearance at baseline.[32,33] In contrast, our sample had a large number of eyes with suspicious optic disc appearance at baseline. It is likely that some of these eyes already had baseline structural damage and, although their baseline visual fields still were statistically normal, they would be more prone subsequently to develop abnormalities shortly thereafter.

Our study has limitations. Patients were not randomized for treatment or no treatment, and the decision as to whether to initiate treatment might have been based on disc assessment results and other risk factors. It is likely that patients deemed at higher risk were those who received more treatment. This may have contributed to underestimate the predictive abilities of the baseline factors in our cohort. Although, we do not expect this to have influenced the comparison of the predictive performances of the different models in our study, it limits the comparison of our results to those from previous studies, including only untreated patients.[22,23] It should be emphasized, however, that the longitudinal models included information on IOP measurements available during follow-up, which provided adjustment for treatment differences over time. Another limitation of our study is that for estimation of structural damage, we used OCT measurements based on the time-domain version of this technology. The use of spectral-domain OCT has resulted in faster and more reproducible scans compared to time-domain OCT.[34] However, because of the relatively recent introduction of spectral-domain OCT, longitudinal data were not available to perform the current study using this technology.

In conclusion, the results of our study demonstrated that baseline and longitudinal estimates of RGC counts using a combination of structural and functional tests performed better than conventional approaches for prediction of progression in glaucoma suspects. With further validation, this approach may prove useful for risk stratification of patients suspected of having glaucoma.

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