The Relationship Between Cup-to-Disc Ratio and Estimated Number of Retinal Ganglion Cells

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PURPOSE. To investigate the relationship between cup-to-disc ratio (CDR) and estimates of retinal ganglion cell (RGC) number.

METHODS. This cross-sectional study included 156 healthy eyes, 53 glaucoma suspects, and 127 eyes with glaucoma. All eyes had standard automated perimetry (SAP), Cirrus SD-OCT, and stereoscopic optic disc photography within 6 months. CDR was determined from stereoscopic photographs by two or more masked graders. The number of RGCs in each eye was estimated using a published model that combines estimates of RGC number from SAP sensitivity thresholds and SD-OCT retinal nerve fiber layer measurements.

RESULTS. The mean estimated RGC count was 1,063,809 in healthy eyes; 828,522 in eyes with suspected glaucoma; and 774,200 in early, 468,568 in moderate, and 218,471 in advanced glaucoma. Healthy eyes had a mean vertical CDR of 0.45 ± 0.15 vs. 0.80 ± 0.16 in glaucomatous eyes. There was good correlation between stereophotographic vertical CDR and SD-OCT vertical CDR ($R^2 = 0.825$; $P < 0.001$). The relationship between estimated RGCs and vertical CDR was best represented using a third degree polynomial regression model, including age and optic disc area, which accounted for 83.3% of the variation in estimated RGC counts. The nonlinear relationship between RGC estimates and CDRs indicated that eyes with a large CDR would require loss of large RGC numbers for a small increase in CDR.

CONCLUSIONS. The relationship between estimated RGC counts and CDR suggests that assessment of change in CDR is an insensitive method for evaluation of progressive neural losses in glaucoma. Even relatively small changes in CDR may be associated with large losses of RGCs, especially in eyes with large CDRs. (ClinicalTrials.gov numbers, NCT00221923, NCT00221897.)

Keywords: glaucoma, ganglion cell, optic disc, optical coherence tomography, perimetry

Glaucoma is characterized by loss of retinal ganglion cells (RGCs), which results in visual field impairment and structural changes to the retinal nerve fiber layer (RNFL) and optic disc.¹ Evaluation of the optic disc is therefore an essential component of detecting and following glaucomatous damage.^{1,2} The optic disc is composed of RGC axons, which at the surface of the disc bend acutely to exit the eye through the lamina cribrosa. The optic disc can be divided into the optic cup and the neuroretinal rim.^{3,4} The cup is a central depression representing an absence of axons and partial exposure of the lamina, while the neuroretinal rim, which is composed of RGCs, neuroglia, astrocytes, and capillaries, is the tissue between the cup and disc margin.⁵ The limit of the cup is defined as the first discernible change in surface contour as the RGC axons course posteriorly.^{3,5}

In the 1960s, Snyderaker⁶ and Armary⁷ noted that the size of the cup relative to the size of optic disc, or the cup-to-disc ratio (CDR), was a useful measure of glaucomatous damage. Progressive enlargement of CDR occurs in glaucomatous eyes due to the loss of RGCs and their axons, with a consequent thinning of the neuroretinal rim. CDR may be measured horizontally or vertically, but because loss of the neuroretinal

rim tends to occur first in the superior and inferior poles, the vertical CDR has become a more commonly used parameter in clinical practice.⁸

The ability of CDRs to detect glaucoma is limited due to the wide variability of CDRs in the normal population. Such variability is explained, at least in part, by the significant relationship between the CDR and the size of the optic disc.^{9–12} Eyes with large optic discs tend to have large cups, whereas eyes with small discs tend to have small cups. Despite these limitations, CDRs are still used extensively in clinical practice to document the optic disc status during follow-up of glaucoma patients or those suspected of having the disease.¹ By documenting CDRs, clinicians hope to be able to better diagnose the disease and detect progressive structural damage. However, relatively large intra- and interobserver variability has been reported for CDR measurements.^{13–19} Such variability would most likely allow only large changes in this parameter to be detected over time, such as CDR changes of at least 0.2.^{14,20} Although such detectable changes are likely to correspond to loss of large amounts of neural tissue, no study has yet evaluated the relationship between CDR measurements and the number of RGCs.

Direct counting of RGCs is not yet possible in vivo in humans, however empirical formulas derived from clinical structural and functional tests may be used to estimate the number of RGCs. These estimates have been shown to correlate well with histologic counts in experimental glaucoma models.²¹ In recent studies, we proposed a method for estimating RGC loss from a combination of standard automated perimetry (SAP) and RNFL assessment with optical coherence tomography (OCT).^{22–24} The estimates of RGC counts performed significantly better than isolated structural and functional parameters for staging the disease and monitoring glaucomatous progression.

The purpose of this study was to evaluate the relationship between CDR and the underlying estimated number of retinal ganglion cells in healthy, suspect, and glaucomatous eyes.

METHODS

This was a cross-sectional study involving participants 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 three-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. Methodologic details have been described previously.²⁵ Informed consent was obtained from all the participants, and the institutional review boards and human subjects committees of all three sites approved all the methods. All methods adhered to the tenets of the Declaration of Helsinki for research involving human subjects.

At each visit during follow-up 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 the Swedish interactive threshold algorithm (SITA Standard testing algorithm 24-2). Only subjects with open angles on gonioscopy were included. Subjects were excluded if they presented with a BCVA < 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 336 eyes of 209 participants, including 156 healthy eyes, 53 eyes with suspected glaucoma, and 127 eyes with glaucoma. Classification was by eye, rather than by patient, and eyes were classified as glaucomatous if they had repeatable (≥ 2 consecutive) abnormal SAP test results on the 24-2 program of the visual field analyzer (Humphrey Field Analyzer [HFA II-i]; Carl Zeiss Meditec, Inc., Dublin, CA). An abnormal SAP result was defined as having a pattern SD outside the 95% confidence limits or a glaucoma hemifield test result outside the reference range. Glaucoma suspects were defined as eyes with suspicious appearing optic discs (presence of suspicious neuroretinal rim thinning or RNFL defects) by masked stereophotograph assessment without repeatable abnormal SAP results. Glaucoma suspects also included eyes with IOP > 21 mm Hg, but with healthy appearing optic discs and without repeatable abnormal SAP results. Healthy subjects were recruited from the general population through advertisements and from the staff and employees of the University of California-San Diego. Healthy eyes had IOP ≤ 21 mm Hg, with

no history of increased IOP and a normal SAP result. Eyes that have undergone glaucoma surgery may experience reversal of cupping and disc swelling in cases of relative hypotony.^{26,27} The improvement in CDR from hypotony could confound the relationship between CDRs and estimated RGC counts. For this reason we excluded eyes that had undergone trabeculectomy or glaucoma drainage device surgery.

Optical Coherence Tomography

The Cirrus spectral domain OCT (SD-OCT) (software version 6.0; Carl Zeiss Meditec, Inc.) was used to acquire RNFL measurements in the study. Cirrus SD-OCT was also used to analyze disc area and vertical and average CDRs. Cirrus SD-OCT 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 μ m. The optic disc cube 200×200 protocol was used to acquire RNFL thickness measurements and measurements of disc area and CDR. This protocol is based on a 3-dimensional (3D) scan of a 6×6 mm area centered on the optic disc in which information from a 1024 (depth) $\times 200 \times 200$ point parallelepiped is collected. The parapapillary RNFL thickness measurements were calculated from a 3.46-mm-diameter circular scan (10.87-mm length) automatically placed around the optic disc. The average RNFL thickness corresponds to the 360° measure automatically calculated by the OCT software (software version 6.0; Carl Zeiss Meditec, Inc.). Because information from the whole region is obtained, it is possible to modify the position of the scan after the examination is conducted. The optic nerve head (ONH) algorithm was used to determine features of the ONH, including disc size and CDR, from the same 3D data cube. The disc margin is identified as the termination of Bruch's membrane and the limit of the optic cup is automatically determined from the change in the thickness of the neuroretinal rim tissue as it exits the opening in Bruch's membrane.

The Cirrus SD-OCT images were reviewed and included if the signal strength was >7 , if movement artifacts and segmentation errors were absent, and there was good centering on the optic disc.

Standard Automated Perimetry

All the patients underwent SAP testing using the SITA Standard 24-2 strategy within 6 months of imaging. All visual fields were evaluated by the UCSD Visual Field Assessment Center (VisFACT).²⁸ 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. Visual fields exhibiting a learning effect (i.e., initial tests showing consistent improvement on visual field indices) were also excluded. Visual fields were further reviewed for the following artifacts: eyelid and rim artifacts, fatigue effects, inappropriate fixation, and evidence that the visual field results were caused by a disease other than glaucoma and inattention. The UCSD Visual Field Assessment Center requested repeats of unreliable visual field test results, and these were obtained whenever possible. The severity of glaucoma was determined by glaucomatous visual field loss using the Hodapp-Anderson-Parrish (HAP) classification.²⁹

Stereophotograph Grading

All patients had simultaneous stereoscopic optic disc photography performed within 6 months of the SD-OCT. Stereoscopic photographs were reviewed with a stereoscopic viewer (Asahi

Pentax Stereo Viewer II; Asahi Optical Co., Tokyo, Japan) by two or more experienced graders. Each grader was masked to the subject's identity and to the other test results. The graders visually estimated the vertical and horizontal CDRs on the basis of the contour of the cup. The mean vertical CDR, mean horizontal CDR, and the average of the vertical and horizontal CDRs assigned by the graders were used for the analysis. If the CDR assigned by each grader differed by >0.15 the photographs were reviewed by a masked adjudicator. Details of the methodology used to grade optic disc photographs at the UCSD Optic Reading Center have been provided elsewhere.^{25,30,31}

Estimation of Retinal Ganglion Cell Number

The estimates of RGC counts were obtained according to the model developed by Medeiros et al.^{22–24} based on empirical formulas derived by Harwerth et al.²¹ for estimating ganglion cell counts from SAP and OCT. The model uses information from structural and functional tests to derive a final estimate of the RGC count in a particular eye. The details of the model and the empirical formulas used to derive RGC counts have been described in detail in previous publications.^{22–24} The initial step of the model consists of translating SAP sensitivity values into RGC counts using empirical formulas derived from experimental research in monkeys and subsequently translated to normal and glaucomatous human eyes. 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:

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

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

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

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

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 (s) 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 ($SAPrgc$) was obtained by adding the estimates from all locations in the visual field. The structural part of the model consisted of estimating the number of RGC axons from RNFL thickness measurements obtained by optical coherence tomography. The model took into account the effect of aging on 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. To derive the total number of RGC axons from the global RNFL thickness measurement obtained by OCT ($OCTrgc$), we applied the following formulas:

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

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

$$a = \text{average RNFL thickness} \cdot 10870 \cdot d$$

$$OCTrgc = 10^{\{\log(a) \cdot 10 - c\} \cdot 0.1}$$

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 nonaxonal composition. These calculations provide an estimate of the number of RGCs from two sources, one functional and one structural. A combined calculation of RGC counts was performed according to the following formula:

$$\text{Estimated RGC count} = (1 + MD/30) \cdot OCTrgc + (-MD/30) \cdot SAPrgc$$

The rationale for using a weighting system for deriving the final RGC count is described by Medeiros et al.,^{22–24} but in essence it relies on the fact that the accuracies of clinical perimetry and imaging tests are inversely related to disease severity.

Statistical Analysis

Normality assumption was assessed by inspection of histograms and using Shapiro–Wilk tests. Student's t -tests were used for group comparison for normally distributed variables and Wilcoxon rank-sum test for continuous nonnormal variables. The relationship between estimated RGC count, vertical CDR, and average CDR was initially examined using scatterplots and locally weighted scatterplot smoothing (LOWESS) curves. LOWESS is a modeling method that combines the linear least squares regression with nonlinear regression.³² It does this by fitting simple models to localized subsets of the data to build a function that describes the deterministic part of the variation in the data, point by point. After LOWESS plots were examined, polynomial regression fits of increasing order were applied to the data until the coefficients of the polynomial variables were not significant. Determination of best fit was also performed by evaluating improvement of adjusted R^2 statistics with models of increasing complexity and agreement with LOWESS plot. Multivariable models adjusted for age and optic disc area.

Observations from two eyes of the same subject are likely to be correlated, which can lead to underestimation of true variance.³³ A between-cluster variance estimator was therefore used in the regression analysis to account for correlations between eyes of the same subject and calculate robust variance estimates.³³ 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

The mean age of participants was 59.3 ± 15.9 years. The participants with healthy eyes were significantly younger than glaucoma suspects and those with early, moderate, and advanced glaucoma ($P < 0.001$ for all comparisons). In all, 149 participants (71.3%) were Caucasian, 44 were of African ancestry (21.1%), and 16 (7.2%) were of other racial groups; 115 (55.0%) were female. The demographic and clinical characteristics, including the results of estimation of retinal ganglion cell number for all eyes, are summarized in Table 1.

The mean estimated RGC count was 1,063,809 in healthy eyes; 828,522 in eyes with suspected glaucoma; 774,200 in early glaucoma, 468,568 in moderate glaucoma, and 218,471 in advanced glaucoma. Differences in the estimated number of RGCs between groups were statistically significant ($P < 0.001$ for all comparisons), except for the difference between glaucoma suspects and eyes with early glaucoma ($P = 0.110$).

TABLE 1. Summary of Demographic and Clinical Characteristics of Healthy Eyes Compared With Glaucoma Suspects and Those With Early, Moderate, and Advanced Glaucoma

Characteristic	Healthy, <i>n</i> = 156	Glaucoma Suspects, <i>n</i> = 53	Early Glaucoma, <i>n</i> = 47	Moderate Glaucoma, <i>n</i> = 52	Advanced Glaucoma, <i>n</i> = 28
Age, y	48.3 ± 15.3	65.6 ± 10.0	65.6 ± 14.7	66.9 ± 12.1	64.1 ± 9.8
MD, dB	0.1 ± 1.2	−0.2 ± 1.3	−1.4 ± 1.2	−8.0 ± 4.1	−21.2 ± 4.1
VFI, %	99.5 ± 0.6	99.3 ± 0.9	97.2 ± 1.2	78.9 ± 13.1	39.0 ± 14.2
Mean RNFL thickness, μm	94.0 ± 9.0	84.0 ± 10.2	83.1 ± 11.6	66.8 ± 12.7	58.0 ± 7.6
Estimated RGC count, cells	1,063,809 ± 174,254	828,522 ± 137,395	774,200 ± 196,786	468,568 ± 167,049	218,471 ± 91,319
Disc area, mm ²	1.79 ± 0.32	1.95 ± 0.41	1.99 ± 0.32	1.89 ± 0.36	1.93 ± 0.39
Vertical CDR, Cirrus	0.45 ± 0.16	0.65 ± 0.11	0.65 ± 0.12	0.75 ± 0.12	0.86 ± 0.07
Average CDR, Cirrus	0.47 ± 0.16	0.67 ± 0.11	0.66 ± 0.12	0.75 ± 0.12	0.84 ± 0.06
Vertical CDR, Photos	0.45 ± 0.15	0.73 ± 0.11	0.69 ± 0.15	0.83 ± 0.13	0.94 ± 0.07
Horizontal CDR, Photos	0.46 ± 0.16	0.72 ± 0.09	0.67 ± 0.15	0.79 ± 0.13	0.88 ± 0.06
Average CDR, Photos	0.45 ± 0.15	0.72 ± 0.09	0.68 ± 0.14	0.81 ± 0.13	0.91 ± 0.06

MD, mean deviation; VFI, visual field index.

The mean (± SD) stereophotographic vertical CDR was 0.62 ± 0.23 , ranging from 0.10 to 1.0. Eyes with glaucoma and suspected glaucoma had significantly larger stereophotographic vertical CDRs than healthy eyes ($P < 0.001$ for both comparisons). There was no significant difference in stereophotograph vertical CDR between glaucoma suspects and eyes with early glaucoma ($P = 0.162$) but eyes with moderate or advanced glaucoma had larger stereophotograph vertical CDRs than glaucoma suspects ($P < 0.001$ for both comparisons). The mean horizontal stereophotograph CDR was 0.62 ± 0.21 (range, 0.1–0.98) and the mean average stereophotograph CDR was 0.62 ± 0.21 (range, 0.10–0.99).

The mean Cirrus vertical CDR was 0.59 ± 0.20 and the mean Cirrus average CDR was 0.60 ± 0.19 . Stereophotographic vertical CDR values were strongly correlated with Cirrus vertical CDR measurements ($R^2 = 0.825$; $P < 0.001$) (Fig. 1A); however, stereophotographic vertical CDR tended to be larger than Cirrus vertical CDR (stereophotograph vertical CDR = $1.07 \times [\text{Cirrus vertical CDR}] - 0.01$). There was also strong correlation between stereophotographic and Cirrus average CDR ($R^2 = 0.798$, $P < 0.001$) (Fig. 1B).

Figure 2 shows the relationship between estimated RGC counts and stereophotograph vertical CDR. The LOWESS plots indicated a nonlinear relationship between stereophotograph CDR and RGCs. The relationship between estimated RGC counts and stereophotograph vertical CDR was best fit by a third degree polynomial regression model ($R^2 = 0.833$, $P < 0.001$). A second degree polynomial model had an R^2 of 0.822, $P < 0.001$, and fit the LOWESS plot less well. More complex models did not significantly improve the fit. The relationship between estimated RGC number and stereophotograph vertical CDR was best represented using the following model (Table 2):

$$\begin{aligned} \text{Estimated retinal ganglion cell number} \\ = 1300173 - 92863vcdr + 25486vcdr^2 - 2543vcdr^3 \\ + 165469da - 8218age \end{aligned}$$

where *vcdr* is the stereophotograph vertical CDR in 0.1 unit, *da* is disc area in mm², and age is measured in years. The variables *vcdr*, *vcdr*², *vcdr*³, disc area, and age accounted for 83.3% of the variation in estimated RGC counts. The regression coefficients for all variables were significantly different from zero ($P < 0.05$ for all variables). A similar function was used to examine the relationship between estimated RGC counts and average CDR. Similar results were

obtained, with the model accounting for 82.8% of the variation in estimated RGC numbers ($R^2 = 0.828$, $P < 0.001$). The results were also similar when Cirrus vertical CDR ($R^2 = 0.824$, $P < 0.001$) and Cirrus average CDR ($R^2 = 0.828$, $P < 0.001$) were investigated.

FIGURE 1. Scatterplots and ordinary least squares (OLS) regression lines showing the relationship between (A) stereophotograph vertical CDR and Cirrus OCT vertical CDR and (B) stereophotograph average CDR and Cirrus OCT average CDR.

FIGURE 2. Scatterplot showing the relationship between estimated RGC number and stereophotograph vertical CDR. The locally weighted scatterplot smoothing (LOWESS) curve is shown.

Figure 3 shows the modeled relationship between estimated RGC count and stereophotograph vertical CDR with age and disc area set at their mean values in the sample. The results for a 60-year-old subject with a disc area of 2.0 mm² are tabulated in Table 3. Figure 4 shows the modeled relationship between estimated RGC count and stereophotograph vertical CDR for arbitrary values of age (60 and 80 years), and for eyes with small (1.5 mm²), medium (2.0 mm²), and large (2.5 mm²) optic discs. Examples of two eyes included in the study, including the estimates of RGC numbers and CDR measurements, are shown in Figures 5 and 6.

DISCUSSION

This study has quantified the relationship between CDR and retinal ganglion cell number. Healthy eyes had a mean stereophotograph vertical CDR of 0.45 ± 0.15 , which corresponded to a mean RGC count of $1,063,809 \pm 174,254$ cells (Table 1). The healthy eye RGC count estimate is consistent with the number of RGCs observed in previous histologic studies.^{34–37} For example, Jonas and colleagues³⁴ found a mean optic nerve fiber count of $1,158,000 \pm 222,000$ in healthy eyes.

Our results showed that eyes with glaucoma had larger stereophotograph vertical and average CDRs, and significantly lower estimated numbers of RGCs than healthy eyes (Table 1, $P < 0.001$ for all comparisons). The mean RGC estimate for

FIGURE 3. Relationship between estimated RGC number and stereophotograph vertical CDR as fitted by the polynomial regression model. Bars include 95% confidence interval for mean values of estimated RGC number. Results are given for age and optic disc area corresponding to average values in the sample (age = 57.7 years and disc area = 1.87 mm²).

glaucomatous eyes was $530,475 \pm 267,241$ cells. The stereophotograph vertical and average CDR increased with worsening severity of disease, and there was a corresponding decrease in RGC number, although the relationship between CDR and RGC count was not linear (Fig. 2). Similar results were found for Cirrus vertical CDR and Cirrus average CDR. A consequence of nonlinearity is that in eyes with a small CDR, loss of a relatively small number of RGCs could result in a large increase in CDR. In contrast, in eyes with a large CDR, the same loss of RGCs will result in a relatively smaller and perhaps undetectable change in CDR. This relationship has important implications for clinical practice, and suggests that CDR is a relatively insensitive measure of RGC loss in eyes with moderate to large CDRs. For example, it can be seen from Table 3 that a change in CDR from 0.5 to 0.6 could occur with a loss of just approximately 44,000 RGCs. In contrast, it would take a loss of close to 300,000 RGCs for an eye to have a change in CDR from 0.9 to 1.0. Therefore, in an eye with advanced disease and large CDR, it will be difficult to detect further changes in CDR unless the loss of RGCs is substantial. These results may explain the widely held belief, also supported by some studies,^{2,20,38,39} that evaluation of the optic disc by biomicroscopy or photographs may be relatively insensitive to detect disease progression in moderate to advanced stages of damage.

The nonlinear relationship between RGC count and stereophotographic CDR may be explained by considering the relationship between CDR and neural tissue at the optic nerve head. The neuroretinal rim is composed of RGC axons, in addition to nonneuronal tissue. When RGC axons are lost there is also a reduction in neuroretinal rim area.^{34,40} Following a histologic study, Jonas and colleagues³⁴ proposed that the relationship between neuroretinal rim area and the number of RGC axons is linear and that a 175,000 decrease in RGC axons is equivalent to approximately a 0.30 mm² decrease in rim area.³⁴ The neuroretinal rim may be considered as an annulus of tissue within the optic canal. Vertical CDR is a measure of neuroretinal rim in a single meridian and represents the ratio of the diameters of the inner and outer arc of the annulus. The relationship between the diameters of the annulus and the area of the annulus is described by the formula $\pi(R^2 - r^2)$, where R is the radius of the outer circle of the annulus and r is the

TABLE 2. Results of the Polynomial Regression Model Evaluating the Relationship Between Estimated Retinal Ganglion Cell Counts and Stereophotograph Vertical CDR

Factor	Coefficient	95% Confidence Interval	P
<i>vcd</i>	−92,863	−185,060 to −666	$P = 0.048$
<i>vcd</i> ²	25,486	7,299–43,673	$P = 0.006$
<i>vcd</i> ³	−2,543	−3,628 to −1,458	$P < 0.001$
Disc area, mm ²	165,469	119,222–211,715	$P < 0.001$
Age, y	−8,218	−9,294 to −7,143	$P < 0.001$
Constant	1,300,173	1,132,075–1,468,271	$P < 0.001$

vcd, stereophotograph vertical CDR in 0.1 units; *vcd*², vertical CDR squared; *vcd*³, vertical CDR cubed.

Table 3. Estimated Number of RGCs for Arbitrary Values of Stereophotograph Vertical CDR for an Eye of a 60-Year-Old Subject With a Disc Area of 2.0 mm²

Vertical CDR	0.4	0.5	0.6	0.7	0.8	0.9	1.0
RGC estimate*	1,011,600	993,010	949,113	864,654	724,377	513,026	215,346
95% confidence interval	980,754–1,042,446	968,372–1,017,648	928,286–969,941	842,089–887,219	700,547–748,207	487,466–538,585	164,075–266,614

* P < 0.001 for all predicted RGC estimates.

FIGURE 4. Expected number of RGCs for given values of CDR based on the results of the polynomial regression model for arbitrary values of age (60 and 80 years) and for small (1.5 mm²), medium (2.0 mm²), and large (2.5 mm²) optic disc areas.

radius of the inner circle, and is therefore nonlinear. An eye with a disc area of 2.0 mm² will have an approximate radius of 0.8 mm. Therefore, if this eye has a CDR of 0.8, the annulus of neuroretinal rim would be $\pi(0.8^2 - [0.8 \times 0.8]^2) = 0.72$ mm². With a CDR of 0.9, the calculated annulus of neuroretinal rim would be $\pi(0.8^2 - [0.9 \times 0.8]^2) = 0.38$ mm². The loss of neuroretinal rim in this eye progressing from CDR of 0.8 to 0.9 would be estimated as $0.72 - 0.38 = 0.34$ mm². Using the relationship of Jonas et al.³⁴ of 175,000 RGCs per 0.30 mm² rim area, a loss of 0.34 mm² would correspond to 198,000 RGCs. This number is remarkably similar to that calculated by our model (Table 3), which shows that a change from CDR of 0.8 to 0.9 in an eye with 2.0 mm² of disc area would correspond to a loss of 211,000 RGCs. Such agreement with histologic findings provides further validation to our analyses. Although the neuroretinal rim is not an annulus as the optic disc is usually vertically oval and rim loss is not concentric, the model supports the finding of a nonlinear relationship between CDR and RGC number.

Although CDR has been commonly used as a clinical measure for diagnosis and staging of glaucoma, the high intra- and interexaminer variability in gradings^{2,13–20} would suggest that relatively large CDR changes (0.2 or more) would be necessary for changes to be flagged as significant, especially if documentation is performed only by drawings or descriptions of the optic disc. Such changes would represent very large losses in RGC numbers, even in relatively early stages of disease. For example, a change in CDR from 0.5 to 0.7 would correspond to close to 130,000 RGCs (Table 3). In more advanced stages of damage, larger losses would be necessary. A 0.2 change in CDR from 0.7 to 0.9 would imply a loss of close to 350,000 RGCs. Such findings strongly imply that clinical assessment of CDR measurements should not be used as the sole method for detection of glaucomatous progression. In contrast to CDR changes, assessment of RNFL measurements with imaging technologies seems to be able to detect much smaller losses of RGCs over time.^{22,23} Interestingly, eyes with CDR of 1.0 still had an estimated RGC number close to 200,000 cells (Table 3). A similar “floor effect” has also been described with structural assessment by imaging technologies^{21,41,42} and it would indicate an inability of these methods to detect further progression once very advanced stages of damage have been

FIGURE 5. Example of a glaucomatous left eye of a 76-year-old patient included in the study. The figure shows the stereophotograph of the optic disc (**A**), the Cirrus OCT RNFL thickness and deviation maps (**B**), and the SAP gray scale and threshold values (**C**). The stereophotographic vertical CDR was graded as 1.0. The disc area was 1.82 mm² and the mean RNFL thickness was 61 μ m. The estimated number of RGCs for this eye was 117,621.

FIGURE 6. Example of a healthy right eye of a 54-year-old subject included in the study. The figure shows the stereophotograph of the optic disc (**A**), the Cirrus OCT RNFL thickness and deviation maps (**B**), and the SAP gray scale and threshold values (**C**). The stereophotographic vertical CDR was graded as 0.35. The disc area was 1.99 mm² and the mean RNFL thickness was 87 μ m. The estimated number of RGCs for this eye was 961,783.

reached. For CDR measurements, the reasons for such floor are likely to include preservation of neuroretinal rim in meridians not measured by CDR, the inability of examiners to discern small quantities of residual neural tissue or supporting elements, or artifact.

The shallow slope in the relationship between CDR and RGCs in eyes with small CDRs suggests that one might expect an increase in CDR with aging, particularly because aging is associated with a decrease in RGCs of 4000 to 8000 cells, or 0.3% to 0.5% per year.^{34-37,43} In clinical practice, significant enlargement of CDR with aging is usually not seen. This could be due to a lack of long-term studies investigating optic disc changes in healthy eyes. Alternatively, the lack of change in CDR with age-related loss of RGCs may be due to an increase in nonneural tissue in the neuroretinal rim. Previous studies have suggested that neural tissue losses are replaced by nonneural tissue and that axonal density decreases with aging.^{43,44} Because these different types of tissue cannot be distinguished by clinical exam, CDR changes would not be apparent. In addition, the shallow relationship between RGCs and vertical CDR in eyes with small CDRs may also be due to the variability of CDRs in normal eyes, with similar RGC counts. To investigate this possibility, we performed a separate analysis using only the glaucomatous eyes in the study but found a similar nonlinear relationship between RGCs and CDR.

Disc size was also a significant influence on the relationship between CDR and the number of RGCs (Table 2). There is a wide range of normal optic disc area, from smaller than 1 mm² to as large as 5 mm² in population-based studies using confocal ophthalmoscopy.⁴⁵⁻⁴⁷ Eyes with large optic discs have larger CDRs⁹ and also tend to have greater globe dimensions, larger neuroretinal rim area,⁴⁸ larger retinal surface area, and a higher number of retinal photoreceptors and RPE cells.^{5,49} Disc area has also been proposed to influence glaucoma susceptibility.^{50,51} We found that eyes with large optic discs had a greater number of RGCs than eyes with smaller discs. The estimated number of RGCs increased by 165,000 for each 1 mm² increase in disc area (Table 2). This estimate shows good agreement with the findings of a histologic study, which found that the number of RGC axons increased by 175,000 for a 1 mm² increase in disc area.³⁴ Given that eyes with large optic discs tend to have large CDRs, change in CDR may be a particularly insensitive measure of RGC loss in eyes with large discs.

We acknowledge the limitations of this study. Empirically derived formulae were used to estimate the number of RGCs and the original formulae were derived from studies in a primate model of glaucoma.²¹ Furthermore, the formulae were developed from studies using time-domain OCT and there may be differences in the RGC estimates obtained using SD-OCT. Despite these limitations, the formulae have been validated in multiple external human cohorts.²²⁻²⁴ Also, the RGC estimates we obtained were closely related to the findings of human histologic studies.³⁴⁻³⁷

In conclusion, the results of our study suggest that assessment of CDR is an insensitive method for evaluation of progressive neural losses in glaucoma. Even relatively small changes in CDR may be associated with large losses of RGCs, especially in eyes with large CDRs.

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