Visual Field Defects and Retinal Ganglion Cell Losses in Patients With Glaucoma

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**Objective:** To determine whether the structure-function relationships for glaucoma in humans and experimental glaucoma in monkeys are similar.

**Methods:** The study was based on retinal ganglion cell density and visual thresholds in patients with documented glaucoma. Data were analyzed with a model that predicted ganglion cell density from standard clinical perimetry, which was then compared with histologic cell counts.

**Results:** The model, without free parameters, produced accurate and relatively precise quantification of ganglion cell density associated with visual field defects. For 437 sets of data, the unity correlation for predicted vs measured cell density had a coefficient of determination of 0.39. The mean absolute deviation of the predicted vs measured values was 2.59 decibels (dB), and the mean±SD of the distribution of residual errors of prediction was −0.26±3.22 dB.

**Conclusions:** Visual field defects based on standard clinical perimetry are proportional to neural losses caused by glaucoma.

**Clinical Relevance:** The evidence for quantitative structure-function relationships provides a scientific basis for interpreting glaucomatous neuropathy from visual thresholds and supports the application of standard perimetry to establish the stage of the disease.

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GLAUCOMA IS A DISEASE that causes progressive loss of vision from the death of retinal ganglion cells, and it is reasonable that the degree of vision loss would be proportional to the amount of ganglion cell loss. Traditionally, this relationship between structure and function in glaucoma has been applied in clinical perimetry to establish the clinical stage, or severity, of the disease, but the quantitative relationship between visual sensitivity and ganglion cell density was established only recently. The quantitative structure-function model, which was developed from data on experimental glaucoma in monkeys, was accurate and relatively precise in predicting the retinal ganglion cell density underlying a given sensitivity and location in the visual field. Although based on experimental glaucoma, the model should be applicable to clinical glaucoma because the visual systems in humans and monkeys are essentially identical. However, direct empirical evidence of the structure-function relationship for clinical glaucoma is an important verification of the scientific basis of interpreting glaucomatous optic neuropathy from visual thresholds.

Previous investigations of ganglion cell density and visual sensitivity for glaucoma in humans have suggested a highly variable relationship. For example, in a study with a relatively large number of patients with glaucoma, Kerrigan-Baumrind et al reported that linear regression analysis of the pointwise correlation between visual sensitivity and ganglion cell loss accounted for only 3% of the total variance. A clearer relationship between neural and visual losses was established by their finding that statistically significant visual field abnormalities occurred if neural losses at the corresponding retinal location exceeded 25% to 35%. In addition, the relationships between structure and function were highly significant, with more global measures of visual sensitivity and neural loss, for example, average visual sensitivity losses or the mean deviation perimetry indices vs mean ganglion cell losses. Thus, although the study demonstrated a clinically significant structure-function relationship, it was not so quantitative for the pointwise translation of clinical perimetry measurements to retinal ganglion cell losses as was found for experimental glaucoma. There are several possible explanations for the differences between the data in monkeys and humans, such as true species differences, variations...
TABLE. Characteristics of Patients and Data From Structure-Function Model

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Abbreviations: dB, decibels; HFA, Humphrey Field Analyzer; MAD, mean absolute deviation; MD, mean deviation; MRE, mean residual error; NA, not available; PSD, pattern standard deviation; r^2, coefficient of determination; SD, standard deviation; TBD, time before death for collection of perimetry data.

*Eyes with the same superscript symbols (a, b, c, and d) indicate pairs of eyes from the same patient.

METHODS

The control data for normal retinal ganglion cell density were from 17 eyes in 17 patients (mean ± SD age, 76.4 ± 11.0 years) without detectable ocular disorders that would affect retinal ganglion cells. Data for patients with glaucoma were from 17 eyes in 13 patients (mean age, 72.2 ± 9.3 years). Each patient had a documented history of glaucoma with full-threshold visual field data obtained within the last 2 years of life. All of the clinical data were obtained with a Humphrey Field Analyzer (HFA)-1 perimeter (Humphrey Field Analyzer; Carl-Zeiss Meditec, Dublin, Calif.) and no Swedish Interactive Threshold Algorithm fields were included. The characteristics of the individual patients with glaucoma and the global indices from their perimetry data (if available) are presented in the Table.

Histologic preservation of all eyes was achieved within 24 hours of death (usually within 12 hours) using aldehyde fixative. Tissue samples were collected from retinal areas that corresponded to 28 test field locations (Figure 1) for the HFA C24-2 program, using a conversion ratio of 1 mm of retinal distance per 3.5° of visual angle. The actual number of samples per eye that were suitable for histologic analysis varied from 26 to 28 for the control eyes and from 17 to 28 for the glaucomatous eyes (the number of samples for each patient with glaucoma is shown in the Table). The retinal tissue samples were sectioned (1-µm thickness) and stained with 0.1% thionine for histologic cell counts. The number of nuclei thought to represent retinal ganglion cells in the ganglion cell layer of each section, as well as the length of the section, was determined for 4 sections at each sample location. Data from the 4 sections were averaged to provide mean cells per unit length (ganglion cells per millimeter). For the present study, the original linear density data were converted to cell density per unit area (ganglion cells per square millimeter) for comparison with other published data and for application of the model. Cell density was estimated using Ambergromie’s method for deriving density from sectioned tissue,15 using the section thickness of 1 µm and an average cell body diameter of 9 µm for the calculations. The cell density was then transformed to a decibel (dB) scale by 10 times the logarithm of the calculated cell density for comparison of the corresponding HFA measurements of visual sensitivity. Thus, for example, a cell density of 10 000 cells/mm² is equal to 40 dB.

The structure-function model was developed to determine retinal ganglion cell density at specific retinal locations from the corresponding HFA visual sensitivity. The specific details for the model have been described previously.20 In brief, the model was derived from the concept that visual thresholds represent a nonlinear pooling of the outputs of the neural detectors. The statistical properties of the pooling of neural responses should be independent of ganglion cell density; thus, visual thresholds vary with the number of neural detectors when
the number of detectors varies from retinal eccentricity, normal aging, or stage of glaucoma.16

In agreement with others,25-27 the general expression between the neural density and visual sensitivity data was exponential, and logarithmic transforms of both variables produced linear relationships for prediction of structural losses from functional measurements.16 However, the parameters of the linear function varied with eccentricity, and, thus, the model required equations to determine the slope and y-intercept as a function of eccentricity, which in turn provided the parameters for the function for predicting ganglion cell density from visual sensitivity. The 3 equations are the slope of the function \( m \) at eccentricity \( e \), or \( m = [0.054 \times e] + 0.95 \); the intercept of the function \( b \) at eccentricity \( e \), or \( b = [-1.5 \times e] - 14.8 \); and the predicted ganglion cell density \( gc \) for a sensitivity \( s \), or \( gc = (s - b)/m \).

The application of the model for human glaucoma was evaluated by comparison of the predicted ganglion cell density as a function of the histologically measured ganglion cell density. The predicted vs measured cell density was compared with a perfect one-to-one relationship, and the goodness-of-fit was evaluated using 3 simple, intuitive statistics: the coefficient of determination \( (r^2) \), the mean absolute deviation (MAD), and the distribution of residual errors (DRE). Each statistic evaluated a different property of the model. For example, \( r^2 \) as a measure of variability explained by the model was determined by the difference between the normal variation of predicted cell density and the variation of predicted cell density for the unity function. The second statistic, MAD, is a measure of the mean error between the predicted and measured cell density, without regard for the direction of error. The third evaluation of the goodness-of-fit, the DRE, is based on the signed errors, with errors of greater predicted than measured cell density considered as negative errors and errors in the opposite direction considered as positive errors. The DRE is a useful analysis for densely clustered data to determine whether the residual errors are systematic and as an assessment of the precision based on the 95% confidence limits of agreement between the model’s predicted and measured values.

**RESULTS**

The results of applying the model to data for patients with glaucoma are shown in Figure 2A and B. Figure 2A compares the ganglion cell density predicted from perimeter measurements of visual sensitivity with the histologic measurements of cell density. The various symbols designate different locations in the visual field, as indicated by the sites of retina samples in Figure 1. Data represent 437 sets of histologic measurements from retinal locations with visual sensitivity greater than zero. The empirical data can be compared with the line of equality that is superimposed on the data, which by visual inspection appear to be scattered unsystematically around the line of unity correlation. The visual goodness-of-fit is verified by statistics for the coefficient of determination, indicating that the unity correlation accounts for 39% of the total variance, and by the MAD, indicating that the mean error of the predicted ganglion cell density is less than 3 dB. Thus, the predicted one-to-one relationship for the structure-function model is only marginally worse than statistical linear regression \( (m = 0.8 \text{ dB/db} \) and \( b = 8 \text{ dB} \) which accounted for 42% of the variance.

The \( r^2 \) and MAD statistics demonstrate that the model is relatively accurate but do not indicate the extent of systematic errors in the predicted values. The DRE (Figure 2B) demonstrates that the errors are not system-atic, because the distribution is centered near zero with an SD of 3.22 dB and, as shown by the 3 darker bars, about 65% of the data are within ±3 dB of zero. The magnitude of errors across the range of ganglion cell density is illustrated by the dashed lines in Figure 2A, which represent the 95% confidence limits for agreement between the predicted and measured values. Thus, the goodness-of-fit statistics indicate that, in the units of standard clinical measurements, the model is accurate, and distribution of errors indicates that the model is relatively precise.

The full data set confirms a strong correlation between structure and function for the patients as a group, but it is also important to determine whether the model produces accurate results for individual patients. To illustrate the range of results, data from the model for each patient or eye are listed in the Table and examples from 6 patients are shown in Figure 3. Data listed in the Table show that for most eyes the model was quantitative, with MADs of less than 3 dB for 12 of the 17 eyes and a mean of the DREs less than 2 dB for 11 of the 17 eyes, although the range of individual variation was large, with \( r^2 \) values ranging from zero to 0.71. For individual examples, patients 10 and 5 (Figure 3A and B) demonstrate the best examples of an accurate and precise relationship between predicted and measured ganglion cell density. For both patients, the perimeter data were collected more than a year before the tissue samples, and patient 10 had more advanced disease than did patient 5 (mean deviation, -9.10 dB and -4.75 dB, respectively). Data for these 2 patients are distinguished by the goodness-of-fit statistics (Table) showing that the relationships for
these individuals are more accurate and precise than for the group data. In contrast, data for patients 15 and 12 (Figure 3C and D) exhibit systematic errors, and the variance of these data with respect to the model is larger than the basic variance of the predicted cell density. Because none of the variance can be explained by the model, the coefficient of determination ($r^2$) for the model prediction of unity correlation is shown by the one-to-one line superimposed on the data. Goodness-of-fit statistics for the mean absolute deviation (MAD) and coefficient of determination ($r^2$) are shown as insets, and the limits of agreement (95% confidence limits) are illustrated by the dashed lines on each graph. B and D, Distribution of residual errors of the model with respect to the one-to-one relationship, with errors of greater predicted than measured cell density designated as negative errors and errors for greater measured than predicted cell density designated as positive errors. The mean ± SD of the distributions are shown in insets, and the percentage of errors that are less than ±3 dB are indicated by the darker bars.

The sources of variability in these data are unknown, but it may be that much of the imprecision is due to experimental error rather than the basic structure-function relationship or the model. This suggestion is based on a comparison of the data for clinical glaucoma and experimental glaucoma. The precision of clinical data are affected by elderly patients who might be less motivated field-takers, by the normal aging effects on the state of the retinal tissue, and by the delay between the visual field tests and the tissue collection. With experimental glaucoma, the subjects are highly competent field-takers, with young eyes and immediate tissue processing. The effect of reducing these sources of error is demonstrated by comparison of data from humans and monkeys (Figure 3). Figure 2C and D shows the results from experimental glaucoma using the relatively accurate relationship between structure and function from clinical perimetry and, most likely, the precision is limited by nonsystematic variations within and across individuals.

Figure 2. Application of the model for structure-function in 437 patients with glaucoma (A and B) and 343 monkeys with experimental glaucoma (C and D). A and C, Relationships between ganglion cell density predicted from perimetry measurements of visual sensitivity as a function of the histologic measurements of cell density. The various symbols designate different locations in the visual field, as indicated by the sites of retinal samples in Figure 1. The model prediction of unity correlation is shown by the one-to-one line superimposed on the data. Goodness-of-fit statistics for the mean absolute deviation (MAD) and coefficient of determination ($r^2$) are shown as insets, and the limits of agreement (95% confidence limits) are illustrated by the dashed lines on each graph. B and D, Distribution of residual errors of the model with respect to the one-to-one relationship, with errors of greater predicted than measured cell density designated as negative errors and errors for greater measured than predicted cell density designated as positive errors. The mean ± SD of the distributions are shown in insets, and the percentage of errors that are less than ±3 dB are indicated by the darker bars.
same model of structure-function relationships as for human beings (Figure 2A and B). The statistical data for the model show a considerably greater precision in the relationship for experimental glaucoma than for clinical glaucoma, with an $r^2$ value of 0.85, compared with 0.39 for clinical glaucoma, and a narrower error distribution, with about 75% of the data, compared with 65% for clinical glaucoma, falling within ±3 dB of zero error. This comparison shows that when experimental variables are better controlled, the structure-function relationship becomes more precise. Thus, the overall results of the study confirm the fundamental assumption of clinical perimetry that the degree of vision loss is representative of the amount of ganglion cell loss.

The most important outcome of the present study was to demonstrate a quantitative relationship between ganglion cell density and visual sensitivity for human clinical glaucoma. Data originally published by Kerrigan-Baumrind et al. were reanalyzed using a model that provided a point-by-point estimation of ganglion cell density from perimetric measurements of visual sensitivity in corresponding retinal and test field locations. The model, without free parameters, produced an accurate and relatively precise quantification (in standard decibel units) of retinal ganglion cell density associated with visual field defects in eyes with glaucoma. The structure-function relationship for in-

**COMMENT**

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dividual patients with glaucoma was generally as good or better than for the grouped data.

The studies of structure-function relationships for clinical perimetry have demonstrated that 2 factors effectively reduce the variability of estimated neural losses: logarithmic scaling of structure and function variables and defining the parameters for the structure-function relationship by eccentricity. The significance of reduced variability, however, is most applicable for established visual field defects, and the quantitative model does not improve the estimation of early neural losses using clinical perimetry. The detection of early neural losses with perimetry is constrained by inherent intersubject variation of psychophysical measures of normal thresholds. Because the relationship between the visual threshold and number of neural mechanisms is logarithmic, a relatively large proportion of ganglion cells (40%-50%) must be lost before the threshold measurement exceeds the normal variability and reaches statistical significance. Therefore, these results do not contradict the previous reports that statistically significant visual field abnormalities occur only after 25% to 35% of ganglion cells have died or that the relationship between sensitivity and neural losses is systematic only after about 50% of the ganglion cells have died.7

The detection of early ganglion cell losses with perimetry might be improved by linearization of the structure and function data, which, in comparison with the logarithmic scaling, will accentuate mild visual field defects and compress the range of defects associated with deep defects from advanced ganglion cell death.28 Several investigators have proposed linear models to improve the accuracy of perimetry for early visual field defects,29,30 but when logarithmic and linear transformations were compared for correlating perimetric defects and neural losses from experimental glaucoma, it was found that the relationship in linear units was less systematic in 2 respects.28 First, the relationship exhibited considerable scatter in these data for small losses in visual sensitivity, and, second, visual sensitivity losses became saturated with larger losses in ganglion cell density. Thus, although linear scaling of perimetric defects and ganglion cell losses potentially could improve the structure-function relationship for visual defects associated with small amounts of cell loss, the usefulness of the relationship is limited because of the high variability in that range. The comparatively greater variability with linear-loss functions is a likely consequence of the logarithmic scale of stimulus intensity for perimetry measurements and because the relationship between visual sensitivity and the number of neural detectors is nonlinear.

In conclusion, the study confirmed that visual field defects measured using standard clinical perimetry are a direct expression of the neural losses caused by glaucoma, in which the quantitative relationship varies with retinal eccentricity. The eccentricity dependence of structure-function relationships is a consequence of the normal variation in ganglion cell density with retinal eccentricity, whereas at any given distance from fixation, the standard perimetric measures of visual sensitivity (decibel units) provide an accurate estimation of ganglion cell density in comparable decibel units. Although the present results suggest that the precision of estimates of ganglion cell density may be somewhat lower for clinical glaucoma than for experimental glaucoma, it is likely that the apparent imprecision is related to difficulties in obtaining perimetry data and postmortem retinal tissue from patients rather than to a difference in the fundamental structure-function relationship. In addition, for either experimental or clinical glaucoma, the precision of estimation is approximately equal across the entire range of ganglion cell loss, but logarithmic scaling of the relationship compresses small losses of both visual sensitivity and ganglion cell density and expands the ranges of larger losses.26 Consequently, the accuracy and precision of the structure-function relationship is best for moderate to advanced glaucomatous neuropathy, which is the range in which subjective measurements by perimetry are generally considered more accurate than objective structural measurements.35-36 For this reason, computer-automated perimetry has become and is likely to remain the standard for assessment of the stage of neural damage from glaucoma.37

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