A Novel Method to Detect Local Ganglion Cell Loss in Early Glaucoma Using Spectral-Domain Optical Coherence Tomography

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PURPOSE. To test the glaucoma-discriminating ability of a new method for detecting local ganglion cell loss using spectral-domain optical coherence tomography (OCT).

METHODS. This study included 58 glaucomatous and 48 healthy eyes from Japanese subjects. Combined thickness of the ganglion cell layer and inner plexus layer (GCIPL) was measured on a macular cube scan in Cirrus HD-OCT. Average GCIPL thickness within a macular elliptical annulus and minimum GCIPL thickness on 360 spokes extending from the inner to the outer radius of the elliptical annulus were calculated. Area under the receiver operating characteristic curve (AROC) to discriminate between healthy eyes and early (mean deviation [MD], $\geq -6$ dB) advanced (MD, $<-6$ dB) glaucomatous eyes were compared between parameters.

RESULTS. Forty-three were normal-tension glaucoma, and 15 were high-tension glaucoma. The mean minimum GCIPL thickness was 77.0 μm in healthy eyes and 60.6 μm in glaucomatous eyes ($P < 0.001$). For the intersession repeatability, the coefficients of variation for average GCIPL and minimum GCIPL were 0.98 and 1.85 in glaucomatous eyes, and 0.89 and 1.85 in healthy eyes, respectively. Minimum GCIPL thickness AROC (0.896) was significantly higher ($P = 0.0062$) than average GCIPL thickness (0.821) for early glaucoma, whereas minimum GCIPL AROC (0.991) was comparable ($P = 0.105$) to average GCIPL (0.964) for advanced glaucoma. The minimum GCIPL thickness AROC was comparable ($P = 0.861$) to average circumpapillary retinal nerve fiber layer (cprNFL) thickness (0.890) for early glaucoma.

CONCLUSIONS. In Japanese patients with 74.1% of normal-tension glaucoma, the minimum GCIPL on spokes may be useful for detecting early glaucoma. (Invest Ophthalmol Vis Sci. 2012; 53:6904–6913) DOI:10.1167/iovs.12-10210

G laucomatic eyes suffer from progressive loss of retinal ganglion cells (RGCs); this leads to clinically detectable structural and functional changes such as glaucomatous optic disc changes and visual field defects. The human retina contains more than 1 million RGCs, with substantial interindividual variability, and approximately 50% of these cells are concentrated within 4.5 mm of the foveal center.1 It has long been suggested that glaucoma-induced structural changes start in the macula because of the dense population of RGCs in this region. This assumption is supported by experimental models of glaucoma that show substantial loss of RGCs in the parafoveal region.2,3 Histological studies of enucleated eyes of patients with glaucoma by Quigley et al.4 and Kerrigan-Baumrind et al.5—and primate eyes with experimental glaucoma by Harwerth et al.6—indicate that considerable loss of macular RGCs occurs when visual field defects are detectable. Taken together, these studies suggest that advances in the methods to assess early loss of macular RGC would improve detection of early glaucoma.

The advent of optical coherence tomography (OCT) has allowed assessment of RGC axons by measuring optic disc topography and circumpapillary retinal nerve fiber layer (cprNFL) thickness. In addition, OCT allows measurement of the macula, which includes RGC axons, cell bodies, and a large amount of other neuronal cells. The advancement of OCT technology into the spectral-domain detection technique, combined with the development of the intraretinal boundary segmentation algorithm, has enabled more selective assessment of macular RGCs. These advancements include the ability to automatically measure the combined thickness of the RNFL, ganglion cell layer (GCL), and the inner plexiform layer (IPL); these three layers are collectively termed the “ganglion cell complex” [GCC]7–9; the combined thickness of the GCL and the IPL10–12; and the manual measurement of the thickness of individual layers such as the GCL and RNFL.3 Earlier studies showed that GCC thickness measurements improved the glaucoma-discriminating ability of the macular measures compared with the total retinal thickness measures.7–9

Despite advancements in the techniques for measuring macular structures, the strategy to detect local loss of the RGCs based on thickness measurements still uses the average and regional thicknesses in the macula. Although the average thickness is advantageous as a global parameter to detect structural abnormalities throughout the macula, it is likely to be less advantageous for detecting the RGC loss limited to a local area. This is because averaging ($\pm$ thickness divided by area) will tend to underestimate the local RGC loss as it takes into account the area with a normal or less affected RGC population. Regional thickness using predefined sectors, such as the Early Treatment Diabetic Retinopathy Study (ETDRS) and glaucoma sector charts, has been used to detect local macular RGC loss.14–21 However, the same averaging effects may be
present within the sectors, and developing a predefined sector that encloses the entire area with local RGC loss is difficult because areas of RGC loss differ from patient to patient.

We explored a new approach for detecting local loss of macular RGCs by using the lowest ganglion cell-inner plexiform layer thickness (GCIPL) among the 360 spokes (termed “minimum GCIPL”) based on ganglion cell analysis in a macular elliptical annulus developed on a macular cube scan with OCT software (Cirrus HD-OCT; Carl Zeiss Meditec, Inc., Dublin, CA). Because this method is based on the relatively symmetric macular RGC density,1 and GCL thickness,13 it presumably detects the location with most severe RGC loss, which should theoretically represent the location with local RGC loss in early glaucoma. The purpose of this study was to determine whether this new approach effectively discriminates between eyes with early glaucoma and healthy eyes as compared with the conventional average and regional thickness measures.

**METHODS**

Study subjects who met the eligibility criteria and underwent examination by Cirrus HD-OCT were enrolled from a database of Japanese patients who were examined for glaucoma between April 17, 2008 and September 25, 2011, at the Department of Ophthalmology, Kyoto University Hospital. If both eyes of a subject were eligible for the study, one eye was chosen randomly for inclusion. Data for candidate control eyes were retrospectively collected from our database of Japanese normal volunteers who were determined by our department to have at least one healthy eye and who agreed to undergo the examinations described in this study.

All patients underwent a comprehensive ophthalmic examination that included measurement of uncorrected and best-corrected visual acuity using the 5-meter Landolt chart, slit-lamp examinations, intraocular pressure (IOP) measurements using a Goldman applanation tonometer, gonioscopy, dilated stereoscopic examination of the optic nerve head (ONH), stereo disc photography with a 3-Dx simultaneous stereo disc camera (Nidek; Gamagori, Japan), red-free fundus imaging using angiograph equipment (Heidelberg Retina Angiogram II [HRA2]; Heidelberg Engineering, Heidelberg, Germany); standard automated perimetry (SAP) using a visual field analyzer (Humphrey Visual Field Analyzer; Carl Zeiss Meditec, Inc.); and SD-OCT examination with OCT equipment (Carl Zeiss Meditec, Inc.).

All investigations in this study adhered to the tenets of the Declaration of Helsinki and the study was approved by the Institutional Review Board and Ethics Committee of Kyoto University Graduate School of Medicine. Informed consent was obtained from all participants.

For inclusion, subjects were required to have a best-corrected visual acuity of 20/20 or better in the Snellen equivalent, a spherical equivalent refractive error ≥−6 diopters (D) and <3 D, a normal open anterior chamber angle, macular cube scans (Carl Zeiss Meditec, Inc.)

**FIGURE 1.** Schema showing a macular elliptical annulus and the spokes and sectors in ganglion cell analysis. (A) Dimension of an elliptical annulus (dimensions: vertical inner and outer radius of 0.5 mm and 2.0 mm, horizontal inner and outer radius of 0.6 and 2.4 mm, respectively) centered on the fovea within a macular area scanned with a macular cube scan. The average GCIPL thickness was calculated on each spoke. Red lines indicate a portion of 360 spokes that extended from the inner radius to the outer radius of the elliptical annulus. The black line indicates the spoke with minimum thickness value. (B) Six sectors (S1–S6) within the elliptical annulus. (C) Boundary lines drawn to measure GCIPL thickness. The purple line indicates the boundary between the RNFL and GCL and the yellow line indicates the boundary between the GCL and IPL.
of good quality, and reliable visual field tests. Exclusion criteria were evidence of media opacities, such as corneal opacity, cataract, and vitreous opacity, vitreoretinal disease, uveitis, or nonglaucomatous optic neuropathy, or a history of intraocular surgery. Patients with neurological and systemic diseases that could affect retina and visual field results—such as cerebral infarction, cerebral tumor, uncontrolled hypertension, and blood disorders—were also excluded.

Glaucomatous eyes were defined by the presence of evident diffuse or localized rim thinning on stereo disc photography regardless of the presence (perimetric glaucoma)/absence (preperimetric glaucoma) of glaucomatous visual field defects or the presence of RNFL defects associated with glaucomatous visual field defects. Healthy subjects were enrolled by advertisement. Healthy controls had an IOP ≤21 mm Hg with no history of increased IOP; an absence of glaucomatous optic disc appearance on stereo disc photography, no RNFL defects on red-free fundus imaging, normal visual field testing results, and no family history of glaucoma.

Eyes were diagnosed as normal-tension glaucoma when all known untreated IOPs were ≤21 mm Hg, and otherwise as high-tension glaucoma.

Eyes with a mean deviation (MD) ≥−6 dB or <-6 dB were classified as early glaucoma or advanced glaucoma, respectively, based on the MD grouping of the Hodapp-Parrish-Anderson grading scale of severity.

The appearance of the optic disc on fundus photographs, including stereoscopic photographs, was evaluated by three glaucoma specialists (MH, HOI, and AN) who were masked to all other information about the eyes. If all three examiners did not agree on the classification, the eye was not used for analysis.

Visual Field Testing

Reliable SAP results obtained within 4 months from the OCT (Carl Zeiss Meditec, Inc.) examination were used. The reliability was defined as fixation loss <20%, false-positive ≤15%, and false-negative ≤33%. Visual field defects resulting from glaucoma were defined using the 24-2 Swedish Interactive Threshold Algorithm standard program as: abnormal range on the glaucoma hemifield test; consistent presence of a cluster of three or more nonedge points on the pattern deviation plot with a probability of occurrence in fewer than 5% of the normal population (P < 5%), with one of these points having P < 1% of the normal population; or a pattern standard deviation (PSD) <5% of the normal reference confirmed on two successive tests.

Spectral-Domain Optical Coherence Tomography (SD-OCT) Macular and Optic Disc Cube Scan

A well-trained examiner obtained a 3-dimensional OCT dataset based on stereoscopic and optic disc cube 200 × 200-scan protocols after pupillary dilatation with tropicamide 1% and phenylephrine 2.5%. These protocols perform 200 horizontal B-scans comprising 200 A-scans per B-scan over 1024 samplings within a cube of 6 × 6 × 2 mm centered at the foveal and optic disc centers, respectively. The examiner discarded poor quality images with a signal strength <6 and any scans with visible eye motion or blinking artifacts (discontinuous jump), or poor centration. To calculate the test-retest measurement variability, three scans per eye with acceptable images were obtained in the same visit.

Ganglion Cell Analysis in a Macular Elliptical Annulus

The prototype ganglion cell analysis (GCA) algorithm developed for the OCT software (Cirrus 6.0; Carl Zeiss Meditec, Inc.) was described in detail elsewhere.10,11 Briefly, this algorithm identifies the outer boundary of the RNFL and the outer boundary of the IPL on a macular cube scan in the OCT equipment (Carl Zeiss Meditec, Inc.). The difference between the RNFL and the IPL outer boundary segmentations yields the combined thickness of the GCL and the IPL (termed “GCIPL”), which is indicative of RGC health. An elliptical annulus (dimensions: a vertical inner and outer radius of 0.5 mm and 2.0 mm and a horizontal inner and outer radius of 0.6 and 2.4 mm, respectively) centered on the foveal center was used as the software analysis field (Fig. 1). The size of the inner ring in the annulus was chosen to exclude the foveal area, where the GCL is thin and difficult to detect. However, the dimension of the outer ring was selected to conform closely to the real anatomy of the macular region, where the GCL is thickest in a healthy eye.1,13 These parameters were tested in a preliminary analysis using GCIPL maps of 47 healthy eyes.10,11

We checked the quality of GCIPL segmentation in all of the B-scans in each cube scan by using the criteria of Ishikawa et al.23 In detail, segmentation failures were defined as obvious disruption of the detected border, and/or border wandering (detected border jumping to and from different anatomic structures), within >5% consecutively (i.e., an uninterrupted error) or 20% cumulatively (i.e., adding up all errors amounted to 20% of the image width) of the entire image. If a cube scan had segmentation failures in >5% of B-scans (6 of 200 B-scans), the scan was excluded from analysis.

Measurement of Macular GCIPL Thickness on a Cube and Spokes

The average GCIPL thickness within the elliptical annulus was calculated in the OCT software (Carl Zeiss Meditec, Inc.; Fig. 1). The FoveaFinder function of this software identifies the foveal center based on the well-known anatomical fact that highly reflective inner retinal layers—such as the RNFL, inner plexiform layer (IPL), and inner nuclear layer (INL)—are never present in the foveal center, and that consequently, a large part of the retinal thickness in the foveal center is comprised of the hyporeflective outer nuclear layer and three highly reflective lines representing the external limiting membrane, photoreceptor inner and outer segment junction, and cone outer segment tip. The software function creates an en face image from the 3D data below the ILM plane to the appropriate plane determined by a cost-function-based method, so that the en face image does not include the three highly reflective lines in the fovea. Because the reflectivity from the inner retinal layers is absent in the foveal center, the center of the fovea of the partial en face image appears as a dark spot. The center of the dark spot is automatically identified as the foveal center. Because the FoveaFinder function is built-in in the commercial OCT software (Carl Zeiss Meditec, Inc.), the disclosure of more detailed method description is not permitted. We checked the accuracy of the detection of the foveal center by manually comparing the automatically selected foveal center on the software screen and serial B-scans in the vicinity of the foveal center, and also by comparing the automatically selected fovea and the GCIPL thickness map, but in no cases was it found necessary to adjust the fovea.

The elliptical annulus was segmented to six sectors that subtended 60°: the superotemporal (S1), superior (S2), supronasal (S3), inferonasal (S4), inferior (S5), and inferotemporal (S6) sectors. Regional GCIPL thicknesses were calculated in each sector. Here, 7500 and 11000–13000 sampling points were used to calculate average and regional GCIPL thicknesses, respectively.

The OCT software (Carl Zeiss Meditec, Inc.) also calculated the mean GCIPL thickness on each of the 360 spokes for every 1° that extended from the inner radius to the outer radius of the elliptical annulus. The resampling method on spokes was nearest neighbor sampling, and 50–60 sampling points were used to calculate mean GCIPL thickness on each spoke. The spoke with the lowest GCIPL thickness (termed minimum GCIPL) was assumed to indicate the location of the most severe local thinning of the GCL, like a compass. The directional angle was defined as starting at the temporal horizontal (−0°) line in the clockwise direction in the right eye (0°–360°) and in the counterclockwise direction in the left eye (Fig. 1).
FIGURE 2. Photographs of the left eye of a 42-year-old woman with glaucoma with an intraocular pressure of 14 mm Hg and an MD of SAP of −2.74 dB. (A) Color. (B) Red-free fundus photographs. (C) A macular vertical scan of SD-OCT. (D) Visual field result. (E) Thickness map of the macular GCIPL thickness in macular elliptical annulus. Localized neuroretinal rim thinning and a RNFL defect are evident in the inferotemporal region in (A) and (B). Visual field defects corresponding to this feature were found in the superior hemifield (D). A disc hemorrhage and slit-like RNFL defects were seen in the superotemporal region, but corresponding neuroretinal rim thinning was not evident and the visual field in the inferior hemifield was normal (A, B, D). The green line in (A) and (B) indicates the scan line for the SD-OCT image in (C). The red square in (C) indicates the area of...
macular elliptical annulus. The \textit{white} and \textit{red arrowheads} in (C) indicate the GCIPL in the corresponding three points in the inferior (damaged) and superior hemispheres (opposite). \textit{Red arrowheads} point out a peaked thinning of the GCIPL. The thinning of the RNFL and GCIPL in the inferior macula compared with that on the superior macula was apparent in the vertical OCT image. The thinning of the GCIPL is focal and abrupt compared with diffuse thinning of the RNFL. The minimum GCIPL thickness was 56.37 μm and the average GCIPL thickness of the elliptical annulus was 76 μm. The \textit{red line} indicates the spoke that showed minimum GCIPL thickness.

**Measurement of cpRNFL Thickness**

The OCT software (Carl Zeiss Meditec, Inc.) extracts from the cube dataset 256 A-scan samples along the path of a calculation circle of 3.46 mm in diameter that was positioned automatically around the optic disc. The built-in algorithm located the center of the optic disc, even if it was not well-centered in the scan image. The disc center was identified by finding a dark spot near the center of the scan that had a shape and size consistent with a range of optic discs.

**Angular Location of Minimum GCIPL and RNFL Defect**

To correlate the angular locations of minimum GCIPL and localized RNFL defects, we calculated the angle of the minimum GCIPL location from the temporal spoke at 0° and measured the angle of localized RNFL defects from a line passing the disc center and foveal center in eyes with localized RNFL defects. The angular locations of the RNFL defects were measured as previously described in detail, with modifications. First, a circle 3.46 mm in diameter, centered on the ONH, and a line from the center of the optic disc to the foveal center, referred to as the reference line, were drawn on the red-free photographs. Next, the two borders of each RNFL defect were defined by drawing lines from the center of the optic disc to each of the points at which the borders of the RNFL defect intersected the circle. The angle of the RNFL location was defined as the angle formed by the reference line and the median line of the two border lines of each RNFL defect. Measurements were made using imaging software (ImageJ; provided in the public domain by National Institutes of Health, Bethesda, MD; http://rsweb.nih.gov/ij/). Only the RNFL defects in the same hemisphere as the minimum GCIPL location were analyzed. When more than two RNFL defects were present, the RNFL defect nearest to the reference line was analyzed, because the GCA is based on an elliptical annulus smaller than the macula.

**Statistical Analysis**

Differences in the age and spherical equivalent of refractive errors between the healthy and glaucoma groups were compared using unpaired \(t\) tests. Differences in sex between the healthy and glaucoma groups were compared using the chi-square test. The statistical significance of differences between the visual field severities of the groups was assessed with ANOVA and subsequent post hoc least significant difference test, as appropriate. The area under the receiver operating characteristic curve (AUC) was calculated to compare the glaucoma discriminating ability of minimum GCIPL thickness on spokes with that of the average and regional GCIPL thicknesses measured on a cube and that of the cpRNFL thickness. The method of Delong et al. was used to calculate the standard error of the area under the characteristics operating curve and the difference between two AUCs. McNemar's test was used to calculate the sensitivity at specificity \(\geq 95.0\%\) for detecting glaucoma. The intraclass correlation coefficient (ICC), coefficient of variation (COV), and pooled within-subject test-retest standard deviation (TRTSD) were calculated to assess the measurement repeatability. The \(t\) test, chi-square test, ICC, COV, TRTSD, and ANOVA were performed using a statistical software program (SPSS 17; SPSS Inc., Chicago, IL) and the AROC comparisons were performed using statistical software (MedCalc12; MedCalc Software, Mariakerke, Belgium). A \(P\) value \(< 0.05\) was considered statistically significant.

**RESULTS**

**Subjects**

Two and eight eyes were excluded because of incorrect segmentation and inadequate signal strength, respectively. Finally, 92 eyes from 58 patients were eligible for this study. After one eye was selected at random from the patients with two eligible eyes, 58 eyes of 58 patients (31 men and 27 women) were included. The glaucomatous subjects ranged in age from 29 to 79 years (mean ± SD, 58.5 ± 12.3 years). In glaucomatous eyes, the spherical equivalent of the refractive error ranged from \(-6.0\) to \(+2.2\) D (advanced glaucoma) and the MD of SAP ranged from \(-2.94\) to \(+1.1\) dB (normal-tension glaucoma) and \(15\%\) of eyes were high-tension glaucoma.

The control group consisted of 47 eyes from 47 healthy volunteers (26 men and 21 women). The healthy subjects ranged in age from 40 to 82 years (55.5 ± 11.0 years). The spherical equivalent of the refractive error ranged from \(-6.0\) to \(+2.5\) D (advanced glaucoma). The mean MDs were \(-2.33\pm 1.8\) for the “early” group and \(-14.2\pm 6.57\) for the “advanced” group. Forty-three (74.1\%) of 58 eyes were normal-tension glaucoma, and 15 (25.9\%) of 58 eyes were high-tension glaucoma.

The control group consisted of 47 eyes from 47 healthy volunteers (26 men and 21 women). The healthy subjects ranged in age from 40 to 82 years (55.5 ± 11.0 years). The spherical equivalent of the refractive error ranged from \(-6.0\) to \(+2.5\) D (early glaucoma) and 20 had an MD \(> -6\) dB (advanced glaucoma). The mean MDs were \(-2.08\) to \(+3.34\) dB (normal-tension glaucoma) and \(15\%\) of eyes were high-tension glaucoma.

**Case**

The left eye of a 42-year-old woman who received follow-up for glaucoma is shown in Figure 2. The intraocular pressure was 14 mm Hg and the MD of SAP was \(-2.74\) dB. Localized neuroretinal rim thinning and a corresponding RNFL defect were observed in the inferotemporal region on color fundus photography and red-free angiograph (Heidelberg Engineering) imaging. On the red-free image, two other RNFL defects were seen on either side of the inferotemporal large vessels. These RNFL defects appeared to be outside the macular scan area of the SD-OCT. Visual field defects corresponding to this feature were found in the superior hemifield. Although a disc hemorrhage and slit-like RNFL defects were observed in the superotemporal region, corresponding neuroretinal rim thinning was not evident and the visual field in the inferior hemifield was normal. Thinning of the RNFL and GCIPL was apparent on the macular vertical OCT image. The RNFL thinning appeared to be diffuse, whereas the GCIPL thinning was focal and abrupt. The minimum GCIPL thickness was 56 μm and the average GCIPL thickness of the elliptical annulus was 76 μm. The spoke on which the minimum GCIPL thickness was detected was located at the inferotemporal region (the red line in Fig. 2E).

**Interobserver Measurement Variability and Reproducibility**

The interobserver means, TRTSDs, COVs, and ICCs of GCIPL thickness measurements in healthy eyes and in glaucomatous eyes are listed in Table 1. In glaucomatous eyes, the ICcS for average GCIPL and minimum GCIPL were 96 and 92,
respectively, and ranged between 80 and 95 for regional GCIPL. In healthy eyes, the ICCs for average GCIPL and minimum GCIPL were 97 and 98, respectively, and ranged between 96 and 97 for regional GCIPL. In glaucomatous eyes, the COVs for average GCIPL and minimum GCIPL were 0.98 and 1.85, respectively, and ranged between 1.44 and 2.46 for regional GCIPL in glaucomatous eyes. The COVs for average GCIPL and minimum GCIPL were 0.89 and 1.85, respectively, and ranged between 1.14 and 1.99 for regional GCIPL in healthy eyes.

Variability of GCIPL Thickness among 360 Spokes

Because the RGC density in the macula is not perfectly symmetrical in normal eyes, it is possible that GCIPL thickness will have some variation with spokes. To determine how variable the GCIPL thicknesses were among the 360 spokes of each eye, we calculated the difference with spokes in normal eyes. The difference between maximum and minimum GCIPL thicknesses among 360 spokes ranged from 5.1 to 20.5 μm (10.0 ± 3.0 μm, mean ± SD) in normal eyes. The maximum percent differences of GCIPL thicknesses against mean GCIPL thickness of each eye were from 6% to 27% (12 ± 4%, mean ± SD).

GCIPL and cpRNFL Thickness

Average and regional GCIPL thicknesses measured on a cube and minimum GCIPL thickness obtained on spokes for healthy eyes and glaucomatous eyes are shown in Table 2. All GCIPL parameters were significantly smaller in eyes with early glaucoma than in healthy eyes. Moreover, all GCIPL parameters were significantly smaller in eyes with advanced glaucoma than in eyes with early glaucoma, with the exception of regional GCIPL thickness in the superior (S2) and superonasal (S3) sectors. The frequency distribution profiles of the directional angle for the spokes with minimum GCIPL thickness were as follows: the minimum GCIPL thickness was found most frequently (38 eyes [65.5%]) between 270° and 360° compared with nine eyes between 0° and 90°, three eyes between 90° and 180°, and eight eyes between 180° and 270°.

Average cpRNFL thickness was significantly smaller in eyes with early glaucoma than in healthy eyes and in eyes with advanced glaucoma than in eyes with early glaucoma (Table 2).

Correlation of the Angular Locations for Minimum GCIPL and RNFL Defects

Of 58 eyes, 36 had localized RNFL defects. The angular locations of the minimum GCIPL and RNFL defects showed a significantly negative correlation (P = 0.022, r = 0.379); the farther the RNFL defects were from the line passing the disc and foveal centers, the closer the spokes of the minimum GCIPL locations were to the temporal spoke at 0°.

Comparison of AROC and Sensitivity with Fixed Specificity at ≥95%

To discriminate between healthy and early/advanced glaucomatous eyes, AROCs and sensitivities with fixed specificity at ≥95% were calculated for average and regional GCIPL thicknesses on a cube and minimum GCIPL thickness on spokes (Fig. 3, Table 3). The AROC and sensitivity with specificity at ≥95% for the average GCIPL thickness was significantly larger than that of the average GCIPL between healthy and early glaucomatous eyes (P = 0.0062 and 0.0034, respectively), but not between healthy and advanced glaucomatous eyes. No significant differences were found for the

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A comparison was made with fixed specificity at a level of $P < 0.001$. The AROC and sensitivity were superior for minimum GCIPL thickness on spokes compared to average cpRNFL to discriminate healthy eyes from any glaucomatous groups. The AROC for the minimum GCIPL thickness between healthy and early glaucomatous eyes was significantly larger than those of regional GCIPL thicknesses for the S1 to S5 sectors ($P < 0.001$), but not for S6. The sensitivity with fixed specificity at $P < 0.001$ for the minimum GCIPL thickness between healthy and early glaucomatous eyes was significantly larger than those of regional GCIPL thicknesses for the S1 to S6 sectors ($P < 0.0001$). The AROC and sensitivity with fixed specificity at $P < 0.001$ for the minimum GCIPL thickness between healthy and advanced glaucomatous eyes was significantly larger than those of regional GCIPL thicknesses for the S1 to S4 sectors ($P = 0.0225$ to $P < 0.0001$), but not for S5 or S6.

### DISCUSSION

This study tested a new strategy based on the concept that the minimum GCIPL thickness may be a parameter for accurately detecting early glaucoma because the spoke with minimum GCIPL thickness should act as a compass to indicate the location with a local RGC loss in eyes with early glaucoma. This approach does not depend on averaging the thickness of the area ($= \text{thickness divided by area}$) that has been used in most of the earlier studies for glaucoma detection using OCT. $^7$-$^21$

In six human retinas obtained from eye bank donors, reported that the isodensity contours of the RGC density in the macula form a horizontally oriented elliptical ring in all eyes. Based on this finding, the elliptical annulus centered on the fovea that was stretched horizontally by 20% was designed for ganglion cell analysis. Earlier studies using SD-OCT showed similar symmetrical thicknesses of the GCL, IPL, and GCL+IPL of healthy eyes in an elliptical annulus. $^7$-$^13$, $19$, $20$ The GCIPL thicknesses on all 360 spokes should be equivalent if the symmetry is perfect; and, consequently, the initial RGC loss limited to a small local area would be detectable as a decrease of the GCIPL thickness on the spokes, leading to minimum GCIPL thickness. Thus, this parameter should theoretically detect early or initial RGC loss with high sensitivity. However, Curcio et al. also reported that the macular RGC density is not completely symmetrical: the temporal RGC density was slightly thinner than the nasal GCL, while the superior and inferior GCL thicknesses were almost equivalent.

One expected disadvantage of this new approach was a lower reproducibility for measuring GCIPL on spokes compared to measuring average and regional GCIPL on a cube because the former is calculated from only 50 to 60 sampling points in each B-scan compared with 7500 sampling points in the macular elliptical annulus and 1100 to 1300 sampling points in the six sectors. However, the reproducibility of the minimum GCIPL thickness appeared to be permissible. Although the intersession reproducibility results in our study were slightly lower for minimum GCIPL compared to average GCIPL, the reproducibility was still excellent both for healthy and glaucomatous eyes.

Our method depends on the anatomical characteristics of the GCL and IPL; namely, the high symmetry in an elliptical ring form. Curcio et al., $^1$ who studied the topography of RGCs in six human retinas obtained from eye bank donors, reported that the isodensity contours of the RGC density in the macula form a horizontally oriented elliptical ring in all eyes. Based on this finding, the elliptical annulus centered on the fovea that was stretched horizontally by 20% was designed for ganglion cell analysis. Earlier studies using SD-OCT showed similar symmetrical thicknesses of the GCL, IPL, and GCL+IPL of healthy eyes in an elliptical annulus. $^7$-$^10$, $13$, $19$, $20$ The GCIPL thicknesses on all 360 spokes should be equivalent if the symmetry is perfect; and, consequently, the initial RGC loss limited to a small local area would be detectable as a decrease of the GCIPL thickness on the spokes, leading to minimum GCIPL thickness. Thus, this parameter should theoretically detect early or initial RGC loss with high sensitivity. However, Curcio et al. also reported that the macular RGC density is not completely symmetrical: the temporal RGC density was slightly lower than the nasal GRC density, whereas the inferior and superior RGC densities were almost equivalent. $^1$ Nakano et al. $^{12}$ measured the GCL thickness on SD-OCT images and found identical symmetry between the macular GCL thickness and the RGC density; that is, the temporal GCL was thinner than the nasal GCL, while the superior and inferior GCL thicknesses were almost equivalent. Therefore, we estimated the variability of GCIPL measurements among 360 spokes and found mild variations (maximum difference, 12% of average GCIPL thickness on 360 spokes) among 360 spokes even in healthy eyes. Although this variation among spokes may have lowered...
the glaucoma discriminating ability of the current method, the AROC and sensitivity with fixed specificity $\geq 95\%$ were still better for discriminating between early glaucomatous and healthy eyes using this method than when using the average macular GCIPL thickness.

In the current study, no significant differences were found for AROC and sensitivity with fixed specificity $\geq 95\%$ between minimum GCIPL and average cpRNFL thickness. However, these two parameters would have different characteristics. A limitation of the current GCA method is that RGC loss that is limited to the area outside the elliptical annulus, which is smaller than the macular area, may be missed. However, the GCIPL parameter in the GCA method would be advantageous to sensitively detect local RGC loss in the macula because the macular elliptical annulus used for the GCA method is designed to cover the highest density of RGCs$^{1}$ and the thickest GCL,$^{13}$ and because GCIPL parameters can detect damage to both RGC axons and cell bodies. By contrast, cpRNFL encompasses all RGC axons that assemble to the optic disc. A limitation of the cpRNFL parameters is that cpRNFL can detect only RGC axons, but not cell bodies. These limitations would apply when only either parameter is used as a structural parameter for glaucoma.

**Figure 3.** Receiver operating characteristics curves (ROC) to discriminate between healthy and glaucomatous eyes by the GCIPL and cpRNFL thicknesses as measured with SD-OCT. (A, B) Healthy eyes versus all glaucomatous eyes. (C, D) Healthy eyes versus eyes with early glaucoma. (E, F) Healthy eyes versus eyes with advanced glaucoma. (A, C, E) ROC in Minimum GCIPL, average GCIPL, and cpRNFL. (B, D, F) ROC in each sector. S1 to S2 correspond to the sectors 1 to 6 as shown in Figure 1B.
diagnosis. For the practical detection of glaucoma, the macular parameters should be used together with the cpRNFL parameters. From this point of view, development of parameters with as high a glaucoma detection ability as possible is desirable in each category of the GCA and cpRNFL methods.

In the current study, the six sectors showed different thinning in GCIPL with disease progression, and subsequently, different glaucoma discriminating ability. The GCIPL thinning was most severe in the inferotemporal (S6) sector and mildest in the superior (S2) and superonasal (S3) sectors. In particular, there were no significant differences found in the GCIPL thickness of the S2 and S3 sectors between eyes with early and advanced glaucoma. These results are consistent with those of earlier studies, in which the inferotemporal area in the macula was found to be most susceptible to glaucomaous damages. It is generally thought that the RGC axons in the inferior hemisphere are more vulnerable to glaucomatous damages. By contrast, the papillomacular bundle is usually spared until the end stages of glaucoma. This indicates that a greater number of RGCs in the nasal part of the macula are less susceptible to glaucomatous damages. Such difference in vulnerability may be responsible for the different degrees of thinning in the GCIPL with disease progression among sectors. The highest susceptibility of the GCIPL in the inferotemporal sector appears to favor the glaucoma discriminating ability of the S6 sector in early glaucoma.

Among the six sectors, the S6 sector had the best glaucoma discriminating ability in AROC analysis and in the sensitivity with fixed specificity \( \geq 95\% \). The AROC of the S6 sector was comparable with that of the minimum GCIPL. However, when the specificity was fixed at \( \geq 95\% \), the sensitivity of the S6 sector was less than that of the minimum GCIPL. As mentioned above, the GCIPL in the S6 sector was most susceptible to thinning with disease progression. This sector cannot detect glaucomatous eyes with RGC damages outside of the S6 sector. This would explain the lower sensitivity with fixed specificity \( \geq 95\% \).

Our results in early glaucoma are consistent with those of a recent study. In our study, however, the ability to discriminate between healthy and advanced glaucoma eyes was not significantly different for the minimum GCIPL and average GCIPL. Because the area of RGC loss would be much larger in advanced glaucoma than in early glaucoma, the underestimation that results from averaging the thickness with the area including normal or less affected region consequently may be lower. Thus, our results suggest that the advantage of the minimum GCIPL over the average GCIPL is in early glaucoma detection.

This study had limitations. First, in the current study—probably because all of our participants were Japanese—74.1% of the participants with glaucoma had normal-tension glaucoma. It is generally known that the paracentral region is more frequently involved in glaucomatous visual field defects in eyes with normal-tension glaucoma. To interpret these results, we need to take into consideration the possibility that the GCIPL measurement within the macular elliptical annulus, in which maximum diameter of the ellipse was 4.8 mm, may favor detection of normal-tension glaucoma. Further studies with other ethnic groups are required. Second, the sample size was small. Given this limitation, the absence of ethnic differences would be beneficial.

In conclusion, the current study in Japanese patients primarily with normal-tension glaucoma showed that the minimum GCIPL on the spoke showed a better ability to discriminate between healthy and early glaucomatous eyes than did the average GCIPL thickness. Measurement of minimum GCIPL may provide a useful method that does not require averaging thickness with area for detecting early macular RGC loss limited to a local area.

### References

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