Impact of Diabetic Retinopathy on Quantitative Retinal Nerve Fiber Layer Measurement and Glaucoma Screening

Hirokazu Takahashi and Etsuo Chihara

PURPOSE. To investigate the impact of diabetic retinopathy on quantitative retinal nerve fiber layer (RNFL) assessment and diagnostic power for glaucoma by scanning laser polarimetry (GDx-VCC) and optical coherence tomography (StratusOCT).

METHODS. The individual RNFL parameters of GDx and OCT were obtained for 170 eyes (one eye from each of 170 subjects [45 healthy, 47 glaucoma, 40 diabetes, and 38 glaucoma with diabetes]) and were compared among the four groups. Diabetic eyes had mild to moderate nonproliferative diabetic retinopathy (NPDR) without maculopathy. In glaucomatous eyes with or without diabetes, the ability to discriminate glaucoma was assessed by the areas under the receiver operating characteristic curves (AUROCs) and the sensitivities at more than 80% and 90% of specificities for each technique.

RESULTS. Using GDx-VCC, significant differences \( P < 0.05 \) in RNFL measurement parameters were found for all comparisons except those between glaucomatous eyes with diabetes and without diabetes. StratusOCT parameters did not detect significant differences between age-matched healthy and diabetic eyes. Among the parameters included, the nerve fiber indicator (NFI) of GDx-VCC and the inferior quadrant thickness (IQT) of StratusOCT had the largest AUROCs and sensitivities at specificities greater than 80%; NFI (0.912, 86%) and IQT (0.902, 85%) in glaucomatous eyes with diabetes; NFI (0.935, 92%) and IQT (0.921, 91%) in simple glaucomatous eyes.

CONCLUSIONS. Mild to moderate NPDR causes a quantitative discrepancy in RNFL measurements between GDx-VCC and StratusOCT in simple diabetic eyes. However, mild to moderate glaucomatous optic neuropathy can be highly discriminated by the two imaging devices in eyes with diabetic retinopathy. (Invest Ophthalmol Vis Sci. 2008;49:687–692) DOI:10.1167/iovs.07-0655

Various ocular imaging instruments have evolved to provide quantitative estimates of the retinal nerve fiber layer (RNFL) thickness around the optic disc. Scanning laser polarimetry (SLP; GDx access with variable corneal compensator [VCC] mode; Carl Zeiss Meditec, Dublin, CA) and optical coherence tomography (StratusOCT; Carl Zeiss Meditec) are major examples with a high degree of sensitivity and specificity in discriminating between glaucomatous and healthy subjects.\(^1,2\)

Substantial modifications of both quantitative imaging techniques have improved the ability to detect glaucomatous optic nerve damage. The prototype SLP was converted to the VCC mode, which provides individualized adjustment of anterior segment birefringence.\(^3,4\) The current third generation of OCT has an increased scan rate and scan resolution and also can be used for macular and optic nerve analysis.\(^5\)

Investigators, however, should recognize additional conditions that cause unexpected differences in measurement from a clinical standpoint. Recent studies have invited attention to errors in quantitative retinal thickness evaluation generated by StratusOCT.\(^6,7\) These errors may be related to image artifacts, operator error, and failure to detect accurate retinal boundaries by OCT software algorithms. An atypical birefringence pattern associated with older patient age may produce an artifactual increase in quantitative RNFL measurements by GDx VCC.\(^8\) Peripapillary atrophy that is evident in the scanning circle may lead to inaccurate measurements.\(^9\)

Those findings prompted us to ask whether quantitative RNFL thickness measurements and the power of GDx-VCC and StratusOCT to detect glaucoma also can be affected by retinal structural changes. Diabetic retinopathy, a major cause of distorted retinal architecture, often coexists with glaucomatous optic neuropathy. Moreover, diabetes mellitus is considered an important risk factor for primary open angle glaucoma (POAG).\(^11\) This considerable issue still has not been investigated clinically, but we have numerous opportunities to evaluate the reliability and vulnerability of obtained results to more accurately discriminate glaucoma in persons with and without diabetes. The purpose of this study was to determine the impact of mild to moderate non-proliferative diabetic retinopathy (NPDR) on quantitative RNFL measurements and glaucoma screening with GDx-VCC and StratusOCT.

SUBJECTS AND METHODS

In this cross-sectional study, we enrolled 170 subjects older than 50 years of age. One eye per participant was randomly selected for inclusion. All subjects were examined at the outpatient clinic of the Senshokai Eye Institute in Kyoto, Japan. Of the 170 eyes, 45 were healthy (defined by intraocular pressure [IOP] <22 mm Hg, no history of diabetes or elevated IOP, a healthy optic disc, and no repeatable abnormal visual field results); 47 subjects were receiving medical treatment for POAG without diabetes mellitus, and 78 subjects (40 without POAG and 38 with POAG) had diabetes mellitus diagnosed on the basis of the diabetes diagnostic criteria of the World Health Organization and were under medical treatment by an experienced endocrinologist.

All GDx-VCC and StratusOCT scans were obtained between September 28, 2005, and March 26, 2007. Informed consent was obtained from all subjects in accordance with the tenets of the Declaration of Helsinki. The institutional ethics committee approved the methodology. All subjects underwent comprehensive ophthalmologic examination, including best-corrected visual acuity (BCVA), applanation tonometry, slit-lamp examination, dilated funduscopy, 45° high-quality fundus color photography (CF-PU2; Canon Inc., Tokyo, Japan) for evaluation of cup and disc size, and automated perimetry using the full-threshold G1 program (Octopus 301, version 2.04; Haag-Streit, Schlieren, Switzerland) within 3 months of the GDx-VCC

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and StratusOCT scans. IOP measurements were repeated on at least three consecutive visits with the use of Goldmann applanation tonometry. Two experienced graders measured each fundus color photograph independently and were masked to the test results of the other. Graders visually estimated the horizontal and vertical cup-to-disc ratios based on the contour of the cup. The mean value of the two graders was recorded as the final grade.

All subjects had a logMAR BCVA of 0.1 or better, spherical equivalent refractive error within ±5.00 diopters, astigmatism within ±3.00 diopters, and clear media without clinically relevant cataract.

We excluded eyes with coexisting neuro-ophthalmologic disease, uveitis, macular disease (including macular degeneration and macular edema), retinal artery or vein occlusion, retinal detachment, history of refractive or intraocular surgery, and degenerative myopia that could lead to less reliable assessment of RNFL thickness and poor quality of obtained images.

Glaucomaticous optic disc changes were defined as undermining of the cup, notching, and focal or diffuse thinning of the rim area; splinter hemorrhages; nasal shifting of the retinal vessels; and asymmetric enlargement of the cup (cup-to-disc asymmetries > 0.2). A glaucomatous visual field defect using the field program (Octopus G1; Haag-Streit) was defined as three consecutive point depressions exceeding 5 dB more than the age-matched controls and at least one of three consecutive points with a depression greater than 10 dB or two consecutive points depressed greater than 10 dB and two adjacent points across the nasal horizontal meridian with a difference of greater than 5 dB. Visual field defects had to be repeatable on at least two consecutive tests. Glaucomaticous eyes with visual field defects on fundus photography caused by retinal hemorrhages or exudates were excluded from the study. Glaucomaticous visual field defects were evaluated by the Hodapp-Parrish-Anderson grading scale of severity of visual field defects, and each enrolled subject was classified as having glaucomatous visual field defect using the field program (Octopus G1; Haag-Streit).

Enrolled subjects with diabetes had mild to moderate NPDR based on the International Clinical Diabetic Retinopathy Disease Severity Scale. Fluorescein angiography (TRC-50X; Topcon Instrument Corp., Tokyo, Japan) was performed in subjects with mild to moderate NPDR or maculopathy to classify the stages of retinopathy and to exclude subjects with diabetic macular edema. Two trained ophthalmologists determined, in a masked fashion, the classifications of diabetic retinopathy. Of 40 eyes of subjects with diabetes, 22 eyes were classified as having mild disease and 19 as having moderate disease. Of 38 eyes with glaucoma and coexisting diabetes, 23 were classified as having mild disease and 15 as having moderate disease.

Enrolled subjects with diabetes had mild to moderate NPDR based on the International Clinical Diabetic Retinopathy Disease Severity Scale. Of 40 eyes with simple diabetes, 22 eyes were classified with mild disease and 19 as having moderate disease. Of 38 eyes with glaucoma and with diabetes, 22 eyes were classified as having mild disease and 19 as having moderate disease.

To evaluate the quality of the GDx-VCC and StratusOCT images, two experienced graders measured each fundus color photograph independently and were masked to each other’s conclusions. Contradictory cases were classified after further discussion. Poor images from 36 subjects were considered unacceptable and were excluded from this study.

**Instrumentation**

To evaluate the quality of the GDx-VCC and StratusOCT images, two trained ophthalmologists who were masked to each other’s conclusions and to other ophthalmologic results judged them independently. Contradictory cases were classified after further discussion. Poor images from 36 subjects were considered unacceptable and were excluded from this study.

**SLP (GDx-VCC).** SLP (GDx-VCC, software version 5.5.0) is an imaging method that measures the slowing of reflected light caused by the birefringence of the RNFL. Full details regarding GDx-VCC have been reported.

Briefly, GDx-VCC assesses RNFL thickness in the peripapillary retina by measuring RNFL birefringence with a near-infrared diode laser that detects the amount of phase shift (retardation)
between incoming and outgoing polarized light that strikes the parallel birefringent microtubules of the axon. GDx-VCC automatically compensates for corneal birefringence, as described by Zhou and Weinreb.\(^5\) GDx parameters studied were the temporal-superior-nasal-inferior-temporal ellipse (TSNIT) average, superior average (25°-144°), inferior average (215°-334°), and nerve fiber indicator (NFI). NFI is calculated using a support vector machine algorithm based on several RNFL measures, and a number from 0 to 100 is assigned to each eye. The reproducibility of the GDx-VCC data at our institution has been reported elsewhere.\(^13\)

In the present study, because the quality of the GDx-VCC images was considered especially important and retinal hemorrhages, exudates, or subclinical macular edema might have affected quantitative data,\(^16\) only high-quality images were included. A baseline image composed of two or three good-quality individual scans was created using GDx-VCC software. A high-quality image was defined as a well-focused and uniformly illuminated reflectance image with a centered optic disc that had minimal residual anterior segment retardation without an atypical retardation pattern. To assess the corneal compensation linked to the birefringence of the Henle fiber layer and lens, the corneal polarization axis and magnitude and macular birefringent images were evaluated in all cases. According to our criteria, the mean image also had to have residual anterior segment retardation less than 13 nm (corresponding to an image quality score of 7) so that a more accurate measurement could be obtained.

**StratusOCT.** StratusOCT (version 4.0.1) measures RNFL thickness with a low-coherence light source projected onto the retina. This reflected light source of the measurement beam is compared with the reflectance of a reference beam reflected from a reference mirror at a known position to determine retinal thickness. An edge-detection algorithm is used to define the posterior border of the RNFL (the anterior border is defined by the large difference in reflectance along the vitreoretinal interface). Details of this technique have been described.\(^19\) Only well-focused and centered scans with a signal strength of 7 or greater were included and subjectively evaluated to confirm that the RNFL detection algorithm closely followed the anterior and posterior RNFL borders. Eyes with artifactual errors and remarkable regression of echo beam caused by hard or soft exudates and retinal hemorrhages in the scan circle were excluded.

RNFL thickness measurements were obtained for 256 circumpapillary points of 1.406\(^\circ\) using a 3.4-mm scan diameter. The landmark option was used to facilitate placement of the scan circle at the same location on repeated scans. Parameters evaluated were global average thickness (360°), superior quadrant thickness (46°-135°), and inferior quadrant thickness (226°-315°).

### Statistical Analyses

Statistical analyses were performed using the Statistical Package for Social Sciences II software (SPSS Inc., Tokyo, Japan). \(P < 0.05\) was considered significant. We compared all investigated parameters from the GDx-VCC and StratusOCT using ANOVA with the post hoc Tukey honest significant difference (HSD) pairwise comparisons. The receiver operating characteristic (ROC) curve was used to describe the ability of each parameter to discriminate between glaucomatous and healthy or diabetic eyes. A perfect test would have an area under the curve (AUC) of 1; a test without diagnostic value would have an AUC of 0.5. Sensitivity and specificity for detecting glaucomatous eyes were determined by obtaining the highest sensitivity values, with the target specificities set at greater than 90% and greater than 80%.

### RESULTS

Table 1 shows the demographic data from the 170 enrolled subjects. Age and refractive error that might have affected RNFL thickness\(^17,18\) did not differ significantly among the groups.

Comparisons of the mean GDx-VCC and StratusOCT measurements among the study groups are shown in Table 2. All the GDx-VCC and StratusOCT parameters were significantly different (ANOVA; \(P < 0.001\)) among the study groups. For GDx parameters, significant differences (Tukey HSD, \(P < 0.001\)) were found between any combination of groups and between glaucomatous eyes with and without diabetes. For OCT parameters, there were no significant differences (Tukey HSD, \(P > 0.05\)) between age-matched healthy and diabetic eyes. TSNIT, superior and inferior average thicknesses, and NFI were reduced on GDx-VCC; however, all three OCT parameters were statistically similar compared with parameters in healthy subjects. AUCs from the GDx-VCC and StratusOCT are summarized in Table 3. The data show the discriminating power of these methods for mild to moderate glaucoma in eyes with or without diabetes. GDx-VCC and StratusOCT parameters with the largest AUCs were the NFI and the inferior quadrant thickness (IQT), respectively, in both groups. At a specificity of 86%, the NFI on GDx and the IQT on OCT detected 92% and 91% of glaucomatous eyes without diabetes, respectively. At a specificity of 92%, 80% and 78% of glaucomatous eyes could be

### Table 2. Retinal Nerve Fiber Measurements by GDx-VCC and StratusOCT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy(^a) (n = 45)</th>
<th>Glaucoma(^a) (n = 47)</th>
<th>Glaucoma with Diabetes(^a) (n = 40)</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDx-VCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSNIT average (μm)</td>
<td>59.5 ± 4.6</td>
<td>50.3 ± 5.3</td>
<td>54.0 ± 4.3</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Superior average (μm)</td>
<td>72.2 ± 7.2</td>
<td>58.4 ± 8.5</td>
<td>64.2 ± 7.3</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Inferior average (μm)</td>
<td>71.3 ± 7.3</td>
<td>53.9 ± 7.9</td>
<td>60.2 ± 7.2</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>NFI</td>
<td>15.2 ± 6.0</td>
<td>41.2 ± 11.2</td>
<td>24.6 ± 7.3</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>StratusOCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (μm)</td>
<td>98.4 ± 7.8</td>
<td>82.2 ± 12.1</td>
<td>99.6 ± 9.6</td>
<td>&lt;0.0001‡</td>
</tr>
<tr>
<td>Superior quadrant (μm)</td>
<td>120.1 ± 11.2</td>
<td>99.4 ± 25.2</td>
<td>122.0 ± 14.2</td>
<td>&lt;0.0001‡</td>
</tr>
<tr>
<td>Inferior quadrant (μm)</td>
<td>127.2 ± 12.5</td>
<td>90.8 ± 20.1</td>
<td>126.4 ± 15.1</td>
<td>&lt;0.0001‡</td>
</tr>
</tbody>
</table>

\(\text{TSNIT, temporal-superior-nasal-inferior-temporal;}\)

\(\text{NFI, nerve fiber indicator;}\)

\(\text{RNFL, retinal nerve fiber layer;}\)

\(\text{OCT, optical coherence tomography;}\)

\(\text{GDx-VCC, GDx access with variable corneal compensator;}\)

\(\text{ANOVA, analysis of variance with Turkey-Kramer post-hoc test.} \)

\(\text{\(^a\) Data are mean ± SD.} \)

\(\text{†Significant differences (} P < 0.01\text{) are found between all combinations but between glaucoma with and without diabetes.} \)

\(\text{‡There are no significant differences between age-matched healthy and diabetes, and between glaucoma with and without diabetes.} \)

\(\text{Significant differences are found between other combinations (} P < 0.05\text{).} \)

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TABLE 3. Sensitivities and Specificities for the Parameters with Areas under the ROC Curve

<table>
<thead>
<tr>
<th>RNFL Thickness</th>
<th>Healthy (n = 38)</th>
<th>GDx-VCC</th>
<th>StratusOCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior average (μm)</td>
<td>82.0</td>
<td>76.0</td>
<td>76.0</td>
</tr>
<tr>
<td>Inferior average (μm)</td>
<td>82.0</td>
<td>76.0</td>
<td>76.0</td>
</tr>
<tr>
<td>NFI (um)</td>
<td>93.0</td>
<td>93.0</td>
<td>93.0</td>
</tr>
<tr>
<td>Average (μm)</td>
<td>82.0</td>
<td>76.0</td>
<td>76.0</td>
</tr>
<tr>
<td>Superior quadrant (μm)</td>
<td>82.0</td>
<td>76.0</td>
<td>76.0</td>
</tr>
<tr>
<td>Inferior quadrant (μm)</td>
<td>82.0</td>
<td>76.0</td>
<td>76.0</td>
</tr>
</tbody>
</table>

ROC, receiver operating characteristic; RNFL, retinal nerve fiber layer; AUC, area under the ROC curve; GDx-VCC, GDx access with variable corneal compensator; OCT, optical coherence tomography; G, glaucoma; GD, glaucoma with diabetes; TSNIT, temporal-superior-nasal-inferior-temporal; NFI, nerve fiber indicator.

DISCUSSION

GDx-VCC and StratusOCT are quantitative imaging devices with a highly discriminating power for glaucoma in otherwise healthy subjects, however, the basic principles for measuring RNFL differ between the two imaging methods. The present study suggests that this difference in principle and the presence of diabetic retinopathy might affect quantitative RNFL measurements of the two devices during glaucoma screening.

Defects in or reductions of RNFL in diabetic eyes without glaucoma have been reported from an early stage of vasculopathy. The present study supports the previously reported evidence using GDx-VCC but could not confirm the previously reported reductions of RNFL with OCT.

In the present study, RNFL measurements by GDx-VCC, which analyzes retardation of only the retinal nerve fibers after corneal compensation of the retardation from the macula, corresponded to the histopathologic evidence in eyes with diabetes. Intracellular or extracellular edema, hemorrhages, fibrin reaction, or gliosis surrounding the optic nerve head might affect the parallel structure of the microtubules and their birefringent property. Meanwhile, GDx-VCC algorithms might have selectively identified RNFL birefringence arising from microtubules within the individual nerve fibers not affected by retinal thickening in which intraretinal fluid accumulation or exudates occur. A previous experimental report describes a certain relationship between actual RNFL thickness and a change in retardation under normal conditions, but the birefringence change might not have correlated with a thickness change under retinal morphologic changes.

In contrast, the RNFL thickness measured by StratusOCT, which includes not only ganglion cell axons but also Müller cell processes and astrocytes, did not show a significant reduction in subjects with diabetes compared with age-matched healthy subjects. The neural retina, including the RNFL, may be substantially thickened by intraretinal fluid accumulation, exudates, and hemorrhages from leaking blood vessels in eyes with mild to moderate NPDR. This prospect might explain the significantly reduced RNFL thickness on a previous OCT report, in which only diabetic eyes without retinopathy were analyzed. It may also contribute to changes in reflectance or contrast characteristics in the reconstructed b-scan of OCT and age-related retinal nerve fiber loss only between diabetic eyes and diabetic eyes with glaucoma.

Regarding the ability to detect glaucoma in otherwise healthy or diabetic eyes, both GDx-VCC and StratusOCT provided excellent results in the present study (Table 3; Fig. 1). In the death of the RGCs occurs early in diabetic eyes. Other cells (RGCs) are enhanced in the sensory retina in diabetes, and found. The apoptosis-promoting factors in retinal ganglion cells (RGCs) are enhanced in the sensory retina in diabetes, and the death of the RGCs occurs early in diabetic eyes. Other morphologic studies using TUNEL (terminal dUTP nick end labeling) staining have reported that enhanced apoptosis of neuroglial elements may affect the early onset of diabetes-associated RNFL loss.
the presence of moderate diabetic retinopathy, sensitivity and specificity ranged from 74% and 83% to 80% and 92% by GDx-VCC and 75% and 82% to 78% and 92% by OCT, respectively. Compared with previous reports, the high AUCs (NFI on GDx-VCC, 0.912; IQT by OCT, 0.902) in eyes with glaucoma and coexisting diabetes in the present study may be attributed to the moderate severity of glaucomatous optic neuropathy (GON) in the study subjects. Another factor is that diabetes-associated RNFL loss may enhance the cumulative damage of GON. Considering a significant optic nerve loss induced by GON, intraretinal architectural changes may have little impact on the detection of glaucoma in diabetic eyes without evident maculopathy. Although visual field losses in eyes with glaucoma and coexisting diabetic retinopathy often have amorphous patterns that are affected by retinal hemorrhage, exudates, and diabetes-associated retinal nerve fiber impairment, subjective optic nerve head assessment in clinical practice, combined with supplemental quantitative RNFL measurements, can have higher diagnostic value in glaucoma screening.

One limitation of our study is related to the inclusion of subjects with moderate GON, which likely increased the difference in the RNFL measurements between subjects with glaucoma and subjects with or without diabetes. In the presence of moderate glaucomatous optic neuron damage, retinal nerve fiber loss analyzed by GDx-VCC and StratusOCT might have been evident despite the presence of diabetic intraretinal edema, exudates, or hemorrhages. However, in subjects with very early glaucoma or in glaucoma suspects, the discriminating power of OCT might have been decreased because of thicker RNFL measurements affected by increased vascular permeability and changes in blood flow in diabetic retinopathy. Moreover, the small sample size in each subgroup and the interplay between the subdivisions of glaucoma and diabetic retinopathy might have affected our results. Therefore, additional larger studies are necessary to support our results and to provide supplementary data.

Another limitation of the present study was that we investigated only clinical scales of RNFL measurements by GDx-VCC and StratusOCT. Regarding GDx images, excluding subjects with incomplete compensation of the anterior segment birefringence, attributable to macular disease, might not have avoided all errors in quantitative RNFL thickness measurements. Meanwhile, RNFL measurements using StratusOCT might have been affected by potentially unexpected artifacts, errors of boundary detection and thickness measurement by OCT algorithms, and corneal dryness, even after experts determined that the image quality was substantially adequate. A histopathology study should be conducted to determine the actual architectural morphology of the RNFL in diabetic eyes with or without glaucoma.

In summary, GDx-VCC and StratusOCT can effectively differentiate mild to moderate glaucoma in eyes without diabetes or in eyes with mild to moderate diabetic retinopathy. Quan-
tative dissociation of RNFL thickness measurements was found between GDx-VCC and StratusOCT in subjects with simple diabetes. Importantly, investigators should recognize that the RNFL thickness reported by each instrument may demonstrate changes of optical properties, not actual thickness.

References