Reproducibility of Peripapillary Retinal Nerve Fiber Layer Thickness and Optic Nerve Head Parameters Measured with Cirrus HD-OCT in Glaucomatous Eyes

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Purpose. To assess the reproducibility of peripapillary retinal nerve fiber layer (RNFL) thickness and optic nerve head (ONH) parameters measured with Cirrus HD-OCT in glaucomatous eyes.

Methods. Fifty-five glaucomatous eyes were included in the study. The optic disc cube 200 × 200 protocol was used to obtain three scans during the same visit to evaluate the intravisit reproducibility. One scan on 4 additional days within a 2-month period of the first session was obtained to assess intervisit reproducibility. Intraclass correlation coefficient (ICC), coefficient of variation (CV), and test-retest SD (TRT SD) were calculated for each RNFL and ONH parameter. The formula $1.645 \times \sqrt{2} \times$ intervisit TRT SD provides an upper tolerance limit to variability beyond which nonphysiologic change should be considered.

Results. All ICCs were excellent, ranging from 83.9% to 99.2% for intravisit measurements and from 80.8% to 99.1% for intervisit measurements. Cup/disc area ratio had the lowest CV (1.1%) in either type of measurement, followed by average RNFL thickness (1.9% and 2.7%). Nasal clock hours and quadrants showed the poorest reproducibility as did the clock hour directly temporally. The intervisit tolerance limit for average RNFL thickness was 3.89 μm.

Conclusions. Intravisit and intervisit measurements of peripapillary RNFL thickness and ONH parameters with Cirrus HD-OCT showed excellent reproducibility, indicating that this instrument may be useful in monitoring glaucoma progression. When comparing two measurements from the same eye on two different visits, a reproducible decrease in average RNFL thickness of approximately 4 μm or more may be considered a statistically significant change from baseline. (*Invest Ophthalmol Vis Sci.* 2010;51:5724–5730) DOI:10.1167/iovs.10-5222

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Supported by National Institutes of Health Grant P30 EY014801, an unrestricted grant from the Research to Prevent Blindness, and unrestricted research support from Carl Zeiss Meditec.

Submitted for publication January 17, 2010; revised May 14, 2010; accepted June 10, 2010.

Disclosure: **J.-C. Mwanza**, Carl Zeiss Meditec (F); **R.T. Chang**, Carl Zeiss Meditec (F); **D.L. Budenz**, Carl Zeiss Meditec (F); **M.K. Durbin**, Carl Zeiss Meditec (F, E); **M.G. Gendy**, Carl Zeiss Meditec (F); **W. Shi**, Carl Zeiss Meditec (F); **W.J. Feuer**, Carl Zeiss Meditec (F)

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Progressive death of retinal ganglion cells and their axons is the hallmark of glaucomatous optic neuropathy. There is evidence that these structural changes precede visual field (VF) deficits as measured by standard automated perimetry.[1] The ability to detect subtle changes in the retinal nerve fiber layer (RNFL) and optic nerve head (ONH) over time is critical in the management of glaucoma. Until very recently, glaucomatous structural changes have been assessed through ophthalmoscopic examination and fundus photography, but small changes are difficult to detect with these techniques and their interpretation is entirely subjective. To provide more accurate, quantitative, and reproducible methods of detecting and following glaucoma-related structural changes, new computerized imaging methods of assessing the RNFL have been developed. Optical coherence tomography (OCT) is a noncontact, noninvasive imaging technique that exploits the property of coherence interferometry and optical back-scattered light to obtain in vivo, high-resolution, cross-sectional images of microstructure in biological tissues. Until recently, retinal and ONH measurements were acquired with time-domain (TD) OCT (Stratus OCT; Carl Zeiss Meditec, Inc., Dublin, CA). The reproducibility of RNFL thickness with TD-OCT has been extensively investigated. Only a few reports are available on the reproducibility of TD-OCT ONH measurements, and they have yielded variable results.[2–6]

Several OCT devices that use spectral domain (SD) technology, one of which is Cirrus HD-OCT (Carl Zeiss Meditec), are commercially available and in their early stage of clinical use. Because both early diagnosis of glaucoma and detection of its progression through subtle changes in RNFL and ONH measurements may be useful for glaucoma diagnosis and management, reproducibility of such measurements must be demonstrated before this technique can be used successfully. Several studies have examined the reproducibility of peripapillary RNFL thickness measurements with different SD-OCT systems.[7–12] Only one study has reported on the repeatability of ONH measurements obtained with SD-OCT (RTVue; Optovue, Inc., Freemont, CA).[8] The purpose of the present study was to assess the intravisit and intervisit reproducibility of peripapillary RNFL thickness and ONH parameters measured with Cirrus HD-OCT in glaucomatous eyes.

Subjects and Methods

Subjects

This study received approval from the Human Subjects Research Office of the Institutional Review Board of the Miller School of Medicine, University of Miami, complied with the Health Insurance Portability and Accountability Act (HIPAA), and adhered to the tenets of the Declaration of Helsinki. Written informed consent and HIPAA consent were obtained from all subjects. Glaucoma subjects enrolled in this

study were recruited from outpatients seeking care at the Glaucoma Service of the Anne Bates Leach Eye Hospital, Bascom Palmer Eye Institute, at the Miller School of Medicine, University of Miami. All subjects underwent recent complete ophthalmologic examination, including determination of best-corrected visual acuity, slit lamp biomicroscopy, intraocular pressure (IOP) measurement, dilated fundus examination, and VF quantification with the Humphrey Visual Field (HVF) Analyzer (Carl Zeiss Meditec) using the Swedish Interactive Threshold Algorithm (SITA) Standard 24–2 program.

Subjects were included if they had any type of glaucoma with controlled IOP and ONH abnormalities with VF deficits typical for glaucoma. They were required to have undergone at least two reliable SITA Standard 24–2 HVF examinations, with the most recent test within 12 months of enrollment date. A VF was considered glaucomatous if the glaucoma hemifield test result was outside normal limits, the pattern SD had $P < 5\%$ or a cluster of three or more points in the pattern deviation plot in a single hemifield (superior or inferior) with $P < 5\%$, one or more of which had a $P < 1\%$.[13] Subjects were classified as having mild, moderate, or severe glaucoma based on the Hodapp-Anderson-Parrish criteria.[14] Other inclusion criteria included age 18 years or older, best-corrected VA ≥20/40, refractive error <5 diopters of sphere or 3 diopters of cylinder, clear media, no history of retinal disease (e.g., diabetic retinopathy, macular degeneration, or retinal detachment), optic nerve abnormalities (e.g., drusen, tilted disc), nonglaucomatous optic neuropathy, or ocular surgery within 1 month of enrollment date. Only one eye randomly chosen for each participant was included in the study.

Optical Coherence Tomography Imaging

All scans were acquired by the same operator with the same Cirrus HD-OCT (software version 3.0.0.64) device using the optic disc cube 200 × 200 protocol in eyes dilated with tropicamide 1% and phenylephrine 2.5%. The optic disc cube 200 × 200 protocol is designed to position the cube scan on the ONH and to be used primarily for glaucoma analysis. After properly seating and aligning the subject, the iris was brought into view using the mouse-driven alignment system, and the line scanning ophthalmoscopic image was focused. The ONH was then centered on the live image before the centering, and enhancement was optimized. After the scanning process was launched, the instrument's 840-nm wavelength laser beam generated a cube of data measuring 6 × 6 mm after scanning a series of 200 B-scans with 200 A-scans per B-scan (40,000 points).

Three scans were acquired in each eye 5 to 10 seconds apart on the first visit to assess intravisit variability, and one scan was acquired on four subsequent visits within 2 months of the initial session to provide measurements for intervisit variability. The first scan from the initial session was used for intervisit calculations. Thus, a total of five scans obtained on five different days were used to calculate intervisit variability. Only scans with signal strength ≥6 and without eye movements or blinking artifacts within 1.73-mm radius around the ONH were used for analysis. All en face images were checked for quality control. No cases of algorithm failure were detected. The Cirrus HD-OCT automated built-in algorithms find the center of the optic disc and extract a B-scan in the shape of a circle of 3.46-mm diameter using the center of the optic disc as the center of the circle. This simulates the scan circle previously obtained using Stratus TD-OCT. The anterior and posterior boundaries of the RNFL are delineated, and the thickness is determined on each 200 × 200 A-scan. The system calculates the RNFL thickness at each point on the circle and reports the measurements for overall average, quadrants, and clock-hours on the printout. With regard to ONH parameters, the algorithm identifies the termination of Bruch's membrane as the disc edge (Figs. 1A, 1B). The rim width around the entire circumference of the optic disc is then determined by measuring the thickness of the neuroretinal tissue in the optic nerve as it turns to exit through the opening in Bruch's membrane. Measured within three-dimensional volume, this constitutes a single area measure. With this method, measurements remain unaffected despite changes if the same disc is viewed from a different angle caused by entering the pupil at a different location. Additionally, the disc and rim area measurements correspond to the anatomy as would be viewed along the axis of the nerve exit. In contrast, when the ONH exit is excessively oblique or in extremely tilted discs, areas determined from ophthalmoscopic examination, photographs, or other imaging techniques will be foreshortened, difficult to quantify, or erroneously quantified. Measuring the neuroretinal rim area in the plane of the ONH addresses the foreshortening and ties the results to the anatomy. The following ONH parameters (Fig. 1C) were analyzed: disc area, rim area, vertical rim thickness (VRT; total rim thickness, in microns, measured in the vertical meridian and corresponding to the summation of the rim width at the superior and inferior positions), horizontal rim

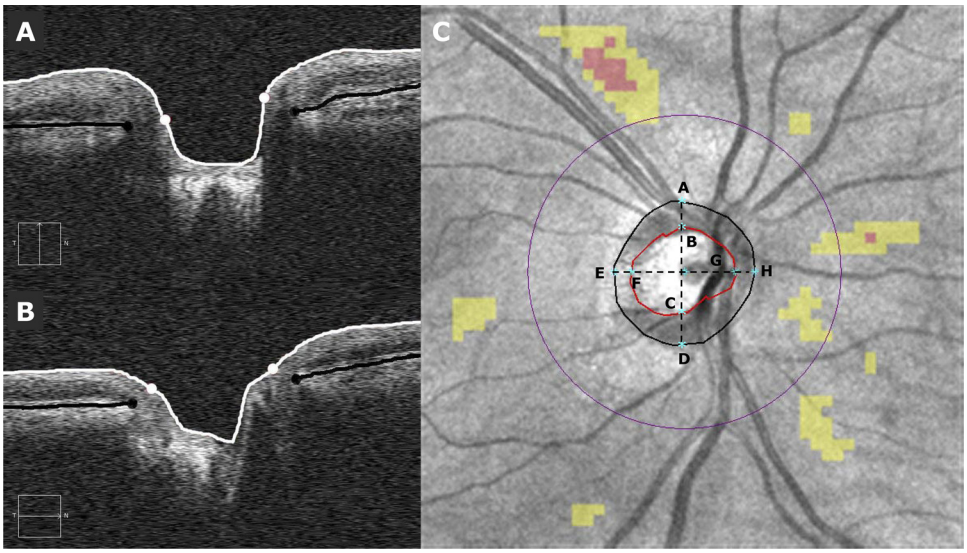

FIGURE 1. Vertical (**A**) and horizontal (**B**) tomograms of the same eye showing the end of the Bruch's membrane (*black dots*) as determined by the Cirrus HD-OCT ONH analysis software. The end of the Bruch's membrane corresponds to the disc margin, whereas *white dots* represent reference points for the cup margin. The distance between the *black dots* and the *white dots* represents the rim width or precisely VRT (**A**) and HRT (**B**). En face image (**C**) showing the disc margin (*outer black*) and the cup margin (*inner red*) as detected and drawn by with Cirrus HD-OCT 5.0 software. The region between disc and cup margins represents the neuroretinal rim area (mm^2); the region inside the cup margin corresponds to the cup area (mm^2); the disc area (mm^2) is the rim area the plus the cup area. The CDR is given by the square root of the ratio of the area of the cup to the area of the disc. The VCDR is the ratio of the *vertical line* through the cup center to the same *vertical line* extending to the disc margin: BC/(AB+BC+CD) or BC/AD. The HCDR is the ratio of the *horizontal line* through the cup center to the same line extending to the disc margin: FG/(EF+FG+GH) or FG/EH. VRT (μm) is the total rim thickness measured in the vertical meridians: AB-BC or simply AB+CD. HRT (μm) is the total rim thickness measured in the horizontal meridian: EH-FG or simply EF+GH.

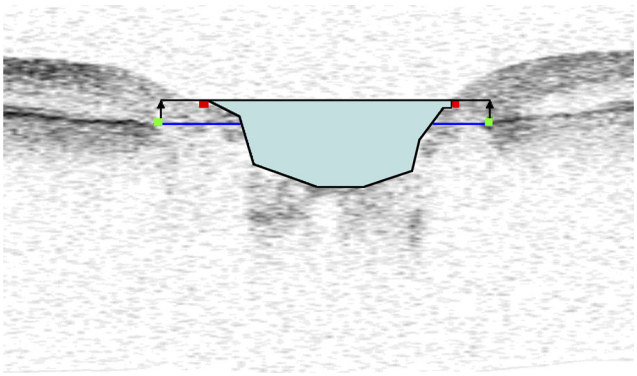

FIGURE 2. Tomogram of an optic disc showing the cup volume (*light blue shaded area*, mm^3) as determined and calculated by Cirrus HD-OCT 5.0 software. *Red spots* indicate reference points to cup margin; *green spots* represent the end of the Bruch's membrane. The *blue horizontal line* is the RPE/Bruch's membrane plane or simply the optic disc plane. The *black horizontal line* is located 200 μm above the disc plane. The cup volume is a three-dimensional measurement defined as the volume between a plane at a fixed offset located 200 μm from the plane of the optic disc and the vitreoretinal interface/inner limiting membrane.

thickness (HRT; total rim thickness measured in the horizontal meridian and representing the summation of the rim width at the nasal and temporal positions), cup-to-disc area ratio (CDR; ratio of cup area to disc area), vertical cup-to-disc ratio (VCDR; ratio of vertical line through the cup center to the same vertical line extending to the disc margin), horizontal cup-to disc ratio (HCDR; ratio of the horizontal line through the cup center to the same line extending to the disc margin), and cup volume (Fig. 2). These RNFL and ONH parameters were automatically generated by a Carl Zeiss Meditec analysis algorithm recently developed for Cirrus HD-OCT (version 5.0), which does not involve user interaction.

Statistical Analysis

For each parameter, the total variability of all measurements was partitioned into variance components because of differences between subjects, differences between days within subjects, and differences within repeated measurements made on a single day. Analyses were performed with statistical software (version 17.0; SPSS Inc., Chicago, IL) using the MinQUE method. Reproducibility was assessed by calculating the intraclass correlation coefficient (ICC), coefficient of variation (CV) and the pooled within-subject test-retest SD (TRT SD) for intravisit and intervisit measurements. The ICC is a statistic that summarizes the reproducibility of a measurement process for a given group of subjects. It is based on variance components analysis and expresses the variance attributed to real differences between subjects as a fraction of the total variation, which also includes sources of measurement variability. The ICC is small when the within-person variation caused, for example, by measurement fluctuations occurring within the same visit or between visits is large compared to the real between-person differences an instrument is designed to detect. A large ICC indicates small fluctuations among repeat measurements on the same subjects. The maximum value of the ICC is 1 (or 100%), whereas its minimum value is theoretically 0.[15] The within-visit CV was calculated by dividing the square root of the within-visit variance component by the average of the measurements and was expressed as a percentage. The numerator of the between-visit CV, the intervisit TRT SD, was calculated as the square root of the summed within- and between-visit variance components. A measurement with a CV <10% was considered as having a good reproducibility. To assess the ability of each parameter to detect differences between stages of glaucoma from mild to moderate glaucoma and from moderate to severe glaucoma, the numerators of the between-visit CVs were expressed as percentages of the difference between each pair of glaucoma group means. As a measure of test-retest variability that represents the expected amount of long-term variability in measurements in most (95%) glaucoma subjects and concerning ourselves only with, for example, a decrease in RNFL thickness or an increase in cup volume (one-tailed test), we applied the formula $1.645 \times \sqrt{2} \times Sw$, where Sw is the square root of the intervisit TRT SD, as previously described.[16]

RESULTS

Demographic Parameters

A total of 81 subjects with stable glaucoma were enrolled in the study based on the inclusion criteria. Of these, 26 were excluded: 23 for not completing the required five visits, two for signal strength <6, and one because of the development of postoperative endophthalmitis during the course of the study. Thus, the analyses presented in this study are based on data from 55 subjects ranging in age from 46 to 87 years (mean, 70.7 ± 11.1 years)—26 with mild, 11 with moderate, and 18 with severe glaucoma. Other demographic and clinical characteristics of the included subjects are shown in Table 1.

Reproducibility of RNFL Thickness and ONH Parameters

Mean values of RNFL and ONH parameters, ICCs, CVs, and TRT SD for intravisit and intervisit measurements as well as intervisit tolerance limits for changes are displayed in Table 2. Intravisit and intervisit ICCs were excellent for all RNFL pa-

TABLE 1. Demographic and Clinical Characteristics of Participants

	All Glaucoma (n = 55)	Early Glaucoma (n = 26)	Moderate Glaucoma (n = 11)	Severe Glaucoma (n = 18)
Male	26	12	3	11
Female	29	14	8	7
Age, y (SD)	70.7 (11.1)	70.5 (10.8)	70.5 (15.6)	71.2 (8.6)
POAG	46	22	7	17
PXF	3	2	1	—
NTG	3	1	2	—
CACG	2	1	—	1
Uveitic glaucoma	1	—	1	—
IOP, mmHg (SD)	13.7 (5.0)	13.9 (4.3)	15.0 (3.7)	12.6 (2.3)
VF MD, dB (SD)	−9.1 (7.3)	−2.9 (1.5)	−8.8 (1.2)	−18.3 (4.5)

POAG, primary open-angle glaucoma; PXF, pseudoexfoliation glaucoma; NTG, normal tension glaucoma; CACG, chronic angle-closure glaucoma; VF MD, visual field mean deviation.

TABLE 2. Intravisit and Intervisit Means and ICC, CV, and TRT SD for RNFL and ONH Parameters

		Intravisit			Intervisit			
Parameter	**Mean**	**ICC* (%)**	**CV (%)**	**TRT**	**ICC* (%)**	**CV (%)**	**TRT**	**Tolerance Limit**
Average RNFL	62.83	98.6 (96.7)	1.9	1.18	97.2 (93.4)	2.7	1.67	3.89
TP quadrant	47.05	96.1 (91.2)	4.6	2.16	92.9 (84.3)	6.3	2.97	6.91
SP quadrant	75.67	97.9 (95.1)	3.2	2.40	96.7 (92.4)	4.0	3.02	7.03
NS quadrant	58.92	87.9 (74.7)	4.8	2.84	82.8 (65.6)	5.9	3.49	8.13
IF quadrant	69.73	97.7 (96.4)	3.7	2.55	97.2 (93.5)	4.0	2.82	6.56
Clock-hour 9R	52.84	88.2 (75.2)	6.0	3.18	81.8 (64.1)	7.8	4.11	9.57
Clock-hour 10R	67.19	89.8 (78.3)	5.5	3.72	85.2 (69.7)	6.9	4.61	10.73
Clock-hour 11R	76.78	95.3 (89.5)	5.6	4.31	94.4 (87.2)	6.2	4.77	11.09
Clock-hour 12R	76.15	96.8 (92.8)	4.6	3.51	92.9 (84.2)	7.1	5.39	12.55
Clock-hour 1R	74.10	97.3 (93.7)	5.6	4.14	97.3 (93.6)	5.6	4.14	9.63
Clock-hour 2R	52.14	98.0 (95.4)	4.3	2.25	97.7 (94.6)	4.7	2.43	5.66
Clock-hour 3R	43.47	85.6 (70.5)	10.0	4.36	83.7 (66.9)	10.8	4.70	10.92
Clock-hour 4R	45.53	96.2 (91.4)	4.9	2.22	89.0 (76.9)	8.6	3.93	9.15
Clock-hour 5R	64.19	98.5 (96.5)	4.2	2.67	96.5 (92.0)	6.4	4.11	9.57
Clock-hour 6R	74.88	96.8 (92.6)	6.0	4.51	96.8 (92.5)	6.0	4.51	10.50
Clock-hour 7R	70.13	95.9 (90.7)	4.9	3.42	93.6 (85.7)	6.2	4.32	10.04
Clock-hour 8R	56.74	83.9 (67.5)	6.4	3.62	80.8 (62.2)	7.1	4.02	9.34
Disc area	1.90	95.1 (89.0)	4.4	0.084	95.1 (88.9)	4.4	0.084	0.195
Rim area	0.68	96.6 (92.2)	6.6	0.045	96.6 (92.1)	6.6	0.045	0.104
Cup volume	0.54	99.2 (98.1)	5.9	0.032	96.9 (92.7)	11.7	0.063	0.147
CDR	0.77	99.2 (98.1)	1.1	0.009	99.2 (98.0)	1.1	0.009	0.020
HCDR	0.76	98.3 (96.1)	2.2	0.017	97.1 (93.2)	2.9	0.022	0.051
VCDR	0.78	97.7 (94.8)	1.7	0.014	97.2 (93.4)	1.9	0.015	0.035
HRT	323.06	98.5 (96.6)	6.7	21.62	98.0 (95.3)	7.8	25.23	58.70
VRT	312.05	97.1 (93.3)	7.6	23.71	97.1 (93.2)	7.6	23.71	55.15

Intervisit TRT SD is the square root of the sum of the intervisit plus intravisit variance components. Tolerance limit is defined as the amount of change expected because of TRT variability alone in less than 5% of subjects, as calculated by the formula $1.645 \times \sqrt{2} \times$ TRT SD. TP, temporal; SP, superior; NS, nasal; IF, inferior.

* Values in parentheses are lower 95% confidence intervals.

rameters, with clock hour 4 showing the lowest values (83.9% and 80.8%) and average RNFL the highest values (98.6% and 97.2%). Among quadrants, the nasal quadrant had the lowest ICC of 88.3% and 82.6% for intravisit and intervisit measurements, respectively. ICCs were also excellent for all ONH parameters and ranged between 95.1% and 99.4% and between 97.1% and 99.2% for intravisit and intervisit measurements, respectively. CVs were all under 10%, with the exception of RNFL thickness of clock hour 3 in the right eye and 9 in the left eye for both intravisit and intervisit measurements and cup volume in intervisit measurements. Among RNFL parameters, average RNFL thickness showed the lowest intravisit (1.9%) and intervisit CV (2.7%). CDR was the ONH parameter with the lowest CV (1.1%) in either type of measurement. The intervisit TRT SD was approximately 2 μm for average RNFL thickness and ranged between 3 μm and 3.5 μm for quadrants and between 4 μm and 5 μm for clock hours, except for clock hour 2 (2.4 μm).

Table 3 displays the TRT SD of each parameter, expressed as a percentage of the difference between glaucoma group

TABLE 3. Intervisit Mean Peripapillary RNFL and ONH Parameters in Early, Moderate, and Severe Glaucoma, Mean Differences, Within-Subject TRT SD, and TRT SD as Percentage of Difference between Early and Moderate and between Moderate and Severe Glaucoma

	Mean by Stage of Glaucoma			Difference between			TRT SD as % Difference between	
Parameters	**Early**	**Moderate**	**Severe**	**Early and Moderate**	**Moderate and Severe**	**TRT SD**	**Early and Moderate**	**Moderate and Severe**
Average RNFL	67.21	61.64	57.56	5.57	4.08	1.67	30	41
TP quadrant	47.58	49.27	45.10	1.69	4.17	2.97	176	71
SP quadrant	82.78	76.82	64.84	5.96	11.98	3.02	51	25
NS quadrant	61.37	53.93	58.92	7.44	4.99	3.49	47	70
IF quadrant	77.04	66.67	61.31	10.37	5.36	2.82	27	53
Disc area	1.94	1.92	1.84	0.02	0.08	0.08	418	105
Rim area	0.74	0.71	0.58	0.03	0.13	0.04	149	34
Cup volume	0.57	0.49	0.53	0.08	0.04	0.06	79	158
CDR	0.75	0.77	0.81	0.02	0.04	0.01	43	22
HCDR	0.76	0.74	0.77	0.02	0.03	0.02	109	73
VCDR	0.75	0.79	0.83	0.04	0.04	0.02	38	38
HRT	312.78	362.33	312.93	49.55	49.4	25.23	51	51
VRT	371.16	305.69	233.00	65.47	72.69	23.71	36	33

Abbreviations as defined in Table 2.

means. Of all parameters, inferior quadrant RNFL and average RNFL thickness showed the best ability to detect differences between mild and moderate glaucoma, with TRT SD as a percentage of stage difference of 27% and 30%, respectively. CDR (22%) and superior quadrant RNFL (25%) appeared to be best for differentiating moderate from severe glaucoma, whereas VRT seemed to perform better for detecting differences throughout all glaucoma stages.

Discussion

Reproducibility of any diagnostic test is important both for diagnostic accuracy and for monitoring changes in disease status. In the case of glaucoma, reproducibility is critical if one is going to use an instrument to monitor for progression. One of the frustrations with the use of visual field testing to detect glaucoma progression is the long-term fluctuation of results.[17] Although serial optic disc photography can be used to monitor for glaucoma progression, the subjective nature of the interpretation can make it difficult to be certain that progression has occurred.[18] OCT is a relatively new technology that is being used clinically to help with the diagnosis of glaucoma and the determination of glaucoma progression, primarily through measurements of RNFL. Improvements in this technology, including SD-OCT, have made it possible to measure ONH parameters as well. Although other studies have been published on the reproducibility of RNFL thickness using SD-OCT, the uniqueness of the present study lies in the fact that it reports for the first time the repeatability and reproducibility not only of RNFL, but also of ONH parameters using the Cirrus HD-OCT 5.0 software in glaucoma patients. This new software version incorporates an algorithm for the measurement of ONH parameters, and the automatic circle placement uses the center of the new disc segmentation, making it even more reliable for RNFL measurements compared with older versions. In addition, our study design (multiple measurements over 5 days), study group (glaucomatous eyes), and parameters tested (RNFL and ONH parameters) make this study unique and adds value to the reproducibility studies on RNFL in normal eyes that have been published using SD-OCT to date. In addition, this is the first study to compare Cirrus HD-OCT RNFL and ONH reproducibility in glaucomatous eyes, which is valuable information when deciding which parameters might be most useful for monitoring glaucoma progression. As such, we believe the results are worth reporting.

The present study found excellent intravisit and intervisit reproducibility of RNFL measurements based on ICCs, CV, and TRT SD. Leung et al.[10] evaluated the repeatability and reproducibility of peripapillary RNFL measurements obtained with Cirrus HD-OCT in 97 normal and 83 glaucomatous eyes and reported similar results. However, it should be noted that their intervisit measurements were obtained on only two separate occasions. The intravisit and intervisit ICC values were all greater than 80%, and CVs were generally <10%. Comparable findings for intravisit reproducibility were also reported by Gonzalez-Garcia et al.,[8] who assessed the repeatability of RNFL with SD-OCT (RTVue; Optovue, Inc.) in 60 eyes from subjects without glaucoma and 38 eyes from subjects with glaucoma. The temporal quadrant had the lowest ICC 86% and the highest CV (4.72%), whereas average RNFL had the highest ICC (97%) and the lowest CV (1.9%). Our intravisit ICC, CV, and TRT SD values were similar to those reported by Vizzeri et al.[12] in glaucomatous eyes. In both studies, clock hours 3, 4, and 9 showed lower ICCs compared with other parameters. Garas et al.[7] used the CV to assess the intravisit reproducibility of peripapillary RNFL thickness measured with SD-OCT (RTVue; Optovue, Inc.) in 37 eyes, including 14 normal or ocular hypertensive eyes and 23 eyes with moderate to severe glaucoma. CVs for average and quadrants RNFL thickness were all <10% in eyes with moderate to severe glaucoma. A study by Kim et al.[9] on the intravisit variability of RNFL thickness measurements with Cirrus HD-OCT using two different methods of ONH centering in 14 normal eyes found an ICC of 98.4% for average RNFL thickness and ICCs <90% in half of sectoral measurements. Average RNFL thickness also performed better than all other RNFL thickness parameters. Overall, it appears that average RNFL showed the highest or one of the highest ICCs, the lowest CV, and lowest TRT SD across studies. From a practical standpoint, this suggests that average RNFL thickness rather than sectoral RNFL thickness may be the best RNFL parameter for monitoring glaucoma progression.

We also found that all measurements of ONH parameters showed excellent intravisit and intervisit reproducibility using Cirrus HD-OCT. There were no differences between intravisit and intervisit ICCs. Our intravisit results agree with those recently reported for SD-OCT (RTVue; Optovue, Inc.) by Gonzalez-Garcia et al.,[8] though their CVs were greater than 10% for cup area, rim volume, cup volume, and CDR. The reproducibility of OCT ONH measurements has been previously evaluated with TD OCT and yielded variable results.[2–6] Paunescu et al.[5] examined 10 young healthy subjects and found that the CDR and VCDR showed the best ICCs of 97% and 90%, respectively, whereas disc area and vertical integrated rim width showed the worst ICCs of 52% and 51%. However, they failed to specify whether these ICCs were for intravisit or intervisit measurements. In another study by Kamppeter et al.[2] involving 10 healthy subjects, ONH measurements showed relatively good reproducibility, with the vertical integrated rim area having the lowest CV and the cup area and disc area the highest. Olmedo et al.[4] evaluated the reproducibility of ONH parameters in 10 normal and 10 glaucomatous eyes. They observed that ICCs for normal eyes were greater than 81% for all parameters, but disc area (64.7%), rim area (33.3%), and horizontal integrated rim area (23.1%) had relatively poor reproducibility. ICCs in glaucomatous eyes ranged from 85.4% to 95.2%, except for disc area (68.1%). No significant differences in reproducibility were found between normal and glaucomatous eyes. Pueyo et al.[6] compared the reproducibility of ONH measurements in 32 healthy, 41 ocular hypertensive, and 33 glaucoma subjects. High reproducibility, with CVs <10%, was observed for disc area, cup area, CDR, horizontal CDR, and vertical CDR, whereas rim area, vertical integrated rim area, and horizontal integrated rim width were less reproducible. More recently, Lin et al.[3] found ONH measurements to be highly reproducible, with ICCs ranging between 86% and 95.9%, with the exception of disc area (73%). It should be noted that unlike Stratus OCT–based reports showing low reproducibility of disc area measurements and variable results with regard to the other parameters, both the study by Gonzalez-Garcia et al.[8] using SD-OCT (RTVue; Optovue, Inc.) and the present study found that disc area measurement is as reproducible as other ONH and RNFL parameters. Disc area is a weak risk factor for glaucoma and is not influenced by IOP level or extent of glaucomatous damage.[19] Therefore, it does not change as glaucoma progresses. Its clinical relevance in determining glaucoma progression is likely to be weak irrespective of its reproducibility. However, because rim area, CDR, VCDR, HCDR, VRT, and HRT are directly dependent on disc area, it is essential that disc area measurements be highly reproducible. Variability in disc area may indicate failure to correctly find and delineate the disc margin, as previously reported with the Stratus OCT ONH analysis algorithm.[20,21] The Stratus OCT algorithm uses a default reference plane located 150 μm above the level of the RPE to define the cup margin. The drawback to this method is that the reference plane is not

stable, so that the default reference plane may be above the "actual" reference plane in optic discs with significant cupping. Leung et al.[22] observed significant changes in ONH measurements when the reference plane was placed 55 μm above or below the default reference plane, indicating the variability of measurements if the reference plane was not stable. The ONH analysis software used for the present study made direct measurements of both the disc and the cup that depended only on the anatomy delineated rather than on a reference plane.

As can be seen in Table 3, on average some parameters measure larger differences between mild and moderate glaucoma than between moderate and severe glaucoma (for example, average, inferior, and nasal RNFL), while others (superior RNFL as well as rim area and CDR) manifest larger differences between moderate and severe glaucoma. The usefulness of a parameter for detecting progression depends on the relative size of these differences between stages compared with the TRT SD, the variability of parameter measurements occurring in the absence of true change. Table 3 expresses the TRT SD as a percentage of these between-disease-stage differences, similar to a coefficient of variation. Smaller percentages indicate a higher likelihood of ability to detect a difference. Despite its subjective nature and intraobserver and interobserver variability when assessed with ophthalmoscopy and photography, CDR is still used as an indicator for glaucoma assessment. The finding that automated CDR calculation with the Cirrus HD-OCT ONH software was highly reproducible may have favorable clinical implications and holds promise for a longitudinal study of change.

Good reproducibility and low variability are required to accurately measure glaucoma progression. A consensus has yet to be reached on the average RNFL thinning limit over which glaucoma progression should be considered. However, based on the intervisit TRT SD for average RNFL thickness found in this study (Table 2), we estimated that intervisit thinning of at least 4 μm may be considered as suspicious and may result from glaucoma progression rather than from inconsistencies in the measurements because of other factors. This was similar to the 4.86-μm change recently suggested by Leung et al. [10] but was much lower than the 8.0-μm and 11.67-μm changes in studies conducted by Budenz et al.[16] and Leung et al.,[23] respectively, using Stratus OCT. The improved reproducibility of average RNFL thickness and even quadrant and clock hour measurements of RNFL are most likely the result of the improved method for measuring the scan circle with Cirrus HD-OCT compared with Stratus TD-OCT. Stratus TD-OCT requires the operator to manually place the scan circle, whereas Cirrus HD-OCT places the scan circle automatically without operator input. Although the operator may not place the scan cube in the same location each time with the Cirrus HD-OCT, the instrument will extract data from the same scan circle each time. These findings may have applications in longitudinal monitoring of glaucoma and other conditions characterized by progressive thinning of the peripapillary RNFL.

The results of this study must be interpreted by recognizing some limitations. First, only good quality scans with signal strength $\geq$6 were included in the analyses, which might have influenced the upper limit of the variability of our measurements. Therefore, the results reported herein may only be valid in patients with good quality scans, and caution should be exercised when diagnosing glaucoma progression based on series including both good and poor quality scans. It would be interesting to know how Cirrus HD-OCT performs when poor quality scans attributed to media opacities are taken into consideration, which likely represents the scenario in daily clinical practice. However, in our own clinical practice, we disregard the numbers for any scans with signal strength $<$6. In our experience, these patients are better monitored with other technologies. Second, some participants had experience with OCT technology testing, which might have contributed to the low variability of the observed measurements. Although this seems unlikely, confirmatory studies are needed. Third, despite that fact that we made an effort to include a wide range of glaucoma severities, our data may only pertain to the population we analyzed and may not be generalizable to all clinical situations.

In conclusion, both intravisit and intervisit measurements of peripapillary RNFL and ONH parameters obtained with Cirrus HD-OCT were found to have excellent reproducibility, indicating that this instrument may be useful in the longitudinal assessment of glaucoma progression.

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