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Comparing measurements of retinal nerve fiber layer thickness obtained on scanning laser polarimetry with fixed and variable corneal compensator

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PURPOSE. *To compare retinal nerve fiber layer (RNFL) thickness measurements obtained on scanning laser polarimetry (SLP) with commercially available instruments coupled with fixed (FCC) and variable corneal compensator (VCC).*

METHODS. *Forty-two eyes of 42 patients underwent a complete ophthalmologic evaluation and achromatic automated perimetry (24-2 program, SITA standard strategy). Nineteen eyes were healthy (average mean deviation: -0.12 dB ± 2.26) and 23 glaucomatous (average mean deviation: -4.92 dB ± 6.49). All patients underwent SLP with both FCC and VCC. Adequate compensation of corneal birefringence on FCC-SLP was checked acquiring macular retardation map (MRM). RNFL thickness was evaluated considering superior and inferior maximum (SM, IM), average thickness and ellipse average (AT, EA), and superior and inferior average (SA, IA). Mean values (±SD) for each parameter measured by the two polarimeters were compared and linear regression calculated. The ability of each parameter to discriminate between normal and glaucomatous eyes was evaluated on both polarimeters calculating area under ROC curve.*

RESULTS. *A significant linear correlation for all parameters was noted (r range: 0.65-0.78). VCC produced slightly higher thickness values than FCC, both in normal and glaucomatous eyes. On both polarimeters, area under ROC curve for all parameters discriminated adequately healthy from glaucomatous eyes (range: 0.68-0.81).*

CONCLUSIONS. *In a highly comparable and selected group of normal and glaucomatous eyes, FCC-SLP and VCC-SLP showed considerable concordance in measuring peripapillary RNFL thickness, both for sectorial and global parameters. Proper corneal birefringence compensation provided separation of normal from glaucomatous eyes on both polarimeters.*
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KEY WORDS. *Glaucoma, Scanning laser polarimetry, Automated perimetry, Birefringence*

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INTRODUCTION

Glaucomatous optic neuropathy is characterized by an acquired, progressive loss of retinal ganglionary cells (RGC) that is thought to precede functional loss (1-4). In fact, up to 40% of RGC may be lost before any glaucoma-related visual field change can be detected (2). Progress in imaging technology allows clinicians to obtain in vivo, quantitative, and reliable measurements of peripapillary retinal nerve fiber layer (RNFL) thickness by means of scanning laser polarimetry (SLP). Retardation (phase shift) of a polarized laser light passing through tis-

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sue possessing the physical property of form birefringence is linearly related to thickness in a primate model (5). Recently, SLP technology has been improved since a variable corneal compensator (VCC) has been coupled with the instrument (6). With respect to the previous model, coupled with a fixed corneal compensator (FCC), SLP-VCC provides a custom compensation of corneal birefringence whose incomplete removal represents a source of spurious measurements on SLP-FCC in most eyes (6-11), so limiting the clinical significance of RNFL thickness measurements caught by that instrument (12-14). Several studies have confirmed that SLP-VCC has improved significantly both the structure-function relationship and the ability to discriminate normal from glaucomatous eyes, with respect to SLP-FCC (15-20). The aim of this study was to describe analogies and differences in RNFL thickness measurements conducted in a sample of normal and glaucomatous eyes by means of both FCC-SLP and VCC-SLP.

METHODS

We selected patients from those referred to the Glaucoma Unit of Trieste Eye Clinic of Trieste University from January through May 2004 for periodical scheduled visits.

Criteria for inclusion in the study population were as follows: RNFL evaluation by SLP with GDx units coupled with FCC and VCC; a properly compensated corneal birefringence on FCC-SLP as imaged on a macular retardation map (MRM), acquired before FCC session; good quality polarimetric images (quality score ≥ 90 for FCC-SLP and ≥ 8 for VCC-SLP); availability of a good quality automated achromatic perimetry (AAP) performed at ± 1 month from SLP; and refractive error within the ±4 spherical diopters range, with less than ±2 cylinder diopters.

Exclusion criteria were as follows: best-corrected visual acuity (BCVA) <20/40; corneal or lens opacity significantly interfering with clinical, AAP, or SLP examination; peripapillary atrophy, tilted disc, uveitis, significant vitreous floaters, diffuse or localized retinal or macular disease; and inability to perform reliable AAP (more than 20% of fixation losses, false-positive or negative rates) or SLP (poor fixation, inattentive patients).

Each patient underwent a complete ophthalmologic examination. BCVA was assessed on a standard ETDRS chart. At slit lamp, anterior segment was examined, and gonioscopy and Goldmann applanation tonometry performed. The optic disc was studied on stereo biomicroscopy with the aid of a +90-D lens after pupil dilation. Glaucomatous optic neuropathy was defined as diffuse or focal rim thinning, cupping, or optic disc hemorrhage. AAP was performed with the Humphrey Field Analyzer (Humphrey Systems, Dublin, CA), 24-2 program, SITA standard strategy. Defects were classified as glaucomatous when glaucoma hemifield test (GHT) was labeled as outside normal limits or pattern standard deviation (PSD) probability of less than 5% was detected. All glaucomatous eyes had at least two previous AAP and all visual field defects were reproducible.

RNFL was evaluated on GDx-FCC through undilated pupils with commercially available instrumentation (Nerve Fiber Analyzer, Laser Diagnostic Technology, Software v2.0.09, San Diego, CA). Three consecutive images in a 15° x 15° field were taken and from them a mean image was obtained, looking for an average deviation <8 µm. This image was used to determine peripapillary RNFL thickness, centering manually the ellipse on inner margin of peripapillary scleral ring on the fundus image. Then the software placed automatically a 10-pixel-wide elliptical band, concentric with the optic disc at 1.75 disc diameters from its center. Before any session, the presence of a properly compensated corneal birefringence was checked acquiring the macular retardation map (MRM), as suggested by Greenfield et al (7). Only eyes with homogeneous, all-blue MRM pattern (i.e., full compensation of anterior segment birefringence) were included in the study. Parameters considered were average thickness (AT), superior average (SA), inferior average (IA), superior maximum (SM), inferior maximum (IM), and ellipse average (EA).

SLP-VCC was performed with a commercially available device (GDx-VCC, software 5.3.4; Laser Diagnostic Technology) using a small calculation area centered on the optic disc. The first reading was obtained to compensate corneal birefringence and proper positioning of macular circle was checked after its acquisition. Then, the second reading provided values of RNFL parameters under the calculation area. Before accepting any reading, ellipse correct placement on inner margin of peripapillary scleral ring was checked on reflectance image. On both readings, maximum effort was paid to obtain high-quality scans (i.e., centered optic disc, well-focused, evenly illuminated image, no motion artifact).

The same parameters chosen for the FCC-SLP were collected. SLP sessions with the two polarimeters were

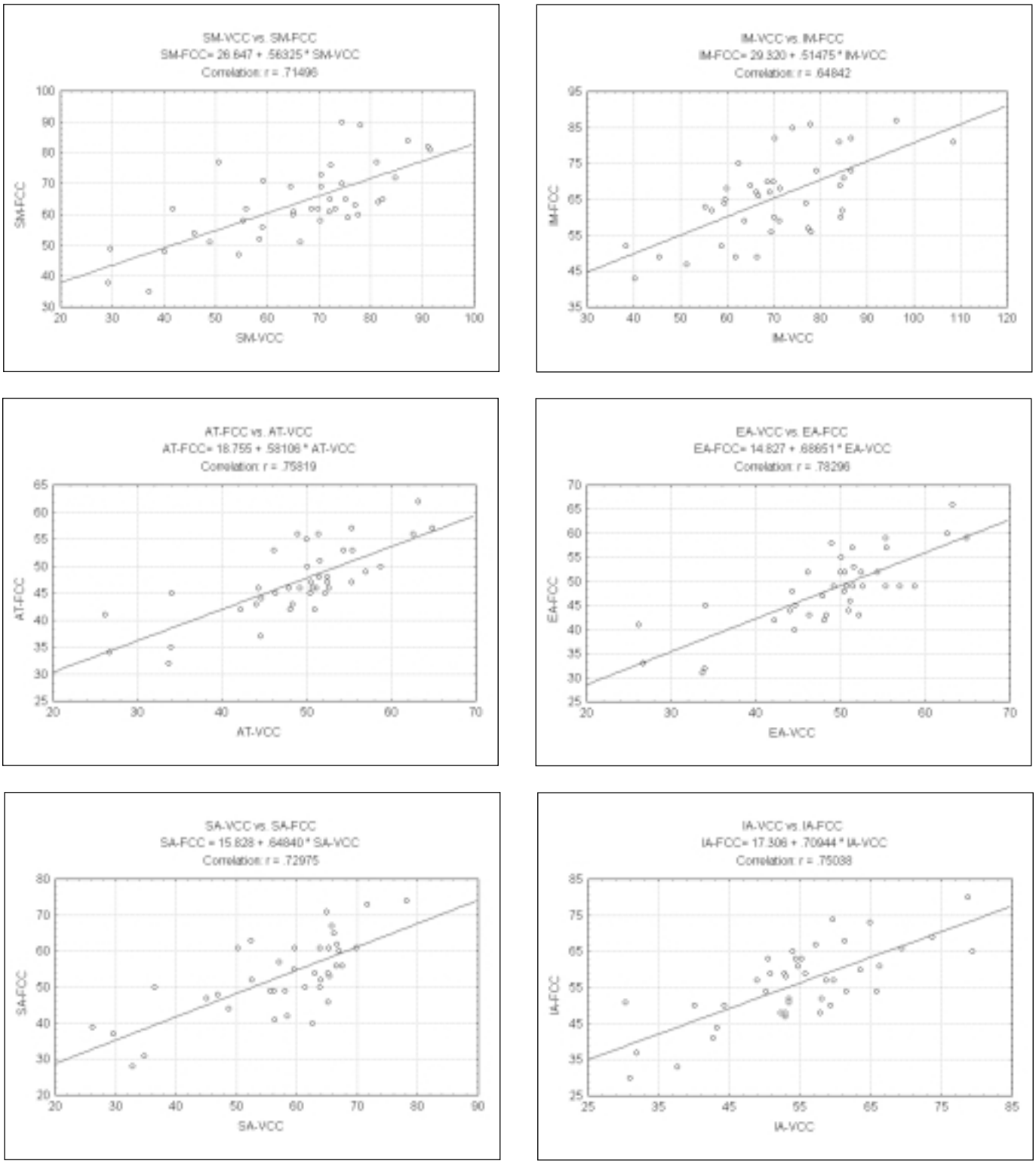

Fig. 1 - *Scattergrams showing the relation between fixed corneal compensator (FCC)-scanning laser polarimetry (SLP) (y-axis) and variable corneal compensator (VCC)-SLP (x-axis) for retinal nerve fiber layer (RNFL) thickness (µm) main parameters. Superior maximum (top left), inferior maximum (top right), average thickness (center left), ellipse average (center right), superior average (bottom left), and inferior average (bottom right). SM = Superior maximum; IA = Inferior average.*

performed on the same day. One single eye from each enrolled subject was selected randomly for inclusion, if both met the eligibility criteria.

Statistical analysis was performed using STATISTICA for Windows (Release 4.3, Copyright StatSoft, Inc., 1993). Pearson coefficient to correlate FCC and VCC measurements of each parameter, paired and unpaired t-test with Bonferroni's correction for multiple comparisons, area under the ROC curve (plotting sensitivity by 1-specificity) to evaluate discrimination ability of all parameters on both machines were employed. Patients were aware of the aim of the study and signed an informed consent to participation.

TABLE I - MAIN DEMOGRAPHIC DATA ABOUT RECRUITED PATIENTS

p value	Normal (n = 19)	Glaucoma (n = 23)	
Age, yr, mean ± SD	59.5 ± 11.5	65.6 ± 11.5	0.095*
Female	14	15	0.099†
Male	5	8	
Visual field MD, dB, mean ± SD	-0.12 ± 2.26	-4.92 ± 6.49	0.003*
Visual field PSD, mean ± SD	2.00 ± 1.05	5.37 ± 4.25	0.001*

*t-Test.
†Chi-square test.
PSD = Pattern standard deviation

RESULTS

Forty-two eyes of 42 subjects met the inclusion and exclusion criteria and then were enrolled. Twenty-nine were women and 13 were men. Mean age was 62.9 years ± 11.7 (range: 31-82). On the basis of AAP, 19 eyes were healthy and 23 glaucomatous. Table I shows demograph-

TABLE II - COMPARISON OF MEAN VALUES (µm) ± SD OF ALL THICKNESS PARAMETERS OBTAINED WITH GDX-FCC AND -VCC IN NORMAL AND GLAUCOMATOUS EYES, SEPARATELY

	SM	IM	AT	EA	SA	IA
			Normal (19)			
FCC	70.37 ± 11.13	69.21 ± 11.15	50.11 ± 6.11	52.16 ± 6.73	58.95 ± 9.62	60.21 ± 10.15
VCC	73.92 ± 9.22	75.48 ± 12.58	53.01 ± 5.58	53.01 ± 5.58	64.2 ± 5.57	59.63 ± 10.5
Mean variation (%)	+5.0	+9.0	+5.8	+1.6	+8.9	-1.0
p value*	0.291	0.113	0.135	0.565	0.047	0.863
			Glaucoma (23)			
FCC	57.86 ± 10.58	62.14 ± 10.51	44.55 ± 5.8	45.05 ± 6.49	47.95 ± 8.79	52.45 ± 9.9
VCC	58.62 ± 17.0	65.49 ± 14.1	45.2 ± 9.0	45.2 ± 9.0	51.54 ± 13.0	50.27 ± 10.25
Mean variation (%)	+1.3	+5.4	+1.5	+0.3	+7.5	-4.2
p value*	0.859	0.377	0.777	0.950	0.288	0.477

Mean percentage variation of VCC values with respect to FCC ones is provided.
*Paired t-test with Bonferroni correction for multiple comparisons.
SM = Superior maximum; IM = Inferior maximum; AT = Average thickness; EA = Ellipse average; SA= Superior average; IA = Inferior average; FCC = Fixed corneal compensator; VCC = Variable corneal compensator

TABLE III - AREAS UNDER THE ROC CURVE (SE) FOR DISCRIMINATION BETWEEN HEALTHY (N = 19) AND GLAUCOMATOUS (N = 23) EYES ON GDX-FCC AND -VCC, FOR EACH PARAMETER

	SM	IM	AT	EA	SA	IA
FCC	0.77 (0.07)	0.70 (0.09)	0.76 (0.08)	0.79 (0.08)	0.81 (0.07)	0.72 (0.09)
VCC	0.76 (0.08)	0.68 (0.08)	0.77 (0.07)	0.77 (0.07)	0.81 (0.07)	0.71 (0.08)

SM = Superior maximum; IM = Inferior maximum; AT = Average thickness; EA = Ellipse average; SA= Superior average; IA = Inferior average; FCC = Fixed corneal compensator; VCC = Variable corneal compensator

ic features (age and sex) as well as main AAP mean indexes, separately for healthy and glaucomatous eyes. According to Hodapp-Parrish-Anderson grading scale for visual field severity (21), among 23 glaucomatous eyes 9 had early, 7 moderate, and 7 severe defects. In Figure 1 and Table IV, for each thickness parameter, the linear correlation between values measured by FCC- and VCC-SLP is shown. Pearson correlation coefficient ranges from 0.65 for IM to 0.78 for EA ($p<0.001$).

In Table II mean values (± SD) of each thickness parameter obtained on the two polarimeters, in normal and glaucomatous eyes, are shown. After applying Bonferroni correction for multiple comparisons, p adjusted significance level was set at 0.008 and then no significant difference was noted. In Table III both instruments were evaluated in their ability to separate normal from glaucomatous eyes. The area under the ROC curve for all parameters was in the 0.68 to 0.81 range, with very close figures among the two polarimeters.

DISCUSSION

FCC-SLP produces inaccurate RNFL thickness values in most eyes. This is due to the fact that corneal polarization axis is frequently oriented at a different angle from the ideal position of 15° nasally downward (10) and incomplete compensation of corneal birefringence creates a too wide range of normal values in the normative database (15). The lower variability of VCC-SLP normative database improved the ability to discriminate between normal and glaucomatous eyes (9, 12, 16, 20). Data obtained from FCC-SLP correlated poorly with visual field defects (17, 22-26). A source of error in these studies was likely introduced by erroneous compensation of anterior segment birefringence. The introduction of VCC-SLP allowed description of significant structure-function relationship (15-20).

The current study aimed to compare the commercially available versions of two different polarimeters, one coupled with FCC, the other with VCC. The majority of available articles dealing with custom compensation of corneal birefringence employed a modified GDx-FCC coupled with slit-lamp mounted device to perform compensation. We selected eyes that on MRM acquired with FCC-SLP had their corneal birefringence properly compensated. Preselection of eyes according to this criterion was a necessary step for our study because our goal was to evaluate the correspondence of thickness values between the two instruments. Then we needed to create the ideal conditions to perform such a comparison and this influenced the results greatly. Thickness measurements obtained on the two machines were linearly correlated (Fig. 1 and Tab. IV). Points around correlation line for AT and EA showed a minimal spread with r values of 0.758 and 0.783, respectively (Fig. 1). On the contrary, data spread around correlation line was greater for IM and SM, with the lowest r value (0.648 and 0.715, respectively). A speculative explanation may be that EA and AT values were extracted from a similar number of pixels on both machines, whereas IM and SM values were evaluated on the 1500 thickest pixels for SLP-FCC and on the 210 thickest ones for SLP-VCC.

On average, we observed a VCC-SLP tendency to provide slightly higher mean measurements than FCC-SLP for all parameters, but IA (Tab. II), in both healthy and affected eyes, without any significant difference. Mean percentage difference was within the acceptable limits of measurement variability, except for IM and SA (Tab. II). Among glaucomatous eyes, mean difference from FCC-SLP measurements was smaller than for normal eyes (Tab. II).

Then, on average, different polarimeters with different corneal compensators measured similar RNFL thickness, with minimal differences. These findings re-emphasize the importance of adequate compensation of corneal birefringence when reliable measurements of RNFL thickness are needed. Previous studies conducted on eyes with uncompensated birefringence on FCC-SLP demonstrated that a significant decrease of thickness parameters ranging from 10 to 20% may be obtained repeating measurements with VCC-SLP (9, 15). The ability to discriminate

TABLE IV - CORRELATION COEFFICIENT (R) FOR EACH THICKNESS PARAMETER WITHIN LINEAR CORRELATION FCC vs VCC

	r value
Superior maximum	0.715
Inferior maximum	0.648
Average thickness	0.758
Ellipse average	0.783
Superior average	0.730
Inferior average	0.750
All	$p < 0.001$

FCC = Fixed corneal compensator;
VCC = Variable corneal compensator

adequately between normal and glaucomatous eyes for all the six considered parameters was tested calculating areas under the ROC curve (Tab. III). Values were similar to those reported by Weinreb et al (9) and slightly worse than those described by Medeiros et al (20). Differences are probably related to a different average severity of glaucoma in the patient population, being lower among eyes described in the present study.

In summary, we collected a sample of normal as well as early, moderate, and advanced glaucomatous eyes sharing the uncommon feature of a full compensation of corneal birefringence on FCC-SLP. In these eyes we measured RNFL thickness with both FCC-SLP and VCC-SLP. Agreement between the two polarimeters was overall good both in terms of linear correlation and similarity of mean values. Under these ideal conditions both machines separated adequately normal from affected eyes. Our results do not authorize considering RNFL thickness measurement with FCC- or with VCC-SLP as procedures of similar clinical value. When accurate and reliable RNFL quantification is requested, the use of VCC-SLP is preferable. However, it is encouraging that FCC-SLP measurements of RNFL thickness also may be considered reliable but only when anterior segment birefringence appears adequately compensated on MRM. This latter condition is found in a minority of eyes and this is the reason for a relatively small sample of eyes that represents a possible flaw in our report.

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