Scanning Laser Polarimetry for Measurement of Retinal Nerve Fiber Layer in Absolute, Advanced and Early Glaucoma

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Background: To detect differences in retinal nerve fiber layer (RNFL) measurements in absolute, advanced and early glaucoma with scanning laser polarimetry (The Nerve Fiber Analyzer GDx), and to assess the usefulness and limitations of this technique for longitudinal follow-up of glaucoma patients.

Methods: This is a prospective, cross-sectional study. Twenty-one eyes of 21 patients with absolute glaucoma, twenty-six eyes of 26 patients with advanced glaucoma and twenty-four eyes of 24 patients with early glaucoma were imaged using scanning laser polarimetry. The twelve standard GDx measurement parameters were compared using ANOVA (analysis of variance) and the Tukey test.

Results: No significant differences were demonstrated for any of the twelve GDx measurement parameters between absolute and advanced glaucoma cases. There were significant differences for some GDx parameters, including the GDx number ($p < 0.0001$) superior ratio ($p < 0.0001$), inferior ratio ($p < 0.0001$), superior/nasal ratio ($p < 0.0001$), maximum modulation ($p < 0.0001$), ellipse modulation ($p < 0.0001$) and inferior average ($p = 0.001$) between early and advanced glaucoma, and, between early and absolute glaucoma. Significant differences were demonstrated for the superior average ($p = 0.01$) parameter between early and absolute glaucoma, but not between early and advanced glaucoma.

Conclusions: For follow-up of glaucoma progression, RNFL measurements using scanning laser polarimetry are more useful in the early stage than in the advanced stage.

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Key words: scanning laser polarimetry, GDx, retinal nerve fiber layer, absolute glaucoma, primary open-angle glaucoma.

Assessment of the retinal nerve fiber layer (RNFL) should be considered in clinical trials of glaucoma neuroprotection, as it is directly correlated with loss of ganglion cells, which is assumed to be a primary event in glaucomatous damage.[1-2] Scanning laser polarimetry (The Nerve Fiber Analyzer GDx) is a computerized laser scanning device designed for the objective and quantitative

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measurement of RNFL thickness and loss. Good sensitivity and specificity have been demonstrated for this instrument in normal and glaucomatous eyes.[3] Although not widely used, GDx also offers excellent prospects for longitudinal assessment of RNFL to monitor glaucoma in terms of progression and assessment of neuroprotection treatment.[4]

Differences in GDx RNFL measurements between various types of glaucoma and healthy eyes have been reported.[5-7] Nevertheless, differences in RNFL measurement using GDx between different stages of glaucoma must be understood, as these are important for longitudinal assessment of RNFL during treatment and follow-up in glaucomatous patients, especially in end stage or absolute glaucoma. As far as we are aware, the RNFL thickness measurement for absolute glaucoma using scanning laser polarimetry has not been investigated.

The purpose of this study was, therefore, to detect any differences in RNFL measurements using GDx to compare absolute, advanced and early glaucoma. It may also be helpful in assessing the usefulness and limitations of the GDx instrument in the longitudinal follow-up of glaucomatous patients.

METHODS

This was a prospective and comparative study in which cross-sectional observations were made using RNFL measurements obtained with scanning laser polarimetry between 2001 and 2003. Patients were recruited from those undergoing treatment in the Department of Ophthalmology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan.

Twenty-one eyes of 21 patients with absolute glaucoma, twenty-six eyes of 26 patients with advanced glaucoma and twenty-four eyes with 24 patients with early glaucoma were enrolled in the study. The definitions and inclusion criteria are detailed below.

Early and advanced glaucoma was defined as primary open-angle glaucoma (POAG), with a typically glaucomatous optic disc appearance, diffuse or focal neuroretinal rim thinning, a visual field defect, normal open angle and untreated intraocular pressure (IOP) greater than 21 mmHg.

Early glaucoma was defined by a mean deviation (MD) of visual field loss no worse than – 6 dB and a corrected pattern standard deviation (CPSD) no worse than the 1% probability level. Advanced glaucoma was defined by an MD worse than –15 dB.[8]

Absolute glaucoma was defined as IOP above 21 mmHg, total loss of all neuroretinal rim, and no light perception.

The exclusion criteria were as follows: (1) refraction of more than ± 5.0 D (sphere) or 2.5 D (cylinder) with a Topcon KR-8100 autorefractometer; (2) pseudophakia or aphakia; (3) coexisting retinal disease; (4) nonglaucomatous optic neuropathy; (5) corneal edema or corneal opacity; (6) lens opacity worse than NC3/NO3 (nuclear color and nuclear opalescence); C3 (cortical cataract); and, P2 (posterior subcapsular cataract) according to the lens opacities classification system (LOCSIII);[9] (7) in early and advanced glaucoma: best-corrected visual acuity (BCVA) < 20/50, reliability criteria for the visual fields were a false negative more than 33%, or false positive more than 33%; (8) in advanced glaucoma: MD of visual field loss worse than – 30 dB.

The automated visual fields were completed within 3 months of the GDx examination. The automated perimetry was performed using a Humphrey Field Analyzer 30-2 full threshold program (Humphrey Instrument, San Leandro, CA, USA).

The RNFL measurements were obtained using scanning laser polarimetry with a fixed corneal compensator (The Nerve Fiber Analyzer GDx, Laser Diagnostic Technologies Inc. San Diego, CA, USA). The details of this technique have been described previously.[10-11] A total of 65,536 retinal locations were measured to create a retardation map corresponding to RNFL thickness over a 15° (256 × 256 pixel) retinal area. Pupil dilation was not required while undergoing the scan. The optic disc margin was approximated by an ellipse placed around the inner margin of the peripapillary scleral ring by an experienced operator. A measuring ellipse was then generated by the instrument at 1.75 disc diameters concentric with the margin of the disc. Default quadrant positions were applied: the peripapillary band was divided into superior and inferior segments of 120° each, a temporal segment of 50°, and a nasal segment of 70°. The measurements for each eye were obtained from a minimum of three images of good quality (well-focused, a centered optic disc in the image, equal and total illumination in all segments and rated as “pass” by the internal GDx soft-

ware), with the best of these then analyzed.

The following twelve standard GDx parameters were chosen for analysis: the GDx number, symmetry, superior ratio, inferior ratio, superior/nasal ratio, maximum modulation, ellipse modulation, average thickness, ellipse average, superior average, inferior average and superior integral.

The definitions of these parameters have been described elsewhere.[12-13] For the GDx number, a trained neural network assesses all pixels and assigns a number from 0 to 100 (0 indicates normal; glaucoma was considered when greater than 30) to an eye. A total of 1500 pixels per quadrant peripheral to an ellipse 1.75 disc diameters from the center of the disc was used to calculate ratio and maximum measures. The symmetry was the ratio of the average of the 1500 thickest pixels in the superior quadrant divided by the average of the 1500 thickest pixels in the inferior quadrant. The superior ratio was the ratio of the average of the 1500 thickest pixels in the superior quadrant divided by the average of the 1500 median pixels in the temporal quadrant. The inferior ratio was the ratio of the average of the 1500 thickest pixels in the inferior quadrant divided by the average of the 1500 median pixels in the temporal quadrant. The superior/nasal ratio was the average of the 1500 thickest pixels in the superior quadrant divided by the average of the 1500 median pixels in the nasal quadrant.

For maximum modulation, first, the average was calculated for the 1500 thickest points in the superior and inferior quadrants. Next, the 1500 median points in the nasal and temporal quadrants were calculated. The lowest of the 4 values was subtracted from the highest, and then divided by the lowest value. Average thickness was the average of all pixels outside the disc margin.

The ellipse modulation, ellipse average, superior average, and inferior average were calculated using pixels within the 10-pixel-wide elliptical band that was automatically positioned concentric with the disc margin outline and 1.75 disc diameters from the center of the optic disc. Ellipse modulation was calculated by taking the thickest pixel within the elliptical band, subtracting the thinnest pixel within the band, and dividing the total by the value of the thinnest pixel. The ellipse average was calculated using the average thickness of the pixels within the elliptical band surrounding the optic nerve. The superior average was the average thickness of the pixels within the elliptical band in the superior quadrant. The inferior average was the average thickness of the pixels within the elliptical band surrounding the inferior quadrant. The superior integral was the total area under the curve and within the superior portion of the elliptical band surrounding the optic nerve.

Statistical analysis was performed using JMP software (SAS institute, Cary, North Carolina, USA). ANOVA (analysis of variance) and the Tukey test were used to compare differences between study groups for the twelve standard GDx parameters. ANOVA and the chi-square test were used to compare the mean age and male-female ratio in the study groups. A p value of less than 0.05 was considered to be statistically significant. In patients with bilateral POAG, one eye was randomly selected for investigation.

RESULTS

The characteristics of the study population, including mean age and male-female ratio, are listed in Table 1.

No significant differences were demonstrated in any of the twelve standard GDx parameters between the absolute and advanced glaucoma groups.

There were significant differences in seven GDx parameters between early and advanced glaucoma, and between early and absolute glaucoma. These parameters were the GDx number, superior ratio, inferior ratio, superior/nasal ratio, maximum modulation, ellipse modulation and inferior average.

Significant differences were demonstrated for the superior average parameter between early and absolute glaucoma, but not between early and advanced glaucoma.

There were no significant differences in the

Table 1. Characteristics of the Study Population

	Absolute glaucoma (n = 21)	Advanced glaucoma (n = 26)	Early glaucoma (n = 24)	p-value
Age (years)	62.8 ± 9.2 (44-78)	63.8 ± 9.6 (45-78)	59.3 ± 9.6 (40-74)	0.22
Male/Female	13/8	14/12	16/8	0.64

symmetry, average thickness, ellipse average and superior integral parameters among the patients with absolute, advanced and early glaucoma. The results are listed in Table 2.

DISCUSSION

Lee and Mok reported that the total average value, superior value and inferior value were all found to be significantly lower in glaucomatous patients than in those without glaucoma, with the exception of the nasal and temporal values.[6]

Nguyen *et al.* demonstrated that the GDx number, superior ratio, inferior ratio, maximal modulation and ellipse average were significantly different between normal eyes and those in the early, moderate and advanced stages of glaucoma.[14]

In our study, significant differences were demonstrated between early and advanced glaucoma, and between early and absolute glaucoma for seven GDx measurement parameters, the GDx number, superior ratio, inferior ratio, superior/nasal ratio, maximum modulation, ellipse modulation and inferior average. A significant difference for the superior average was only demonstrated between early and absolute glaucoma, but not between the early and advanced glaucoma.

Based on these results, it seems reasonable to suggest that these seven GDx parameters are sensitive for follow-up of early glaucomatous eyes, although the specificities have not been clearly defined. Of the GDx parameters, the inferior average thickness is more sensitive than the superior average thickness, which may reflect that the RNFL thickness distribution pattern is a double hump configuration with the highest mean thickness in the inferior quadrant at the optic disc border.[15-16]

Eyes that deviate widely are characterized by strong peripapillary retardation that artifactually increases the apparent RNFL thickness. By contrast, where the corneal polarization axis is closer to the compensator axis, eyes are characterized by weaker peripapillary retardation, resulting in lower measured RNFL thickness.[14,17] In cases of far- advanced to absolute glaucoma, central visual acuity is markedly decreased and fixation is poor. Therefore, absolute glaucomatous eyes may deviate widely when GDx is performed, and strong peripapillary retardation that artificially increases the apparent RNFL thickness may occur.

Histomorphometrical measurement of the RNFL thickness in eyes with absolute glaucoma has revealed that a mean thickness for the remainder of the RNFL was 40 μm with no marked differences between the disc regions. The mean overall thickness of glial and other tissues representing the remnant RNFL was 40 μm.[15] The measured thickness of approximately 40 μm for the remainder of the RNFL in absolute glaucoma corresponds to the finding that the glial content of the RNFL in monkeys is about

Table 2. Retinal Nerve Fiber Layer Measurements Using Scanning Laser Polarimetry

Parameters	Absolute glaucoma (n = 21)	Advanced glaucoma (n = 26)	Early glaucoma (n = 24)	*p* - value
The GDx number	72.0 ± 15.6 (38-98)	65.6 ± 13.4 (42-94)	37.5 ± 18.1 (11-88)	< 0.0001*
Symmetry	0.90 ± 0.05 (0.8-1.0)	0.93 ± 0.10 (0.74-1.1)	0.91 ± 0.10 (0.75-1.1)	0.41
Superior ratio	1.29 ± 0.22 (0.82-1.77)	1.371 ± 0.15 (1.13-1.71)	1.78 ± 0.29 (1.35-2.64)	< 0.0001*
Inferior ratio	1.44 ± 0.25 (0.94-1.99)	1.49 ± 0.20 (1.2-2.02)	1.97 ± 0.35 (1.24-2.62)	< 0.0001*
Superior nasal	1.17 ± 0.09 (1.05-1.42)	1.22 ± 0.14 (1.19-1.87)	1.55 ± 0.20 (1.19-1.87)	< 0.0001*
Maximum modulation	0.48 ± 0.21 (0.21-0.99)	0.51 ± 0.19 (0.21-1.02)	0.99 ± 0.34 (0.36-1.64)	< 0.0001*
Ellipse modulation	0.79 ± 0.30 (0.33-1.48)	0.9 ± 0.37 (0.36-1.98)	1.83 ± 0.58 (0.83-3.18)	< 0.0001*
Average thickness (μm)	65.0 ± 9.15 (42-78)	66.4 ± 12.9 (41-107)	69.2 ± 11.6 (47-87)	0.82
Ellipse average (μm)	64.6 ± 10.0 (42-82)	67.9 ± 14.2 (42-110)	72.7 ± 12.8 (53-98)	0.10
Superior average (μm)	63.8 ± 10.8 (40-80)	67.5 ± 15.3 (43-113)	74.0 ± 14.8 (52-107)	0.01†
Inferior average (μm)	69.5 ± 11.7 (46- 85)	73.1 ± 14.7 (44-114)	84.4 ± 13.6 (57-107)	0.001*
Superior integral	0.19 ± 0.04 (0.11-0.27)	0.22 ± 0.15 (0.12-0.31)	0.21 ± 0.04 (0.14-0.29)	0.32

* Significant differences demonstrated between early and advanced glaucoma, and between early and absolute glaucoma from the Tukey test.

† Significant differences demonstrated between early and absolute glaucoma from the Tukey test.

20-30% of the total retinal nerve fiber bundle.[18]

This may explain why all twelve GDx measurement parameters were not significantly different between absolute glaucoma and advanced glaucoma in our results. Factors affecting image acquisition with scanning laser polarimetry include anterior and posterior segment pathologies.[19] Anterior segment pathologies, particularly those local to the cornea and lens, may influence birefringence and produce spurious RNFL measurements with the GDx. To prevent errors in RNFL measurements with GDx in this study, we excluded the posterior segments and eyes with a corneal abnormality, and cataracts were matched using LOCSIII grading.[9] Patients with lens opacities worse than NC3/NO3, C3, P2 were excluded.

A major limitation of this study was the failure to correct for the slow axis of corneal birefringence. The commercial GDx used in our study employs a fixed corneal compensator (FCC) that assumes all persons have a slow axis of corneal birefringence 15° nasally downward at a magnitude of 60 nm. The magnitude and axis are highly variable, however. The wide range of these measurements represents a source of error in RNFL assessment using the current FCC- GDx. It has been demonstrated that custom correction for the axis of corneal birefringence using a variable corneal compensator (VCC) improves the discriminating power of GDx.[20] Nevertheless, further study is necessary to determine whether VCC-GDx is able to differentiate between absolute glaucoma and advanced glaucoma.

Although there are some shortcomings, these results are helpful in assessing the usefulness and limitations of the quantitative RNFL imaging instrument in diagnosis and longitudinal follow-up of glaucomatous patients.

In conclusion, no significant differences were demonstrated for any of the GDx measurement parameters between absolute glaucoma and advanced glaucoma cases. There were significant differences, however, for some GDx parameters between early and advanced glaucoma, and, between early and absolute glaucoma. We suggest that scanning laser polarimetry is useful in monitoring progression of glaucoma from its early stage to a more advanced stage, especially in patients with poor reliability in visual field tests. The most sensitive parameters are the GDx number, superior ratio, inferior ratio, superior/nasal ratio, maximum modulation, ellipse modulation and inferior average.

REFERENCES

1. Blumenthal EZ, Weinreb RN. Assessment of the retinal nerve fiber layer in clinical trials of glaucoma neuroprotection. Surv Ophthalmol 2001;45:S305-12.
2. Johnson CA, Cioffi GA, Liebmann JR, Sample PA, Zangwill LM, Weinreb RN. The relationship between structural and functional alteration in glaucoma: a review. Semin Ophthalmol 2000;15:221-33.
3. Choplin NT, Lundy DC. The sensitivity and specificity of scanning laser polarimetry in the detection of glaucoma in a clinical setting. Ophthalmology 2001;108:899-904.
4. Poinoosawmy D, Tan JCH, Bunce C, Membrey LW, Hitching RA. Longitudinal nerve fibre layer thickness change in normal-pressure glaucoma. Graefes Arch Clin Exp Ophthalmol 2000;238:965-9.
5. Kamal DS, Hitchings BRA. Use of the GDx to detect differences in retinal nerve fiber layer thickness between normal, ocular hypertensive and early glaucomatous eyes. Eye 2000;14:367-70.
6. Lee VW, Mok KH. Retinal nerve fiber layer measurements by nerve fiber analyzer in normal subjects and patients with glaucoma. Ophthalmology 1999;106:1006-8.
7. Tsai JC, Chang HW. Scanning laser polarimetry in patients with acute angle-closure glaucoma. Eye 2004;18:9-14.
8. Hodapp E, Parrish R, Anderson D, eds. Clinical decisions in glaucoma. St. Louis: CV Mosby. 1993:11-63.
9. Chylack LT Jr., Wolfe JK, Singer DM, Leske MC. The lens opacities classification system III. Arch Ophthalmol 1993;111:831-6.
10. Cesar SG, Bowd C, Blumenthal EZ, Gokhale PA, Zangwill LM, Weinreb RN. Using optical imaging summary data to detect glaucoma. Ophthalmology 2001;108:1812-8.
11. Poinoosawmy D, Fontana L, Wu JX, Fitzke FW, Hitchings RA. Variation of nerve fibre layer thickness measurements with age and ethnicity by scanning laser polarimetry. Br J Ophthalmol 1997;81:350-4.
12. Weinreb RN, Zangwill L, Berry CC, Bathija R, Sample PA. Detection of glaucoma with scanning laser polarimetry. Arch Ophthalmol 1998;116:1583-9.
13. Colen TP, Tjon-Fo-sang MJ, Mulder PG, Lemij HG. Reproducibility of measurements of the nerve fiber analyzer (NFA/GDx). J Glaucoma 2000;9:363-70.
14. Nguyen NX, Horn FK, Hayler J, Wakili N, Junemann A, Mardin CY. Retinal nerve fiber layer measurements using laser scanning polarimetry in different stages of glaucomatous optic nerve damage. Graefes Arch Clin Exp Ophthalmol 2002;240:608-14.
15. Dichtl A, Jonas JB, Naumann GOH. Retinal nerve fiber

thickness in human eyes. Graefes Arch Clin Exp Ophthalmol 1999;237:474-9.
16. Jonas JB, Gusek GC, Naumann GOH. Optic disc morphometry in chronic primary open-angle glaucoma. Graefes Arch Clin Exp Ophthalmol 1988;226:522-30.
17. Greenfield DS, Knighton RW. Stability of corneal polarization axis measurements for scanning laser polarimetry. Ophthalmology 2001;108:1065-9.
18. Ogden TE. Nerve fiber layer of the primate retina: thickness and glial content. Vision Res 1983;23:581-7.
19. Collur S, Carroll AM, Cameron BD. Human lens effect on in vivo scanning laser polarimetric measurements of retinal nerve fiber layer thickness. Ophthalmic Surg Lasers 2000;31:126-30.
20. Bagga H, Greenfield DS, Feuer W, Knighton RW. Scanning laser polarimetry with variable corneal compensation and optical coherence tomography in normal and glaucomatous eyes. Am J Ophthalmol 2003;135:521-9.

以雷射掃描極光儀測量輕、重度與絕對性青光眼的視網膜神經纖維層厚度

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背 景： 用雷射掃描極光儀 (神經纖維分析儀：GDx) 測量及比較輕度，重度與絕對性青光眼的視網膜神經纖維層厚度，以評估此儀器對於青光眼病患追蹤治療的實用性及限制。

方 法： 我們以雷射掃描極光儀測量 24 個輕度青光眼病患 (24 隻眼睛)，26 個重度青光眼病患 (26 隻眼睛) 與 21 個絕對性青光眼病患 (21 隻眼睛) 的視網膜神經纖維層厚度。用 ANOVA 和 Tukey test 來比較這三個族群於 12 個 GDx 標準測量參數是否有統計上的差別。

結 果： 我們發現重度青光眼與絕對性青光眼病患之間於 12 個 GDx 標準測量參數均沒有統計上的差別。而輕度與絕對性青光眼之間，和輕度與重度青光眼之間有 7 個 GDx 標準測量參數有統計上的差別包括：the GDx number ($p < 0.0001$)，superior ratio ($p < 0.0001$)，inferior ratio ($p < 0.0001$)，superior/nasal ratio ($p < 0.0001$)，maximum modulation ($p < 0.0001$)，ellipse modulation ($p < 0.0001$) and inferior average ($p = 0.001$)。另外 superior average ($p = 0.01$) 測量參數在輕度與絕對性青光眼之間有差別，而在輕度與重度青光眼之間則沒有差別。

結 論： 用雷射掃描極光儀追蹤測量青光眼病患的視網膜神經纖維層厚度是否有變化，在輕度青光眼病患比重度青光眼病患較有幫忙。

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關鍵字： 雷射掃描極光儀，視網膜神經纖維，青光眼。

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