SCIENTIFIC CORRESPONDENCE

Comparison of algorithms for detection of localised nerve fibre layer defects using scanning laser polarimetry

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Aims: To evaluate different algorithms used to analyse retinal nerve fibre layer thickness (RNFL) data obtained by scanning laser polarimetry, in order to compare their relative abilities to discriminate between patients with glaucomatous localised nerve fibre layer defects and normal subjects.

Methods: 48 eyes of 48 glaucomatous patients with localised RNFL defects and 53 eyes of 53 healthy subjects were included in this study. The localised RNFL defects were identified by RNFL photography and/or slit lamp biomicroscopic examination. All patients were submitted to RNFL examination using scanning laser polarimetry (GDx nerve fibre analyser, Laser Diagnostic Technologies, Inc, San Diego, CA, USA). Three methods of analysis of polarimetry data were used: GDx software provided parameters; RNFL thickness measurements in 16 equal sectors around the optic disc (sectoral analysis); and Fourier analysis of the curve of distribution of RNFL thickness measurements. Linear discriminant functions were developed to assess sensitivity and specificity of the sectoral based analysis and Fourier analysis and were compared to the GDx parameters. In addition, areas under the receiver operating characteristic (ROC) curves were compared.

Results: At a fixed specificity of 91%, the sensitivity of the linear discriminant function from sectoral data (LDF sectoral) was 81%, with an area under the ROC curve of 0.93. The linear discriminant function from Fourier measures had a comparable performance, with an area under the ROC curve of 0.93, and sensitivity of 71% for specificity at 91%. At the same specificity, the sensitivities of the GDx software provided parameters ranged from 15% to 40%. The areas under the ROC curves for the LDF sectoral and LDF Fourier were significantly greater than the ROC curve area for the single best GDx parameter.

Conclusion: The sectoral based analysis and the Fourier analysis of RNFL polarimetry data resulted in an improved detection of eyes with glaucomatous localised nerve fibre layer defects compared to the GDx software provided parameters.

Retinal nerve fibre layer (RNFL) abnormalities have been shown to precede the development of visual field defects in glaucomatous patients.^{1,2} Hence, the recognition of RNFL defects is crucial for the early diagnosis of this disease. Glaucomatous changes in the retinal nerve fibre layer can be classified as localised, diffuse, or as a combination of the two.³ A localised RNFL defect appears as a wedge-shaped dark area in the nerve fibre layer with its tip touching the optic disc border.^{3–5} Localised RNFL defects are considered to be very rare in normal individuals, being a specific sign of optic nerve disease. They are found in approximately 20% of glaucomatous eyes and are most often seen in the early stages of the disease.⁶

Although red-free photographs have been established as a standard method for detection of nerve fibre layer defects, the qualitative and subjective nature of this assessment, as well as the requirement for maximal pupillary dilatation and optimal media clarity, limits its applicability.^{7,8} Furthermore, histological studies have shown that as much as 50% of the nerve fibres can be lost without the appearance of nerve fibre layer defects in red-free photographs.⁹

Scanning laser polarimetry (SLP) is a diagnostic tool developed to quantitatively measure the thickness of the peripapillary RNFL.^{10–12} This technology is based on the principle that polarised light passing through a birefringent medium undergoes a measurable phase shift, known as retardation, which is directly proportional to the thickness of the medium. The measure of RNFL thickness is based on the linear relation between the retardation of reflected light and histologically measured RNFL tissue thickness.¹⁰ The GDx nerve fibre analyser (Laser Diagnostic Technologies Inc, San Diego, CA, USA) is a scanning laser polarimeter which has been shown to produce objective and reproducible RNFL thickness measurements with no need for pupillary dilatation.^{13–16}

Previous reports have shown that GDx software provided parameters have a limited ability to discriminate between normal and glaucomatous eyes.^{17–22} To improve upon these results, several studies have used different methods to analyse the RNFL thickness measurements provided by the equipment. Weinreb *et al*¹⁷ combined some of the GDx parameters in a linear discriminant function and found detection of glaucoma to have been improved compared to the single best parameter, the number. In another study, Greaney and colleagues²² found that the best results with scanning laser polarimetry were obtained from discriminant analysis of 30° sectoral data.

In another approach, suggested by Essock *et al*,^{23–27} Fourier analysis was applied to the RNFL thickness measurements from scanning laser polarimetry resulting in an increased sensitivity and specificity for glaucoma detection compared to the GDx parameters. Because Fourier analysis is based on the overall shape of the RNFL thickness distribution curve, it may be more robust than methods that emphasise local measures or single parameters. In addition, by comparing relative differences in the shape of RNFL thickness distribution curve, the Fourier method may better identify glaucomatous patients with localised nerve fibre layer defects.

In this study, we evaluated different algorithms used to analyse nerve fibre layer thickness data obtained by scanning laser polarimetry, in order to compare their relative abilities to discriminate between patients with glaucomatous localised nerve fibre layer defects and normal subjects.

PATIENTS AND METHODS

Fifty three healthy subjects and 48 glaucomatous patients meeting entry criteria were enrolled in this study. All patients were evaluated at the department of ophthalmology, University of São Paulo, São Paulo, Brazil, and informed consent was

obtained from all participants. Mean age (SD) for normal subjects and glaucoma patients was 50 (15) years and 59 (12) years, respectively ($p=0.001$; Student's t test). Among the healthy subjects, 36 (68%) were female and 17 (32%) were male. Among the glaucoma patients, 23 (48%) were female and 25 (52%) were male.

Each subject underwent a comprehensive ophthalmological examination including review of medical history, best corrected visual acuity, slit lamp biomicroscopy, intraocular pressure (IOP) measurement using Goldmann applanation tonometry, gonioscopy, dilated funduscopy examination using a 78D lens, and automated perimetry using 24-2 full threshold test (Zeiss-Humphrey field analyser, Dublin, CA, USA). To be included, all subjects had to have a best corrected visual acuity of 20/40 or better; spherical refraction within plus or minus 3 D, and cylinder correction within plus or minus 3 D. Eyes with coexisting retinal disease, uveitis, or non-glaucomatous optic neuropathy were excluded from this study. When both eyes of a patient were eligible for the study, one eye was randomly chosen for inclusion.

Normal controls eyes had intraocular pressures of 22 mm Hg or less with no history of increased IOP, healthy appearance of the optic disc, and retinal nerve fibre layer (no diffuse or focal rim thinning, cupping, optic disc haemorrhage, or RNFL defects indicative of glaucoma or other ocular pathologies), and a normal visual field test. Normal visual field was defined as a mean deviation (MD) and corrected pattern standard deviation (CPSD) within 95% confidence limits, and a glaucoma hemifield test (GHT) within normal limits.

Glaucomatous patients had localised nerve fibre layer defects detected by slit lamp biomicroscopic examination or RNFL red-free photographs according to previously defined criteria.⁶ Although the criteria to identify glaucomatous patients were not based on the results of Humphrey visual field testing, most localised nerve fibre layer defects corresponded to localised deep Humphrey 24-2 visual field defects with elevated CPSD values. Average MD and CPSD of the glaucomatous eyes on the visual field test nearest the imaging date were -5.32 (3.81) dB and 6.94 (3.70) dB, respectively. Intraocular pressure was not used as a criterion for inclusion or exclusion of glaucomatous patients.

Scanning laser polarimetry measurements

All patients were imaged using a scanning laser polarimeter, the GDx nerve fibre analyser. The GDx nerve fibre analyser is a confocal scanning laser ophthalmoscope coupled with an integrated polarisation modulator. Details of its operation have been reported previously.¹³ The technique is based on birefringence properties of the RNFL. The ganglion cell axons contain microtubules, cylindrical intracellular organelles with diameters much smaller than the wavelength of the illuminating light, which are form birefringent. The RNFL birefringence induces a change (retardation) in the state of polarisation of an illuminating laser beam that passes through it. The retardation has been shown to be correlated to the thickness of the RNFL.

Three to six retardation maps of the peripapillary retina of each eye were acquired and a mean map was created and used for analysis by averaging the three best images. The images were evaluated by both subjective and software generated image quality assessment. Poor quality images were discarded.

The disc margin was established by an experienced operator who used an ellipse to outline the inner margin of the peripapillary scleral ring. A 10 pixel-wide elliptical band was automatically positioned concentric with the optic disc margin outline and at 1.7 disc diameters from the centre of the optic disc. This elliptical band was divided into 16 sectors (22.5° each) and the average retardation was recorded for each of the 16 sectors. There were eight sectors for the superior hemiretina and eight sectors for the inferior hemiretina. These

Figure 1 Representation of the sectoral division used in the study. The sectoral division is shown superimposed on the reflectance image obtained from the GDx nerve fibre analyser. The 10 pixel wide elliptical band was divided into 16 sectors (22.5° each) numbered from the temporal region in clockwise fashion for the right eye and in anticlockwise fashion for the left eye.

sectors were numbered from 1 to 16 according to the representation shown in Figure 1. The GDx software automatically converts retardation measurements into μm using a conversion factor, based on previous histological comparison in monkey eyes.¹⁰

Data analysis

The following three methods of analysis of RNFL polarimetry data were used in this study.

GDx software provided parameters

The GDx software calculates summary parameters based on quadrants which are defined as temporal (335°–24° unit circle), superior (25°–144°), nasal (145°–214°), and inferior (215°–334°). The GDx software provided parameters investigated in this study were the number (a score generated by a neural network in this system); symmetry (superior quadrant thickness/inferior quadrant thickness); superior ratio (superior quadrant thickness/temporal quadrant thickness); superior/nasal ratio; maximum modulation (thickest quadrant/thinnest quadrant)/thinnest quadrant; average thickness; ellipse average, ellipse modulation, superior average; inferior average and superior integral. The latter five parameters are measured relative to the 10 pixel-wide elliptical band. All these parameters have been described in detail elsewhere.¹⁷

The value of a discriminant analysis model proposed by Weinreb *et al*¹⁷ (LDF Weinreb) was also investigated. Details of this model are given elsewhere.¹⁷

Sectoral based analysis

In this analysis, RNFL thickness in each of the 16 sectors around the optic disc was compared between glaucomatous patients with localised RNFL defects and normal subjects and a stepwise discriminant analysis was used to identify and combine the most useful sectors separating the two groups.

Fourier analysis

In normal subjects, the distribution of RNFL thickness around the optic disc has a characteristic pattern known as double hump curve. This pattern is the result of a greater RNFL

Table 1 Mean (SD) μm of retinal nerve fibre layer (RNFL) thickness measurements in the 16 sectors around the optic disc in glaucomatous eyes with localised RNFL defects and normal eyes

Sectors*	Glaucoma (n=48)	Normals (n=53)	p Value†
S1	37.5 (13.4)	38.4 (16.2)	0.779
S2	38.8 (14.0)	40.8 (16.2)	0.522
S3	50.1 (17.7)	60.6 (21.1)	0.008
S4	68.9 (22.8)	81.8 (23.5)	0.006
S5	76.1 (22.6)	93.9 (27.9)	0.001
S6	78.6 (21.0)	95.7 (23.9)	<0.001
S7	63.8 (18.5)	72.7 (20.0)	0.023
S8	42.9 (12.6)	45.4 (16.6)	0.398
S9	38.9 (11.5)	44.5 (15.9)	0.049
S10	55.0 (17.7)	66.6 (21.8)	0.005
S11	73.9 (21.5)	94.8 (24.0)	<0.001
S12	78.2 (20.2)	106.3 (24.8)	<0.001
S13	69.2 (19.0)	100.8 (22.5)	<0.001
S14	55.1 (16.1)	80.1 (20.6)	<0.001
S15	44.2 (14.0)	52.4 (18.6)	0.013
S16	40.2 (13.2)	43.4 (16.9)	0.289

*For definition of the sectors around the optic disc, see Figure 1.

†Significant differences, after adjusting for multiple comparisons (Holm test), are in italics.

thickness in the superior and inferior regions compared to the temporal and nasal ones. RNFL loss in glaucoma leads to a change in this curve, either by reducing its amplitude or changing its shape. However, as RNFL thickness is known to vary widely among healthy subjects, the usefulness of absolute thickness values to separate glaucomatous from normal subjects is limited. Rather than emphasising thickness itself, an analysis of the global shape of the RNFL thickness distribution around the optic disc may be more effective in detecting RNFL loss in glaucoma. This analysis can be accomplished by a mathematical procedure known as Fourier analysis.²⁸ Using Fourier analysis, a complex waveform pattern (such as the double hump curve of the RNFL) can be broken down and described as a series of harmonically related sinusoids of specific frequencies, amplitudes, and phases. These sinusoids, when summed together point by point, reproduce the original pattern. Fourier analysis provides the amplitudes and phases of the sinusoidal components which can then be used to study quantitatively the original waveform. The fast Fourier transform (FFT) was used to determine the coefficients—that is, the amplitude and phase of each sinusoid. The software MATHEMATICA V 4 (Wolfram Research, Inc, Champaign, IL, USA) was used to perform the FFT algorithm on the data from scanning laser polarimetry. Details from the FFT procedure have been published elsewhere.^{29–30} In brief, when Fourier analysis is performed on a set of discrete data that describe some waveform, the number of sine waves generated equals half the number of data points. The lowest frequency component, also called fundamental (F_{FUN}), corresponds to a sine wave pattern with one cycle (that is, one hump). The other components are sine waves whose frequencies are integer multiples of the fundamental.

The pattern of thickness measurements from SLP in the eight sectors around the optic disc of each hemiretina was analysed in order to obtain the Fourier coefficients. Because the analysis is performed on eight data points it will yield four sine waves (F_{FUN} , F2, F3, and F4) for each hemiretina. The amplitude value of each component sine wave is the peak to trough distance and indicates its relative contribution to the shape of the composite curve fitting the RNFL thickness measurements. Also produced by Fourier analysis is a component called DC, which represents the overall level of all amplitude measures. In our application the DC component is equivalent to the mean RNFL thickness.

The Fourier components (DC component and all amplitude values) from all subjects were entered into a stepwise

discriminant analysis to develop a classification rule (linear discriminant function, LDF) designed to identify and combine the best measures to differentiate glaucomatous eyes with localised RNFL defects from normal eyes.

Comparison of methods

Student's *t* tests were used to evaluate GDx software provided parameters, sectoral RNFL thickness values, and Fourier coefficients differences between glaucomatous and healthy eyes. The Holm test was used to adjust for multiple comparisons.³¹

Receiver operating characteristic (ROC) curves were used to describe the ability to differentiate glaucomatous patients with localised RNFL defects from normal eyes of each GDx software provided parameter and also of the LDF obtained from sectoral based analysis (LDF sectoral) and of the LDF obtained from Fourier measures (LDF Fourier). The ROC curve shows the trade-off between sensitivity and 1 – specificity. An area under the ROC curve of 1.0 represents perfect discrimination whereas an area of 0.5 represents chance discrimination. The method of DeLong *et al*³² was used to compare areas under the ROC curve. Minimum specificity cut-offs of 80% and 90% were used to calculate the sensitivities of the GDx parameters, sectoral based LDF, and Fourier based LDF.

The use of the same population to identify and test a discriminant function can lead to a significant bias or optimism with respect to the predictive ability of the model when applied to an independent dataset. To provide an internal validation estimate, we used a computer intensive bootstrap approach.^{33–34} The bootstrap procedure provides stable and nearly unbiased estimates of performance, and has been claimed to be the most efficient validation method available, comparing favourably with standard cross validation techniques.^{33–35} The superiority of bootstrap estimates over traditional methods seems to be particularly evident for error rate estimation in two class discrimination problems when samples sizes are small.³⁶ Bootstrapping is a resampling method that allows one to make inferences about the population that the sample originated from by drawing *B* samples (*B* = 1000 in the current study) with replacement from the original dataset, of the same size as the original dataset. In the .632 bootstrap method, a model is built for each bootstrap sample and evaluated only in those subjects not sampled. The prediction errors are then averaged over all bootstrap samples (test performance). Since the evaluation is based on an independent dataset, this method can be seen as a direct extension or as a smoothed version of cross validation. As the dataset is sampled with replacement, on average 63.2% of the subjects

Figure 2 Receiver operating characteristic (ROC) curves for the linear discriminant function obtained from Fourier analysis (LDF Fourier), sectoral based analysis (LDF sectoral), and for the linear discriminant function from Weinreb *et al*¹⁷ (LDF Weinreb).

are included at least once in a bootstrap sample, giving the method its name.³⁴ The estimated performance is a weighted combination of the apparent performance (resubstitution error estimate on the full dataset) and test performance. The .632+ bootstrap is an extension of the .632 method applying a regularisation coefficient based on the amount of overfitting and has the advantage of performing well even when there is severe overfitting.³⁴ In our study, the area under the ROC curve was used as a measure of predictive performance of the linear discriminant functions while the .632+ bootstrap method was applied to estimate the optimism or bias of this measure. The application of the .632+ method in a similar situation has been reported elsewhere.³⁵

Statistical analyses were performed using S-Plus 2000 software (MathSoft, Inc, Seattle, WA, USA).

RESULTS

The mean RNFL estimates for the 16 sectors around the optic disc in normal and glaucomatous eyes are shown in Table 1. Significant differences, after adjustment for multiple comparisons, were observed for the sectors S3, S4, S5, and S6 in the superior hemiretina and for the sectors S10, S11, S12, S13, and

S14 in the inferior hemiretina. Discriminant analysis of RNFL thickness in the 16 sectors identified the following three sectors as being the most discriminating: S13, S3, and S16. The combination of these sectors in a linear discriminant function (LDF sectoral) had an area under the ROC curve of 0.93 (Fig 2). The bootstrap estimate of bias under the ROC curve was 0.009. With specificity set at 91%, this LDF had a sensitivity of 81%. With specificity of 81%, the sensitivity was 90%.

Fourier analysis of thickness measurements in the eight sectors of each hemiretina resulted in five components (four harmonics and the DC component). The mean values of the amplitudes of the Fourier components in normal and glaucomatous eyes are shown in Table 2. Significant differences, after adjustment for multiple comparisons, were found for the amplitudes of the fundamental and DC components, in the superior as well as in the inferior hemiretina. Stepwise discriminant analysis applied to the Fourier measures resulted in a linear discriminant function (LDF Fourier) containing the following components: F_{FUN} (superior), F_{FUN} (inferior), F4 (superior), and F4 (inferior). This LDF had an area under the ROC curve of 0.93 (Fig 2), with a bootstrap estimative of bias of 0.017. With specificity set at 91%, the sensitivity was 71%. With specificity at 81%, the sensitivity was 97%. There was no significant difference between the areas under the ROC curves for the LDF sectoral and LDF Fourier ($p = 0.83$).

Table 3 shows the measures for the GDx software provided parameters, and also for the LDF from Weinreb *et al*,¹⁷ in normal and glaucomatous eyes. Significant differences were observed for all evaluated parameters, after adjustment for multiple comparisons. Table 4 shows the ROC curve areas and sensitivities (at fixed specificities) for all parameters and also for the LDF Weinreb. ROC curve areas for these measures ranged from 0.63 to 0.86 and, among them, the LDF Weinreb had the largest area under the ROC curve (Fig 2). For a specificity set at 91%, the sensitivity of the LDF Weinreb was 52%. With specificity at 80%, the sensitivity was 77%. The area under the ROC curve for this LDF was significantly inferior to the area under the ROC curve for the LDF sectoral ($p=0.033$) and LDF Fourier ($p = 0.028$).

DISCUSSION

In this study, the GDx software provided parameters showed low power to discriminate eyes with glaucomatous localised RNFL defects from healthy eyes. With a specificity of at least 90%, the sensitivity of the GDx parameters ranged from 15% to 40%. The linear discriminant function proposed by Weinreb *et al*,¹⁷ combining some of the GDx parameters, performed better than any of the single GDx parameters evaluated in this study, which confirms previous findings by several other

Table 2 Mean (SD) of the amplitudes of Fourier coefficients in glaucomatous eyes with localized nerve fibre layer defects and normal eyes

Fourier measures*	Glaucoma (n=48)	Normals (n=53)	p Value†
Superior hemiretina			
DC	57.1 (13.7)	66.1 (17.8)	0.005
F_{FUN}	12.7 (4.42)	16.4 (4.15)	<0.001
F2	2.71 (1.43)	2.71 (1.60)	0.999
F3	1.76 (0.89)	2.07 (0.97)	0.101
F4	1.02 (0.90)	0.88 (0.94)	0.446
Inferior hemiretina			
DC	56.7 (13.8)	73.6 (18.1)	<0.001
F_{FUN}	10.9 (3.83)	17.6 (3.61)	<0.001
F2	2.96 (1.49)	2.45 (1.32)	0.069
F3	1.45 (0.75)	1.75 (0.76)	0.054
F4	0.94 (0.68)	0.96 (0.75)	0.941

*DC = scaling factor corresponding to the overall level of all amplitude measures and equivalent to the mean retinal nerve fibre layer thickness measurements in this application. F_{FUN} = amplitude of the fundamental component. F2, F3, F4 = amplitudes of the second, third and fourth harmonics, respectively.

†Significant differences, after adjusting for multiple comparisons (Holm test), are in *italics*.

Table 3 Mean (SD) of GDx software provided parameters and linear discriminant function scores from Weinreb *et al*¹⁷ (LDF Weinreb), in glaucomatous eyes with localised nerve fibre layer defects and in normal eyes

	Glaucoma (n=48)	Normals (n=53)	p Value*
LDF Weinreb	-0.477 (0.823)	0.773 (0.852)	<0.001
The number	44.7 (28.9)	22.6 (17.6)	<0.001
Symmetry	1.02 (0.12)	0.93 (0.13)	<0.001
Superior ratio	1.99 (0.49)	2.27 (0.52)	0.005
Inferior ratio	1.96 (0.54)	2.51 (0.58)	<0.001
Superior/nasal	1.80 (0.36)	1.98 (0.39)	0.015
Max modulation	1.14 (0.49)	1.54 (0.51)	0.001
Ellipse modulation	2.28 (0.79)	2.84 (0.97)	0.002
Average thickness	54.8 (12.7)	65.2 (16.5)	0.001
Ellipse average	56.7 (13.2)	69.5 (17.5)	<0.001
Superior average	63.2 (15.7)	75.6 (19.9)	0.001
Inferior average	63.8 (15.6)	85.2 (19.7)	<0.001
Superior integral	0.180 (0.045)	0.215 (0.059)	0.001

*Significant differences, after adjusting for multiple comparisons (Holm test), are in *italics*.

Table 4 Areas under the receiver operating characteristic (ROC) curves for discriminating between healthy (n=53) and glaucomatous (n=48) eyes with localised nerve fibre layer defects. Sensitivities at fixed specificities

	Area under ROC curve (SE)	Sens/Spec (spec=90%)	Sens/Spec (spec=80%)
LDF sectoral*	0.93 (0.03)	81/91	90/81
LDF Fourier†	0.93 (0.03)	71/91	97/81
LDF Weinreb‡	0.86 (0.04)	52/91	77/81
The number	0.76 (0.05)	40/91	50/81
Inferior ratio	0.78 (0.05)	40/91	58/81
Inferior average	0.82 (0.04)	35/91	75/81
Ellipse average	0.75 (0.05)	27/91	44/83
Max modulation	0.73 (0.05)	29/91	46/81
Average thickness	0.71 (0.05)	23/91	42/81
Superior average	0.71 (0.05)	25/91	29/81
Superior integral	0.69 (0.05)	19/91	46/81
Ellipse modulation	0.69 (0.05)	15/93	46/83
Symmetry	0.69 (0.05)	23/92	46/83
Superior ratio	0.64 (0.06)	29/91	33/81
Superior/nasal	0.63 (0.06)	19/91	31/81

*Linear discriminant function obtained from sectoral based analysis.

†Linear discriminant function obtained from Fourier analysis.

‡Linear discriminant function from Weinreb *et al*.¹⁷

authors.^{17 19 20} However, for a specificity of 91%, the sensitivity of this LDF was only 52%. The lower diagnostic ability of the GDx parameters may be related to the wide variability of absolute nerve fibre layer thickness measurements in healthy subjects. Like several other biological measures, the RNFL thickness has been found to vary widely in the normal population.^{37 38} This may limit the identification of glaucomatous patients who have had loss of nerve fibres, but whose absolute RNFL thickness values are still within the normal population limits. Furthermore, the fact that GDx software provided parameters are usually calculated by thicknesses averaged over a large region (for example, a quadrant) may also limit the detection of certain localised nerve fibre layer defects.

Our study showed the sectoral analysis of RNFL thickness data to be of superior performance in identifying glaucomatous patients with localised RNFL defects compared to the GDx parameters. By analysing thickness measurements in sectors comprising 22.5°, the chance of missing localised RNFL defects is reduced compared to measurements averaged over larger regions. In fact, the combination of the sectors S13, S3, and S16 in a linear discriminant function had the highest sensitivity (for specificity at 90%) among all the measures evaluated in our study. The inclusion of the sectors S13 and S3 in this discriminant function is easily understandable as these

sectors correspond to the inferior temporal and superior temporal areas, respectively, where most of the localised nerve fibre layer defects were present. The inclusion of the sector S16 in this discriminant function may be related to corneal polarisation aspects of the GDx instrument. Inadequate compensation of anterior segment birefringence may cause the total retardation measured by the GDx to reflect not only the RNFL retardance, but also the retardation of the combined cornea and corneal compensator. The magnitude of the minimum retardation around the optic disc (in the temporal and nasal parts of the peripapillary retina) is related to the magnitude of retardation arising from anterior birefringent structures.³⁹ In fact, Garway-Heath *et al*³⁹ showed that adjusting peripapillary retardation measurements by using temporal retardation values resulted in improved discrimination between normal and glaucomatous eyes. In our analysis, it is possible that the incorporation in the discriminant function of the sector S16, which corresponds to the temporal area (see Fig 1), provided a partial correction for the erroneous compensation of anterior segment birefringence in some patients. In a study comparing optic nerve imaging methods to distinguish normal eyes from those with glaucoma, Greaney and colleagues²² found that the best results from scanning laser polarimetry were obtained by using a discriminant function combining RNFL thickness sectoral data. In their discriminant

function, a sector corresponding to the temporal thickness ($0-30^\circ$) was also included, along with sectors from the inferior temporal ($270-300^\circ$), inferior nasal ($240-270^\circ$), and superior nasal ($150-180^\circ$) regions.

The application to our data of the Fourier analysis of RNFL thickness measurements resulted in a better diagnostic ability than that of any of the GDx software provided parameters. The linear discriminant function combining some of the Fourier measures resulted in a discriminative power comparable to that of the sectoral based analysis. However, although the ROC curve areas were almost identical for these two methods, the sensitivities to detect eyes with glaucomatous localised RNFL defects were different depending on the chosen level of specificity. For higher levels of specificity ($=90\%$), the sectoral based analysis performed better, whereas for moderate specificity ($=80\%$), the Fourier method proved to be superior.

For both hemiretinas, the relevant Fourier measures entered in the linear discriminant function were the fundamental and fourth harmonic amplitudes. The inclusion of the fundamental component is an expected result as the fundamental component has one hump and hence contributes the most to the shape of the RNFL in each hemiretina. The other components serve to shape the pattern provided by the fundamental, so that the composite curve matches the original RNFL pattern. It is likely that the incorporation of higher frequency components in the LDF Fourier may be involved in the identification of localised RNFL defects.

The application of Fourier analysis to the RNFL polarimetry data was first reported by Essock *et al.*²³⁻²⁷ These authors found a sensitivity and specificity of 96% and 90%, respectively, in the differentiation of glaucomatous from normal subjects.²³ The present study yielded a lower level of sensitivity (71%) for a similar level of specificity. Several possible explanations may account for the differences between these studies. In the work by Essock *et al.*,²³ a fundamentally different method of combination of Fourier measures was used, including measures based on the amplitude of the fundamental component, sum of the amplitudes of the Fourier coefficients, and also two asymmetry measures, one to reflect superior-inferior asymmetry within an eye and another to reflect asymmetry between the eyes of the same individual. Different population characteristics may also be related to the different results. Our study only included glaucomatous patients with localised nerve fibre layer defects. This type of defect is most often found in the early stages of the disease. Hence, one of the reasons for the discrepancies in the results of the two studies may be related to differences in severity of glaucoma between patient populations. In fact, in the study by Essock *et al.*,²³ the average visual field MD of glaucomatous patients was -8.9 dB, considerably higher than the average MD of the glaucomatous patients included in our study (-5.32 dB). The incorporation of Fourier measures into a linear discriminant function was also reported by Essock and colleagues.^{26, 27} They found an area under the ROC curve of 0.928 for the discrimination of glaucomatous patients with early visual field defects (average MD $= -4.0$ dB) from normal subjects, a finding very similar to ours. In short, all these studies seem to confirm the potential of the Fourier analysis of polarimetry data to improve detection of glaucoma compared to standard GDx parameters.

Other attempts to improve detection of localised and diffuse NFL loss with scanning laser polarimetry have been tried. Sinai *et al.*⁴⁰ developed an analysis method in which diffuse loss was defined as a reduction in the peak to trough amplitude of the double hump RNFL pattern, and localised loss was defined as a lowering of the correlation of thickness values between local regions shown previously to have good correlation in normal individuals. Their method had a sensitivity and specificity of 94% and 91%, respectively, to discriminate glaucoma patients from normal subjects.

Although different methods of analysing thickness measurements from SLP result in an improved detection of eyes

with glaucomatous localised RNFL defects compared to the GDx parameters, a persistent source of error may be related to the erroneous compensation of anterior segment birefringence.⁴¹ The RNFL is not the only birefringent structure in the eye. The cornea and Henle fibre layer of the macula, and to a lesser extent the lens, are also birefringent. To address anterior segment birefringence, the GDx employs a fixed corneal compensator that assumes that all individuals have a slow axis of corneal birefringence 15 degrees nasally downward with a magnitude of retardance of 60 nm. However, there is a wide variation both in the axis and in the magnitude of corneal birefringence in normal and glaucomatous individuals.⁴²⁻⁴⁴ An improvement of the scanning laser polarimetry technology consisting of the variable compensation of anterior segment birefringence has recently been described.⁴⁵ The diagnostic abilities of the GDx software provided parameters and of the other methods employed in this study to detect localised nerve fibre layer defects remain to be investigated in the setting of SLP with variable corneal compensation.

The use of a structural inclusion criterion (localised RNFL defect) in a study evaluating a structure based test (scanning laser polarimetry) may lead to a bias or overoptimism in the diagnostic ability of this test.⁴⁶ Ideally, functional tests should be used as inclusion criteria when evaluating diagnostic precision of structural tests. However, the primary purpose of this study was to compare the relative abilities of several algorithms to evaluate RNFL polarimetry data in order to discriminate eyes with localised nerve fibre layer defects from normal eyes. In this regard, the Fourier based LDF and sectoral based LDF were clearly superior to the GDx software provided parameters. Furthermore, previous studies have found that sectoral based methods and Fourier analysis also performed better than GDx software provided parameters in diagnosing glaucomatous eyes defined strictly by means of visual field results.^{22, 23}

In conclusion, both the sectoral based analysis and the Fourier analysis of RNFL polarimetry data resulted in an improved detection of eyes with glaucomatous localised nerve fibre layer defects compared to the GDx software provided parameters. Further studies using larger samples and different populations are required to develop and validate standard methods to be applied in the clinical practice of diagnosis and follow up of glaucoma.

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