Retinal nerve fiber layer measured by Heidelberg retina tomograph and nerve fiber analyzer

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PURPOSE. *To compare retinal nerve fiber layer (RNFL) thickness measured by Heidelberg retina tomograph (HRT) and nerve fiber analyzer (GDx).*
METHODS. *Twenty eyes of 20 consecutive healthy subjects were recruited for this study. Each subject had a normal visual field and a normal optic nerve head, which was assessed by slit-lamp biomicroscopy using a 90° lens. Using the HRT and GDx, RNFL measurement was calculated as for software vs 2.01 and vs 1.0.14, respectively. Retinal nerve fiber layer thickness was evaluated for the entire annulus surface every 5° degrees. RNFL was assessed by HRT and GDx. HRT RNFL measurement was calculated at 0 μm from the edge, while GDx RNFL measurement at 1.75 disc diameter as for software. The difference between the highest points and the deepest points was calculated and compared. Furthermore, because of the possibility of different scales in the two systems, the following ratio was calculated: superior/inferior, superior/temporal, superior/nasal, inferior/temporal, and inferior/nasal.*
RESULTS. *When the entire RNFL thickness was considered, a significant (p<0.001) difference was found between the HRT and GDx measurements. A difference of 200 μm was found between the highest and the deepest HRT points while a difference of 40 μm was found between the highest and the deepest GDx points.*
CONCLUSIONS. *HRT and GDx RNFL measurements were statistically different in each sector. However, ratio parameters showed no difference between the obtained values except for superior/temporal ratio and inferior/temporal ratio. (Eur J Ophthalmol 2005; 15: 246-54)*

KEY WORDS. *Retinal nerve fiber layer (RNFL), RNFL thickness, RNFL height, Confocal scanning laser, Polarimeter, Glaucoma*

INTRODUCTION

Ganglion cells from the entire retina are directed towards the scleral canal where they change their direction and create the optic nerve. Axonal glaucoma loss can be detected both by the optic nerve head assessment and by retinal nerve fiber layer (RNFL) evaluation (1-4). Detection of the earliest structural change in peripapillary retinal nerve fibers might facilitate the diagnosis of glaucoma and improve the monitoring of progressive glaucomatous damage. Instead of evaluating all the ganglion cell fibers in the scleral canal where they are crowded, some authors have proposed over the last 15 years to evaluate the RNFL around the optic disc either by red free photography or ophthalmoscopy (5, 6). Over the last 10

years new computerized systems have tried to assess the RNFL.

Using confocal scanning lasers such as Heidelberg Retina Tomograph (HRT, Heidelberg Eng, Heidelberg, Germany), a significant difference in RNFL height (RNFLH) between normal and glaucomatous patients has been found (4, 7).

However, this technique is based on the capacity of the system to find a reference plane 50 µm below the retinal surface at about -7 degrees temporally. The reference plane is theoretically located within the papillomacular bundle, which is the least involved part in glaucomatous damage (8). However, the position of this plane can change from one subject to another and the glaucoma RNFL loss may affect the analysis.

The scanning laser that uses a polarized light such as the GDx (Laser Diagnostic Technologies, Inc., San Diego, CA) should be able to quantify the RNFL thickness (RNFLT) around the optic nerve head by measuring the retardation of the polarized light when it passes through the ganglion cell axon microtubuli (9,10).

This technique should thus be able to assess the thickness of the microtubuli of ganglion cell layer and then to convert the results into pseudo-µm (or µm). The system converts the results in µm or pseudo-µm based on a calibration study in which 1 retardation degree corresponded to approximately 7.4 µm (11).

Thus, HRT and GDx are able to measure peripapillary RNFL but using different methods; the results of their measurements have been called RNFLH or RNFLT, respectively. Furthermore, these two systems have different location to assess RNFL: the former assesses RNFLH just around ONH on the outer edge of optic discs using a reference plane, the latter assesses RNFLT around the optic disc from 1.1 disc diameter to 2.5 disc diameters. The aim of this study was to compare quantitatively and qualitatively RNFL measurements analyzed by two different and independent tools, such as the Heidelberg Retina Tomograph and the GDx, to better understand what they are measuring.

PATIENTS AND METHODS

Twenty eyes of 20 healthy subjects were recruited for this study. Each subject came to the hospital because of a family history of glaucoma or ocular hypertension, but they were classified as normal because of the lack of any pathologic clinical signs.

Subjects had a normal visual field and a normal optic nerve head, which was assessed by slit-lamp biomicroscopy using a 90-diopter lens by one observer (A.M.). The mean age of the 20 subjects was 67.5±6.2 years (mean±standard deviation [SD]) and the refractive error was -1.24±0.9 diopters, ranging from -5 to +2.5 diopters.

Visual field test was performed by Octopus 1-2-3, program Gd1X (Interzeag AG, Schlieren, CH), and was considered normal when the visual field was reliable and no defects were present (mean defect and within normal limits corrected loss variance). Reliable visual field was considered only when false-negative and false-positive responses were less than 30% and fixation losses less than 20%. Analyzing the visual field of all the patients, the mean sensitivity was 32.02±3.1 dB, the mean defect was 1.54±1.1 dB, and the corrected loss variance was 1.4±3 dB.

At stereoscopic slit-lamp biomicroscopy, optic nerve head was considered normal when no neuroretinal rim notch, no disc hemorrhages, and/or no cup/disc diameter ratio asymmetry unexplained by a difference in disc area size between the two eyes was present. The mean disc area was 2.4±0.2 mm^2 calculated by HRT.

After completion of the tests, only one eye from each subject was randomly selected. RNFL was assessed using the HRT and GDx software versions 2.01 and 1.0.14, respectively, by one observer (M.I.).

RNFLT assessed by GDx

The GDx is a scanning laser polarimeter, which uses a 780 nm polarized light source and can quantify the RNFLT or the nerve fiber thickness by measuring the retardation of the reflected light (12). Its reproducibility has been shown and the actual software is able also to ignore the retinal vessel when assessing the RNFLT (13-16).

The obtained RNFLT measurements are calculated in retardation degree which is the amount of shift phase of polarized light after having passed through the RNFL and being reflected back from a deeper layer, or in µm or pseudo-µm (11).

To measure the RNFLT the user has to position a 10 pixel green ellipse which is usually at 1.75 disc diameter (DD) as for software GDx version 1.0.14. The

map consisted of 256 x 256 pixels and the value of each pixel represented the amount of retardation at a particular location.

The mean of three images was used to calculate all RNFL measurements. Only good quality images were selected for analysis as for software version 1.0.14. Good quality was defined on the basis of image intensity, image vignetting, image even illumination, and contrast.

The average point value had to be over 96.

Then retardation information was obtained for a 10-pixel width circle concentric with the disc margin. Using the standard circle, which appears as a 10 pixel green circle on the GDx display, the system assessed the RNFLT at 1.75 DD from the outer edge of the optic nerve head.

It is possible to modulate the position of the analyzed circle at various distances from the optic disc edge ranging from 1.1 DD to 2.5 DD, with 0.1 DD stepwise. All RNFL values were obtained in integral of retardation degree and automatically changed in μm as for software.

RNFLH assessed by HRT

The optic disc of each eye studied was analyzed by Heidelberg Retina Tomograph, version 2.01. Using this confocal scanning laser, which used a diode laser at 670 nm as a light source, we obtained a series of 32 confocal images, each 256 x 256 pixels.

The field of each image was 10 degrees. Three 10° field images were obtained for each eye. The computer converted the 32 confocal images to a single topographic image in approximately 90 seconds.

The mean of the three topographic image height measurements was used.

After drawing a line on the outer edge of the ONH using the mouse and observing an ONH slide to identify better the Elschnig ring, the program measured 12 predefined parameters.

These parameters, which were extracted automatically from the ONH topography image, were calculated using the height of the retinal surface at the papillo-macular bundle as reference plane (as per software version 2.01) (17-21).

As for the single parameters, RNFLH was measured using the standard reference plane that was placed between 350° and 356° at 50 μm posterior and parallel to the retinal surface.

The RNFLH measurement values are considered negative when the points moved away from the reference plane (to the vitreous) and positive when they were closer to the sclera by the HRT system.

In this way the well described peaks in the superior and inferior sectors (double hump) are two deeps. Thus all the RNFLH values were multiplied for (-1).

Furthermore, because GDx measurements were in μm, all the HRT measurements, which were in mm, were multiplied for (1000). Thus all the HRT measurements were corrected as follows: final HRT values = -1000 * original HRT values.

The value -0.174 mm between 0 and 5 degrees was corrected to 174 μm. In this way HRT RNFLH values had theoretically the same unit and sign of GDx measurements.

RNFL analysis

The 360° circumference was divided into 72 segments measuring 5° each both for HRT and GDx, but HRT RNFLH was assessed at 0 μm from the edge, while GDx RNFLT at 1.75 DD (22).

The difference between the peakest points and the deepest points was calculated and compared and also the following ratio was calculated: superior/inferior, superior/temporal, superior/nasal, inferior/temporal, and inferior/nasal. The sectors were calculated on the basis of the following:

- GDx program (superior sector between 25° and 145°, nasal sector between 150° and 210°, inferior sector between 215° and 335°, and temporal one between 340° and 20°) (12).

- Jonas et al's studies (superior sector from 25° to 125°, nasal sector from 130° to 230°, inferior sector from 235° to 335°, and temporal sector from 340° to 0° to 20°) (23).

A descriptive analysis of all the data was calculated. When the distribution of the data was normal, Student's t-test and Pearson's r correlation were used; when the data were not normally distributed, Mann-Whitney test and Spearman correlation were used.

To evaluate whether there was a statistical agreement between HRT and GDx measurements, Bland-Altman test was used. A p value ≤ 0.05 was considered to be statistically significant.
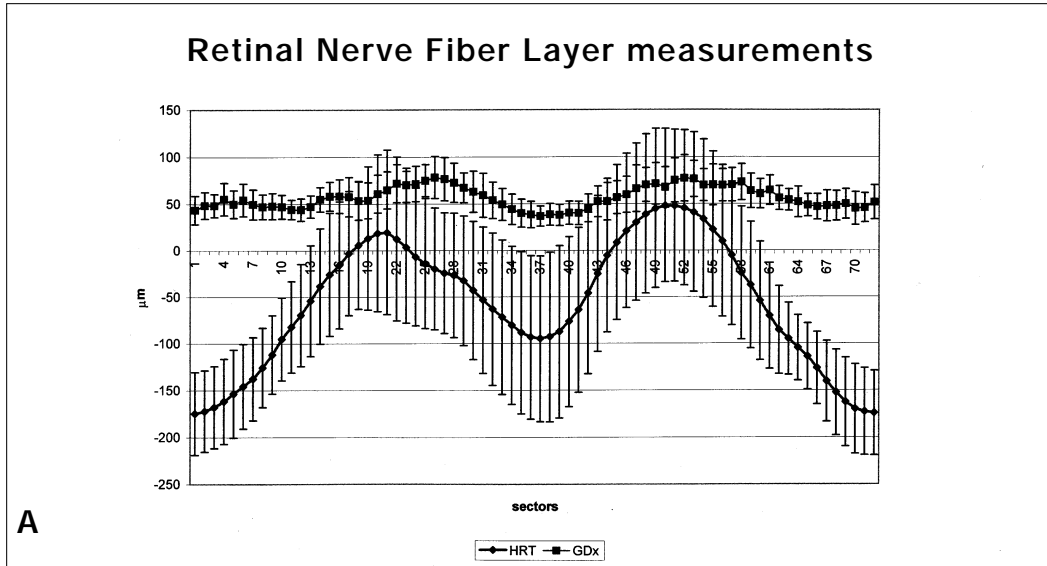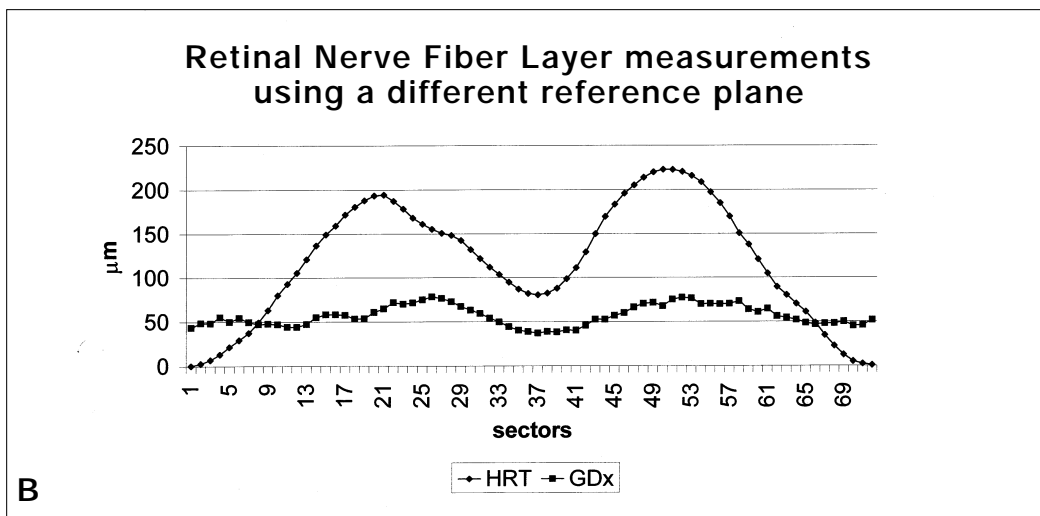
Fig. 1 - (A) *Retinal nerve fiber layer (RNFL) measured by Heidelberg Retina Tomograph (HRT) and nerve fiber analyzer (GDx).* **(B)** *RNFL measured by GDx and HRT with a different reference plane.*

RESULTS

When the entire RNFL was considered, a significant ($p<0.001$) difference was found between the HRT and GDx measurements (-56.51 ± 48.73 µm and 56.55 ± 10.1 µm, respectively).

A difference of 200 µm was found between the highest and the deepest HRT points vs a difference of 40 µm between the highest and the deepest GDx points (Tab. I, Fig. 1).

Furthermore, HRT and GDx calculated both the highest and the deepest points of the RNFL: different positions of the highest and deepest RNFL points were found between the two techniques.

A significant ($p<0.001$) difference was found for all the 5° segments calculated by using HRT and GDX (Tab. II). When the four sectors were calculated using the two different methods, significant ($p<0.001$) differences were found between HRT and GDx sectors (Tab. III).

No difference existed for the ratio parameters between the two systems except for superior/temporal and inferior/temporal, which were significantly ($p < 0.001$) different.

TABLE I - LOCATION OF THE HIGHEST AND DEEPEST RNFL POINTS

Chosen	Degree	Corresponding	
Highest HRT		**HRT values**	**GDx values**
	245	47.97	67.55
	250	47.77	75.00
	255	45.46	77.25
	240	44.94	71.45
Deepest HRT			
	5	-172.09	48.50
	350	-172.72	45.80
	355	-174.28	51.55
	0	-174.64	43.60
		Difference	**p<0.001**
Highest GDx		**GDx values**	**HRT values**
	125	78.25	-19.71
	255	77.25	45.46
	260	76.50	40.89
	130	76.45	-24.33
Deepest GDx			
	185	38.70	-92.80
	175	38.50	-93.28
	190	38.05	-87.26
	180	36.85	-94.69
		Difference	**p<0.001**

RNFL = Retinal nerve fiber layer; HRT = Heidelberg Retina Tomograph; GDx = Nerve fiber analyzer

Using Bland-Altman method, no agreement was found between the RNFL measurements obtained from the two systems. A strong correlation was found between the two techniques (r=0.98, p<0.001).

DISCUSSION

Histologically, Varma et al. found that NFLT ranged from 405 µm to 316 µm (24), while Dichtl et al showed RNFLT ranging from 400 µm to 131 µm (25), but both studies showed that inferior and superior sector were the thickest.

Using a nerve fiber analyzer, Weinreb and coworkers found that superior and inferior sectors had the highest retardation and no difference for retardation was found between nasal and temporal sectors. They also reported a retardation decrease with increasing distance from the optic nerve head margin reflecting a thinning of the RNFL peripherally (26). Iester and Mer-

moud showed similar results; they found a significant difference between the measurements just around the ONH and those at 2.0 disc diameter. No significant difference was found between the RNFLT at 1.1 DD and at 1.75 DD, whose change was about 10 µm (22).

In this study, the mean HRT RNFLH was -56.51±48.73 µm while the mean GDx RNFLT was 56.55±10.1 µm, and when the HRT results were compared to the GDx values, RNFL measurements were significantly different. The difference was greater than 60 to 70 µm in each quadrant, which was greater than 10 µm found in our previous study (22). Thus the different position of RNFL measurements between the two considered systems was not considered a bias for the study. The different magnitude between the two techniques was well shown when the HRT reference plane was moved under the deepest point of the double hump curve and was parallel to the retinal surface as for software (Fig. 1b): HRT RNFL was higher than GDx value, GDx showed thinner RNFLT values. Furthermore, the difference be-

TABLE II - RNFL MEASUREMENT VALUES BY HRT AND GDX

Degree	HRT Mean	SD	GDx Mean	SD	Degree	HRT Mean	SD	GDx Mean	SD
0-5	-174.64	44.13	43.60	15.16	180–185	-94.69	88.68	36.85	10.84
5–10	-172.09	43.31	48.50	14.26	185–190	-92.80	90.81	38.70	11.67
10–15	-167.98	43.67	48.30	12.38	190–195	-87.26	92.37	38.05	11.28
15–20	-161.73	45.39	55.30	17.37	195–200	-76.42	91.20	40.40	12.55
20–25	-153.53	46.93	49.90	14.85	200–205	-63.87	88.51	40.10	12.62
25–30	-145.70	45.13	54.45	17.39	205–210	-45.92	86.76	45.45	14.86
30–35	-137.64	44.37	49.60	16.01	210–215	-24.99	83.84	52.45	16.52
35–40	-125.60	42.46	47.15	13.22	215–220	-5.44	82.53	52.45	19.95
40–45	-111.77	42.02	47.75	13.97	220–225	8.48	83.13	56.80	17.55
45–50	-95.09	44.37	47.10	12.77	225–230	20.92	82.78	59.80	19.31
50–55	-82.16	49.07	44.15	10.38	230–235	30.17	84.15	66.20	24.79
55–60	-69.47	54.94	43.95	12.02	235–240	38.88	85.23	70.30	18.11
60–65	-54.06	59.71	47.20	11.87	240–245	44.94	85.08	71.45	22.15
65–70	-38.33	62.05	54.90	12.97	245–250	47.97	81.97	67.55	21.56
70–75	-25.82	66.15	58.10	15.33	250–255	47.77	81.15	75.00	23.45
75–80	-15.71	68.18	58.20	17.98	255–260	45.46	82.89	77.25	24.66
80–85	-3.06	66.77	57.55	20.92	260–265	40.89	85.25	76.50	19.26
85–90	5.67	68.68	53.35	20.33	265–270	33.74	84.80	69.95	17.06
90–95	12.98	76.81	53.60	19.21	270–275	22.39	83.28	70.15	21.79
95–100	18.50	84.04	60.70	20.14	275–280	10.05	81.31	69.75	17.19
100–105	19.39	88.16	64.65	19.76	280–285	-5.24	75.33	70.25	17.79
105–110	12.29	87.86	71.80	20.29	285–290	-24.40	71.44	73.10	19.26
110–115	3.47	81.44	70.05	18.35	290–295	-37.32	68.06	63.80	18.36
115–120	-6.95	73.94	71.35	18.78	295–300	-54.25	63.74	60.70	15.60
120–125	-13.98	69.92	74.90	17.18	300–305	-70.42	56.83	64.50	15.77
125–130	-19.71	65.47	78.25	22.37	305–310	-85.61	47.15	56.10	12.87
130–135	-24.33	64.91	76.45	23.10	310–315	-94.97	38.64	54.20	11.55
135–140	-26.74	66.87	72.60	20.85	315-320	-104.75	34.94	51.95	16.54
140–145	-32.49	69.71	67.20	15.83	320–325	-113.95	35.39	48.45	12.00
145–150	-42.96	74.25	63.05	21.95	325–330	-126.39	38.57	46.75	13.65
150–155	-53.27	78.69	59.25	23.60	330–335	-140.48	42.93	47.75	16.63
155–160	-63.06	81.77	53.85	19.51	335–340	-152.40	45.52	47.90	15.99
160–165	-71.54	82.86	49.45	17.12	340–345	-162.58	47.23	49.95	15.98
165–170	-80.35	84.73	44.20	16.36	345–350	-170.00	47.60	45.05	17.63
170–175	88.28	86.94	40.00	15.01	350–355	-172.72	46.21	45.80	15.60
175–180	-93.28	87.53	38.50	14.14	355–0	-174.28	44.82	51.55	18.31

All p<0.001.
RNFL =Retinal nerve fiber layer; HRT = Heidelberg Retina Tomograph; GDx = Nerve fiber analyzer

Possible explanation of different RNFL assessments

HRT RNFLH = GDx RNFLT + CONNECTIVE TISSUE

A

Retinal Nerve Fiber Layer measurements obtained by using HRT together GDx values
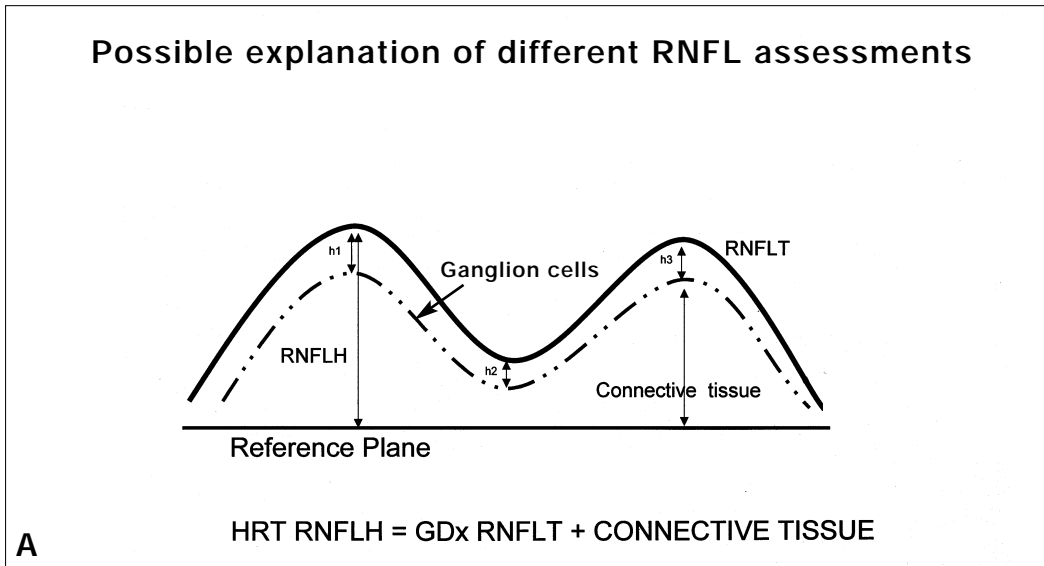
B

Fig. 2 - (A) *Retinal nerve fiber layer (RNFL) assessed by Heidelberg Retina Tomograph (HRT) and nerve fiber analyzer (GDx). HRT RNFL height (RNFLH) is measured by using a reference plane. GDx RNFL thickness (RNFLT) has been drawn below the HRT curve. HRT could assess ganglion cell layer and the connective tissue, while GDx could evaluate just fiber layer. For this reason HRT had higher values. The HRT RNFLH had a great difference in thickness between the peak and the deepest points; GDx RNFLT had mild changes in the thickness all around the double hump (h1 = h3 > h2) if RNFL thickness was considered.* **(B)** *HRT and GDx RNFL calculated using the results of this study with this theory.*
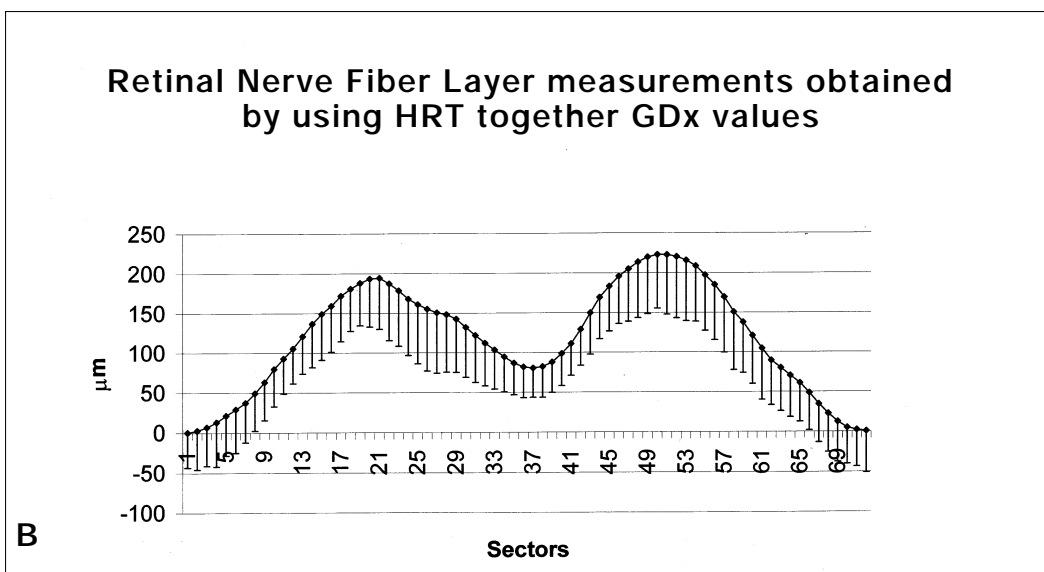
tween the highest point and the deepest one was calculated and the HRT measurements had a difference of 200 μm vs GDx of 40 μm. Some possible explanations could be the HRT reference plane position or the GDx retardation unit that should not be converted in μm, or some unknown technological error.

An interesting interpretation of the results that tries to use both RNFL measures could be that RNFLH assessed by HRT is the result of RNFLT, which is assessed by GDx, plus the connective tissue. For this reason HRT RNFLH is thicker than GDx, which is able

to assess just ganglion cell layer without measuring connective tissue and the other retinal layers (Fig. 2).

When the peakest and deepest points were considered, HRT and GDx showed a different location. In particular for HRT the highest points were located at the inferior sector and the deepest ones were temporal; when GDx was used, the deepest points were nasal and the highest ones were both in the superior and inferior sector. This mild shift could be also due to the cornea polarization; indeed it has been shown that cornea polarization can influence RNFL results

TABLE III - RNFL SECTOR MEASUREMENTS

	HRT values Superior sector	GDx values	HRT values Inferior sector	GDx values
GDx sectors	-35.24	60.17 *	-20.27	63.45 *
Standard sectors	-41.56	58.61 *	-32.29	63.52 *
	Nasal sector		**Temporal sector**	
GDx sectors	-65.61	45.77 *	-164.33	49.27 *
Standard sectors	-48.01	51.70 *	-167.73	49.16 *
	Sector ratio parameters			
	HRT values Superior/inferior	GDx values	HRT values Nasal/temporal	GDx values
GDx sectors	0.91	0.96 n.s.	0.37	1.11 n.s.
Standard sectors	1.61	0.95 n.s.	0.26	0.98 n.s.
	Superior/nasal		**Inferior/nasal**	
GDx sectors	0.73	1.35 n.s.	1.87	1.42 n.s.
Standard sectors	0.50	1.16 n.s.	0.30	1.23 n.s.
	Superior/temporal		**Inferior/temporal**	
GDxsectors	0.20	1.30 *	0.12	1.35 *
Standard sectors	0.24	1.27 *	0.19	1.34 *

*p<0.001, RNFL = Retinal nerve fiber layer; HRT = Heidelberg Retina Tomograph; GDx = Nerve fiber analyzer; n.s. = Not significant

(27). The passive cornea compensator inside the system (called GDx FCC [GDx fixed corneal compensation]) we used might not be sufficient to compensate the birefringence of the cornea of all the subjects. In particular it has been shown that 20% of the population does not have a standard corneal polarization and the GDx FCC is not able to compensate. To avoid this possible error a new polarimeter (GDx Access VCC [GDx variable corneal compensation], Laser Diagnostic Technologies, Inc., San Diego, CA) has been introduced. This should be able to avoid a possible peak shift evaluating first the macular polarization and then comparing the results to the ONH RNFLT values obtained (27).

When sector measurements were considered, significant differences were found for all four parameters between HRT and GDx. These differences were present when the parameters were calculated both using the GDx software sectors and using Jonas et al's sectors. When the sector ratio parameters were calculated no difference was found between HRT and GDx sector ratio parameters except for superior/temporal and inferior/temporal parameter (Tab. III). These

significant differences could be related to the presence of vessels that HRT is not able to compensate or the reference plane position.

Despite the limitations of this study, which are the statistical power, the impossibility to evaluate histologically the measurements obtained, the presence of vessels that HRT is not able to compensate, or the reference plane position, the fixed corneal polarization, HRT and GDx showed significant correlation. Even if the two different systems are measuring the same cells by using different techniques and anatomic locations, significant differences were found between HRT and GDx RNFL measurements and no statistical agreement was found between the two techniques. This suggests that HRT and GDx are measuring RNFL in a different manner.

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