Acta Ophthalmologica

Glaucoma diagnostic capacity of optic nerve head haemoglobin measures compared with spectral domain OCT and HRT III confocal tomography

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ABSTRACT.

Purpose: **The computer program LAGUNA ONHE determines optic nerve head haemoglobin (ONH Hb) on retinal photographs based on detecting colour differences. This study compares the diagnostic capacity of Laguna ONhE with that of spectral domain optical coherence tomography (OCT) and confocal tomography (HRT III).**

Methods: **In a prospective, observational, cross-sectional study, glaucomatous (n = 66) and healthy (n = 52) eyes were examined by Spectralis OCT, HRT III and Laguna ONhE. The following Laguna ONhE variables were determined: ONH Hb across the vertical disc diameter (8&20 Hb), estimated cup–disc ratio (C/D) and the glaucoma discriminant function (GDF), which combines the slope of Hb amount with the mean in 8&20 Hb. The three diagnostic methods were compared by calculating areas under ROC curves (AUCs). Correlations between variables were assessed through Spearman's rho coefficient.**

Results: **Areas under ROC curves (AUCs) were 0.785 (95% CI: 0.700–0.863) for GDF, 0.807 (95% CI: 0.730–0.883) for OCT retinal nerve fibre layer thickness (OCT-RNFL) and 0.714 (95% CI: 0.618–0.810) and 0.721 (95% CI: 0.628–0.815) for the HRT III variable GPS (glaucoma probability score) and vertical C/D ratio, respectively. Glaucoma discriminant function (GDF) was correlated with OCT-RNFL (0.587, p 0.001; 0.507, p 0.045; and −0.119, p 0.713 for mild, moderate and advanced glaucoma, respectively), mostly so with inferior OCT-RNFL (0.622; p < 0.001). Glaucoma discriminant function (GDF)-HRT III correlations were lower (rim area 0.471, p < 0.0001; rim/disc area 0.426, p < 0.0001; vertical C/D −0.413, p < 0.0001; GPS −0.408, p < 0.0001; rim volume 0.341, p < 0.0001).**

Conclusion: **Similar diagnostic power was observed for Laguna ONhE, Spectralis OCT and HRT III.**

Key words: glaucoma – haemoglobin – HRT – OCT – optic nerve perfusion – perimetry – retinography

Acta Ophthalmol. 2016: 94: 697–704

doi: 10.1111/aos.13050

Introduction

High intraocular pressure (IOP) is one of the most important and the only treatable risk factor identified so far for the development of glaucoma. Among other factors implicated both in the development and in the progression of this disease are blood flow dysregulation and an abnormal haemoglobin (Hb) content of the optic nerve head (ONH) (Flammer et al. 2002; Moore et al. 2008; Gonzalez de la Rosa et al. 2013). Despite the findings of large clinical trials (Gordon et al. 2002, 2007; Leske et al. 2003; Chauhan et al. 2008) indicating that vascular diseases are not proved risk factors of glaucoma onset or progression, some patients with glaucoma show vascular alterations such as vasospasm, systemic hypotension, angiographic vascular perfusion defects and modified blood flow, which could lead to a decreased blood supply to the ONH and retina (Tezel et al. 2010).

Our group has recently proposed the use of software to interpret retinal photographs in terms of the amount of Hb present in the optic nerve disc (ONH Hb) (Gonzalez de la Rosa et al. 2013). This computer program, LAGUNA ONHE (optic nerve head haemoglobin), can be used to colorimetrically analyse ONH photographs to estimate the amount of Hb present at the disc, and this is relatively simple and inexpensive, as only a non-mydriatic fundus

camera is needed. Our studies have shown its high reproducibility, good glaucoma diagnostic capacity and good correlation with most morphologic and functional indices of primary open-angle glaucoma (POAG) (Gonzalez de la Rosa et al. 2013; Mendez-Hernandez et al. 2014). The method also shows excellent intraobserver, interobserver, within-session and between-session reproducibility in pseudophakic patients with and without glaucoma along with high interanalysis reproducibility when performed by a masked examiner (Mendez-Hernandez et al. 2014).

This study was designed to compare the capacity of the Laguna ONhE method to diagnose glaucoma with that of optical coherence tomography (OCT) and heidelberg retina tomograph (HRT).

Patients and Methods

Subjects

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Informed consent was obtained from each participant.

The study sample was comprised of 118 eyes of 66 patients with POAG and 52 healthy subjects. Only one eye was chosen randomly in each participant.

Patients with POAG were screened by an independent observer who examined the subjects including visual field tests and funduscopy. Patients with POAG were consecutively recruited from the glaucoma department of our hospital. Inclusion criteria were focal (localized notching) or diffuse neuroretinal rim narrowing, concentric enlargement of the optic cup, or both, and/or reproducible abnormal visual field test results. The MD visual field index (OCTOPUS G1-program) was used to define the patient subgroups: mild (MD $\leq$ 6 dB), moderate (MD > 6–12 dB) and advanced (MD $\geq$ 12 dB) glaucoma.

Patients with a spherical equivalent greater than 5D or 3D of astigmatism, significant lens opacity, or with an oblique or tilted ONH, a narrow angle, suspected ocular hypertension (OHT) or secondary glaucoma were excluded.

The healthy age-matched subjects were recruited among persons undergoing a routine eye test at our hospital, the relatives of the patients enroled and hospital staff. The inclusion criteria for this study group were as follows: IOP <18 mmHg measured by Goldmann applanation tonometry (GAT); an intact neuroretinal rim with no signs of haemorrhage, notches, excessive cupping defined as concentric enlargement of the optic cup (Jonas et al. 1993; Jonas & Dichtl 1995) or retinal nerve fibre layer (RNFL) defects; normal white-on-white automated perimetry variables (mean deviation (MD) < 2 dB and loss variance (LV) < 7 dB; OCTOPUS G1-program).

Individuals with a history of diabetes, moderate or severe arterial hypertension (patients treated with two or more drugs), cardiovascular or haematologic diseases as well as systemic diseases or ocular diseases that could affect vision or a history of neurologic disease were excluded from the study. Age, and previous cataract and glaucoma surgery were not criteria for exclusion.

Study protocol

In an initial visit, all participants were subjected to a complete ophthalmologic examination including complete clinical history, white-on-white perimetry using the Octopus perimeter TOP G1 program (Haag-Streit AG, Bern, Switzerland), visual acuity measurement and refraction, slit lamp biomicroscopy, Goldmann applanation tonometry (GAT), gonioscopy, fundus examination and retinography (Canon non-mydriatic retinal camera CR-DGi; Canon Inc., Tokyo, Japan). All participants also underwent optical coherence tomography (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany), confocal scanning laser tomography (HRT III, Heidelberg Retina Tomograph Model 3; Heidelberg Engineering) and ONH Hb determination on the retinal photographs taken in the examination. The most relevant parameters of each diagnostic procedure, Spectralis OCT and HRT3, were selected for data analysis. Image quality of each procedure was evaluated. All Spectralis OCT and HRT3 images were acquired with a quality greater than 7/10. Optic nerve Hb contents were measured using the *Laguna ONhE* procedure as described in our previous studies (Gonzalez de la Rosa et al. 2013; Mendez-Hernandez et al. 2014).

ONH Hb measurements

Zones of the ONH with a high Hb content mainly reflect red light while areas of low Hb reflect higher proportions of green and blue light. The LAGUNA ONHE program uses mathematical algorithms for automatic component segmentation to delimit the ONH border and identify the central retinal blood vessels. Two areas of the ONH were defined: the central retinal vessels and the ONH tissue itself. The formulas were then calculated at those pixels corresponding to the vessels as a whole and for every isolated pixel of tissue. The ONH areas with high Hb content mainly reflect red light. In contrast, areas with low Hb content reflect a lower proportion of the red component compared with the green and blue light. The blue, green and red components of the picture were assessed with an image analysis program using MATLAB image processing toolbox (MathWorks Inc., Natick, MA, USA). The method whereby analyses Hb amount has been previously described (Gonzalez de la Rosa et al. 2013).

Figure 1 shows how the LAGUNA ONHE program automatically divides the image of the papilla into 24 sectors shared across three concentric rings. The outer ring mostly aligns with the neuroretinal rim, the central ring corresponds to the transition area, which may include the neuroretinal rim and cupping, and the inner rim mainly comprises the cup area. As with other imaging techniques used in glaucoma diagnosis, this alignment can be more difficult in tilted discs. On the basis of colorimetric analysis of the papilla, it is known that both in control individuals and in patients with glaucoma, Hb levels are greater in the more peripheral sectors, that is, in the outer ring. The middle ring tends to show a lower amount of Hb than the outer ring, and the innermost ring features the least amount of Hb. In other words, tissue Hb levels diminish from the disc periphery towards the centre such that the difference between peripheral and central Hb levels can be calculated. By determining colour changes in the central disc zone, the software estimates the cup and cup–disc ratio. Laguna ONhE also uses an index called the glaucoma discriminant function Glaucoma discriminant function

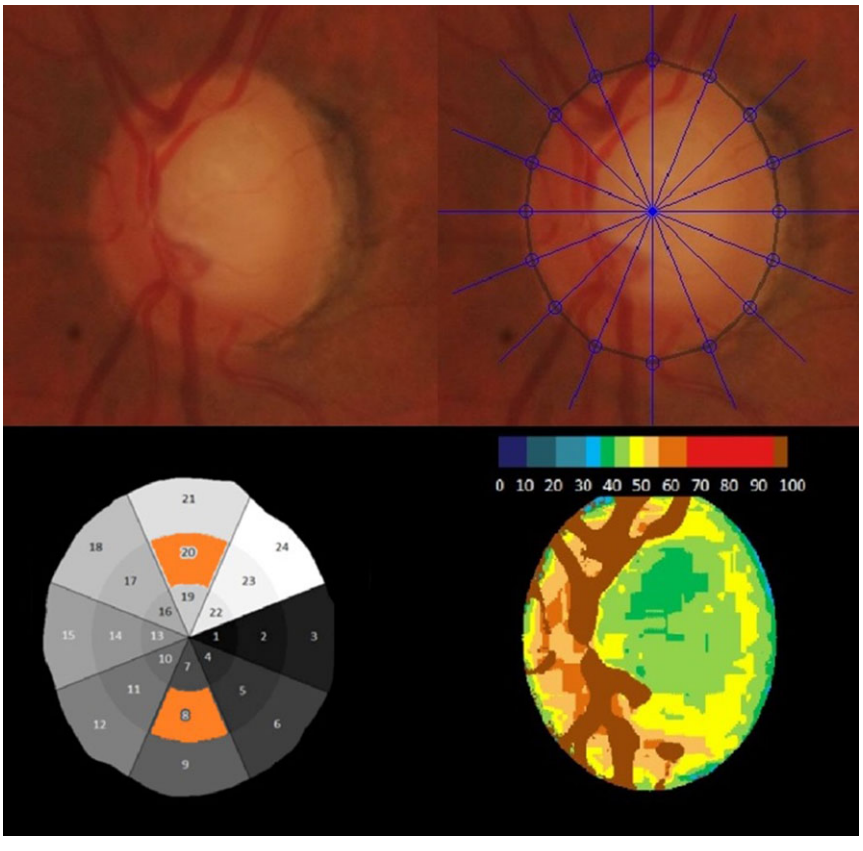

Fig. 1. Image of a glaucomatous papilla. Upper images are the colour fundus photograph of the optic disc (left) and the disc automatically divided in radial sectors (right) whereas the lower images are the disc automatically divided into eight 45-degree radial sectors and three concentric rings (left) and the corresponding pseudo-image representing the amount of haemoglobin (right). A colorimetric scale is shown at the top of the lower left image to assess the amount of haemoglobin.

(GDF), which combines the Hb slope, obtained through multiple regression analysis of Hb estimated for each sector with the mean Hb amount determined in the vertical disc diameter, in sectors 8 and 20 (8&20 Hb). ONH Hb is expressed as a percentage (%).

Glaucoma discriminant function (GDF) is expressed as a whole number from +75 to −90. It describes the probability that the optic nerve disc shows an Hb amount within the normal range (positive values) or in the range consistent with glaucoma (negative values). The relationship is therefore direct such that the further the GDF from zero in either direction, the greater the likelihood will be that the disc belongs to one or the other diagnostic groups.

Data were compared between patients with POAG and healthy subjects using the Student's *t*-test. The chi-squared test was used to compare data among the patient subgroups. It was first checked that all data were normally distributed (Kolmogorov–Smirnov test). Data are presented as means ± SD. Relationships between structural parameters were examined through Spearman correlation. Areas under receiver operating characteristic curves (AUCs) were calculated for all parameters determined in each test. To compare the AUCs obtained with the different devices, the Hanley/McNeil method was used (Hanley & McNeil 1983). All statistical tests were performed using the software package IBM SPSS (version 17.0; IBM Corp., Somers, NY, USA). Significance was set at $p < 0.05$.

Results

The study sample was comprised of 118 eyes of 118 subjects: 52 healthy control eyes and 66 eyes with POAG. All the data analysed were normally distributed. Table 1 provides the main demographic and clinical characteristics of the study subjects, and the variables determined using Spectralis OCT, HRT3 and Laguna ONhE (minimum, maximum, mean and standard deviation). This table also shows comparisons of means between the glaucoma subgroups (Student *t*-test) and levels of statistical significance (p).

There was a slight predominance of women, 68 women (57.6%) versus 50 men (42.4%). No significant differences were detected in age or gender between the study groups including the glaucoma subgroups (chi-squared test).

OCT-measured RNFL thickness (OCT-RNFL) was significantly lower in the patients with glaucoma than in controls ($p < 0.001$).

No significant differences between groups were detected for the HRT3 variables such as disc area, rim area, cup/disc area ratio or rim volume. However, the patients with glaucoma showed abnormal vertical cup/disc ratios and glaucoma probability scores (GPS) ($p < 0.0001$).

The amount of Hb observed in the vertical disc diameter (sectors 8 and 20) (8&20 Hb) was significantly lower in the patients with glaucoma ($p = 0.001$) and in all the glaucoma subgroups ($p = 0.018$ mild, $p = 0.002$ moderate, $p < 0.001$ advanced). The mean GDF value in the control group was positive. In the mild glaucoma subgroup, GDF was negative ($p = 0.001$) and acquired a more negative value with glaucoma severity ($p < 0.001$ for moderate and advanced glaucoma). Estimated C/D ratios did not differ significantly among groups.

Table 2 provides the correlations detected between GDF and the OCT, HRT3 or perimetry data adjusted by age, sex and disc size in the patients with glaucoma and glaucoma patient subgroups.

Glaucoma discriminant function (GDF) was well correlated with disc area determined by HRT3 in moderate glaucoma ($r = -0.587$; $p = 0.008$). Glaucoma discriminant function (GDF) showed greater correlation with OCT-RNFL in normal subjects ($r = 0.322$; $p = 0.024$) and in both mild and moderate glaucoma subgroups ($r = 0.587$; $p = 0.001$ and $r = 0.507$; $p = 0.045$, respectively), and also showed correlation with Octopus G1 MD in moderate glaucoma ($r = -0.609$; $p = 0.012$).

Receiver operating characteristic (ROC) curves were constructed for all the variables determined using each diagnostic procedure, and the cut-off

Table 1. Demographic/clinical characteristics and the disc and visual field variables (mean; SD) recorded in the study groups.

	Control (1) Mean; SD (range)	Mild glaucoma (2) Mean; SD (range)	Moderate glaucoma (3) Mean; SD (range)	Advanced glaucoma (4) Mean; SD (range)	POAG (2,3,4) Mean; SD (range)	*t*-test (p)
n	52	32	19	15	66	
Age (yr)	67.80; 10.13 (52–84)	70.20; 8.35 (50–82)	69.20; 1.11 (50–85)	68.67; 9.10 (50–83)	69.36; 9.13 (50–85)	1 versus 2: 0.986 1 versus 3: 0.875 1 versus 4: 0.876 1 versus POAG: 0.275
Sex, M/F (%)	27/25 (51.91/48.10)	11/21 (34.40/65.64)	10/9 (52.63/47.37)	9/6 (60.00/40.00)	23/43 (34.80/65.10)	1 versus 2: 0.226† 1 versus 3: 0.536† 1 versus 4: 0.224† 1 versus POAG: 0.916†
MD TOP G1, (dB)	0.05; 1.38 (−2.60–0.30)	3.54; 1.55 (3.02–5.96)	8.67; 1.74 (3.10–11.90)	15.53; 5.32 (7.00–26.50)	7.74; 5.57 (3.02–26.50)	1 versus 2: <0.001* 1 versus 3: <0.001* 1 versus 4: <0.001* 1 versus POAG: <0.001*
RNFL thickness OCT (μm)	92.79; 14.11 (68.00–134.00)	81.59; 18.51 (33.00–117.00)	70.05; 12.75 (52.00–92.00)	51.33; 14.00 (36.00–82.00)	71.39; 19.88 (33.00–134.00)	1 versus 2: 0.001* 1 versus 3: <0.001* 1 versus 4: <0.001* 1 versus POAG: <0.001*
Disc area HRT III (mm^2)	1.84; 0.67 (0.65–3.33)	2.02; 0.62 (0.52–3.31)	1.95; 0.52 (0.64–3.09)	2.13; 0.48 (1.58–2.98)	2.02; 0.56 (0.52–3.31)	1 versus 2: 0.227 1 versus 3: 0.509 1 versus 4, 0.124 1 versus POAG: 0.105
Rim area HRT III (mm^2)	1.19; 0.52 (0.35–3.09)	1.07; 0.44 (0.09–1.84)	1.06; 0.46 (0.17–1.85)	0.89; 0.49 (0.24–1.84)	1.15; 0.47 (0.09–3.09)	1 versus 2: 0.291 1 versus 3: 0.355 1 versus 4: 0.050 1 versus POAG: 0.076
Cup/disc area ratio HRT III	0.46; 0.94 (0.06–0.72)	0.43; 0.21 (0.05–0.95)	0.79; 1.51 (0.12–0.72)	0.55; 0.26 (0.12–0.88)	0.56; 0.83 (0.05–0.95)	1 versus 2: 0.830 1 versus 3: 0.271 1 versus 4: 0.709 1 versus POAG: 0.546
Rim/disc area ratio HRT III	0.65; 0.16 (0.30–0.94)	0.56; 0.21 (0.05–0.95)	0.56; 0.18 (0.14–0.93)	0.45; 0.26 (0.12–0.88)	0.54; 0.22 (0.05–0.95)	1 versus 2: 0.041* 1 versus 3: 0.053 1 versus 4: <0.001* 1 versus POAG: 0.002*
Rim volume HRT III (mm^3)	0.41; 0.97 (−0.25–0.70)	0.23; 0.19 (−0.18–0.65)	0.24; 0.22 (−0.15–0.94)	0.16; 0.12 (0.01–0.47)	0.22; 0.19 (−0.18–0.94)	1 versus 2: 0.288 1 versus 3: 0.428 1 versus 4: 0.305 1 versus POAG: 0.102
Vertical C/D ratio HRT III	0.42; 0.25 (0.00–0.94)	0.56; 0.24 (0.01–0.98)	0.63; 0.22 (0.05–0.91)	0.70; 0.21 (0.23–0.98)	0.61; 0.23 (0.01–0.98)	1 versus 2: 0.019* 1 versus 3: 0.003* 1 versus 4: <0.001* 1 versus POAG: <0.001*
GPS HRT III	0.42; 0.32 (0.08–0.93)	0.60; 0.31 (0.08–0.92)	0.73; 0.19 (0.23–0.93)	0.75; 0.23 (0.10–0.93)	0.67; 0.27 (0.08–0.93)	1 versus 2: 0.014* 1 versus 3: <0.001* 1 versus 4: <0.001* 1 versus POAG: <0.001*

Table 1. (Continued).

	Control (1) Mean; SD (range)	Mild glaucoma (2) Mean; SD (range)	Moderate glaucoma (3) Mean; SD (range)	Advanced glaucoma (4) Mean; SD (range)	POAG (2,3,4) Mean; SD (range)	*t*-test (p)
GDF Laguna ON_hE	11.08; 17.68 (−30.00–51.00)	−5.53; 26.23 (−69.00–37.00)	−14.05; 20.38 (−64.00–18.00)	−32.60; 24.03 (−89.00–8.00)	−14.14; 26.13 (−89.00–37.00)	1 versus 2: 0.001*
						1 versus 3: <0.001*
						1 versus 4: <0.001*
						1 versus POAG: <0.001*
Estimated C/D ratio Laguna ON_hE	0.52; 0.10 (0.27–0.73)	0.60; 0.14 (0.38–0.91)	0.65; 0.10 (0.49–0.88)	0.75; 0.11 (0.54–1.00)	0.65; 0.14 (0.38–1.00)	1 versus 2: 0.467
						1 versus 3: 0.376
						1 versus 4: 0.419
						1 versus POAG: 0.315
8&20 Hb Laguna ON_hE	66.46; 11.91 (40.46–84.70)	60.06; 11.60 (29.40–78.00)	57.36; 9.62 (32.00–72.50)	49.60; 11.07 (25.00–66.30)	56.91 11.55 (25.00–78.00)	1 versus 2: 0.018*
						1 versus 3: 0.002*
						1 versus 4: <0.001*
						1 versus POAG: 0.001*

† Chi-square test.
* difference significant at $p < 0.05$.
C/D = cup/disc, GDF = glaucoma discriminant function, GPS = glaucoma probability score, MD = mean defect, M/F = male/female, N = number of cases, POAG = primary open-angle glaucoma.

providing the best balance between sensitivity and specificity was determined in each case (Tables 3 and 4).

OCT-RFNL and GDF had the greatest AUCs. AUC for OCT-RNFL was 0.81, p = 0.001 with a sensitivity of 83.3%, a positive predictive power of 74.3%, a specificity of 63.5% and a negative predictive power of 75.0%. Regarding GDF, the AUC obtained was 0.78, $p < 0.001$, with a sensitivity of 72.7%, a positive predictive power of 76.2%, a specificity of 71.2% and a negative predictive power of 67.3%. In the advanced glaucoma group, AUCs obtained were 0.97, $p < 0.01$ for OCT-RNFL and 0.94, $p < 0.01$ for GDF.

Discussion

The role of ocular blood flow to the optic nerve in glaucoma has been a controversial field partly due to limitations in assessing ocular blood flow accurately (Hayreh 1997; Harris et al. 2001). The supply of oxygen of the ONH depends in part on various factors, such as blood flow, blood oxygen saturation and amount of circulating blood. Blood flow may be indirectly estimated from velocity measured in neighbouring arteries by Doppler or measured locally using complex methods of laser Doppler (Michelson et al. 1995) or laser speckle flowgraphy (Yaoeda et al. 2000). Blood oxygen saturation determination for the study of glaucoma has proven to be difficult (Michelson & Scibor 2006) because of instrument difficulties and contradictory results (Hardarson et al. 2009; Traustason et al. 2009). The third factor, amount of circulating blood, is what the Laguna ONhE method attempts to quantify. In a recent study, this factor was measured by OCT angiography as ONH vascularization (Jia et al. 2012). The method proposed here differs from spectrophotometric analysis of the ONH, targeted at measuring Hb oxygen saturation. Laguna ONhE tries to quantify Hb itself as an indirect measure of the volume of blood that irrigates the papilla. In clinical practice, methods that are efficient and accessible to most users are necessary. Laguna ONhE is a non-invasive simple method that is not expensive as it is performed on retinal photographs and thus only a fundus camera is needed. Many diagnostic devices such as OCT angiography are

Table 2. Correlations adjusted by age, gender and disc size between GDF (Laguna ONhE) and OCT, HRT III or perimetry variables.

			GDF					
	Control		Mild glaucoma		Moderate glaucoma		Advanced glaucoma	
	r	p	*r*	p	*r*	p	*r*	p
Octopus TOP G1								
MD	0.263	0.067	0.314	0.097	**−0.609***	**0.012**	0.194	0.546
LV	0.213	0.142	−0.096	0.620	0.236	0.378	−0.057	0.859
Spectralis OCT								
Average RNFL thickness	**0.322***	**0.024**	**0.587**[§]	**0.001**	**0.507***	**0.045**	−0.119	0.713
HRT III								
Disc area	−0.214	0.127	−0.325	0.069	**−0.587**[§]	**0.008**	−0.400	0.140
Rim area	**0.368**[§]	**0.009**	0.266	0.163	0.200	0.459	0.254	0.425
Rim volume	0.158	0.203	0.363	0.053	−0.277	0.299	0.290	0.361
Rim/disc area ratio	**0.326***	**0.022**	0.208	0.279	0.159	0.557	0.233	0.466
Cup/disc area ratio	−0.158	0.278	−0.215	0.263	0.032	0.905	−0.233	0.466
Vertical cup/disc ratio	**−0.344***	**0.016**	−0.213	0.266	0.123	0.650	−0.172	0.564
GPS	−0.181	0.214	−0.073	0.705	−0.227	0.398	−0.298	0.346

[§] Correlation at a significance level below p = 0.01.
* Correlation at a significance level below p = 0.05.
Statistical significant correlations are shown in bold values.

Table 3. AUCs for the main variables measured using the different methods.

	AUC	SE	Cut-off	S (%)	PPP (%)	Sp (%)	NPP (%)	p value	Comparison between AUCs (p)[†]
OCT: average RNFL thickness	0.81	0.04	88.50	83.3	74.3	63.5	75.0	0.001*	0.59
Laguna ONhE: GDF	0.78	0.04	2.50	72.7	76.2	71.2	67.3	<0.001*	–
HRT III: vertical cup/disc ratio	0.72	0.05	0.52	75.8	71.2	61.5	63.5	<0.001*	0.22
GPS	0.71	0.05	0.67	63.6	75.0	73.1	61.3	<0.001*	0.15
Cup/disc area ratio	0.68	0.05	0.36	62.1	71.9	69.2	59.0	0.001*	0.05

S and Sp = Indicated are the best cut-offs for sensitivity and specificity, PPP and NPP = positive predictive power and negative predictive power.
SE = standard error of the AUC.
* $p < 0.05$.
[†] Compared with Laguna ONhE-GDF AUC (Hanley/McNeil method).

Table 4. AUCs for the main variables measured using the different methods in the patient subgroups.

	Mild glaucoma		Moderate glaucoma		Advanced glaucoma	
	AUC	p	AUC	p	AUC	p
HRT III vert. cup/disc ratio	0.66	0.02*	0.76	0.01*	0.80	<0.01*
HRT III GPS	0.66	0.02*	0.76	0.01*	0.77	0.01*
HRT III cup/disc area ratio	0.63	0.05	0.71	0.01*	0.73	0.01*
OCT average RNFL thickness	0.69	0.01*	0.88	<0.01*	0.97	<0.01*
Laguna ONhE-GDF	0.68	0.01*	0.83	<0.01*	0.94	<0.01*

* $p < 0.05$.

inaccessible to many ophthalmologists. In addition, our software only requires a non-mydriatic retinography while mydriatic drops are needed to obtain high-resolution OCT angiography images. It also has been suggested that changes in ONH reflectance could help identify differences in Hb levels (Crittin & Riva 2004), but no practical methods to quantify such changes have been proposed. Further, the Laguna ONhE method appears to show a greater relationship with other morphological and functional indices for glaucoma diagnosis than the recently described OCT angiograph (Jia et al. 2014). It is known that in patients with advanced glaucoma, besides the disc showing greater cupping, it also appears pale in colour and this pallor is more pronounced as glaucoma damage advances due to the increased excavation. Our clinical experience indicates that the subjective colour of the disc may guide a diagnosis of glaucoma (Gonzalez de la Rosa et al. 2013) and may be useful in other diseases such as optic neuritis where reduced perfusion may be found in different regions from those affected in glaucoma. In multiple sclerosis, reduced perfusion is most frequently observed in the temporal rim (Bambo et al. 2013). In turn, the colour of the papilla is determined by the amount of Hb present relative to that observed on the constant white background provided by myelin. The red colour of Hb is the result of its great capacity to absorb short wavelengths and lower capacity to absorb long wavelengths of the visible light spectrum. Thus, the greater the absorption of short wavelengths, the greater the amount of Hb present. Also, by adequately selecting the wavelengths examined, the degree of tissue oxygenation can also be assessed.

The glaucoma discriminant function, GDF, was able to discriminate between healthy eyes and eyes with POAG, and differences between the two subject groups were greater for an increasing severity of glaucoma. In contrast, the estimated C/D ratio provided by the program, though higher in the patients with glaucoma, failed to differ between the two study groups. This may be attributed to the fact that Laguna ONhE provides only an estimate of this ratio (Rodriguez Una et al. 2014). C/D ratios differed significantly among the three methods tested in our study. The Laguna ONhE C/D ratio is a rough estimate because it is based on 2D photographs. HRT3 provides a more accurate C/D ratio as it performs a 3D analysis of the ONH. Interestingly, the Laguna ONhE C/D ratio was more similar to the HRT3 vertical C/D ratio than the C/D area ratio. Thus, Laguna ONhE could be better at estimating the vertical C/D ratio, which becomes more evident in advanced glaucoma. We are presently working on a new version of Laguna ONhE targeted at improving its measure of the C/D ratio (Pena-Betancor et al. 2015).

As in the present study, most investigations examining correlations between different structural tests and perimetry have mainly focused on overall indices provided by the tests compared (Lan et al. 2003; Danesh-Meyer et al. 2006; Kawano et al. 2006; Yalvac et al. 2009; Zhong et al. 2009; Kaushik et al. 2010; Cvenkel & Sket Kontestabile 2011).

In our patients with glaucoma, correlations between GDF and TOP G1 indices or the other structural variables examined were moderate. Greatest correlation was observed for OCT-RNFL, and slightly lower correlation was detected for the HRT3 variables. Correlations between GDF and OCT-RNFL values for the nasal and temporal quadrants were low while higher correlation was observed at the vertical meridian (GDF showed greatest correlation with OCT-RNFL in the inferior sector, 0.622; $p < 0.001$). This could be due to the important role played by the vertical disc quadrants in glaucoma as the GDF is based on Hb levels in these sectors (Gonzalez de la Rosa et al. 2013; Rodriguez Una et al. 2014). Nouri-Mahdavi et al. (2004) reported that the factor that best distinguished normal eyes from glaucomatous eyes was RNFL thickness in the superior and inferior quadrants. In the LAGUNA ONHE program, the vertical disc quadrants (sectors 8 and 20) are the most discriminant of glaucoma in that they show the greatest difference in Hb between healthy and glaucoma eyes (Gonzalez de la Rosa et al. 2013). This is consistent with the data in the literature, which indicates that the first fibres of the disc rim to undergo damage are those located at the superior and inferior poles such that the cup stretches vertically (Quigley & Addicks 1981). This is why Laguna ONhE uses the mean Hb value for these two sectors and the Hb gradient to construct the factor GDF (Gonzalez de la Rosa et al. 2013).

The variable featuring the largest AUC was OCT-RFNL (AUC = 0.807; $p = 0.001$; S = 83.3%; Sp = 63.5%), followed by GDF (AUC = 0.781; $p < 0.001$; S = 72.7%; Sp = 71.2%) and HRT III vertical C/D ratio (AUC = 0.721; $p < 0.001$; S = 75.8%; Sp = 61.5%). Thus, the AUCs obtained were fairly similar for the different structural diagnostic markers. These values may be classified as moderate, although 95% confidence intervals in some of the ROC curves were analysed and sensitivity and specificity values were within the medium range.

In a prior study (Gonzalez de la Rosa et al. 2013), we observed high correlation and a similar diagnostic capacity of LAGUNA ONHE to other procedures. Here we have compared the LAGUNA ONHE program with Spectralis OCT instead of OCT Cirrus and also stratified the patients with glaucoma by disease stage according to the MD value obtained. The non-mydriatic retinal camera used in this study also differs from the camera used in our earlier study. The findings of both studies indicate that Laguna ONhE offers results that are reproducible and independent of the diagnostic device used as the standard reference or POAG severity.

Our study has limitations. The high variability in normal optic disc morphology, as well as refractive errors, poor fixation and eye movements, may affect measurement accuracy. Thus, to avoid the influence of some of these factors, patients with a spherical equivalent greater than 5D or 3D of astigmatism, or with an oblique or tilted ONH, were excluded. In addition, this is a clinical-based, case–control study, so it may not be appropriate to generalize results to all patients with glaucoma.

In conclusion, Laguna ONhE shows a good diagnostic capacity and diagnostic agreement with other procedures based on morphology. The GDF index was found to correlate well with TOP-G1 perimetry indices and the most clinically meaningful Spectralis OCT and HRT3 variables. Future studies to compare colorimetry with spectrometry and retinal vessel oximetry as well as other automated software which also uses retinal photographs for glaucoma diagnosis may be necessary. This new photographic colorimetric procedure could be useful for an early structural diagnosis of glaucoma.

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Received on March 30th, 2015.
Accepted on February 4th, 2016.

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This study was supported in part by the Instituto de Salud Carlos III grant PI09/90933. Some of the contents of this study were presented at the 2013 ARVO Annual Meeting, Seattle, FL, USA, May 2013. Some of the contents of this study were described as a poster in World Glaucoma Congress, Vancouver, Canada, July 2013.

GDLR. M. has a proprietary interest in the LAGUNA ONHE program. The remaining authors declare no conflict of interest.