Discrimination Between Healthy Eyes and Those With Mild Glaucoma Damage Using Hemoglobin Measurements of the Optic Nerve Head

Livia Studart de Meneses, MD, Lorena Ribeiro Ciariini, MD, Gabriel Ayub, MD, MSc, José Paulo C. Vasconcellos, MD, PhD, and Vital Paulino Costa, MD, PhD

Measurements of optic nerve Hb concentration using a colorimetry photographic device demonstrated good accuracy in discriminating healthy eyes from eyes with mild glaucoma.

Methods: Eyes from patients with mild primary open angle glaucoma (MD > −6 dB) (n = 58) and from healthy subjects (n = 64) were selected. Retinal nerve fiber layer thickness measurements of all eyes were acquired with optical coherence tomography. Optic disc photographs were also obtained, and the images were analyzed using the Laguna ONhE software, which measures the amount of Hb in 24 sectors of the ONH. The software also calculates the Glaucoma Discriminant Function (GDF), an index that expresses the chance of the ONH being compatible with glaucoma. Areas under the receiver operating characteristic curve and sensitivities at fixed specificities of 90% and 95% of each Laguna ONhE parameter were calculated.

Results: The mean retinal nerve fiber layer thickness and vertical cup/disc ratio of the control and glaucoma groups were 90.0 ± 10.6 μm versus 66.28 ± 9.85 μm (P < 0.001) and 0.65 ± 0.09 (P < 0.001), respectively. Total Hb (66.9 ± 4.45 vs. 62.89 ± 4.89, P < 0.001) and GDF (11.57 ± 15.34 vs. −27.67 ± 20.94, P < 0.001) were significantly higher in the control group. The Hb concentration was also significantly higher in 21 of the 24 sectors in the control group compared with the glaucoma group (P < 0.05).

The GDF had the largest areas under the receiver operating characteristic curve (0.93), with 79.3% sensitivity at a fixed specificity of 95%.

Conclusion: Measurements of optic nerve Hb concentration using a colorimetry photographic device demonstrated good accuracy in discriminating healthy eyes from eyes with mild glaucoma. Further studies are needed to understand vascular factors implicated in the development of glaucoma.

Key Words: glaucoma, diagnosis, OCT, optic nerve, software

Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Downloaded from http://journals.lww.com/glaucomajournal by Hl5l9GSG7aJChu94LTdzT+99Q3Lrk1+qBgkEaDIhY
evaluate the diagnostic accuracy of the Laguna ONhE software in Brazilian patients with mild glaucomatous damage.

**METHODS**

We conducted a retrospective, case-control study. This study was performed in accordance with the Declaration of Helsinki and was approved by the University of Campinas Medical Institutional Review Board. Since it was based on a review of medical records, the informed consent was waived.

All subjects had undergone a complete ophthalmologic examination, including best corrected visual acuity, slit lamp examination, Goldmann applanation tonometry, gonioscopy, indirect ophthalmoscopy, optic disc evaluation with a 78-diopter lens, color retinography (Canon CR-1 NM Fundus Camera Mark II, Japan) and Spectral-Domain OCT (version 5.1.1.6; Cirrus, Carl Zeiss-Meditec Inc, Dublin, CA). Glaucoma patients also underwent standard achromatic perimetry (SITA 24-2, Humphrey Visual Field Analyzer; Carl Zeiss-Meditec).

To be included in the glaucoma group, all subjects were required to meet the following criteria: (1) previous diagnosis of POAG, defined as the presence of at least 2 of the following optic disc abnormalities: C/D ratio > 0.6, localized loss of neuroretinal tissue, optic disc hemorrhage, or C/D asymmetry > 0.3, (2) mild visual field damage, defined as MD > −6 dB, (3) open angle on gonioscopy, (4) best corrected visual acuity ≥ 20/40, (5) refractive error < 5 spherical diopters and < 3 cylindrical diopters, (6) no history of ocular or systemic disease or surgery that could interfere with colorimetric measurements. Exclusion criteria included: age below 18 years, secondary glaucomas, unreliable visual fields (false positive errors > 15% or fixation losses > 20%), low-quality OCTs (quality score < 6), and neurological diseases affecting the optic nerve and/or the retina.

Inclusion criteria for the control group were: (1) absence of eye disease, such as age-related macular degeneration, diabetic retinopathy, uveitis and retinal detachment; (2) absence of family history of glaucoma; (3) intraocular pressure < 21 mm Hg by Goldmann applanation tonometry; (4) open angle on gonioscopy, (4) best corrected visual acuity ≥ 20/40; (5) refractive error < 5 spherical diopters and < 3 cylindrical diopters; (6) open angle on gonioscopy; and (7) normal refractive error.

For each Laguna ONhE parameter with MedCalc (version 19.3.1: MedCalc Software Ltd, Ostend, Belgium), the statistical analysis was performed with the Statistical Package for Social Sciences—SPSS (version 22.0; IBM Corporation, Armonk, NY). Normality was assessed by the Shapiro-Wilk test. Categorical variables were compared between the 2 groups using the chi² test or the Fisher exact test, whereas continuous variables were compared using the 1-tailed unpaired t test or the Mann-Whitney U test. Receiver operating characteristic curves were built, and areas under the curve (AUC), sensitivities and specificities at fixed specificities of 90% and 95%, were calculated for each Laguna ONhE parameter with MedCalc (version 19.3.1: MedCalc Software Ltd, Ostend, Belgium). The log-rank test was used to compare AUCs obtained with different parameters. P-values < 0.05 were considered statistically significant. We used the Bonferroni correction when multiple comparisons were performed.

**RESULTS**

One hundred and thirty eyes were found to be eligible, and 122 were included in the analysis. Eight eyes (6.15%) were excluded because retinal images could not be evaluated by the Laguna ONhE software. The control group included 64 eyes and the glaucoma group included 58 eyes. The mean MD in the glaucoma group was −2.85 ± 1.9 dB, and 8 eyes (13.8%) had preperimetric glaucoma. Baseline characteristics of both groups are listed in Table 1.

There were no differences in age, sex, and race distributions between the glaucoma and control groups (P > 0.05). As expected, the glaucoma group had a thinner RNFLT (66.28 ± 9.85 vs. 90 ± 10.6 μm, P < 0.0001). Table 2 displays the Laguna ONhE parameters obtained in both groups. The glaucoma group had larger vertical cup/disc ratios (0.65 ± 0.09 vs. 0.5 ± 0.09, P < 0.0001) and cup/disc ratios area (0.44 ± 0.12 vs. 0.29 ± 0.1, P < 0.0001) than
the control group. Mean total Hb concentration was significantly lower in the glaucoma group (67.9 ± 4.45% vs. 62.89 ± 4.89%, P < 0.0001). Also, mean Hb concentrations in all sectors were lower in the glaucoma group, although the differences were not statistically significant in sectors 2, 3, 6, 12–15, 18, 21, and 24 (P > 0.002 following Bonferroni correction). Mean GDF was significantly higher in the control group (11.57 ± 15.34 vs. −27.67 ± 20.94, P < 0.0001) (Table 2).

Table 3 displays the AUCs, and the sensitivities at fixed specificities of 90% and 95% of all parameters. GDF obtained the largest AUC (0.93), and the best sensitivities at 90% (82.76%) and 95% (79.31%) specificities. Hb in Sector 8 (AUC = 0.888) and Hb total (AUC = 0.804) also showed good diagnostic accuracy. The AUC obtained with GDF was significantly larger than those obtained with Hb Total (P = 0.001) and Hb Cup (P < 0.001). The AUC obtained with Hb in Sector 8 was also significantly larger than those obtained with Hb total (P = 0.06) and Hb cup (P < 0.001). There was no statistically significant difference between the AUCs obtained with GDF and Hb sector 8 (P = 0.154) (Fig. 2).

**DISCUSSION**

Clinical evaluation and documentation of the ONH are essential for the diagnosis and monitoring of glaucoma. Optic disc photographs can be used to monitor eyes that are suspicious or have a diagnosis of glaucoma. However, its interpretation is subjective and shows great intra- and interobserver variabilities. Computerized imaging exams have emerged as an objective way to provide quantitative measurements of the ONH and RNFL, assisting ophthalmologists in the diagnosis and follow-up of glaucoma. As mentioned before, OCT offers high reproducibility and high accuracy for the diagnosis of glaucoma and has been widely used in clinical care. However, it is an expensive tool, with prices varying between USD 40,000 and USD 150,000 which may limit its use in underprivileged environments. Our study shows that Laguna ONhE, an accessible and easy-to-use technology (at a cost of USD 1 per image analyzed, www.retinalyze.com) which requires only a fundus camera to obtain the images and a software that determines the amount of Hb in the ONH, provides high sensitivity and specificity for the diagnosis of early glaucoma.

**TABLE 1.** Baseline Characteristics of Both Groups

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Glaucoma Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>36/28</td>
<td>31/27</td>
<td>0.756*</td>
</tr>
<tr>
<td>Age (y)</td>
<td>60.47 ± 14.55</td>
<td>62.16 ± 14.29</td>
<td>0.339*</td>
</tr>
<tr>
<td>Race (European descent/Asian descent)</td>
<td>64/0</td>
<td>55/3</td>
<td>0.105*</td>
</tr>
<tr>
<td>Latenality (right/left)</td>
<td>31/33</td>
<td>30/28</td>
<td>0.717*</td>
</tr>
<tr>
<td>MD (dB)</td>
<td>—</td>
<td>−2.85 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>RNFLT (μm)</td>
<td>90 ± 10.6</td>
<td>66.28 ± 9.85</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Statistically significant P-values are in bold.

*One-tailed nonpaired t test.

†Mann-Whitney U test.

‡Fisher exact test.

§χ² test.

RNFLT indicates retinal nerve fiber layer thickness.
Optic nerve ischemia and reduced ocular blood flow have been suggested to participate in the development and progression of glaucomatous damage.\textsuperscript{59-61} Optic nerve perfusion depends on 3 factors: oxygen saturation, blood flow, and amount of Hb. It has been advocated that changes in ONH reflectance can detect variations in Hb levels, indirectly measuring ONH perfusion.\textsuperscript{55,62} Previous studies have investigated the diagnostic accuracy of the Laguna ONhE software.\textsuperscript{35,46} Although comparisons between the various published studies investigating the diagnostic ability of the Laguna ONhE software are hampered by demographic differences, diverse inclusion and exclusion criteria, and by different levels of severity of damage, their results are listed below.\textsuperscript{53}

| TABLE 2. | Mean GDF, CDR, CD Area, and Mean Hb Concentrations in Each Sector in the Control and Glaucoma Groups |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sector          | Control         | Glaucoma        | P               |
| GDF             | 11.57 ± 15.34   | −27.67 ± 20.94  | <0.0001†       |
| Vertical CDR    | 0.5 ± 0.09      | 0.65 ± 0.09     | <0.0001†       |
| CD area         | 0.29 ± 0.1      | 0.44 ± 0.12     | <0.0001†       |
| Hb total        | 67.9 ± 4.45     | 62.89 ± 4.89    | <0.0001†       |
| Hb cup          | 58.67 ± 2.72    | 52.68 ± 7.72    | <0.0001†       |
| Hb Sector 1     | 71.63 ± 11.76   | 58.46 ± 13.61   | <0.0001†       |
| Hb Sector 2     | 76.69 ± 4.6     | 74.70 ± 7.42    | 0.0403†        |
| Hb Sector 3     | 77.53 ± 3.69    | 78.62 ± 4.72    | 0.0785†        |
| Hb Sector 4     | 69.97 ± 11.57   | 56.63 ± 13.03   | <0.0001†       |
| Hb Sector 5     | 77.78 ± 3.83    | 70.88 ± 7.16    | <0.0001†       |
| Hb Sector 6     | 79.33 ± 3.64    | 73.9 ± 3.73     | 0.854†         |
| Hb Sector 7     | 61.38 ± 10.77   | 49.55 ± 11.06   | <0.0001†       |
| Hb Sector 8     | 72.38 ± 5.46    | 59.19 ± 9.15    | <0.0001†       |
| Hb Sector 9     | 78.21 ± 4.0     | 73.74 ± 7.1     | <0.0001†       |
| Hb Sector 10    | 51.78 ± 10.62   | 44.66 ± 9.28    | 0.0001†        |
| Hb Sector 11    | 59.15 ± 7.27    | 51.67 ± 7.93    | <0.0001†       |
| Hb Sector 12    | 63.84 ± 4.08    | 61.48 ± 4.92    | 0.0075†        |
| Hb Sector 13    | 48.42 ± 11.04   | 43.26 ± 8.48    | 0.002†         |
| Hb Sector 14    | 53.46 ± 7.38    | 50.29 ± 7.48    | 0.0108†        |
| Hb Sector 15    | 57.59 ± 4.68    | 58.82 ± 5.32    | 0.00185†       |
| Hb Sector 16    | 52.19 ± 11.92   | 45.83 ± 9.46    | 0.0005†        |
| Hb Sector 17    | 62.17 ± 7.58    | 54.92 ± 9.58    | <0.0001†       |
| Hb Sector 18    | 66.65 ± 3.92    | 67.6 ± 5.33     | 0.375†         |
| Hb Sector 19    | 61.28 ± 13.3    | 51.29 ± 13.17   | 0.0001†        |
| Hb Sector 20    | 75.43 ± 9.28    | 64.62 ± 12.32   | <0.0001†       |
| Hb Sector 21    | 81.26 ± 4.65    | 79.45 ± 4.92    | 0.0195†        |
| Hb Sector 22    | 69.42 ± 14.07   | 56.96 ± 14.37   | <0.0001†       |
| Hb Sector 23    | 61.8 ± 9.07     | 74.49 ± 10.76   | <0.0001†       |
| Hb Sector 24    | 83.58 ± 4.62    | 82.68 ± 4.61    | 0.1418†        |

Statistically significant P-values (P < 0.002) following the Bonferroni correction, are in bold.

*One-tailed nonpaired t test.
†Mann-Whitney U test.
CD indicates cup/disc; CDR, cup/disc ratios; GDF, Glaucoma Discriminant Function; Hb, hemoglobin.

| TABLE 3. | AUCs and Sensitivities at Fixed Specificities of 90% and 95% of Laguna ONhE Parameters |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sector          | GDF             | Vertical CDR    | CD Area         | Hb total        | Hb cup          | Hb Sector 1     |
| AUC             | 0.93            | 0.887           | 0.848           | 0.804           | 0.704           | 0.781           |
| Sensitivity 90% | 82.76           | 76.56           | 73.44           | 56.9            | 29.31           | 53.45           |
| Sensitivity 95% | 79.31           | 56.25           | 42.19           | 48.28           | 10.34           | 70.34           |
| Sensitivity     |                 |                 |                 |                 |                 |                 |

It is noteworthy that the above-mentioned studies did not exclude patients with moderate or severe glaucomas, which tends to increase the diagnostic accuracy of the GDF index as the best diagnostic parameter, associated with a large AUC (0.93), and a high sensitivity (79.3%) at a fixed specificity of 95%. Interestingly, the AUC obtained in this study with the GDF is comparable to those reported in (95% CI: 0.618–0.810) and 0.721 (95% CI: 0.628–0.815) for the HRT III GPS (glaucoma probability score) and the HRT III vertical C/D ratio, respectively. The authors concluded that the diagnostic accuracy of Laguna ONhE was similar to those obtained with OCT and HRT III.

In another paper, Mendez-Hernandez et al\textsuperscript{46} evaluated the performance of the Laguna ONhE software in 67 POAG patients and 41 controls. Mean MD in POAG patients was −4.57 dB, but there is no description of the number of patients with mild, moderate or advanced glaucoma. The AUC obtained with the GDF parameter was 0.92, with a 75% sensitivity at 95% specificity. Pena-Betancor et al\textsuperscript{42} analyzed 87 healthy eyes and 71 glaucoma eyes with the Laguna ONhE software. Glaucomatous eyes had a mean MD of −10.53 ± 9.02 dB, indicating that most had moderate to advanced glaucomatous damage. The GDF parameter showed an AUC of 0.896 (95% CI: 0.84–0.95), with 67% sensitivity at a fixed specificity of 95%.
series including patients with moderate and advanced glaucoma. Furthermore, the AUC reported herein is comparable to those reported with OCT measurements in eyes with early glaucoma, which varied between 0.844 and 0.92.\textsuperscript{65-67} The Hb concentration at sector 8, localized in the inferior ONH rim, was also associated with good diagnostic accuracy (AUC = 0.888). This finding is in accordance with other reports that have indicated that the inferior sectors of the optic nerve and RNFL appear to be affected earlier in glaucoma, with better diagnostic performances than other sectors.\textsuperscript{70,71}

The limitations of this study include those that apply to any retrospective study, including selection bias and the fact that it was conducted in a single center with a relative small sample size. In addition, as in all imaging methods, the Laguna ONhe software is also affected by artifacts. In fact, some retinal scans (6.5%) could not be read by the software, indicating that some photographs may not have enough quality to be analyzed by Laguna ONhE. In fact, the reported proportions of eyes that could not be analyzed in other series varied from 1.3% to 12.\textsuperscript{34,35,42,47} Finally, because this is cross-sectional study, with a selection of cases with well-established glaucoma and clearly normal individuals, the diagnostic accuracy obtained may be overestimated. It would have been preferable to evaluate the performance of the Laguna ONhE software in suspicious patients followed longitudinally to determine those who would progress to glaucoma.

In conclusion, our results suggest that the Laguna ONhE software, an accessible method to diagnose glaucoma based on fundus photographs, shows good diagnostic accuracy to differentiate normal eyes from eyes with mild glaucoma. Longitudinal studies with a larger number of patients are necessary to confirm our findings and to evaluate if this technology can be used in the follow-up of glaucoma patients.

FIGURE 2. Receiver operating characteristic curves of some diagnostic parameters of the Laguna ONhE software: GDF, Hb sector 8, Hb total, and Hb cup. GDF indicates Glaucoma Discriminant Function; Hb, hemoglobin. Figure 2 can be viewed in color online at www.glaucomajournal.com.

REFERENCES


