Provocative tests, functional exams and daily curve of intraocular pressure in glaucoma suspects

Sebastião Cronemberger, MD, PhD, Nassim Calixto, MD, PhD, Hélio de Maria Vieira Filho, MD, Tiago Tomaz de Souza, MD, Camila Araújo Souza, MD, Roberto de Alencar Gomes, MD.

From Glaucoma Service, Federal University of Minas Gerais, Belo Horizonte, Brazil

Funding/Financial Support: None
Proprietary interest: None

Purpose: To assess sensibility and specificity, positive and negative predictive values and probability of false-positive and false-negative of the water-drinking test, ibopamine test, frequency doubling perimetry, short-wave automated perimetry and daily curve of intraocular pressure in glaucoma suspects.

Design: Cross-sectional study

Methods: Glaucoma suspect eyes and normal control eyes from age-matched individuals, both with normal standard achromatic perimetry, were submitted to water-drinking test, ibopamine test, frequency doubling perimetry, short-wave automated perimetry and daily curve of intraocular pressure.

Results: We included 45 Glaucoma suspect and 30 normal control eyes. Sensibility and specificity were respectively 75.6% and 100% for daily curve of intraocular pressure; 35.6% and 80% for ibopamine test; 22.2% and 96.7% for short-wave automated perimetry; 8.9% and 100% for frequency doubling perimetry. Positive and negative predictive values of the same workups were: 100% and 99.5%; 3.5% and 98.4%; 12% and 98.4%; 8.7% and 98.2%; 100% and 98.2%. False-positive and false-negative probabilities were: 0% and 0.5%; 96.5% and 1.6%; 91.3% and 0%; 88.0% and 1.6%; 0% and 1.9%.

Conclusions: The daily curve of intraocular pressure presented the highest sensibility with the highest positive predictive value. Frequency doubling perimetry presented a very low probability of false-negative. Ibopamine test, water-drinking test and short-wave automated perimetry presented a very high probability of false-positive.

Key words: Glaucoma, ocular hypertension, sensibility and specificity, diagnostic tests.

INTRODUCTION

Glaucoma is the second cause of blindness in the world, affecting 67 million people. Even though an early glaucoma diagnosis is crucial, this disease remains a challenge because it is asymptomatic in its initial stage. Glaucoma suspects (GS) are individuals who possess clinical findings or risk factors that indicate an increased likelihood of developing glaucoma. However, not all GS will develop glaucoma.

Glaucoma workups present limitations and no consensus exists about what are the best. They should be rapid, cost-effective and easy to interpret. Workups with high sensibility and specificity are essential to correctly diagnose people who truly have glaucoma and to avoid treatment of normal eyes.

Elevated intraocular pressure (IOP) is the main risk factor for glaucoma development and progression. As a consequence, IOP normalization is the only goal of glaucoma treatment. However, a correct IOP investigation has not been truly done. Some authors have given importance to isolated IOP measurements or IOP curves performed during office hours. Unfortunately, studies have rarely shown that IOP investigation must include IOP measurement at 6:00 a.m. in the daily curve of IOP (DCPo) with the patient in bed and darkness before having stood up. To make an early glaucoma diagnosis some authors have performed the ibopamine test (IT) and others, the water-drinking test (WDT). WDT has also been performed, with controversial results, for studying glaucoma progression. Some authors reported a correlation between IOP peaks in the DCPo and those of WDT, suggesting that this test could substitute the DCPo. Functional tests such as frequency doubling perimetry (FDP) have been performed to detect early functional defects in magnocellular cells. Short-wave automated perimetry (SWAP) has been employed.
for the diagnosis of glaucomatous defects in parvo ganglion cells not discovered with standard achromatic perimetry (SAP).\textsuperscript{14} The association of FDP and SWAP was reported to provide better diagnostic results when compared with the same exams performed separately.\textsuperscript{15}

The aim of this study is to compare sensitivity and specificity, positive and negative predictive values, false-positive and false-negative probabilities of the WDT, IT, FDP, SWAP and DCPo in the same patients.

**METHODS**

This study enrolled GS and normal control (NC) eyes from age-matched individuals from February 2008 to June 2011. It was approved by the Ethics Committee of the Federal University of Minas Gerais, and adhered to the principles enshrined in the Declaration of Helsinki. Informed consent was obtained from all patients.

All subjects underwent the following examinations: medical and ocular history, best corrected visual acuity testing, slit lamp biomicroscopy, Goldmann applanation tonometry - GAT), fundus examination and standard achromatic perimetry (SAP) with Octopus 1-2-3. In this paper, GS were patients with a positive family history of glaucoma that presented IOP in isolated measurements, from 19 to 24 mmHg in one or both eyes and/or optic disc with a vertical cup/disc ratio (CDR)≥0.7 with localized optic disc notching and thinning in one or both eyes and/or CDR asymmetry ≥0.3. GS had visual acuity ≥0.8, transparent dioptric mediums, normal SAP and no clinical or surgical antiglaucomatous treatment. The NC included normal volunteers with visual acuity equal to 1.0, IOP≤18 mmHg; normal anterior segment, eye fundus and SAP; no other risk factor for glaucoma development including family history. In both groups, we performed three central corneal thickness (CCT) measurements using the DGH5100e pachymeter and chose the lowest CCT value. We included only eyes having a CCT from 476 to 612 µ.\textsuperscript{16} Only one eye per patient was included in each group. In NC, the choice was randomized. We excluded patients with active ocular disease, diabetes and/or cardiopathy and those with arterial hypertension requiring three or more medications.

Glaucoma suspects and NC were submitted to the following non-sequential workups in the same manner:

- **DCPo**: performed in GS after patients were in hospital for one day. Their IOP was measured by two authors (SC and NC) at 9:00 a.m., 12:00, 6:00 and 10:00 p.m. with GAT and 6:00 a.m. on the following day in a supine position in bed and in darkness with the Perkins tonometer. We calculated the mean of the IOP measurements (IOPm) and their standard deviation (SD). For NC, we used the IOPm and SD from healthy age-matched individuals studied before, under the same conditions, in our Service by one of the authors (NC).\textsuperscript{17} We compared the values of IOPm and of SD of GS with those of healthy age-matched individuals. In this comparison, each GS presented an abnormal DCPo when their IOPm and/or SD showed higher values than the superior normal limits (IOPm + 2 SD) and/or (SD + 2 SD) of the DCPo from healthy age-matched individuals (Table 1).

- **WDT**: was performed between 8:00 and 9:00 a.m. by five authors (SC, HMVF, TTS, CAS and RAG). The patients had no food or liquid for ten hours. The IOP was taken before drinking a liter of water in five minutes. After drinking the water, the IOP was taken every 15 minutes for one hour. The WDT was considered abnormal when an increase of IOP≥6 mmHg was registered.\textsuperscript{7}

- **IT**: was performed between 8:00 and 9:00 a.m. by one author (SC). Firstly, we measured the IOP. After this, one 2% ibopamine drop was instilled followed by a second drop five minutes later. The IOP measurement was repeated in 15, 30, 45, and 60 minutes after the first drop. The IT was positive when the IOP increase was ≥4 mmHg.\textsuperscript{5}

- **FDP**: was performed by two authors (SC and CAS) with the “Run Threshold N-30” strategy (Welch Allyn FDP Skaneateles Falls, N.Y.). The reliable criteria for FDP were a 25% loss of fixation and false-positive and negative responses lower than 30%. We repeated FDP up to three times when the patients presented any alteration in the first exam. FDP was repeated in a minimum 30-minute interval or within a week after the first exam. FDP was considered abnormal if there was, in the “pattern deviation”, a reduction in the sensitivity contrast with a P-value <0.05 in two or more contiguous quadrants in the central 20 degree field. This reduction must be reproduced in at least two more exams.\textsuperscript{15}

- **SWAP**: was performed by a same expert technician using the Humphrey perimeter (Humphrey Field Analyzer II, Carl Zeiss Meditec, Inc, Dublin, Irlanda). The patients had a near visual correction done minutes before the exam. None of the patients had abnormal color vision at Ishiara test. We used the 30-2 total threshold strategy. All patients had a three to five-minute adaptation period in darkness while the device was calibrated. The reliable criteria for SWAP were a loss of fixation <25% and false-positive and false-negative responses <30%. When the patients presented any alteration in the first SWAP, it was repeated up to three times. SWAP was repeated in a maximum one week interval after the first exam. SWAP was considered abnormal if a significant loss in the visual field was detected with the presence of five or

---

**Table 1. Superior normal limits of mean intraocular pressure (IOPm + 2 SD) and of standard deviation (SD + 2 SD) from the DCPo of normal eyes studied by Calixto\textsuperscript{17}**

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>n</th>
<th>IOPm</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 to 25</td>
<td>24</td>
<td>14.62</td>
<td>2.28</td>
</tr>
<tr>
<td>26 to 35</td>
<td>22</td>
<td>15.93</td>
<td>2.28</td>
</tr>
<tr>
<td>36 to 45</td>
<td>20</td>
<td>16.66</td>
<td>2.63</td>
</tr>
<tr>
<td>46 to 58</td>
<td>20</td>
<td>16.92</td>
<td>2.22</td>
</tr>
</tbody>
</table>

DCPo, daily curve of intraocular pressure; IOPm, mean intraocular pressure; SD, standard deviation; n, number of eyes.
more points with \( P < 0.05 \) or with two or more points with \( P < 0.1 \%), or the glaucoma hemifield test at the frontier limit or outside the normal limits.\(^{15}\) This reduction must be reproduced in at least two more exams.

In the statistical analysis, 17.0 SPSS was used. We calculated the sensibility and specificity, the positive and negative predictive values considering 2% of glaucoma prevalence, and the false-positive and false-negative probabilities for each workup. We used the chi-square test to compare the results of the groups. The level of significance was set at 5% (\( P \)-value < 0.05).

**RESULTS**

We included 45 eyes from 45 GS with an average age of 53.3 ± 12.4 years and 30 eyes from 30 NC with an average age of 53.9 ± 13.8 years and no difference statistically significant (\( t = 1.23 \); \( P = 0.52 \)). In GS, 34 (75.6%) patients were female, and 11 (24.4%) were male. The NC had 20 (66.7%) females and ten (33.3%) males. In GS, 17 (37.7%) patients were Caucasian, 15 (33.3%) mulatto, and thirteen (28.9%) black. In NC, 19 (63.3%) patients were Caucasian, eight (26.7%) mulatto, and three (10%) black. Two GS were excluded due to an incipient cataract that could contribute to an abnormal SWAP result. In GS, 29 (64%) eyes were suspect because of a vertical CDR ≥ 0.7. Fifteen eyes (33.3%) presented an IOP ≥ 19 mmHg and inferior or equal to 24 mmHg. One eye (2.2%) of a patient presented an asymmetric vertical CDR > 0.3. In NC, five (16.7%) eyes presented a CDR = 0.4. The others 25 (73.3%) eyes presented a CDR ≤ 0.3.

The average CCT was 534.3 ± 29.1 µm in GS and 533.2 ± 33.8 µm in NC, with no statistical significance (\( t = 1; \ P = 0 \)).

In GS, FDP was done three times on 15 (33.3%) eyes, twice on five (11.1%) and only once with a normal result on 25 (55.6%) eyes. SWAP was done three times on 11 (24.4%) eyes, twice on eight (17.8%) and only once with a normal result on 26 (57.8%) eyes.

In NC, FDP was done twice on eight (26.7%) eyes and only once with a normal result on 22 eyes (73.3%). SWAP was done three times on three (10%) eyes, twice on five (16.7%) and only once with a normal result on 22 (73.3%) eyes.

Table 2 demonstrates that the sensibility and specificity were respectively 75.6% and 100% for DCPo; 35.6% and 80% for IT; 22.2% and 96.7% for SWAP; 15.6% and 96.7% for WDT; 8.9% and 100% for FDP. The positive and negative predictive values of the same workups were: 100% and 99.5%; 3.5% and 98.4%; 12% and 98.4%; 8.7% and 98.2%; 100% and 98.2%. The false-positive and false-negative probabilities were: 0% and 0.5%; 96.5% and 1.6%; 91.3% and 0%; 88% and 1.6% and 1.9%.

No local or systemic side effects of 2% ibopamine were observed.

Table 3 demonstrates a high difference statistically significant between the DCPo of GS and NC (\( X^2 = 41.5; \ P < 0.001 \)).

Table 4 demonstrates a difference statistically significant between the SWAP of GS and NC (\( X^2 = 5.1; \ P = 0.02 \)).

<table>
<thead>
<tr>
<th>Workup</th>
<th>Normal (n=45 eyes)</th>
<th>Normal (n=30 eyes)</th>
<th>Statistical analysis</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT</td>
<td>29 (64.4%)</td>
<td>16 (35.6%)</td>
<td>24 (80%)</td>
<td>06 (20%)</td>
</tr>
<tr>
<td>SWAP</td>
<td>35 (77.8%)</td>
<td>10 (22.2%)</td>
<td>29 (96.7%)</td>
<td>01 (3.3%)</td>
</tr>
<tr>
<td>FDP</td>
<td>41 (91.1%)</td>
<td>04 (8.9%)</td>
<td>30 (100%)</td>
<td>00</td>
</tr>
<tr>
<td>WDT</td>
<td>38 (84.4%)</td>
<td>07 (15.6%)</td>
<td>29 (96.7%)</td>
<td>01 (3.3%)</td>
</tr>
</tbody>
</table>

\( n \), number of eyes; IT, ibopamine test; P, probability; \( X^2 \), chi-square; SWAP, short-wavelength automated perimetry; FDP, frequency double perimetry; WDT, water-drinking test.
Our findings are in agreement with other authors.3 This has great relevancy as an elevated IOP is the main risk factor in the development and progression of glaucoma. We demonstrate that the DCPo performed with the IOP taken at 6:00 a.m. before the patient stands up.18 He reported that the IOP at 6:00 a.m. is not new. In 1925, Thiel had reported that the IOP peak was discovered at 6:00 a.m.. In 28 (82.4%) out of 34 GS with abnormal DCPo due to a very high SD (average of 3.72±1.41) the IOP peak was discovered at 6:00 a.m.. In 22 eyes (11 patients) with suspicion of DCPo. They reported that in the impossibility of performing a DCPo with the IOP taken at 6:00 a.m. in a supine position in bed and in darkness, they performed the office curve and the WDT analyzing separately their results.16 In relation to WDT, Helal Jr9 as well as Meirelles et al10 took into account the highest IOP values found in WDT, without allowing for the fact that WDT is only considered positive when the IOP increases 6 mmHg or more from baseline. If this criteria had been adopted by Helal Jr9 only ten (45.5%) out of the 22 eyes would have presented a positive WDT. Nevertheless, this positive WDT percentage (45.5%) is far superior to that found in the present work (7%). Certainly, this finding reflects the inadequate manner in which the WDT was performed by that author.9 Helal Jr9 also found, in agreement with this study, an IOP peak at 6:00 a.m. in a supine position in bed and in darkness in the DCPo of 20 (90.9%) out of 22 suspect eyes. Despite having been done correctly in the present study, WDT presented a very low sensibility (7%) and a very high specificity (100%). Therefore, WDT is not indicated for GS. In this study, we always performed WDT in the morning to avoid possible variations in hormone secretion (anti-diuretics and corticosteroids) that can influence water absorption. Moreover, fasting is important in order to avoid the interference of food or liquids in the gastric-intestinal tract. It is important to stress that WDT was done in the past by several authors.9,12-14 This WDT was completely abandoned for the early diagnosis of glaucoma as well as for detecting glaucoma progression because its low sensibility.

Our findings are in agreement with the classic studies of Doughty and Zaman.16 They found a CCT average of 544±34 µm. The average CCT in the present study (544.3±29.1 for GS group and 533.2±33.8 for NC) is very close to the findings of Doughty and Zaman.16

We demonstrate that the DCPo performed with the IOP taken at 6:00 a.m. in bed and in darkness is the workup that presented the highest sensibility (75.6%). This has great relevancy as an elevated IOP is the main risk factor in the development and progression of glaucoma. Our findings are in agreement with other authors.3 They reported that among the methods utilized for IOP investigation, the best way to detect the IOP peaks is the measurement taken at 6:00 a.m. in a supine position in darkness and before the patient has stood up.3 The present study emphasizes that the IOP peak at 6:00 a.m. is the key for an abnormal SD. In this paper, the great majority of GS - 26 eyes (76.4%) - had an abnormal DCPo due to a very high SD (average of 3.72±1.41) in the DCPo. In 28 (82.4%) out of 34 GS with abnormal DCPOS, the IOP peak was discovered at 6:00 a.m.. In three (8.8%) eyes the IOP peak occurred at 9:00 a.m. and in three (8.8%), the IOP peak was registered at 12:00 p.m. But the great importance of measuring the IOP at 6:00 a.m. is not new. In 1925, Thiel had reported for the first time, using a Schiotz tonometer, that the IOP is more elevated in the morning between 5:00 and 7:00 a.m. before the patient stands up.18 He reported that the nocturnal IOP elevation is caused by the supine position that leads to cerebral and ocular venous stasis which reduces the aqueous outflow.18
whose authors performed WDT correctly, thereby demonstrating that WDT presents low sensitivity and high specificity, aside from presenting false-positive and negative results.

Solimon et al reported that the FDP and SWAP combination provides better diagnostic results than these exams performed separately. They conclude that in the objective of researching different neurons, this combination increases the detection of glaucoma. For us, this is difficult to understand due to the anatomical difference and the diverse concentrations of magno and parvo ganglion cells and their significance in the determination of the visual field. It is also very difficult to figure out the damage caused by magno and parvo ganglion cells simultaneously due to glaucoma. A debate by experts in visual fields concluded that so far no real contribution for early glaucoma diagnosis can be obtained by the investigation of the disease by means of FDP and SWAP. In the present paper, the SWAP sensibility, even though low (24.1%), was much higher than FDP (8.9%). On the other hand, the specificity of both exams was very high (100% and 94.7% respectively). Therefore, our data can not endorse the conclusions of Solimon et al. Another very important aspect of the FDP and SWAP is the observation herein that both exams presented the effect of the learning curve in GS as well as in NC. In GS, FDP was confirmed positive after performing three exams in only four (8.9%) eyes, although it was performed three times on 15 (33.3%) out of 45 eyes, and twice on five (11.1%) eyes. In NC, FDP was 100% negative in 30 eyes, but had to be done twice on eight (26.7%) of the 30 eyes. In relation to SWAP, in GS, it was confirmed positive after performing three exams in ten (22.2%) eyes. However, their results were negative after performing the test twice on eight (17.8%) eyes. In NC, SWAP was confirmed positive after performing three exams in only one eye (3.3%) despite having been done three times on three (10%) of the 30 eyes. Even so, the positive SWAP result in one normal eye was false-positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive. The patient presented a cup disc ratio equal to zero and normal IT, WDT positive.

In this paper, SWAP had 22.2% and FDP 8.9% sensibility respectively. The-