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ORIGINAL ARTICLE

Psychophysical and Electrophysiological Testing in Ocular Hypertension

Marcella Nebbioso*, Fabio De Gregorio†, Laura Prencipe†, and Irene Pecorella

ABSTRACT

Purpose. The purpose of this study was to compare psychophysical and electrophysiological testings in early optic nerve dysfunction in a group of clinically asymptomatic subjects with suspect ocular hypertension (OHT).

Methods. Forty eyes of 40 patients with suspect OHT and asymmetrical horizontal cup/disc ratio (0.2/0.4), 22 eyes of 22 patients with open-angle glaucoma (OAG), and 40 eyes of 40 healthy controls were evaluated by using frequency-doubling technology perimetry (FDT), contrast sensitivity (CS), pattern electroretinography (PERG), and pattern visual-evoked potentials (VEP). The VEP were elicited by checkerboard stimuli with large (VEP 120), medium (VEP 45), and small (VEP 15) checks; then the values of the amplitude (A) and latency (L) of P100 peaks were studied. Receiver operator characteristic (ROC) curves were calculated to determine the sensitivity, specificity, and optimal cutoff points of abnormal values. A logistic regression analysis was performed to determine which tests were providing the most useful information. In addition, Kruskal-Wallis test was performed to test the differences between the control group and the OHT group.

Results. VEP P100 peak latency (VEP L15 and VEP L45) and amplitude (VEP A120), PERG N95 peak amplitude, CS at medium spatial frequencies (CS 4SF), and FDT pattern standard deviation (PSD) yielded the greatest sensitivity (85.0 to 60.0%) and specificity (80.0 to 60.0%) ratio, displaying the largest ROC curve areas; whereas PERG N95 peak latency ROC curve had the smallest areas. Kruskal-Wallis test showed that most diagnostic tests were able to differentiate the OHT group from the control group. Stepwise logistic regression analysis identified VEP L15 ($p < 0.001$), CS 4SF ($p = 0.023$), FDT PSD ($p = 0.032$), and VEP A120 ($p = 0.072$) as tests that could be useful to distinguish controls from OHT.

Conclusions. Our data confirm that psychophysical and electrophysiological tests are useful for early detection of patients at risk of developing OAG.

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Key Words: contrast sensitivity (CS), frequency-doubling technology perimetry (FDT), primary open-angle glaucoma (OAG), ocular hypertension (OHT), pattern electroretinogram (PERG), pattern visual evoked potentials (VEP)

Glaucomatous optic neuropathy is a multifactorial disease causing progressive loss of optic nerve fibers. Early open-angle glaucoma (OAG) may present either optic disc or retinal nerve fiber changes despite normal visual fields.[1] Therefore, it is often difficult to differentiate ocular hypertension (OHT) and OAG. Several risk factors such as intraocular pressure (IOP), race, age, central corneal thickness, family history, refractive defects, vascular factors, systemic diseases, and some drugs may play a major role in the evolution from OHT to established glaucoma. Hence, research in the past years has been aimed at defining the most effective means of screening patients at risk and, consequently, sorting out those patients requiring treatment.

The aim of our study was to investigate the sensitivity and specificity of a range of psychophysical and electrophysiological testings in differentiating OHT and OAG subjects from "normals." For this purpose, all patients underwent the following examinations: frequency-doubling technology perimetry (FDT), contrast sensitivity (CS), pattern electroretinogram (PERG), and pattern reversal visual-evoked potentials (VEP).

MATERIALS AND METHODS

In compliance with the Helsinki Declaration, informed consent was obtained from all subjects before enrolment. Three groups were formed: group 1 (40 OHT), group 2 (22 OAG patients), and

*MD
†PhD
Department of Ophthalmology, Centre of Ocular Electrophysiology, University of Rome "Sapienza," Rome, Italy.

group 3 (40 normal controls). All subjects in groups 1 and 3 were free from ocular or systemic disease, including age-related macular degeneration, diabetes, multiple sclerosis, high myopia, previous intraocular surgery, or trauma etc.

Group 1—OHT Suspects

Group 1 included 16 OD and 24 OS of patients with a family history of glaucoma. There were 22 men and 18 women aged 31 to 68 years (mean age, 53.6 ± 11.3 SD). Eligibility was determined through a detailed medical and ocular history, and a comprehensive eye examination. Eye examination included best-corrected visual acuity (BCVA) for far and near vision, slitlamp biomicroscopy, IOP measurements with Goldmann applanation tonometry at four different times, corneal pachymetry, gonioscopy, dilated fundus examination, horizontal cup-disc (C/D) ratio evaluation. Measurements of the visual field (standard automated periphery [SAP]) were performed on the 30 to 2 Swedish interactive threshold algorithm standard program of the Humphrey Field Analyzer (Carl Zeiss Meditec, Inc., Dublin, CA).

Inclusion criteria were as follows:

- IOP in the range of 19 to 22 mmHg, without any treatment;
- refraction values between ±4 D sphere and ±2 D cylinder;
- BCVA for far distance up to 8/10;
- open angle at gonioscopy;
- normal corneal pachimetry (550 to 620 μm);
- horizontal C/D ratio of 0.2 to 0.6 at slitlamp examination;
- asymmetrical horizontal C/D ratio 0.2/0.4 at slitlamp examination
- normal SAP (30 to 2 Swedish interactive threshold algorithm standard program).

In this group, the mean IOP value was 20.86 ± 1.16 mm Hg. BCVA ranged between 8/10 and 10/10 (logMAR 0.14 to −0.3).

Group 2—OAG Patients

This group included 10 OD and 12 OS of 10 men and 12 women, aged 39 to 72 years (mean age 59.3 ± 10.4 SD). All patients were free from other ocular or systemic disease, previous intraocular surgery, or trauma. The mean IOP was 14.73 ± 1.45 SD mm Hg on account of ongoing medication. Refraction values ranged between ±5 D spheres and ±1.5 D cylinder. BCVA for distant vision was between 8/10 and 10/10 (logMAR 0.14 to −0.3). OAG diagnosis was confirmed by

- glaucomatous optic disc changes, open angle at gonioscopy, and no clinically apparent underling primary cause for glaucoma;
- horizontal C/D ratio of 0.5 to 0.8 at slitlamp examination;
- abnormal SAP, defined as three or more adjacent points depressed by 5 dB or more and reproduced on three occasions; or two adjacent points depressed by 10 dB.
- SAP testing was considered reliable only when false negative/positive responses and fixation losses were <20%.

Group 3—Normal Controls

Twenty OD and 20 OS of healthy subjects (19 men and 21 women) aged 31 to 70 years (mean age, 48.7 ± 10.0 SD) were examined. The mean IOP value was 14.78 ± 2.15 mmHg and the horizontal C/D ratio was <0.5, with minimal or no differences between the two eyes. Minimal refraction defects were occasionally detected and BCVA for far vision was between 8/10 and 10/10 (logMAR 0.14 to −0.3). All patients showed normal corneal thickness up to 620 μm and normal visual fields at SAP 30 to 2.

Electrophysiological and Psychophysical Tests

The three groups were subjected with normal pupillary function to the following tests: FDT, CS, PERG, and VEP. Examinations were repeated the following days to minimize the learning effect (Figs. 1 and 2). Data obtained at the last examination were used for statistical analysis.

The Humphrey Matrix FDT perimeter (Welch Allyn, Skaneateles, NY; Zeiss Humphrey, San Leonardo, CA) is a contrast psychophysical test with a more complex target than the standard threshold automated perimetry.[2–4] Its target consists of a pattern of low spatial frequency (SF), 0.50 cycles per degree (cpd), in combination with a high temporal frequency stimulus, 18 Hz, and a mean luminance of 50 candelas/meter squared (cd/m^2). Each target is 5 × 5° square, except for the central target, which is a 5° diameter circle. A video display unit presents to the patient a stimulus that is a monochrome sinusoidal grating of vertical black and white bars. These bars undergo rapid counter phase flickering (rate of 18 times/s). A total of 69 stimulus locations are shown, 17 in each quadrant and one in the fovea for the 30 to 2 threshold program. Each target preferentially stimulates the combined activity of many retinal cell types.[2–7]

The Matrix FDT perimeter uses a Bayesian threshold estimation strategy known as ZEST in which the contrast of the frequency doubling stimulus is increased if the target is not seen and decreased if the target is seen. A severe defect is noted after two successive presentations with a stimulus target at maximum contrast that elicits no response. The age of the subject is entered so that the instrument can choose expected values of contrast from an age-normalized database. The output data from the FDT perimetry include the number, the location, and the severity of the defect for the 69 target zones. Readings appear as a numerical table and as probability maps with five gray tones.[3,5]

The CS test was performed at 200 cm distance, with a specific testing program "Static Contrast photopic" on the optoelectronic stimulator Vision Monitor MonPack 120 by Metrovision (Pérenchies, France). If necessary, an optical correction was placed for the test distance. Testing was monocular. The examination involves threshold measurements performed with an ascending limit technique: the grating was first presented to the patient as "unseen" and the contrast level was increased slowly until a response was obtained. This increase of contrast was made in a progressive manner, by steps of 0.25 dB (decibels) of contrast, so as to avoid responses elicited by abrupt contrast changes. A first presentation was made for each test without recording the response, allowing training of the patient. Each measure was repeated several times to evaluate the reproducibility of responses: five series of six measurements were made. The final graph indicates all the responses obtained for each SF in cpd and at different contrast in dB. Thus, a curve is shown as a graph in green color. Characteristics of tests used in our standard clinical protocol were as follows:

- stimulation field 2.2° horizontal and 1.6° vertical centered on the fovea;

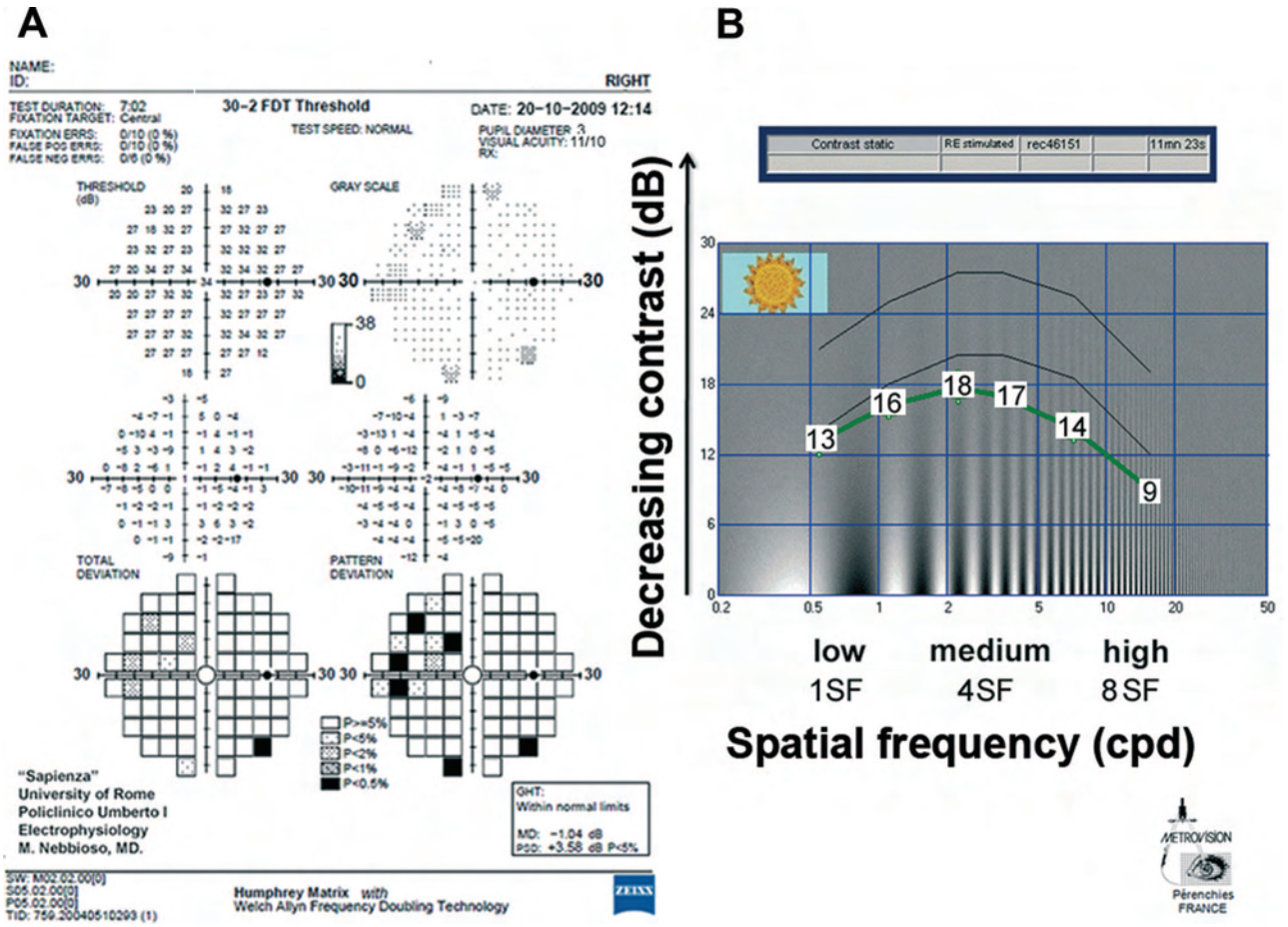

FIGURE 1.

Psycophysical testing. Panel A, Abnormal PSD at FDT perimetry. Panel B, Decreased average responses of CS performed at low (0.8 to 1.6 cpd), medium (3.2 to 6.4 cpd), and high (12.8 to 25.6 cpd) SF, denominated 1, 4, and 8SF, respectively. A color version of this figure is available online at www.optvissci.com.

- stimulation with vertical sinusoidal grating;
- three average values of 6 SF in cpd performed at low (0.8 to 1.6 cpd), medium (3.2 to 6.4 cpd), and high (12.8 to 25.6 cpd) SF, denominated 1SF, 4SF, and 8SF, respectively;
- temporal modulation frequencies "static procedure";
- constant average luminance of 80 to 90 cd/m^2 except for very high contrast levels (>50%).[8–11]

The transient PERG was performed at 33 cm distance, with an alternated 35 program on the optoelectronic stimulator Vision Monitor MonPack 120 by Metrovision. If necessary, an appropriate optical correction was placed for the test distance. The PERG was evoked by alternating contrast reversal of a black and white square checkerboard pattern of constant mean luminance with stimulus field size 78.8 arc degree (deg) centered on the fovea, check size of 47.3 min arc, contrast 100%, mean luminance 45 cd/m^2, and 4.5 reversals/s. The waveform of the transient examination was obtained at low temporal frequencies of the stimuli. The International Society for Clinical Electrophysiology of Vision standard PERG protocols were coded for binocular recording and were identified by a series of N35, P50, and N95 peaks, each characterized by an amplitude (A) and latency (L) (or time-to-peak).[12,13] For the purpose of this study, only N95 peak, A and L (AN and LN), were taken into account.

The transient VEP was performed to 100 cm distance, on the optoelectronic stimulator Vision Monitor MonPack 120 by Metrovision with reference to the International Society for Clinical Electrophysiology of Vision guidelines:[14–16] stimulus field size 23.6 arc deg centered on the fovea, contrast 100%, mean luminance 50 cd/m^2, and 2 reversals/s. The VEP were elicited by checkerboard stimuli with large (106 min arc), medium (44.9 min arc), and small (15.4 min arc) checks. The waveform of the transient examination was obtained at low temporal frequencies of the stimuli; then the values of the A and L (or time-to-peak) of P100 peaks were studied. The standard VEP protocols were coded for a single recording channel with a midline occipital active electrode. VEP was identified by a series of N75, P100, and N135 peaks, each characterized by an A and L.

The FDT mean deviation (MD) and pattern standard deviation (PSD) values, the CS measurements performed at each SF, the PERG A and L (N95 peak), and the VEP A and L (P100 peak) were analyzed in OAG patients and OHT suspects and compared with the normal controls. Criteria for abnormal responses at VEP, FDT, CS, and PERG included values differing 2 SD from the normal average. Only one eye of each subject was used for analysis. The eyes with the higher C/D ratio were selected in the OHT group, whereas control and OAG eyes were randomly selected for data analysis.

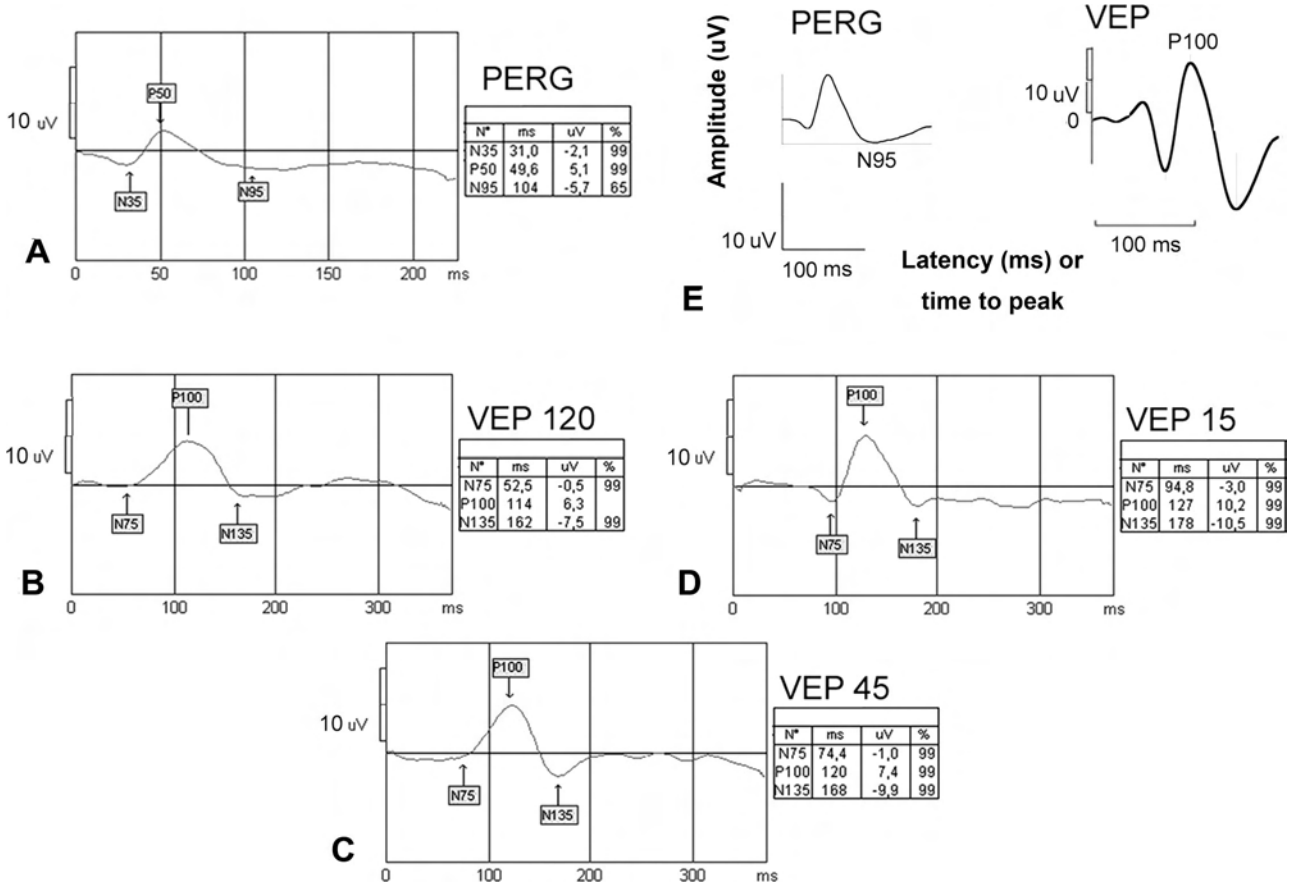

FIGURE 2.

Electrophysiological testing, same case as Fig. 1. Panel A, Abnormal decrease (amplitude) of the N95 peak at PERG. Panels B and C, Abnormal decrease (amplitude) and delay (latency or time-to-peak) of the P100 peak at VEP (large and medium checks, VEP 120 and VEP 45, respectively). Panel D, Abnormal delay of the P100 peak at VEP (with small check, VEP 15). Panel E, Diagram of normal transient PERG and VEP illustrating how measurements of negative and positive waves were made.

TABLE 1.

SE, SP, and AUC ROC of each test, calculated by comparing control subjects (n = 40) and OAG patients (n = 22)

Test	AUC	AUC 95% CI	CC	SE	SP	Cutoff	SE[a] (SP 95%)
FDT MD	**0.997**	0.942–1.00	98.4	95.5	100	<−3.8	100
FDT PSD	**0.997**	0.942–1.00	96.8	100	90	>3.3	100
VEP A120	**0.910**	0.801–0.963	88.7	72.7	97.5	<8.4	72.7
VEP A45	**0.909**	0.801–0.964	85.5	63.6	97.5	<8.3	63.6
VEP A15	0.779	0.650–0.871	74.2	63.6	80.0	<10.3	50.0
VEP L120	**0.846**	0.723–0.920	87.1	63.6	100	>112	68.2
VEP L45	**0.956**	0.865–1.00	91.9	77.3	100	>115	86.4
VEP L15	**0.981**	0.913–1.00	90.3	72.7	100	>124	86.4
CS 1SF	0.754	0.633–0.858	72.6	63.6	77.5	<17.5	27.3
CS 4SF	0.820	0.705–0.908	77.4	72.7	90	<20.5	50
CS 8SF	0.791	0.668–0.883	64.7	68.2	77.5	<14.5	54.6
PERG AN	**0.930**	0.843–0.982	91.9	90.9	92.5	>−5.6	72.7
PERG LN	0.538	0.401–0.660	53.2	50	55	>103	0.0

The optimal cutoff values were chosen considering the highest percentage of correctly classified (CC) subjects with SE and SP more than 60%. The FDT (MD, PSD), VEP (A120, A45, L120, L45, L15), and PERG AN yielded the greatest SE and SP ratio having the largest AUC ROC (values given in bold).

[a]To allow comparison with other studies for each test, the SE of the test was also calculated when the SP was 95%.

Statistical Analysis

Receiver operator characteristic (ROC) curves were constructed to graphically illustrate the diagnostic performance of the above mentioned tests. The plots show test sensitivity (SE) along the y axis vs. its 1-specificity (SP) along the x axis. The optimal cutoff values were chosen considering the highest percentage of correctly classified subjects displaying >60% SP and SE and, if this was not

possible, the best combination of SE and SP. Areas under the curve (AUC) were used to evaluate the best test by comparing controls, OAG patients, and OHT subjects. A logistic regression analysis was then applied to determine which tests were providing the most useful information. In addition, Kruskal-Wallis test was performed to test the differences between the control group and the OHT group. As a multiple comparison was performed, the Bonferroni correction was applied. All p values were given two tailed. Analyses were performed with STATA 10.1 software.

RESULTS

The test results were normal in the control group and, at least to some extent, abnormal in the OHT group. Table 1 shows SE, SP, and AUC ROC of each test, calculated by comparing control

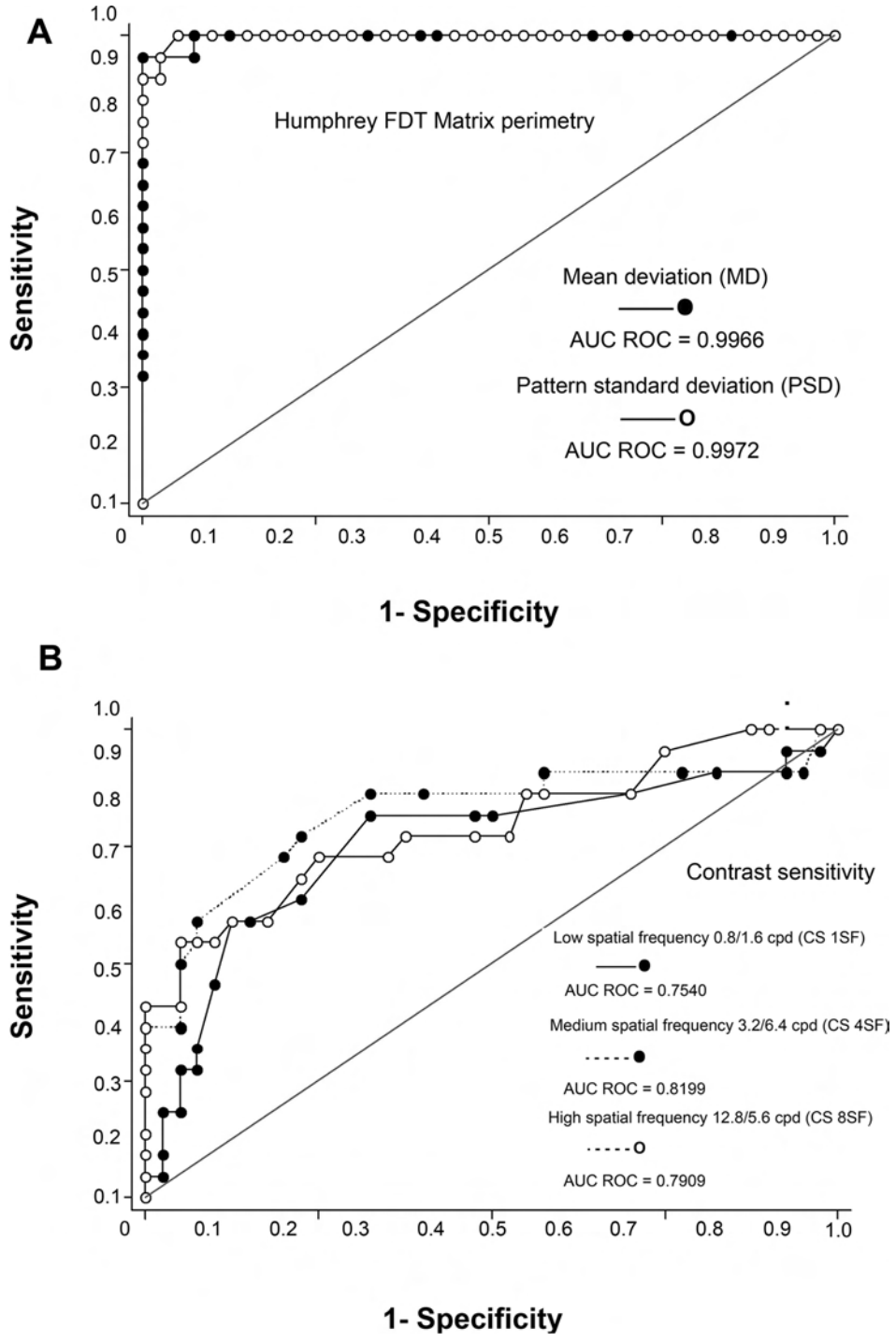

FIGURE 3.

OAG patients vs. control group. Panel A, ROC curves describing sensitivity (SE) and specificity (SP) of perimetric indexes of FDT MD and FDT PSD. Panel B, ROC curves describing SE and SP of CS measurements performed at low, medium, and high SF (1, 4, and 8SF).

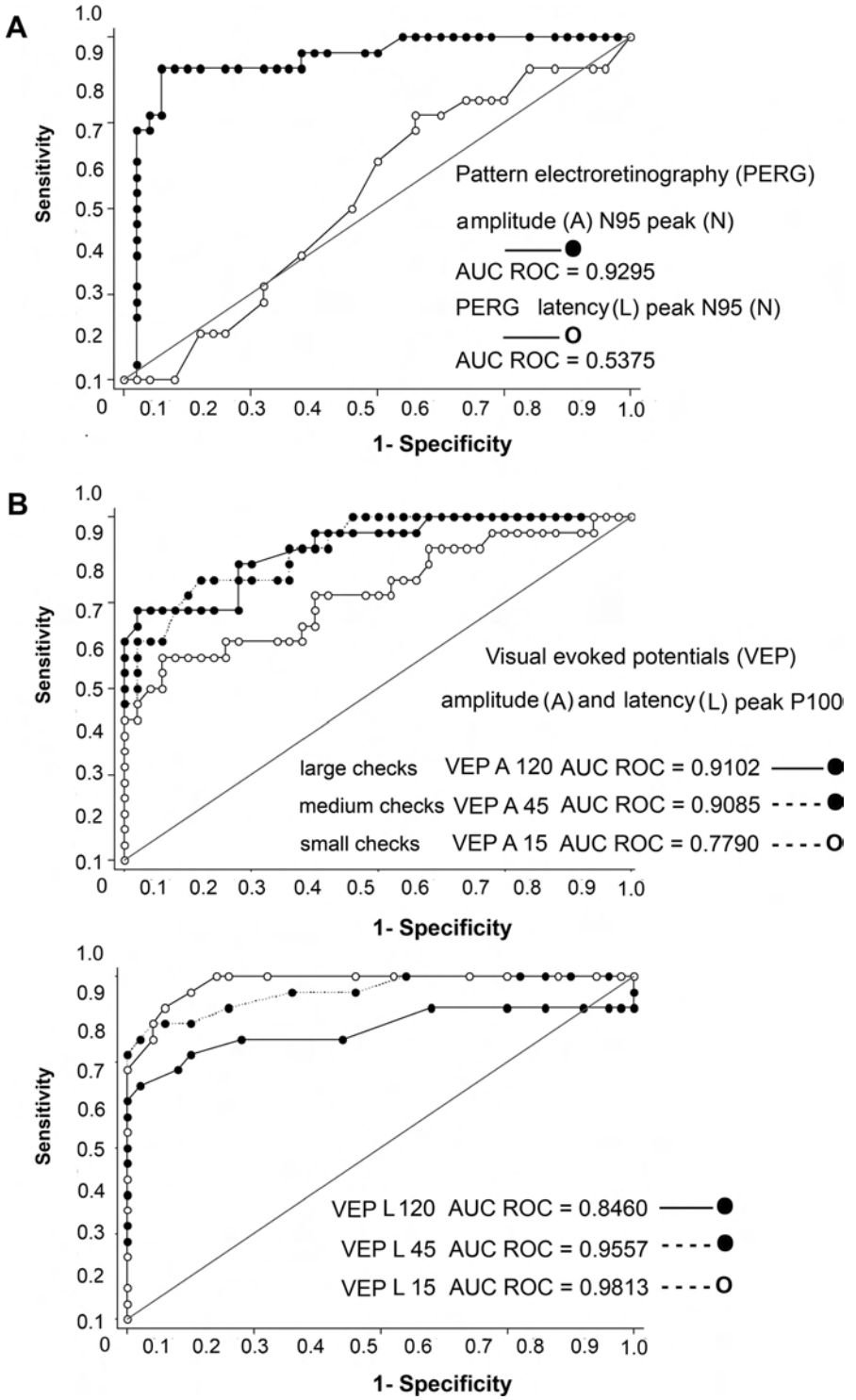

FIGURE 4.

OAG patients vs. control group. Panel A, ROC curves describing SE and SP of PERG amplitude (A) and latency or time-to-peak (L) of the N95 peak. Panel B, ROC curves describing SE and SP of VEP A and L of the P100 peak (120, 45, and 15 min arc: measurements performed with large, medium, and small checks).

subjects (n = 40) and OAG patients (n = 22). Figs. 3 and 4 show the ROC curves of each examination.

OAG vs. Controls

FDT, VEP, and PERG AN yielded the greatest SE and SP ratio, having the largest ROC curve areas (Table 1, values in bold). Particularly, FDT PSD and FDT MD had 100% and 95.5% SE, and 90 and 100% SP, respectively; PERG AN had 90.9% SE and 92.5% SP. VEP L at all SF (15, 45, and 120) and VEP A at low (120) and medium (45) SF of stimulation had SE values ranging from 77.3 to 63.6% and SP values ranging from 100 to 97.5%. Besides, CS at medium SF (4SF) had 72.7% SE

and 90.0% SP (Figs. 3 and 4; Table 1). ROC curves of CS at high SF (8SF) and low SF (1SF), VEP A at small checks (15 min arc), and PERG LN had the smallest areas, thus indicating poor SE and SP (Figs. 3 and 4; Table 1).

OHT vs. Controls

The Kruskal-Wallis test (Table 2; Figs. 5 to 7) indicated that the values of VEP 120 (A and L), VEP 45 (A and L), VEP L15, CS 1 and 4SF, and PERG AN in OHT patients were significantly different from those of normal subjects (Table 2, values in bold), whereas FDT (MD and PSD), PERG LN, VEP A15, and CS 8SF did not show significant differences between the OHT group and control group.

Table 3 shows AUC ROC of controls vs. OHT. As expected AUC derived from OHT subjects were smaller than AUC of OAG patients for each test (Tables 3 and 1). The best test to separate controls from OHT was VEP L15 with an AUC of 0.8747. Comparison with other tests showed that FDT MD, VEP A15, CS 8SF, and PERG LN were significantly worse than VEP L15 (Table 3). By adopting the same cutoff as OAG patients, we correctly classified 65% of subjects having an SE of 30% and SP of 100%. The best cutoff for VEP L15 was 114, because it correctly classified 82.5% of subjects with an SE of 85% and SP of 80%. Stepwise logistic regression analysis was then applied to evaluate what test other than VEP L15 may distinguish controls from OHT suspects. Results showed that FDT PSD ($p = 0.032$), VEP A120 ($p = 0.072$), CS 4SF ($p = 0.023$), and obviously VEP L15 ($p < 0.001$) remained in the model (Table 3, values in bold).

DISCUSSION

The clinical diagnosis of OHT does not include loss of vision and optic disc abnormality, whereas glaucoma represents a typically slow chronic degeneration of retinal ganglion cells (RGCs) function that leads to progressive visual impairment. Progression to OHT can be suspected in individuals at risk for glaucoma (borderline IOP, glaucoma family history, elevated myopia, etc.) when increasing IOP is associated with impairment of some functional test (generally not SAP), negative results at fundoscopy and visual acuity examination. Visual function tests, including SAP, are not generally considered to be very sensitive to early glaucomatous changes.[17,18]

TABLE 2.
Statistical comparison between OHT group and control group

Test	OHT (average ± SD)	Controls (average ± SD)	p
FDT MD	−0.72 ± 3.04	0.80 ± 2.07	0.018
FDT PSD	3.64 ± 1.47	2.70 ± 0.39	0.005
VEP A120	9.74 ± 3.91	13.89 ± 4.39	**0.0001**
VEP A45	10.39 ± 4.11	13.79 ± 4.29	**0.0006**
VEP A15	13.11 ± 5.77	14.89 ± 5.24	0.157
VEP L120	107.53 ± 7.93	103.43 ± 3.56	**0.0013**
VEP L45	109.08 ± 6.01	103.42 ± 4.62	**0.0001**
VEP L15	119.20 ± 6.17	109.67 ± 5.28	**0.0001**
CS 1SF	17.36 ± 1.53	18.45 ± 2.22	**0.0036**
CS 4SF	20.45 ± 1.84	21.96 ± 1.72	**0.0004**
CS 8SF	15.26 ± 2.60	16.36 ± 2.76	0.0537
PERG AN	−6.58 ± 1.45	−8.035 ± 3.54	**0.0001**
PERG LN	101.52 ± 6.89	101.55 ± 5.87	0.7799

VEP (A120, A45, L120, L45, L15), CS (1SF, 4SF), and PERG AN show significant differences (values given in bold).

The aim of our study was to investigate the SE and SP of a range of psychophysical and electrophysiological testings in OHT subjects at risk of developing OAG. Computerized diagnostic examinations appear to be appropriate methods of screening, being non-invasive, reliable, and easy to perform. Optic nerve structural changes may be present with fundoscopy before the development of SAP abnormalities[19–21]; however, visual field defects in the absence of any clinically observable optic nerve damage can also occur.[17]

It is generally believed that FDT evaluates the function of magnocellular (M) RGCs. These cells account for 3 to 5% of ganglion cell fibers in the human retina and appear to be already damaged in the early phase of glaucoma.[3,22,23] Recent studies, however, indicated that FDT results are mediated by the combined activity of many cell types or, most likely, by cortical mechanisms receiving input from RGCs.[6,7] Whatever the cellular pathway involved in the early glaucomatous changes, FDT is so far one of the most useful instruments to investigate glaucoma patients.

Johnson and Samuels[24] compared FDT and SAP in a group of glaucomatous patients and found that FDT had 93% SE and 100% SP and 97% of mean SE and SP for MD and PSD. Similar results in OHT and OAG patients were reported by other authors.[3,25,26] FDT abnormality with normal Humphrey SAP results may suggest that FDT might allow detection of early campimetric defects.

Fan et al.[27] studied 68 OAG patients with unilateral field loss detected by SAP Octopus G2 program; the contralateral normal eye of participants was examined with the FDT Humphrey N-30 threshold program. Visual field examination was followed by further SAP evaluations administered over 3 years. Of the eyes with abnormal FDT results, 51% developed abnormal SAP results after 4 to 27 months, whereas none of the eyes with normal FDT results developed subsequent abnormal SAP results ($p < 0.05$).

Ferreras et al.[28] examined 278 eyes using FDT, SAP, short-wave length automated perimetry (SWAP), optic disc topography (OCT-Heidelberg Retina Tomography II), laser polarimetry (GDx VCC), and optical coherence tomography (Zeiss Stratus 3000) to detect optic disc and retinal nerve fiber alterations. With these examinations, they were able to differentiate preglaucomatous from glaucomatous patients. They demonstrated that at least 20% of patients with normal optic disc and with normal SAP (Humphrey threshold 24-2) had functional damages in both FDT and SWAP examinations. However, Kondo et al.[29] did not find any correlation between FDT and Humphrey SAP in 11 normal tension glaucoma patients.

In addition, Hong et al.[30] criticized Matrix FDT because they showed a large learning effect on MD and PSD measures among the various visual field indices and unstable results in 24 OAG patients evaluated three times within 1 month.

Lately, there has been a renewed interest in electrophysiological testing for the early diagnosis of preglaucomatous changes. Many

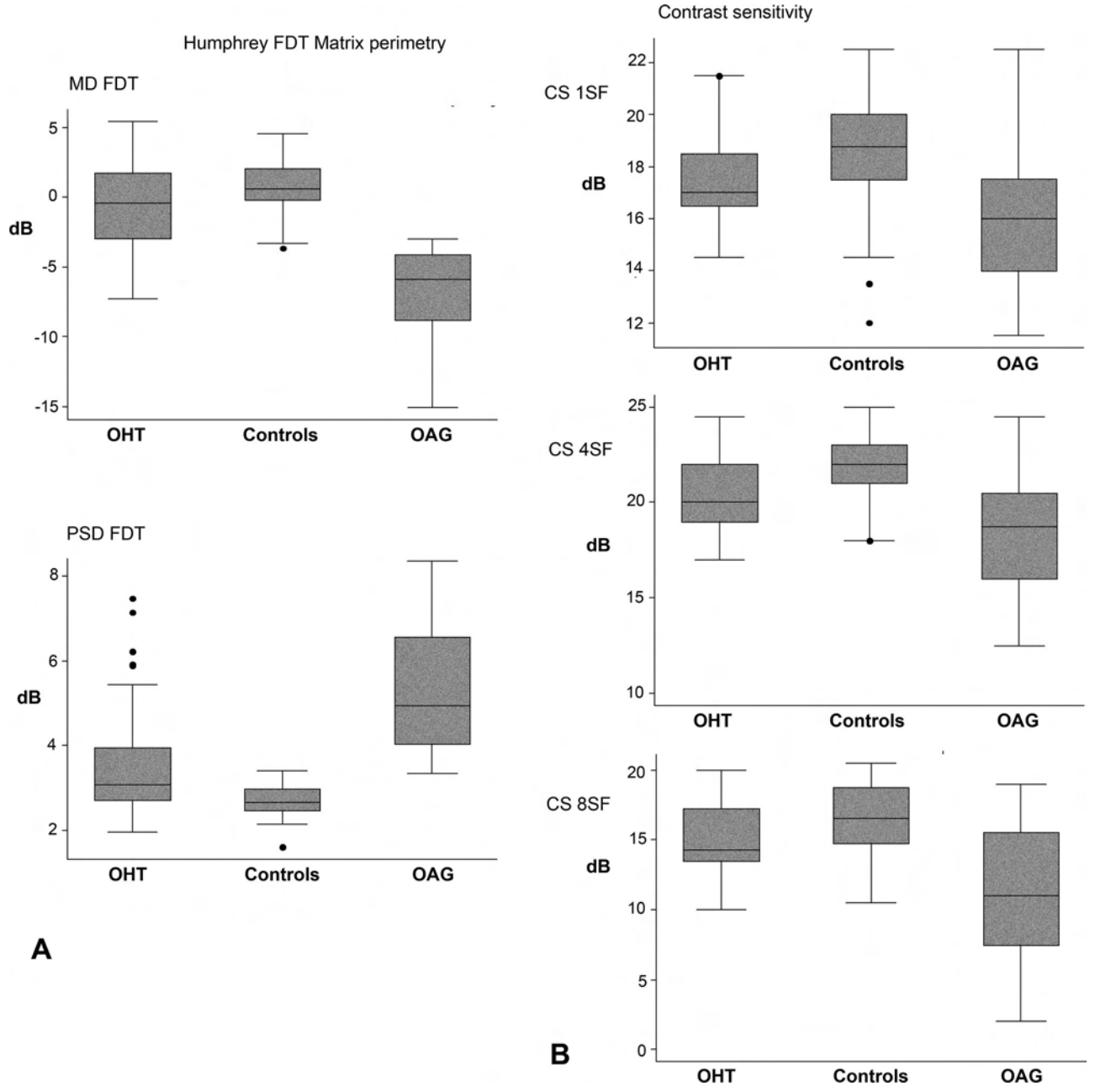

FIGURE 5.

Box and whisker plots comparing OHT group, OAG group, and control group. Panel A, Perimetric indexes of FDT perimetry (MD and PSD). Panel B, CS tests measured at different SF (low = 1SF, medium = 4SF, and high = 8SF).

studies focused on the use of PERG. O'Donaghue et al.[31] found a progressive reduction of the N95 PERG component both in established glaucoma and OHT patients. They examined 216 established and suspect glaucoma eyes for up to 2 years using PERG and demonstrated that an abnormal result is an early indicator of preglaucomatous damage before any scotoma can be detected by perimetry. PERG data showed 45% SE and SP in the high risk group, 25% in the medium risk, and 15% in the low risk group.

Ventura and Porciatti[32] reported an abnormal PERG in 52% glaucoma suspects and in 69% early manifest glaucoma patients; PERG amplitude loss increased with a more negative MD index and with increasing optic nerve head cupping. They suggested that PERG can predict development and progression of glaucoma.

Bach et al.[33,34] examined suspected glaucoma patients with normal visual fields and normal optic disc, at 6-month intervals during a follow-up of >8 years, using PERG. SE and SP of PERG results were 80 and 71%, respectively. Therefore, they suggested that PERG can help to predict stability or progression of glaucoma in suspected glaucoma patients at least 1 year before conversion to patent glaucoma.

According to North et al.,[35] PERG is a sensitive electrophysiological test because it is able to discover a dysfunction of RGCs before cell death caused by OHT or glaucoma: they found that N95 peak amplitude was significantly reduced by 17% in the OHT group and by 30% in the glaucoma group in comparison with the control group. Conversely, they only

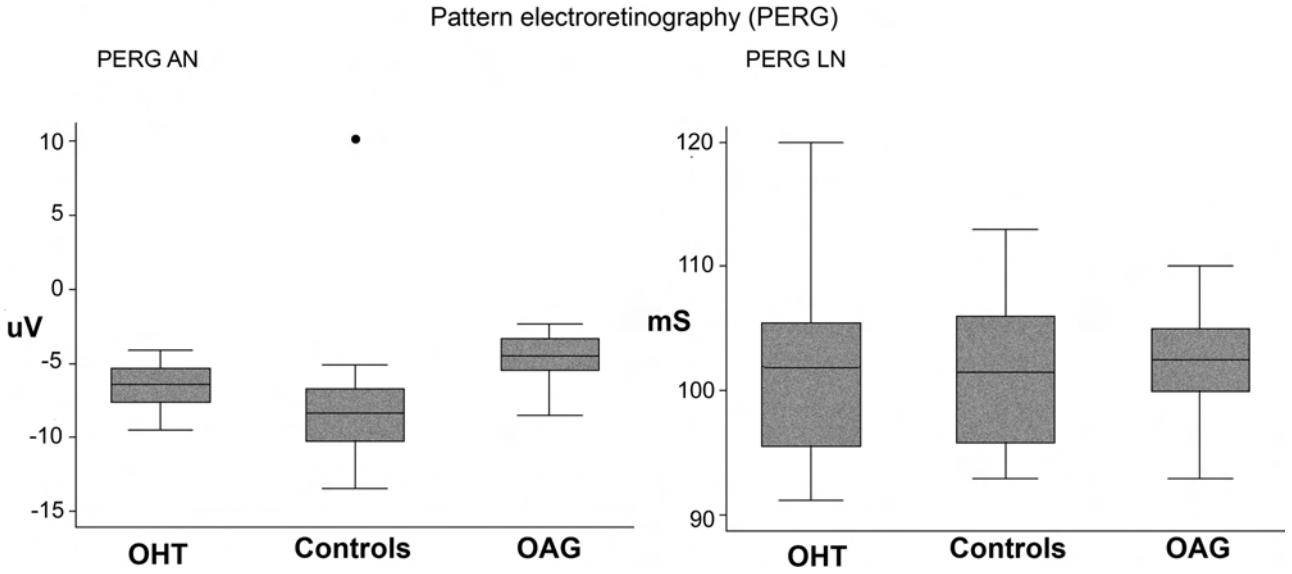

FIGURE 6.
Box and whisker plots of PERG amplitude (A) and latency (L) of N95 peak (AN and LN) comparing OHT group, OAG group, and control group.

found a weak correlation between the OCT and the electrophysiological tests.

Also Forte et al.[36] believe that PERG is able to detect an early functional damage of viable RGCs in presence of a normal retina nerve fiber layer. Positive results of the test were demonstrated in 20.8% eyes with suspected glaucoma and in 21.4% eyes with OHT.

Our results are in agreement with those described in these studies and indicate, in addition, that PERG AN (SE from 90.9 to 80.0% and SP from 92.5 to 60.0%) is a better test than PERG LN (SE from 50 to 60% and SP from 42.5 to 55%) in differentiating both OHT and OAG patients from normal controls.

Vaegan and Hollows[37] reported that both PERG and VEP are very sensitive and more reliable than psychophysics tests to detect early glaucoma changes. Nevertheless, other authors believe that psychophysical tests (SAP, blue-on-yellow SAP, temporal CS, and spatiotemporal CS) have higher SE and SP than electrophysiological tests (VEP, blue-on-yellow VEP, PERG, and color-contrast PERG) even in the early phase of glaucoma.[38–40]

According to Korth et al.,[39,40] CS is the most sensitive test because it is reduced even in preperimetric stages of OHT. In their study, they also indicated that blue-on-yellow VEP is more sensitive than PERG to detect abnormalities in all disease stages, because the latter is altered only in the perimetric phase. In contrast with other authors, they reported that the neuroretinal rim area may detect the glaucomatous damage more reliably than electrophysiological procedures.

Although we did not perform blue-on-yellow VEP, PERG AN and VEP L15/45 were in our study rather specific tests (SP from 92.5 to 100%), second only to FDT in discriminating OAG patients from controls. Besides PERG AN, VEP L15/45, and VEP A120 showed an SP from 60 to 80% and an SE from 60 to 85% in discriminating OHT subjects from controls.

Experimental studies demonstrate direct correlation of PERG AN and RGCs loss. Ben-Shlomo et al.[41] found that unilateral OHT, induced in 17 Lewis rats through laser photocoagulation, caused a reduction in PERG AN of 45% compared with the control eyes, with a significant reduction of RGCs from 2525.0 ± 372.4 to 1542.8 ± 333.8 cells per mm^2. This decrease in RGCs number was significantly correlated with the decrease in PERG A but did not reflect in the flash ERG.

Presently, the "standard" for glaucoma diagnosis relies on SAP that is known to be very insensitive to injury associated with early stage of the disease. This confirms that we currently have only very limited tools to diagnose and track the progression of glaucomatous injury to RGCs.[17,18,42–45]

Our results indicate that most diagnostic tests such as FDT PSD, VEP 120 (A and L), VEP 45 (A and L), VEP L15, CS 1 and 4SF, and PERG AN could be helpful to distinguish OHT suspects from normal subjects. Values comprised among the range for OAG (Table 1) and the range for OHT (Table 3) could be considered as suspicious results. For instance, suspicious values for VEP L15 could range between 114 and 124, whereas values <114 could be considered normal and values >124 would indicate glaucomatous disease.

In this study, also CS test (particularly CS 1 and 4 SF) proved useful, though it appeared to be less sensitive than PERG AN. Early detection of campimetric abnormalities by using new procedures, such as high pass resolution perimetry, short wave automatic perimetry, motion perimetry, and flicker perimetry may prove of uttermost importance, because these procedures do not take into account structural changes, but functional deficits of RGCs.

The results we obtained apparently confirm the diagnostic usefulness of combined psychophysics and electrophysiological tests to detect OHT patients. In agreement with Hong et al.[30] observations, patients, and controls underwent repeat measurements, and we used only the last measurement to minimize the learning effect.

FDT PSD, VEP A120, VEP L15, and CS 4SF provided the highest diagnostic accuracies in distinguishing controls from OHT, based on ROC and stepwise logistic regression analysis. Kruskal-Wallis analysis found that the mean values of most tests

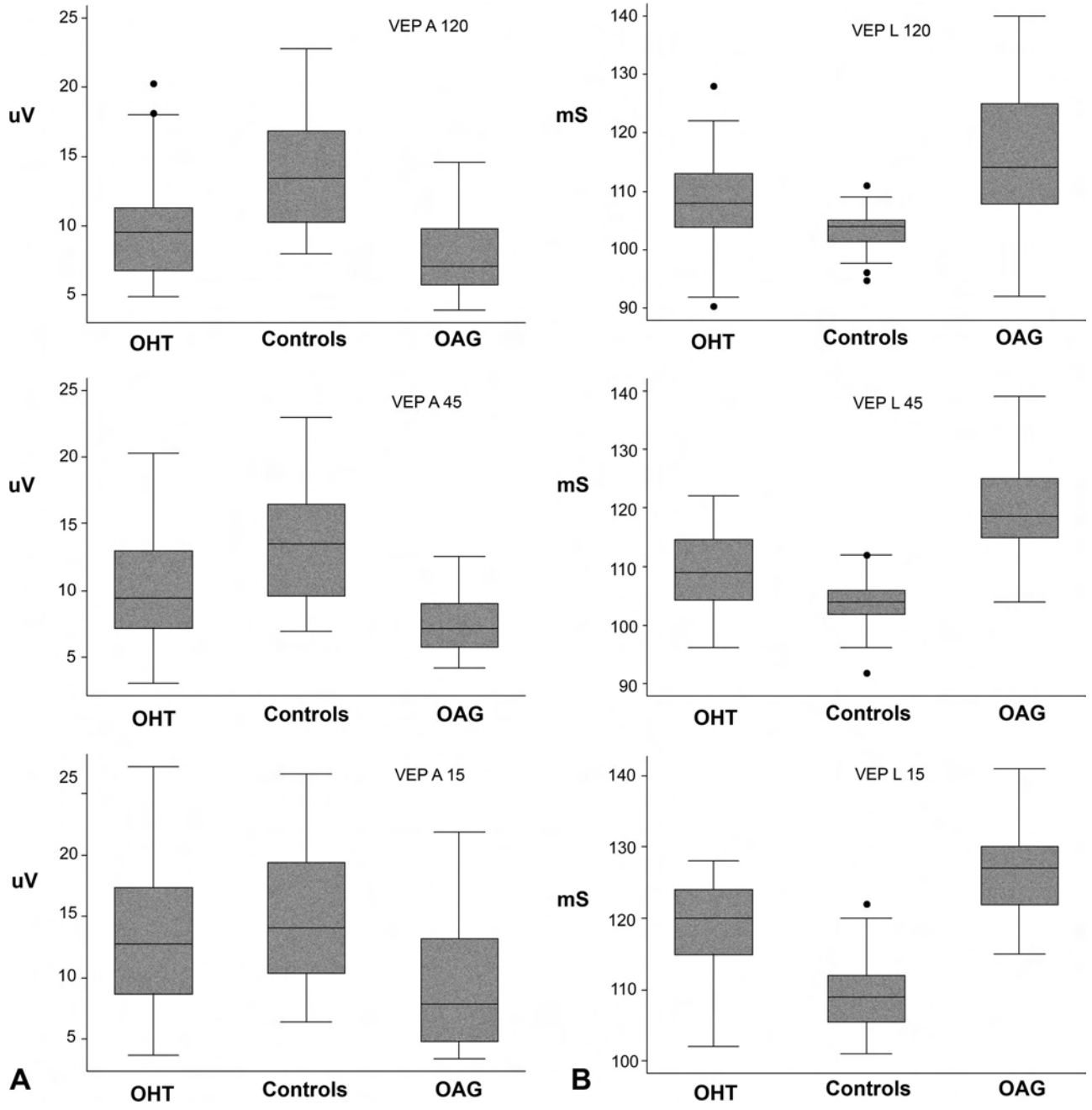

FIGURE 7.

Box and whisker plots comparing OHT group, OAG group, and control group. Panel A, VEP amplitude (A). Panel B, VEP latency (L).

performed in OHT patients significantly differed from those of the control group. The analysis of ROC curves showed that all tests had high SE and SP. Furthermore, the analysis of ROC curves combined with cutoff points allowed the identification of true pathologic and false positive subjects in the OHT group.

In summary, our studies demonstrated that test results were abnormal in the OHT/glaucoma group. Because performance on these tests requires function of specific populations of RGCs, abnormal results in glaucoma are not surprising. Abnormal results in the OHT group (no vision loss at SAP) suggest that at least some tests (FDT, CS and VEP, PERG) are more sensitive in detecting RGCs injury than SAP. But which of these procedures is best cannot be easily determined by our study. For example, the control group might have contained "normotensive glaucoma" subjects with no vision loss on SAP and cup/disc ratios in the normal range. Yet, these subjects could show deficits on the procedures used in this study. Also, some of the OHT suspects could be normal and will never develop vision loss on SAP.

In conclusion, our data appear to confirm the usefulness of the psychophysics and electrophysiological testing for early detection of patients at risk of developing OAG, but only a properly powered longitudinal study can identify which measure(s) can accurately track disease progression and which measure works best at different stages of the disease. Additional longitudinal studies will also be needed to determine how drug treatment can affect OHT patients in the evolution of psychophysical and electrophysiological testing.

TABLE 3.
Comparison of AUC ROC of controls vs. OHT subjects. Stepwise logistic regression analysis (values in bold).

	Controls vs. OHT							
Variable	AUC	AUC 95% CI	p[a]	CC	SE	SP	Cutoff	SE[b] (SP 95%)
FDT MD	0.6541	0.5308–0.7773	0.0234	65.0	62.5	67.5	<0.03	22.5
FDT PSD	**0.7263**	**0.61271–0.83979**	**0.5345**	**68.8**	**72.5**	**65.0**	**>2.8**	45.0
VEP A120	**0.7688**	**0.6654–0.8721**	**0.997**	**71.3**	**65.0**	**77.5**	**<10.1**	45.0
VEP A45	0.7241	0.61303–0.8351	0.1513	65.0	70.0	60.0	<12.2	40.0
VEP A15	0.5919	0.46602–0.71773	0.0016	60.0	57.5	62.5	<13.2	22.5
VEP L120	0.7087	0.58711–0.83039	0.2197	73.8	60.0	87.5	>108	42.5
VEP L45	0.7578	0.65304–0.86259	0.6838	70.0	60.0	80.0	>108	42.5
VEP L15	**0.8747**	**0.79609–0.95329**	**—**	**82.5**	**85.0**	**80.0**	**>114**	40.0
CS 1SF	0.6881	0.56999–0.80626	0.1578	63.8	60.0	67.5	<17.5	0.0
CS 4SF	**0.7281**	**0.61804–0.83821**	**0.5036**	**66.3**	**65.0**	**67.5**	**<21**	15.0
CS 8SF	0.625	0.50067–0.74933	0.0124	63.8	53.5	75.0	<14.5	5.0
PERG AN	0.7556	0.65034–0.86259	0.6507	70.0	80.0	60.0	>−7.8	25.0
PERG LN	0.4819	0.35357–0.61018	<0.001	51.3	60.0	42.5	>100	7.5

[a]Each test was compared with the test that had the best AUC ROC (VEP L15), Bonferroni correction was applied.
[b]To allow comparison with other studies for each test, the SE of the test was also calculated when the SP was 95%.

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Nebbioso Marcella
Viale del Policlinico, 155
00161 Rome
Italy
e-mail: marcella.nebbioso@uniroma1.it