Optimizing the Detection of Preperimetric Glaucoma by Combining Structural and Functional Tests

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Purpose. We evaluated the performance of low contrast achromatic (LLA) multifocal visual evoked potentials (mfVEP) in preperimetric glaucoma and compared its diagnostic performance to other early diagnostic tests. We identified the clinically most useful tests and combinations in preperimetric glaucoma.

Methods. We studied 59 patients with at least one glaucomatous disc, with normal, reliable visual fields in that eye, and 17 normal controls. All participants underwent complete ophthalmic examination including Humphrey visual fields (HVF), short wavelength automated perimetry (SWAP), frequency doubling perimetry (FDT Matrix), Spectralis optical coherence tomography (OCT), Heidelberg retinal tomography (HRT 3), and color stereoscopic optic disc photographs. We recorded mfVEPs using LLA stimulation.

Results. We studied 85 eyes of 59 patients (64.89 ± 8.15 years) and 34 eyes of 17 controls (64.28 ± 13.06 years; $P = 0.64$). Heidelberg retinal tomography and LLA mfVEP demonstrated the best sensitivities (50.6% and 51.8%, respectively) in identifying preperimetric glaucoma, and were not significantly different from each other. Both tests had significantly better sensitivity than all other tests ($P < 0.0001$). Of the eyes, 76.5% were identified by the combination of (any one of) LLA mfVEP and HRT. Sensitivity of the combination was significantly better than any of the individual tests ($P < 0.05$ for all pairs), or any other combinations of tests, with better negative than positive predictive value.

Conclusions. The LLA mfVEP test identified approximately 50.6% eyes with preperimetric glaucoma, which was significantly higher than other perimetric methods, and similar to HRT. The combination of LLA mfVEP and HRT had exceptionally high sensitivity of 76.5% for preperimetric glaucoma.

Keywords: preperimetric, glaucoma diagnosis, multifocal VEP, electrophysiology, imaging

Early glaucoma diagnosis remains a challenge. The realization that significant irreversible nerve loss occurs before defects begin to appear on sensory action potentials (SAP)[1,2] has, over the past decade, led to the evolution of numerous different methods attempting to diagnose very early glaucoma.[3] Some of these methods have focussed on detecting structural deviations of glaucomatous optic nerves (Heidelberg retinal tomography [HRT 3]; Heidelberg Engineering, Heidelberg, Germany) and peripapillary nerve fiber layers (optical coherence tomography [OCT]) from age-matched normals, while others use perimetric methods with novel stimuli (frequency doubling perimetry [FDT] and short wavelength automated perimetry [SWAP]) to isolate specific nonredundant visual pathways.[3–9] The structural measures are less patient dependent and, therefore, more objective, on the other hand, demonstration of functional loss by perimetric methods that corresponds to observed structural changes lends more confidence to the diagnosis.

The multifocal visual evoked potentials (mfVEP) is a form of objective perimetry that involves multifocal visual stimulation and recording of corresponding topographic responses from the occipital cortex. It now is established as an objective tool that compliments subjective testing as a method of visual field examination.[10,11] The conventional mfVEP stimulus is a high-contrast pattern-reversal checkerboard stimulus. We previously reported 2 novel stimulation patterns specifically designed to be of use in very early glaucoma: Blue-on-Yellow (BonY) and Low Luminance Achromatic (LLA) stimulation, both of which showed comparable results in patients with early, perimetric glaucoma, with LLA having slightly, though insignificantly higher sensitivity.[12,13]

The aims of the current study are to evaluate the performance of LLA stimulation mfVEP in identifying visual loss in preperimetric glaucoma, to compare the diagnostic performance of LLA mfVEP with other early diagnostic tests in glaucoma, and to identify the clinically most useful tests and combinations of structural with functional tests in preperimetric glaucoma.

Methods

Participants

We recruited from a glaucoma practice 59 patients with at least one glaucomatous optic disc, and normal and reliable visual fields in that eye and 17 subjects who were normal on a complete ophthalmic examination and volunteered to be

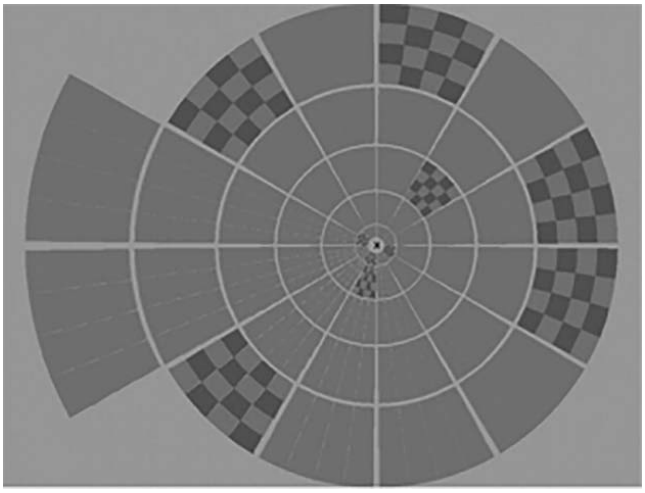

FIGURE 1. Low luminance achromatic stimulus pattern.

normal controls. Approval was obtained from the institutional review board. Written informed consent was obtained from all participants, and the study was conducted in accordance with the tenets of the Declaration of Helsinki.[14]

Inclusion Criteria

Glaucoma Patients. Abnormal discs were identified based on one or more of the following criteria: definite focal rim notching, cup–disc asymmetry $\geq$ 0.2 with no disc size asymmetry, plus rim irregularity, and markedly thinner inferior than superior rim (representing violations of the ISNT rule[15]) of an eye with no abnormal disc configuration (e.g., tilting). Reliable (defined below) and normal SITA-Standard achromatic Humphrey 24-2 (Zeiss Humphrey Systems, Dublin, CA, USA) visual field in at least one eye with glaucomatous disc change on at least two occasions was required for inclusion. Normal visual fields were defined as those where the International Society for Geographic and Epidemiological Ophthalmology (ISGEO) visual field criteria for minimum abnormality were not met[16] (i.e., a cluster of three abnormal patients at $P < 5\%$ with one of them at $P < 2\%$ and Glaucoma Hemifield Test outside normal limits).

Normal Subjects. Participants with normal-appearing optic discs and completely normal and reliable visual fields were included in the study as controls.

Exclusion Criteria

Patients with significant cataract or other media opacities and/or any other ocular abnormality were excluded. Significant cataract was defined as cataract causing visual acuity lesser than 6/9 after best correction, and/or any posterior subcapsular opacities (irrespective of Snellen acuity).

Tests

All subjects underwent visual acuity testing, subjective refraction, standard white on white perimetry (Humphrey 24-2, SITA-Standard), short wavelength automated perimetry (SWAP, 24-2 SITA-SWAP), FDT Matrix perimetry (24-2, full threshold), Spectralis OCT (Heidelberg Engineering), HRT 3, and color stereoscopic optic disc photographs. Multifocal VEP was recorded using low LLA stimulation.[13] The order of tests was randomized.

Subjective Perimetric Tests. White on white SAP and SWAP were performed using a Humphrey Field Analyzer (HFA; 24-2, SITA-Standard). Frequency doubling perimetry was performed with the FDT Humphrey Matrix (Carl Zeiss Meditec, Dublin, CA, USA) using the 24-2 full-threshold strategy. Sensory action potential and SWAP were performed with near correction in place. A test was considered reliable if fixation losses were fewer than 20% and false-positive and false-negative rates were lower than 33% (rates of reliability fixed by the perimeter software). Unreliable tests were repeated. Abnormal tests were defined by the presence of a cluster of 3 or more abnormal points at $P < 5\%$ with at least one point lower than $P < 1\%$ on the pattern deviation plot, and Glaucoma Hemifield Test outside normal limits.

Retinal Nerve Fiber Layer (RNFL) Imaging. The Spectralis spectral-domain OCT (SD-OCT; software v. 5.3.3.0) was used to obtain RNFL thickness measurements. The high-resolution protocol was used, obtaining 1536 A-scans from a 3.45-mm circle centered at the optic disc. Images with quality score >25 dB (range, 0–40 dB) were considered acceptable. For each sector of RNFL (superonasal, superotemporal, inferonasal, inferotemporal, nasal, and temporal), the Spectralis SD-OCT software provided a classification of thickness (within normal limits, borderline, and outside normal limits) based on the comparison with an internal normative database. The parameter was classified as within normal limits if its value fell within the 95% confidence interval (CI) of the healthy, age-matched population. A "borderline" result indicated that the value was between the 99% and 95% CI, and an "outside normal limits" result indicated that the value was lesser than the 99% CI. Abnormality in this study was defined as any sector with RNFL thickness < 99% CI.

Optic Disc Imaging. The Heidelberg Retinal Tomograph III (HRT-III; Heidelberg Explorer Software v. 1.5.10.0; Heidelberg Engineering) was used to acquire confocal scanning laser ophthalmoscopy (CSLO) images in the study. Good images required a focused reflectance image with a standard deviation not greater than 50 μm. The Moorefield's Regression Analysis (MRA) provided a classification for each disc sector (similar to Spectralis OCT) and was classified as within normal limits, borderline, and outside normal limits. Only the MRA was used for analysis in this study. As for OCT, all borderline values were considered normal.

Multifocal Visual Evoked Potentials. Multifocal VEPs were recorded under low LLA stimulation. The visual stimulus was generated on a 19-inch high-resolution LCD display (LG L1954, response time 2 ms; LG Electronics, Seoul, South Korea) with a refresh rate of 60 Hz. All subjects were refracted optimally for near and seated 30 cm from the display. All recordings were conducted monocularly, right eye first.

LLA Stimulus[13] (Fig. 1). A low LLA mfVEP stimulus paradigm based on sparse stimulus presentation[10] was used. The stimulus consisted of a cortically scaled dartboard pattern of 58 segments: 56 segments were arranged in 5 concentric rings (eccentricities 1°–2.5°, 2.5°–5°, 5°–10°, 10°–16°, and 16°–24°), and 2 segments straddled the horizontal nasally (24°–33°). A fixation target occupied the central 1°. Each segment contained a 4×4 grid of gray checks scaled proportional to segment size (luminance, 20 cd/m^2), which appeared briefly on a lighter gray background (luminance, 125 cd/m^2) according to a pseudorandom binary sequence. The temporal characteristics of the stimulus have been detailed previously.[17] Briefly, the pseudorandom sequence had a total length of 440 elements and consisted of 2 types of elements (elements 0 and 1) distributed pseudorandomly. Each element of the sequence lasted nine frames of the monitor ($16.67 * 9 = 150$ ms). Element 1 of the binary sequence was represented by two consecutive states: a pattern-on (checkerboard blue-and-yellow pattern)

Table 1. Sensitivity of Tests

Test	Sensitivity, *n* (%)	*P* Value*
SWAP	18 (21.2)	<0.0001
FDT-matrix	16 (18.8)	<0.0001
LLA mfVEP	43 (50.6)	–
HRT	44 (51.8)	1.00
OCT, Spectralis	20 (23.5)	<0.0001

* *P* value compared to LLA calculated using McNemar test.

state, which lasted two frames, and a pattern-off (diffuse illumination of the entire segment with a light gray color) state, which lasted seven frames. For the element 0 of stimulating sequence, the pattern-off state (diffuse yellow illumination) was active for all nine frames of the element.

Recording.[13] Four gold-cup electrodes (Grass Technologies, Warwick, RI) placed in a custom-built electrode holder were used for bipolar recording: two electrodes positioned 4 cm on either side of the inion, one electrode in the midline 2.5 cm above the inion, and one electrode 4 to 5 cm below the inion.[18] Electrical signals were recorded along four channels, as the difference between superior and inferior, left and right, and obliquely between left and inferior, and between right and inferior electrodes. The ground electrode was placed on an ear lobe. The visual evoked responses were amplified 100,000 times (sampling rate, 512 Hz) and bandpass filtered (1–20 Hz). Recording of each eye lasted 5 to 7 minutes depending on the number of runs required to maximize signal-to-noise ratio. All recordings were performed monocularly. The custom designed software correlated the electrical responses with the stimulus appearance and assigned signals to the corresponding segments. This software also scaled the responses to the background electroencephalogram to reduce the interindividual variability, as described previously.[19]

Analysis[13]

For every segment, the largest peak-to-trough amplitude for each wave within the interval of 60 to 200 ms were determined for each channel. The wave of maximal amplitude from each segment in the field from the four channels was selected automatically, and the software created a combined topographic map. The difference in intereye amplitude for each segment (except the nasal step region) also was calculated and this was compared to the intereye difference in the normal database for each segment. The asymmetry plot was eye-specific; that is, only the eye with lower amplitude was flagged as abnormal, not the other eye. Asymmetry plots were generated for each eye. The two segments constituting the nasal step did not have corresponding segments in the contralateral eye, and, therefore, were not included for asymmetry analysis. Areas of amplitude asymmetry were documented by constructing an asymmetry probability plot. A visual defect for mfVEPs was defined as a cluster of three or more abnormal segments on the amplitude deviation or intereye asymmetry deviation plots with $P < 0.02$, with at least one of them with $P < 0.01$, or two or more zones with $P < 0.005$ on the asymmetry deviation plot.

Photographs

Sequential stereo-photographs (20°, centered on the optic disc) were taken under dilation with a digital fundus camera (Zeiss VISUPAC 450). Two glaucoma specialists, who were masked to clinical information and to each other's findings, graded the optic disc photographs.

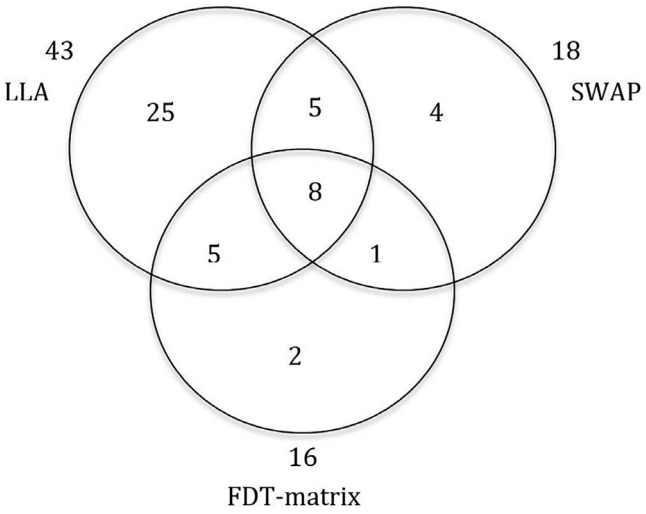

Figure 2. Comparison of perimetric tests. *Upper left circle* includes all eyes positive on LLA mfVEP; *upper right circle* includes all eyes positive on SWAP perimetry; *bottom circle* includes all eye spositive on frequency doubling perimetry.

Both examiners confirmed eligibility based on definition; identified the location of glaucomatous rim changes as right superior, right inferior, left superior and/or left inferior; and identified the location of the worst rim (out of four). Agreement between the two observers was very good ($\kappa = 0.86$). Differences were resolved by discussion.

Results

Demographics

Glaucoma Patients. We recruited 85 eyes of 59 patients with glaucoma satisfying all inclusion criteria in at least one eye. Mean age of participants was 64.89 ± 8.15 years and 28 were women. All patients had binocular disc changes, and a normal visual field in at least one eye (which was the eye[s] included for analysis). Average HFA MD of included eyes was −0.4 ± 1.5 dB, average MD on SWAP was −1.9 ± 3.41 dB, and on FDT-Matrix it was −1.41 ± 3.3 dB. For all structural and perimetric tests, only defects topographically corresponding to disc changes were considered for analyses.

Controls. A total of 34 eyes of 17 control subjects (10 women, 7 men) who satisfied the inclusion criteria for controls also were included. Mean age of the controls was 64.28 ± 13.06 years. There was no significant difference in age between the glaucoma patients and controls ($P = 0.64$).

Table 1 summarizes sensitivities of all the diagnostic tests. Heidelberg retinal tomographyT and LLA mfVEP demonstrated the best sensitivities in identifying patients with preperimetric glaucoma, and were not significantly different from each other. Both tests had significantly better sensitivity than all other tests (Table 1).

As inferred from the Venn diagram above (Fig. 2), LLA mfVEP identified 43 of 50 (86%) eyes identified with any functional defect (corresponding to disc changes). In 39 eyes, no functional defect was demonstrated on any of the three perimetric tests.

Comparing LLA mfVEP with the other objective tests, that is, HRT and OCT (Fig. 3), LLA mfVEP performed significantly better than OCT and comparably with HRT in terms of sensitivity. Of 43 eyes, 22 (51.2%) positive on LLA also were positive on HRT and 22 of 44 eyes (50%) positive on HRT also

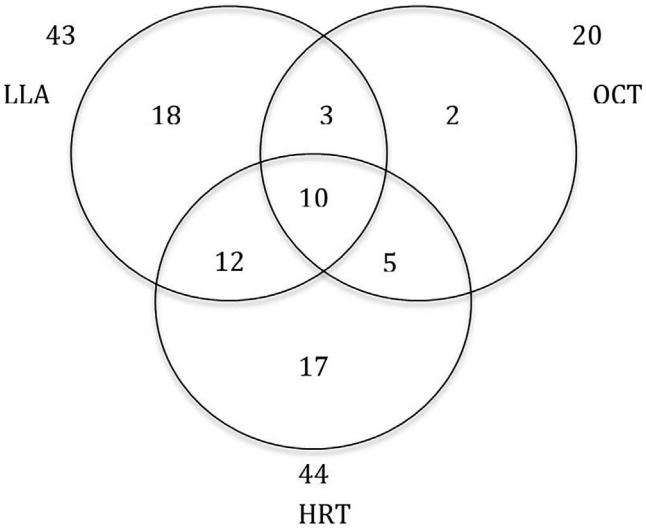

Figure 3. Comparison of LLA mfVEP, HRT, and OCT. *Upper left circle* includes all eyes positive on LLA mfVEP; *upper right circle* includes all eyes positive on OCT; *bottom circle* includes all eyes positive on HRT.

were positive on LLA mfVEP; 22 eyes (25.8%) were positive on both.

Specificity was highest for OCT; however, there was no significant difference in specificities of any of the individual tests (Table 2); 67/85 (78.8%) eyes were identified by the combination of all three tests (i.e., positive on any one or more tests; Fig. 4).

Average disc area (as measured by HRT) was 2.14 ± 0.48 mm^2.

Combinations of Tests

In an attempt to improve diagnostic performance, combinations of one structural with one functional test were examined. The criterion for positivity was at least any one test yielding a positive result.

Of 85 eyes, 65 (76.5%) were identified by the combination of (any one of) LLA mfVEP and HRT. Sensitivity of the combination was significantly better than any of the individual tests ($P < 0.05$ for all pairs), or any other combinations of tests. Specificity of this combination was lower than that of the individual tests, although the loss of specificity was not statistically significant (McNemar's test).

Table 2. Sensitivities, Specificities, Positive and Negative Predictive Values of Individual Tests and Combinations

Test	Sensitivity *n* (%)	Specificity, %	PPV*	NPV*
SWAP	18 (21.2)	85.29	0.49	0.62
FDT-matrix	16 (18.8)	97.05	0.81	0.64
LLA mfVEP	43 (50.6)	91.18	0.79	0.75
HRT	44 (51.8)	88.23	0.75	0.73
OCT, Spectralis	20 (23.5)	100	1	0.66
LLA+HRT	65 (76.5)	82.35	0.74	0.84
LLA+OCT	49 (57.65)	91.18	0.81	0.76
SWAP+OCT	33 (38.82)	85.29	0.64	0.68
SWAP+HRT	51 (60.00)	76.47	0.63	0.74
MATRIX+OCT	30 (35.29)	97.06	0.89	0.69
MATRIX+HRT	51 (60.00)	85.29	0.73	0.76

* Positive and negative predictive values calculated for prevalence of 40%.

Predictive Values

Positive and negative predictive values were calculated for the individual tests as well as combinations. Assuming that the prevalence of early glaucoma among referrals for suspect discs is roughly two of five, or 40%, PPV and NPV were calculated for that prevalence.

As a single test, the OCT had the best PPV (100%), while LLA mfVEP and HRT had similar NPVs (75% and 73%).

The combination of LLA+HRT (any one test positive) had the best NPV among all tests and test combinations put together (84%). The best PPV (89%) for combinations was seen for the combination of Matrix + OCT.

Discussion

In a previous study published in 2009, we examined the diagnostic performance of Blue-on-Yellow pattern onset stimulation and mfVEP in preperimetric glaucoma, and reported nearly 50% sensitivity.[12] Subsequently, to elucidate the mechanism for the effective performance of BonY stimulation (whether luminance or chromatic properties) we compared the sensitivities of pattern-onset high luminance achromatic (HLA), LLA (an achromatic stimulus with luminance contrast similar to the BonY stimulus), and BonY stimulation in a group of patients with early but perimetrically proven glaucoma (MD < 6 dB) and found that LLA was similar to BonY, and both performed significantly better that HLA stimulation.[13] The current study examined the diagnostic performance of LLA mfVEP in preperimetric glaucoma, and also compared it to other commonly used early diagnostic methods in glaucoma.

We found that LLA mfVEP had almost the same sensitivity as HRT, with slightly, though not significantly, better specificity. The advantage of the LLA mfVEP over the HRT, however, is that it is a functional test that is able to demonstrate loss of visual function, lending more confidence to the diagnosis of glaucoma, particularly while contemplating therapy. The demonstration of visual defects, which corresponded topographically to disc changes, added further confidence to the diagnosis. Currently available alternative methods of demonstrating visual field defects in preperimetric glaucoma, that is, SWAP and FDT, demonstrated sensitivities of 21.2% and 18.8%, respectively, similar to what has been reported previously.[20] The LLA mfVEP demonstrated much higher sensitivity (50.6%), which is a fairly high value considering that we are dealing with glaucoma that is so early that defects have not yet been demonstrated on conventional SAP.

In a previous study, where we examined the LLA stimulus for the first time and compared it to high contrast stimulation, we demonstrated that the LLA mfVEP identified defects in 90% eyes with early perimetric glaucoma (MD < 6 dB), with 96% specificity. It performed significantly better than the achromatic high contrast stimulus. Multifocal VEP responses to achromatic stimulation saturate at approximately 40% to 50% luminance contrast,[21–23] which is similar to what we used in this study for LLA. Response at this level of luminance contrast has been described previously to have predominant magnocellular contributions. The parvocellular pathway, which constitutes approximately 80% of retinal ganglion cells is responsible for processing of high contrast, low temporal, and high spatial frequency information (achromatic high luminance contrast stimuli) while the magnocellular pathway conveys information about low contrast and low spatial frequency achromatic images, and has higher temporal resolution. For subjective functional tests, using stimuli that preferentially target the magnocellular pathways, has been shown to enable

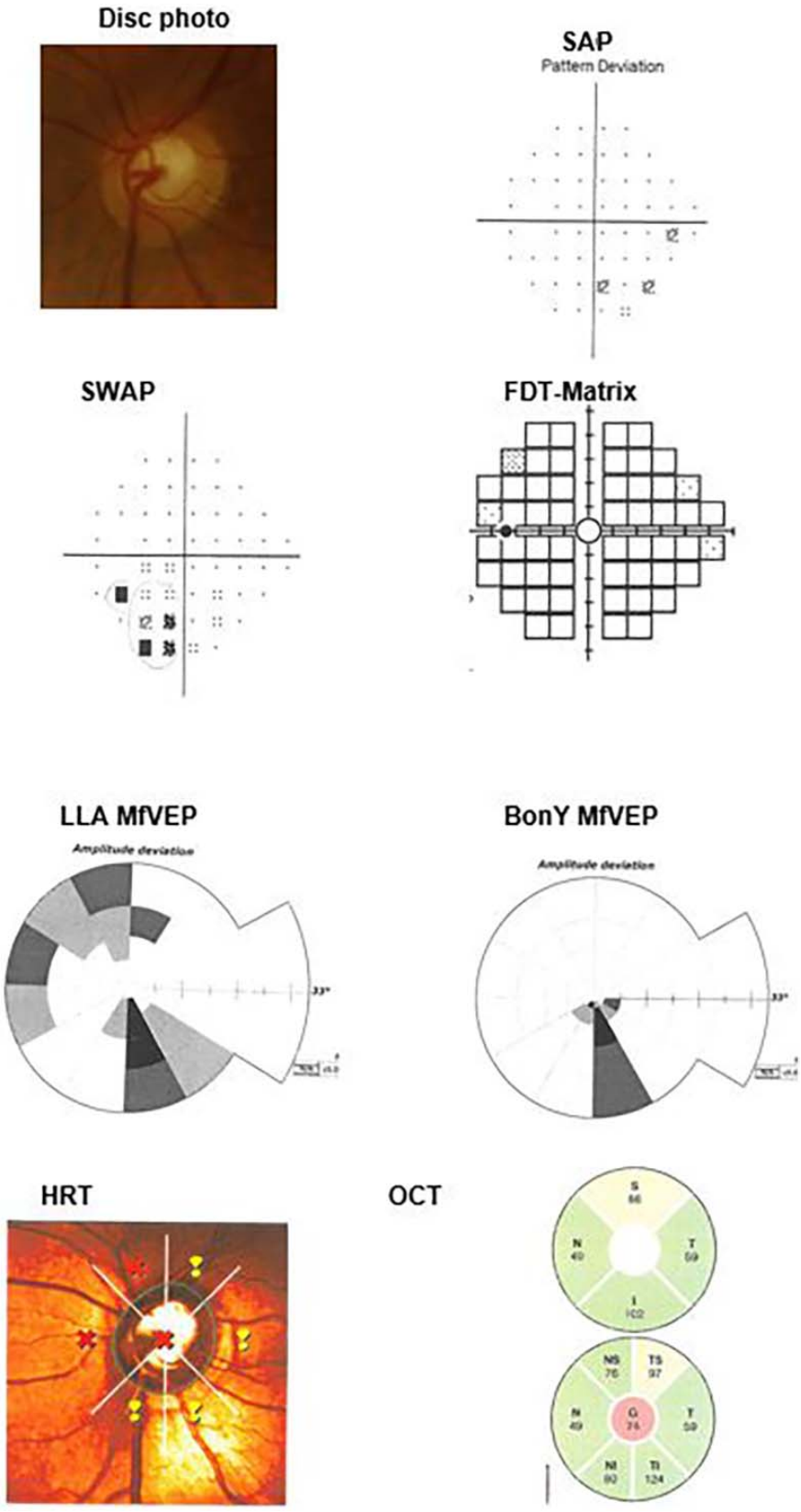

FIGURE 4. Case example. Subject with left superior neuroretinal rim thinning, normal SAP, and frequency-doubling perimetry, corresponding changes on HRT, SWAP, LLA, and BonY mfVEP and borderline changes on OCT (considered normal for analysis in this study).

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earlier identification of scotomas[24] due to lower functional redundancy in cells subserving these pathways,[25,26] since each of these pathways constitutes, on average, only approximately 10% of the ganglion cell population.[27,28] This preferential stimulation of the magnocellular pathway may explain the excellent performance of the LLA stimulus.

The rationale for combining tests to examine their combined diagnostic performance was to try and maximize sensitivity. The combination of one structural with one functional test is commonly practiced and is feasible in most clinical situations. The combination of HRT and LLA mfVEP yielded the best sensitivity of 76.5% while maintaining 82.4% specificity. This combination also had the best negative predictive value of 84% (at a presumed glaucoma prevalence of 40%), and a PPV of 74%, meaning that when both tests are negative, there is an 84% chance that the eye is not glaucomatous, but that if one of the tests is positive, there is a 74% chance that the eye does have glaucoma. Therefore, this combination is of better value in ruling out disease when negative.

The best positive predictive value for any single test or combination was seen with the OCT, which was 100%. However, sensitivity of the test was quite low at 23%. Addition of FDT-Matrix to the OCT improved sensitivity to 35%, while reducing PPV to approximately 90%. This combination may be useful in situations where high PPV is desired, for example, while considering second or third line medications, or when the risks of treating inappropriately outweigh the risks of not treating early disease that may exist.

The fact that we defined abnormality in structural tests as "abnormal" rather than "borderline" classification may have unfairly limited the sensitivity of the Spectralis OCT, but may have favored the HRT, which is more dependent on cup shape. On the other hand one could argue that since the diagnosis was made on a clinical impression from a disc photo, it may reflect some overdiagnosis by us of physiologically large cups as preperimetric glaucoma.

Although the use of both eyes of the patients is not ideal from a statistical viewpoint, it represents a real-life situation that is very common. It is the nature and a fallacy of the asymmetry analysis that it is dependent on both eyes, in this study as well as in real life. However, the use of both eyes of patients, whether preperimetric in both eyes, or representing glaucoma perimetric changes in one eye, if anything, reduces the sensitivity of the technique resulting in underestimation of defects, never overestimation. This is because, if both eyes were affected, but one eye more than the other, as happens typically in glaucoma, the lesser affected eye could be flagged as normal by the asymmetry analysis even though it is not really normal, just lesser affected. Unless amplitude analysis identifies the defect, that particular defect will be missed. There is no way asymmetry analysis will overdiagnose glaucoma in this situation, it will only underdiagnose. However, as already mentioned, bilaterality and asymmetry are inherent attributes of the disease, and preperimetric glaucoma more often than not is present in contralateral eyes of patients with perimetric glaucoma in one eye, and the purpose of the study was to examine the utilities of these tests in real-life situations.

It also was not ideal to use both eyes of control patients. This was done for logistic reasons. However, again this actually reduced specificity of the test rather than increased it. One subject had bilateral scotomas as a result of cortical convolutions, which was counted as a defect nevertheless for specificity calculations, therefore as two defects even though they existed in the same subject.

Preperimetric glaucoma is a challenging clinical situation, and its management is rendered more difficult by the imperfections of diagnostic tests currently available. The strength of the current study, however, is its focus on clinical relevance of results of these tests. The results of this study are directly applicable to clinical situations. The combinations of tests studied are feasible in most situations, and most clinicians would use a combination of at least one structural and one functional test for baseline evaluation and follow-up of these patients.

In conclusion, LLA mfVEP identified 50.6% eyes with preperimetric glaucoma, which was significantly higher than other perimetric methods, and similar to the HRT. The combination of LLA mfVEP and HRT had exceptionally high sensitivity of 76.5% for preperimetric glaucoma and better negative than positive predictive value. The OCT had very high specificity and PPV, with, however, significantly lower sensitivity. The Matrix-OCT combination had somewhat better sensitivity at 35.3%, and high PPV (90%). Longitudinal studies of these patients are currently underway to examine how the different tests predict the future conversion of preperimetric to perimetric glaucoma.

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