Perimetric Measurements With Flicker-Defined Form Stimulation in Comparison With Conventional Perimetry and Retinal Nerve Fiber Measurements

Folkert K. Horn, Ralf P. Tornow, Anselm G. Jünemann, Robert Laemmer, and Jan Kremers

Department of Ophthalmology and University Eye Hospital, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

PURPOSE. We compared the results of flicker-defined form (FDF) perimetry with standard automated perimetry (SAP) and retinal nerve fiber layer (RNFL) thickness measurements using spectral domain optical coherence tomography (OCT).

METHODS. A total of 64 healthy subjects, 45 ocular hypertensive patients, and 97 “early” open-angle glaucoma (OAG) patients participated in this study. Definition of glaucoma was based exclusively on glaucomatous optic disc appearance. All subjects underwent FDF perimetry, SAP, and peripapillary measurements of the RNFL thickness. The FDF perimetry and SAP were performed at identical test locations (G1 protocol). Exclusion criteria were subjects younger than 34 years, SAP mean defect (SAP MD) > 5 dB, eye diseases other than glaucoma, or nonreliable FDF measurements. The correlations between the perimetric data on one hand and RNFL thicknesses on the other hand were analyzed statistically.

RESULTS. The age-corrected sensitivity values and the local results from the controls were used to determine FDF mean defect (FDF MD). The FDF perimetry and SAP showed high concordance in this cohort of experienced patients (MD values, \( R = -0.69, P < 0.001 \)). Of a total of 42 OAG patients with abnormal SAP MD, 38 also displayed abnormal FDF MD. However, FDF MD was abnormal in 28 of 55 OAG patients with normal SAP MD. The FDF MD was significantly \( (R = -0.61, P < 0.001) \) correlated with RNFL thickness with a (nonsignificantly) larger correlation coefficient than conventional SAP MD \( (R = -0.48, P < 0.001) \).

CONCLUSIONS. The FDF perimetry is able to uncover functional changes concurrent with the changes in RNFL thickness. The FDF perimetry may be an efficient functional test to detect early glaucomatous nerve atrophy. (ClinicalTrials.gov number, NCT00494923.)

Keywords: early glaucoma, flicker-defined form (FDF) perimetry, spectral domain OCT, retinal nerve fiber layer thickness

There is a strong relationship between glaucomatous reduction of the nerve fiber layer thickness and defects in routinely performed standard perimetry (SAP).\(^1\)-\(^5\) It was shown that minor visual field defects (expressed in decibels) are accompanied by a substantial retinal nerve fiber layer (RNFL) thickness decrease. According to the linear model of Hood and Kardon,\(^6\) at the upper limit of the normal range (2 dB) of conventional perimetric defects, 37% of the total available nerve fibers may already have been lost. Assuming that such a reduction in number of nerve fibers is accompanied by some sort of functional change, it is expected that more sensitive functional methods to detect glaucoma can be developed. As flickering targets proved to be particularly suitable for the early detection of glaucomatous damages,\(^7\)-\(^9\) new devices for sensory tests using temporally modulating targets have been developed. In these studies, it has been stated that perimetry, such as the frequency-doubling technology (FDT),\(^10\)-\(^13\) flicker tests,\(^7\),\(^14\),\(^15\) or pulsar perimetry\(^16\) may be helpful in glaucoma diagnosis. Recently, the flicker-defined form (FDF) stimulus\(^17\) was proposed to be a useful technique for perimetric measurements in glaucoma.\(^18\) Currently, a comparison of the FDF (using the Heidelberg Edge Perimeter [HEP], Heidelberg Engineering, Heidelberg, Germany) with Octopus G1 perimetry is lacking. The purpose of the present investigation is to study the structure-function relationship between FDF perimetry and RNFL thickness in patients with early signs of glaucomatous nerve atrophy, and to compare these data with the relationship between Octopus SAP and RNFL thickness.

METHODS

Procedures

The study followed the tenets of the declaration of Helsinki for research involving human subjects and was approved by the institutional review board. Informed consent was obtained from all participants. Totals of 64 healthy subjects and 142 patients of the Erlangen Glaucoma Registry (available in the public domain at www.clinicaltrials.gov, NCT00494923) participated in this study. In the frame of this Registry, normal subjects and patients were examined annually over a period between 3 and 18 (10.7 ± 4.2) years using slit-lamp biomicroscopy, tonometry, funduscopy, gonioscopy, pachymetry, perimetry, and papillometry. The individual structural and
functional data, presented here, were obtained within a 6-week period.

**Inclusion and Exclusion Criteria**

All individuals that participated in the study (Table 1) were familiar with psychophysical and perimetric tests. Visual acuity was 20/40 or better, pupil widths were between 2.1 and 5.2 mm, and myopic refractive error was between −8.5 and 5.3 diopters (D). To study the association between FDF and RNFL in patients with early defects, patient eyes with mean defects exceeding 5 dB in SAP were excluded. The presence of cataract, eye diseases, and systemic diseases that possibly are associated with changes in temporal contrast sensitivity (e.g., diabetes mellitus) was an additional exclusion criterion. Criteria for the diagnosis of glaucoma were an open anterior chamber angle and glaucomatous changes of the optic nerve head, including an unusually small neuroretinal rim area in relation to the optic disc size, and vertical cup-to-disc ratios being larger than horizontal ratios. The diagnosis and the optic disc classification, according to the stages given by Jonas et al., were based on 15° optic disc photographs. For evaluation, all optic disc photographs were ordered randomly and inspected by two glaucoma specialists. In the case of conflicting diagnoses a third specialist was consulted. The assessment of the optic disc was performed in a masked fashion so that the examiners were unaware of each other's diagnosis, IOP, and visual field data. All glaucoma patients had glaucomatous optic discs for more than one year at the start of our study. If both eyes fulfilled all inclusion criteria, the eye with a higher Jonas classification or (when this parameter was equal for the two eyes) one randomly selected eye entered the statistical analyses. The information of the two eyes of the normal subjects was used for determination of FDF normal values.

**Standard Perimetry**

All subjects underwent visual field tests with standard white-on-white perimetry using a computerized static projection perimeter that generates age-corrected relative sensitivity values for all test positions (Octopus 900, testing strategy, G1-standard; Interzeag, Köniz, Switzerland). Refraction errors were corrected according to the patient's age. To avoid "ring" scotomas due to the rims of the correcting lenses, trial lenses with thin rims were placed at 13- to 15-mm distance from the eye. In the octopus-G1 procedure a parameter called "Reliability factor" (RF) is routinely determined representing the ratio between the sum of the wrong responses, and the total number of negative and positive catch trials. In the present evaluation of patients from our glaucoma registry, with exclusively experienced participants, the maximum RF was 12%. Definition of a normal white-on-white perimetry was in agreement with previous proposals. To use identical test pattern in SAP and FDF, 5 locations from G1 standard-protocol were omitted (central, and the two uppermost and lowermost targets). The results at 54 test locations were used to obtain the SAP square root of loss variance (sLV), the global SAP mean defect (MD), and the regional mean defects in areas as defined in Figure 1 (left).

**Subjects**

**Healthy Subjects.** The study included 64 healthy subjects from the Erlangen glaucoma registry. Findings with slit-lamp inspection, tonometry without medication, and funduscopy were in the normal range. White-on-white perimetry and optic discs were inspected, and classified as normal. To study the
age-dependency of the FDF sensitivity values, data from subjects with age between 24 and 80 years were analyzed (Fig. 1). In the subsequent analysis, the data of the four healthy controls, who were younger than 34 years, were excluded (Tables 1, 2) to match the age of the control group with those of the patient groups.

**Glucoma Patients.** All subjects of the glaucoma patient group showed glaucomatous abnormalities of the optic discs. The optic disc damage stage according to Jonas was between 1 and 3 (Table 1). Definition of glaucoma was based exclusively on glaucomatous optic disc appearance. A total of 86 patients (88.6%) had elevated IOP (higher than 21 mm Hg) in the medical history, while 11 patients had normal pressure applanation tonometry measurements. All 45 OHT patients had IOPs above 22 mm Hg as revealed by repeated measurements. All 45 OHT patients had normal white-on-white perimetry and normal-appearing optic discs.

**Spectral-Domain Optical Coherence Tomography (SD-OCT)**

An SD-OCT (Spectralis, Heidelberg Engineering) was used to measure the RNFL thickness along a circle of 3.4 mm diameter around the optic disc. A detailed description of the Spectralis SD-OCT technique and the analysis can be found elsewhere. 24 To assess the relationship between RNFL thickness and functional defects in corresponding retinal regions, three peripapillary sectors were defined (Fig. 1) extending between 34° and 79° for the superior retina, between 270° and 315° for the inferior retina, and between 315° and 34° for the papillomacular bundle 24 (with 0° corresponding to 9 o’clock).  

**FDF Perimetry**

The HEP (Heidelberg Engineering) is a device that tests contrast sensitivity using the FDF stimulus. The technology and paradigm have been described in detail previously. 25–27 Briefly, an illusionary contour (edge) can be perceived at the border of two random dot areas that modulate in counter-phase at a temporal frequency of 15 Hz. Here, the FDF stimuli had circular central fields of 3° diameter at central retinal locations or of 5° diameter at peripheral locations (Fig. 1, left). The mean background luminance was 50 cd/m². The luminance of the random dots was modulated around this mean luminance. The Michelson contrast was varied in the perimetric procedure. The sensitivity was proportional to the inverse of the threshold contrast for the perception of an illusionary contour. All tests were performed by two trained examiners. Before FDF testing, the subjects were familiarized with the test procedure and stimulus type. The tests were performed in a darkened room. The FDF stimulus was located optically at infinity and required an according correcting lens. The patients were instructed to fixate a point in the middle of the monitor screen and to press a response button if the circular target appeared anywhere on the monitor. A Standard

*Table 2. Results From the FDF Measurements for Healthy Subjects and Patients (Mean and SD)*

<table>
<thead>
<tr>
<th>Group, N</th>
<th>HEP: FDF-Mean Sensitivity, db (Range)</th>
<th>HEP: FDF MD, dB (Range)</th>
<th>HEP: FDF-sLV, dB (Range)</th>
<th>Maximum of False-Positive Error Rate</th>
<th>Pupil Size HEP, mm (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, 60</td>
<td>18.13 ± 2.0 (11.6–22.9)</td>
<td>0.04 ± 1.8 (−3.7–5.6)</td>
<td>2.02 ± 0.5 (1.3–3.3)</td>
<td>3.3</td>
<td>3.4 ± 0.6 (2.2–4.7)</td>
</tr>
<tr>
<td>Ocular hypertension, 45</td>
<td>17.2 ± 2.2 (15.0–22.0)</td>
<td>0.9 ± 1.9 (−3.2–4.7)</td>
<td>2.53 ± 0.8 (1.3–4.6)</td>
<td>3.9</td>
<td>3.3 ± 0.6 (2.1–4.7)</td>
</tr>
<tr>
<td>Early OAG, 97</td>
<td>12.1 ± 4.4 (2.5–22.3)</td>
<td>5.89 ± 4.2 (−2.7–15.5)</td>
<td>3.98 ± 1.5 (1.6–8.3)</td>
<td>3.8</td>
<td>3.6 ± 0.6 (2.3–5.2)</td>
</tr>
</tbody>
</table>

Numbers in brackets indicate range.
The RNFL thickness versus SAP MD and FDF MD values are shown in Figure 3 for the mean of all visual field data. MD while being within the normal range for FDF MD (lower quadrant), whereas only four patients were abnormal with SAP MD (right upper quadrant), and 28 FDF exclusively (upper left quadrant). The RNFL thickness was not significant after correction for multiple testing. The calculated correlation between functional and structural data. The drawn curves are the fits of Equation 1 for SAP and 0.034 (0.028, 0.04) for FDF perimetry. The RNFL thickness was age-corrected by 0.6 dB/decade at all test locations. Before further statistical analyses were performed. Table 2 summarizes FDF results. Compared to controls, mean FDF MD values were elevated in the glaucoma group (5.9 ± 6.6 dB, P < 0.001) and the OHT group (0.9 ± 1.9 dB, P = 0.02).

The correlation analysis between FDF perimetry and conventional SAP showed significant associations of the two methods (Table 5). A separate analysis in subject groups revealed an association between SAP MD and FDF MD in the patient and control groups. For the OHT group, the correlation was not significant after correction for multiple testing. The plot in Figure 2 shows the FDF MD values as a function of SAP MD for all patients, indicating the strong correlation between the two (R = 0.69, P < 0.001).

Mean defect data from 38 glaucoma patients were out of normal range for both tests (upper right quadrant of the quadrants as defined by the normal limits, depicted by the dashed lines in Fig. 2) and 28 for FDF exclusively (upper left quadrant), whereas only four patients were abnormal with SAP MD while being within the normal range for FDF MD (lower right quadrant).

The RNFL thickness versus SAP MD and FDF MD values are shown in Figure 3 for the mean of all visual field data. Obviously, there is a strong relationship between functional and structural data. The drawn curves are the fits of Equation 1 to the data. The estimated values of parameter \( b \) (with confidence intervals) from the fits were: 0.106 (0.087, 0.125) for SAP and 0.034 (0.028, 0.04) for FDF perimetry. The coefficients of the Spearman rank correlations (Table 4) are significant for the two perimetric tests and larger for FDF MD (R = 0.61) than for SAP MD (R = 0.48), indicating a slightly stronger correlation between RNFL thickness and FDF data. However, this difference was not significant (P = 0.11).
required sample size to obtain nonoverlapping confidence intervals and, therefore, significantly different correlation coefficients was estimated to be 424. In addition to statistical comparisons of correlation coefficients from mean RNFL thickness and total visual field defects, Figure 4 and Table 4 present results for the different bundles as defined in the left plot of Figure 1. The correlation analysis between local RNFL thickness and corresponding FDF defects revealed significant Spearman correlation coefficients for the arcuate bundles of the visual field (inferior: \( R = -0.47 \), superior: \( R = -0.68 \)) and to a lesser degree for the papillomacular bundle (\( R = -0.39, \ P < 0.001 \)). As in the “overall” analysis of all G1-testpoints, the focal correlation coefficients in the different sectors were slightly larger for FDF than for SAP data. The largest differences between the correlation coefficients was found in the superior sector (\( P = 0.045, \text{Table 4} \)), but again, this was statistically not significant after correction for multiple testing.

**DISCUSSION**

Temporally modulating stimuli in visual field tests, such as FDT and flicker perimetry, can detect functional defects in glaucoma earlier than white-on-white targets as applied in conventional SAP.30,31 For the novel FDF stimulus,25 this has been shown less frequently. The FDF stimulus has been implemented in the commercially available HEP (Heidelberg Engineering).32,33 In our study, visual fields in normal subjects and glaucoma patients from the Erlangen glaucoma registry obtained with FDF and SAP (both expressed in decibels) were compared. In these comparisons, the different dynamic ranges of the two methods should be taken into account: FDF MD is calculated from contrast thresholds data that can range from 0.5% to 100%, while the SAP MD is based on a luminance ratio (Weber fraction) from target-on-background-luminance that can exceed a value of 100. Therefore, the total dynamic range is larger for SAP than for FDF perimetry. The FDF may show profound “ceiling effects,” meaning that patients who are not able to detect the FDF stimulus even at maximal contrast still may be discernible with SAP. In our study, however, patients with such severe losses were not included and the influence of the “ceiling effect” probably is small.

The purpose of our study was to compare the data from the new device to results from Octopus SAP at the same test positions. Therefore, we used measurements in healthy subjects to generate a normal database (that was not available for the test positions of the G1 protocol). When results from the new FDF
perimetry were compared to defects by conventional perimetry, a considerable concordance was found between the two techniques. This is true not only for direct comparison of the perimetric results in subjects and patients, but also for the correlation between perimetric defects and structural SD-OCT data. Table 4 indicates that SAP and FDF defects were correlated highly with loss in RNFL thickness, and that confidence intervals of the correlation coefficients from FDF and SAP overlap widely. Figures 3 and 4 strongly indicate that the two perimetric methods can detect functional changes concurrent with changes in RNFL thickness. The fitted curve in our SAP MD data resulted in an estimated value of parameter $b$ of 0.106, which agrees surprisingly well with the linear model by Hood and Kardon\textsuperscript{6} (that assumes $b$ to be 0.1). A similar curve, but

<table>
<thead>
<tr>
<th>Visual Field</th>
<th>FDF MD vs. RNFL-Loss</th>
<th>SAP MD vs. RNFL-Loss</th>
<th>Comparison of the Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>$-0.61 \ (-0.70, -0.50) \ P &lt; 0.001^*$</td>
<td>$-0.48 \ (-0.60, -0.34) \ P &lt; 0.001^*$</td>
<td>$P = 0.111^\dagger$</td>
</tr>
<tr>
<td>Central</td>
<td>$-0.39 \ (-0.53, -0.24) \ P &lt; 0.001^*$</td>
<td>$-0.25 \ (-0.38, -0.05) \ P = 0.006^*$</td>
<td>$P = 0.120^\dagger$</td>
</tr>
<tr>
<td>Inferior</td>
<td>$-0.47 \ (-0.60, -0.30) \ P &lt; 0.001^*$</td>
<td>$-0.40 \ (-0.54, -0.25) \ P &lt; 0.001^*$</td>
<td>$P = 0.445^\dagger$</td>
</tr>
<tr>
<td>Superior</td>
<td>$-0.68 \ (-0.76, -0.57) \ P &lt; 0.001^*$</td>
<td>$-0.52 \ (-0.65, -0.39) \ P &lt; 0.001^*$</td>
<td>$P = 0.045^\dagger$</td>
</tr>
</tbody>
</table>

Pairwise comparisons reveal an overlap of the confidence interval and no statistically significant difference between the correlation coefficients if a correction for multiple testing is applied. $R$, correlation coefficient with confidence interval (2.5%, 97.5%).

* Spearman test.
† Fisher’s $Z$-test.

Flicker-Defined Form Stimulation

### Table 4. The Results of the Correlation Analyses Between Perimetry and RNFL Thickness in 142 Patients Show Significant Association in All Sectors

**FIGURE 4.** The RNFL thickness as a function of SAP loss (left plots) and FDF loss (right plots) in the superior and inferior bundles of the visual field and corresponding optic disc zones (Fig. 1). The data confirm the presence of a strong inverse correlation between field loss and RNFL thickness, and illustrate the reduction of the RNFL thickness when perimetric losses increase ($P < 0.001$, Table 4). The curves are fits of Equation 1 to the data (see also Fig. 3). Dotted lines indicate the residual thickness that was obtained from advanced glaucoma patients for the present RNFL sectors. Open symbols: OHT patients. Filled symbols: glaucoma patients.
with a different value of $b$, was found to fit the data from FDF perimetry (Fig. 3, right).

In conclusion, in this cohort of trained participants the FDF stimulus was able to detect patients with glaucomatous nerve atrophy at an early stage and was correlated strongly with loss of RNFL thickness. This technique might be a new method in diagnosis of glaucoma that should compete against other sensory tests in the same patients to compare feasibility and performance.

Acknowledgments

The authors alone are responsible for the content and writing of the paper.

Disclosure: **F.K. Horn**, None; **R.P. Tornow**, None; **A.G. Jünemann**, None; **R. Laemmer**, None; **J. Kremers**, None

References