A Method for Comparing Electrophysiological, Psychophysical, and Structural Measures of Glaucomatous Damage

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Objective: To develop a method for comparing multifocal visual-evoked potential (mfVEP) responses and behaviorally determined visual fields with structural measures of the optic nerve head.

Methods: Humphrey 24-2 visual fields and mfVEPs were obtained from each eye of 20 patients with open-angle glaucoma. Monocular and interocular analyses were performed to identify locations with abnormal mfVEP responses. Optic discs were assessed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Tomograph II). The image of the optic nerve head was divided into 6 sectors. The rim and disc area measurements for each sector were compared with those in a normal database using Moorfields regression analysis. The optic nerve head measurements for the 6 sectors were related to the Humphrey visual field locations and the 60 sectors of the mfVEP display.

Results: Of 240 sectors tested (40 eyes x 6 sectors), 18.8% on Humphrey visual field, 22.1% on mfVEP, and 10.8% on confocal scanning laser ophthalmoscopic testing were significantly different from those of control subjects. There were 165 sectors with no significant deficits. There was agreement for 86.7% of the sectors when the Humphrey visual field and mfVEP results were compared. The confocal scanning laser ophthalmoscopic results were in agreement for 84.6% of these sectors.

Conclusions: The method used allows for a comparison among measures of visual function and a structural measure of the optic nerve head. In general, the results of the functional and structural measures showed agreement; however, there were clear examples of disagreements that merit further study.

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Glaucoma is a progressive optic neuropathy in which loss of retinal ganglion cells leads to a characteristic pattern of optic nerve head and visual field damage. The importance of detecting the presence and extent of the structural and functional damage cannot be overestimated in the treatment of the patient with glaucoma. Structural deficits can be evaluated quantitatively with fundal imaging techniques such as the confocal scanning laser ophthalmoscope, and functional deficits can be evaluated with static automated achromatic perimetry. Although the latter has become the clinical standard for detecting and monitoring glaucomatous visual field deficits, there are problems with this technique. For some patients, it is difficult, or even impossible, to obtain reliable visual field measures. In addition, a significant loss of ganglion cells can occur before the development of visual field loss.

A new technique, the multifocal visual-evoked potential (mfVEP), has generated considerable interest as a potential solution to these problems. Studies have shown that the technique can detect local damage. However, the extent to which mfVEP results will augment information obtained with static automated perimetry remains to be determined.

The problems of obtaining reliable and repeatable measurements are not unique to techniques that assess functional damage. Techniques that assess structural damage (eg, confocal scanning laser ophthalmoscopy) face similar problems of variability and test-retest reliability. It is only by comparing the results of measures of structural change with those of functional change that our ability to diagnose and monitor glaucoma will be improved.

Here, we provide a method for comparing the results of functional and structural measures in a group of patients with open-angle glaucoma. The aim is to develop an easy way for displaying and comparing the results of tests supplying topographic information about glaucomatous...
damage. The results of 2 techniques for assessing functional damage, standard automated achromatic visual fields and the mfVEP, are compared with the results of a technique for assessing optic disc damage, confocal scanning laser ophthalmoscopy.

METHODS

SUBJECTS

Twenty white patients, aged 40 to 74 years (mean ± SD age, 59.0 ± 8.7 years), with open-angle glaucoma were enrolled. All patients had glaucomatous optic nerve damage in at least 1 eye, defined as a cup-disc ratio of 0.6 or more, cup-disc asymmetry between fellow eyes of greater than 0.2, rim thinning, notching, excavation, and/or retinal nerve fiber layer defects and achronic visual field loss. No specific intraocular pressure was required to diagnose open-angle glaucoma. Of the 20 patients, 9 had primary open-angle glaucoma, 10 had normal-tension glaucoma, and 1 had pigmentary glaucoma. Inclusion criteria were a visual acuity of 20/25 or better (19 patients had a visual acuity of 20/20, and 1 had a visual acuity of 20/25), refractive errors not exceeding 5.00 diopters sph and 2.00 diopters cylinder, undilated pupillary diameters of at least 2.5 mm, reproducible visual field defects, an open anterior chamber angle, and no other posterior segment eye disease.

Thirty control subjects, aged 20 to 62 years (mean ± SD age, 36 ± 13 years), with no history or evidence of ocular disease and a corrected visual acuity of 20/20 or better also participated. All subjects gave written informed consent before participating in the study. The protocol was approved by the committee of the Institutional Board of Research Associates of Columbia University, and procedures followed the tenets of the Declaration of Helsinki.

VISUAL FIELDS

Standard full-threshold or SITA (Swedish interactive threshold algorithm)-standard automated achromatic perimetry was performed with an analyzer (Humphrey Field Analyzer II; Humphrey Systems, Inc, Dublin, Calif) using program 24-2. Both eyes were tested. The mean (median) value of the mean deviation of the Humphrey visual field (HVF) results for this group of patients was −2.72 (−2.72) dB; the range was from 1.36 to −7.84 dB. All patients had prior experience with automated perimetry using the analyzer, and they all had reliable fields in both eyes as determined by the HVF statistics. Reliable visual fields were defined as those with fewer than 33% fixation losses, false-positive results, and false-negative results.

mfVEP RECORDING PROCEDURE

The mfVEP testing was performed on both eyes using computer software (VERIS; Electro-Diagnostic Imaging, San Mateo, Calif). The stimulus is shown in Figure 1A. A, The multifocal visual-evoked potential (mfVEP) stimulus display. The dartboard pattern consists of 60 sectors, each with a checkerboard pattern of 16 checks, 8 white (200 candelas [cd]/m²) and 8 black (<3 cd/m²). The sectors are cortically scaled with eccentricity to stimulate approximately equal areas of visual cortex (ie, central sectors were smaller than peripheral sectors). The entire display subtended a diameter of 44.5°, and the central 12 sectors fell within 2.6° of the foveal center (Figure 1A). The stimulus array was displayed on a black-and-white monitor driven at a frame rate of 75 Hz. The 16-element checkerboard of each sector had a probability of 0.5 of reversing on any pair of frame changes, and the pattern of reversals for each sector followed a pseudorandom m-sequence. Three channels of recording were obtained using gold cup electrodes. The electrodes for the midline channel were placed 4 cm above the inion (active), at the inion (reference), and on the forehead (ground). The same ground and reference were used for the other 2 channels, but the active electrode was placed 1 cm up and 4 cm lateral to the inion on either side. By subtracting different combinations of pairs of channels, 3 additional derived channels were obtained, resulting in effectively 6 channels of recording representing the 6 possible pairs of the 4 recording electrodes.

The VEP records were amplified with the high- and low-frequency cutoffs set at 3 and 100 Hz (preamplifier P511J; Grass Instrument Co, Quincy, Mass), and were sampled at 1200 Hz (every 0.83 milliseconds). The m-sequence had 2¹⁵−1 elements, requiring about 7 minutes of recording. Two 7-minute recordings were obtained during monocular stimulation of each eye. Both eyes were tested twice in ABBA fashion, and the average of 2 recordings was used for analysis. The second-order kernel responses were extracted using computer software (VERIS 4.3; Electro-Diagnostic Imaging). The mfVEPs were low-pass filtered using a sharp cutoff at 35 Hz and a fast Fourier transform technique. This and all other analyses were performed with computer software (MATLAB; MathWorks Inc, Natick, Mass). A refractor/camera (Electro-Diagnostic Imaging) was used to retract the subjects’ eyes and monitor eye position and fixation stability. Subjects were asked to fixate on the center of a black “X” located in the center of the display. Segments contaminated by eye movements, loss of fixation, and/or noise were discarded and rerecorded.

Figure 1. A, The multifocal visual-evoked potential (mfVEP) stimulus display. The dartboard pattern consists of 60 sectors, each with a checkerboard pattern of 16 checks, 8 white (200 candelas [cd]/m²) and 8 black (<3 cd/m²). The sectors are cortically scaled with eccentricity, and the entire display has a diameter of 44.5°. B, An mfVEP record showing the signal and noise windows used for analysis.
RESPONSE ANALYSIS

Root-mean-square (RMS) amplitudes were calculated for each mfVEP response during an interval from 45 to 150 milliseconds. The signal-noise ratio (SNR) was also measured for each response, as previously described. Briefly, to obtain the SNR for an individual response, the RMS of the response from 45 to 150 milliseconds was divided by an estimate of the noise in the records. This noise measure was obtained, for each eye of each individual, as the mean of the 60 RMS amplitudes of the records from 325 to 430 milliseconds, a region of the record virtually without a signal (Figure 1B). The SNR is equal to the following: RMS(signal window)/mean RMS(noise window).

Two analyses were performed on the best of the responses from the 6 channels, a monocular and an interocular analysis. The best responses for an individual were derived differently for the 2 analyses. For the monocular analysis, for each eye and each location, the response with the largest SNR, among the 6 channels, was selected. The 60 responses thus chosen for each eye defined the best array for that eye. For the interocular analysis, at each location, the response with the largest SNR was selected among the 12 responses (2 eyes × 6 channels). The response from the other eye from that same channel made up the pair of responses at that location in the interocular best array. Locations where the larger of the monocular responses had an SNR below 1.7 were excluded from the analysis. This represented 1% of the responses from the healthy controls.

For the monocular test, to determine if the mfVEP responses were significantly smaller, the SNR of an individual response was compared with the mean and standard deviation of the SNRs for the 30 control subjects at that location. Probability plots, resembling the probability plots for the HVF test, were produced by coding whether the SNRs of the responses were significantly different from those of healthy controls. Examples of monocular probability plots for the left and right eyes of a patient are shown in Figure 2A and B, respectively. Each square
locates the center of 1 of the 60 sectors of the stimulus display (Figure 1A). The colored squares indicate the locations with SNR values that fell more than 1.96 (light color) or 2.58 (dark color) SDs below the mean values. Blue indicates that the right eye, and red that the left eye, had significantly smaller SNRs.

For the interocular analysis to determine if the mfVEP response was significantly smaller in one eye compared with the other, the ratio of the RMS amplitudes from the 2 eyes was calculated for each location (ie, log [RMS of the right eye/RMS of the left eye]). The log of the interocular ratio obtained from the patient for each location was then compared with the mean and standard deviation of the log ratio values from the control subjects, and an interocular probability plot was derived. An example of an interocular probability plot and of the mfVEP responses obtained from the right (blue) and left (red) eyes of another patient is shown in Figure 2C and D, respectively. Each square in the probability plot locates the center of 1 of the 60 sectors of the stimulus display. The colored squares indicate the locations with values that fell more than 1.96 (light color) or 2.58 (dark color) SDs below the mean values. For this patient, the right eye had significantly smaller signals than the left eye (the squares are blue), and most of the responses in the inferior hemifield were significantly decreased.

TOPOGRAPHIC OPTIC NERVE HEAD ANALYSIS

The optic nerve head was assessed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Tomograph [HRT] II; Heidelberg Engineering GmbH, Heidelberg, Germany). The size of the field of view was 15° × 15°, and digitization was performed in frames of 384 × 384 pixels. The spatial resolution was 10 µm per pixel. Three 3-dimensional images were obtained for each eye from each patient. With the HRT II, there is an automatic online quality control during image acquisition. If 1 or more of the acquired image series cannot be used for any reason (eg, if the patient were to lose fixation), then additional images are automatically acquired until 3 useful image series have been obtained. The mean topographic result of the 3 scan series was used for analysis. The contour line was drawn around the optic disc by one of us (P.T.). The standard reference plane was used. There are several approaches that can be used to discriminate between healthy and glaucomatous optic discs. These approaches rely on global and/or sectoral topographic indices and have similar sensitivities. We used the Moorfields regression analysis approach. It has the advantage of adjusting the global and sectoral rim areas for disc size and age to improve specificity and to allow for the assessment of regional damage. Regional damage was assessed with HRT II software by dividing the optic nerve head into 6 sectors (nasal, supranasal, supratemporal, temporal, inferotemporal, and inferonasal). The sectors were compared with those in a normal database using Moorfields regression analysis, and then classified into 1 of 3 categories (within normal limits, borderline, or outside normal limits). Briefly, this classification was performed as follows: the rim and disc area for each sector were compared with those in a normal database, and the sectors were then classified depending on the patient’s age and the overall size of the optic disc. The analysis provided a predicted value and an actual value for the rim area of each of the 6 sectors. If the percentage of the rim area for a given sector was larger than or equal to the 93% age-dependent confidence limit, then the sector was classified as being within normal limits. If the percentage of rim area was between the 93% and 99.9% confidence limits, then the respective sector was classified as borderline. Finally, if the percentage of the rim area for a sector was lower than the 99.9% confidence limit, then the sector was classified as being outside normal limits. Only those sectors classified as being outside normal limits were included in our mapping analysis.

The rim area sectors were related to HVF 24-2 locations using a map based on one developed by Garway-Heath et al. The map relates the 6 sectors of the optic nerve head to the visual field test points (Figure 3A and B). The 6 sectors were also related to the 60 mfVEP probability plot locations (Figure 3C).

The HVF probability plots (total deviation plots), HRT rim sector maps, and monocular and interocular mfVEP probability plots were compared for each patient. An example of the format developed for displaying the results is shown in Figure 4. The isodegree contours (radii of 2.6° and 22.2°) have been drawn to make it easier to compare the HVF probability plots (Figure 4A and B), the HRT rim sector maps (Figure 4C and D), and the mfVEP probability plots (Figure 4E-G). For this patient, the HVF probability plots show an upper hemifield defect in the left eye and no significant defects in the right eye (Figure 4A and B, respectively). The saturated colored squares indicate those locations with P < .01 vs the age-matched controls, and the desaturated colored squares indicate those locations with P < .05 vs the controls.

To provide a similar topographic map for the HRT results, the map in Figure 3A was coded as shown in Figure 4C and D. If a sector was classified as being outside the normal limits, then all the corresponding points in the HVF were circled, as in Figure 4C. A colored circle indicates that the percentage of the rim area for the corresponding sector was lower than the 99.9% confidence limit. In this

Figure 3. The visual field (A), the optic nerve head (B), and the multifocal visual-evoked potential probability plot (C) divided into 6 sectors.
Figure 4. All data are for the same patient. A and B, Humphrey visual field total deviation probability plots for the left and right eyes, respectively. The dark red squares (A) indicate those locations with $P < .01$ vs the age-matched healthy control subjects; and the light blue square (B), the location with $P < .05$ vs the control subjects. C and D, Confocal scanning laser ophthalmoscopic (Heidelberg Retina Tomograph II) rim sector maps for the left and right eyes, respectively. The red circles (C) indicate that rim area sectors 4, 5, and 6 were classified as being outside the normal limits. E and F, Monocular multifocal visual-evoked potential (mf VEP) probability plots for the left and right eyes, respectively. G, An interocular mf VEP probability plot. For E-G, the black squares indicate that there was no significant difference vs control subjects; and the colored squares (red for the left eye and blue for the right eye), locations with values that fell more than 1.96 (light color) or 2.58 (dark color) SDs below the mean values.
case, the HRT maps predict a significant defect in the upper hemifield of the left eye and no significant defects in the right eye. The monocular (Figure 4E and F) and interocular (Figure 4G) mfVEP probability plots also show an upper hemifield defect in the left eye and no significant defects in the right eye. For this patient, all 3 tests are in agreement. There are slight differences in the extent of the defect between the 3 measures that can be attributed to differences in the way the visual field is sampled. For example, on the mfVEP plot, the defect extends into the central 2.6°, but some of the outermost sectors appear to be normal. With the mfVEP technique, 12 responses are obtained in the central 2.6°, compared with 1 measure of sensitivity for the fovea with the HVF.

**Figure 5** shows the results obtained from a patient who showed agreement between the 2 measures of visual function. The HVF (Figure 5A and B) and mfVEP (Figure 5E-G) results show significant defects in the right eye (Figure 5B, F, and G), while the HRT results (Figure 5C and D) were classified as being within the normal limits. A lower hemifield defect can be seen in the HVF probability plot for the right eye (Figure 5B), together with 2 points in the upper field with *P* < .01. There are no significant defects in the plot for the left eye (Figure 5A). The HRT maps (Figure 5C and D) predict no significant defects in either eye. In agreement with the HVF, the mfVEP shows a lower hemifield defect for the right eye (Figure 5F), and there is also a cluster of 3 points at *P* < .01 in the upper hemifield on the interocular probability plot (Figure 5G).

The results of the 3 tests for all 20 patients (40 eyes) are summarized in **Figure 6** and in **Table 1** and **Table 2**. In Figure 6 and in Tables 1 and 2, the locations of the HVF and mfVEP defects are related to the 6 rim area sectors shown in Figure 3B. Sectors on the HVF and mfVEP probability plots were classified as being outside the normal limits if they contained either 2 or more adjacent field locations with *P* < .01 or 3 or more adjacent locations with *P* < .05. Of a total of 240 sectors tested (40 eyes × 6 sectors), 45 (18.8%) on HVF, 53 (22.1%) on mfVEP, and 26 (10.8%) on HRT testing were significantly different from those of control subjects. Twice as many sectors were classified as abnormal with the 2 measures of visual function than with the measure of structural damage.

A comparison between these 2 measures of visual function (HVF and mfVEP) shows that there is agreement for 86.7% of the sectors: 175 (72.9%) of the sectors had no significant defects and 33 (13.8%) had abnormalities (Table 1). Of the disagreements between the tests, 20 (8.3%) of the sectors showed significant mfVEP defects and no HVF defects; 13 of these sectors corresponded to the central visual field area, or sector 1. To compare these functional measures with the HRT, the HVF, and the mfVEP, results were separated into 3 groups according to whether (1) there was agreement about the presence of a defect in a sector, (2) there was agreement about the absence of a defect (the sector was normal), or (3) there was disagreement between the measures. The correspondence between these 3 groups and the HRT results is shown in Table 2. Of the 208 sectors showing agreement on the functional measures, the HRT results were in agreement in 176 (84.6%) of the cases; 165 sectors had no significant defects on all 3 measures, while 11 had significant defects. Thus, in 32 (15.4%) of the cases in which the mfVEP and HVF results agreed, the HRT showed discrepant results. This comparison is potentially misleading because only 26 (10.8%) of the 240 sectors had significant defects on HRT testing compared with about 20% for the HVF and the mfVEP. In our HRT mapping analysis, we only included sectors classified as being outside the normal limits. If we were to change our criterion and include sectors classified as borderline, then 76 (31.7%) of the sectors would have defects on HRT testing. However, this would not improve the agreement between the functional and structural tests—it would decrease to 74% (Table 3).

**COMMENT**

The development of new technologies designed to measure the optic nerve and nerve fiber layer and new techniques such as the mfVEP to assess visual function promise to increase our understanding of the relationship between structural and functional abnormalities in glaucoma. However, to realize this promise, we need to develop methods for comparing the results of these new techniques. In addition, a topographic comparison of the results will enhance the clinician’s ability to diagnose and monitor disease progression. The method previously described allows for such a comparison between 2 measures of visual function, the HVF and the mfVEP, and a measure of the optic nerve head integrity, the HRT. Specific HVF and mfVEP locations suggesting glaucomatous damage are related to structural changes in the neural rim of the optic nerve head assessed with HRT.

Previous studies have provided methods for mapping structural damage to functional damage assessed with achromatic automated perimetry or with short-wavelength automated perimetry. A review is provided by Johnson et al. These maps showed that some visual field zones topographically mapped to certain rim sectors (eg, patients with superior hemifield sensitivity loss tended to have inferior rim defects, and vice versa). In addition, one defective visual field zone may be related to several rim sectors.

In this study, we used the map developed by Garway-Heath et al to relate the test locations of the HVF and the mfVEP to sectors of the optic nerve head measured with the HRT II. Despite the fact that there are differences between the 2 functional tests regarding stimulus duration, stimulus size, and adaptation level, and more important differences in the mechanism of response generation and in the nature of the response measure, the agreement between the 2 measures of visual function was quite good. This finding is consistent with previous work. The disagreements between the 2 measures are interesting. Most sectors defined as abnormal on the mfVEP but normal on the HVF were located in the center of the visual field; they corresponded to sector 1. One explanation for this finding is that the field is sampled in a different way by the 2 techniques. In particular, within the central 2.6° (5.2° diameter), there are 12 mfVEP responses but only 1 HVF test point. This ability of the mfVEP technique to sample the central retina in detail may be relevant to findings implicating macular loss in glaucoma. Whether the technique is more sensitive in
Figure 5. All data are for the same patient. A and B, Humphrey visual field total deviation probability plots for the left and right eyes, respectively. In B, the dark blue squares indicate those locations with $P < .01$ vs the age-matched healthy control subjects; and the light blue squares, those locations with $P < .05$ vs the control subjects. C and D, Confocal scanning laser ophthalmoscopic (Heidelberg Retina Tomograph II) rim sector maps for the left and right eyes, respectively. E and F, Monocular multifocal visual-evoked potential (mfVEP) probability plots for the left and right eyes, respectively. G, An interocular mfVEP probability plot. For E-G, the black squares indicate that there was no significant difference vs control subjects; and the colored squares (red for the left eye and blue for the right eye), locations with values that fell more than 1.96 (light color) or 2.58 (dark color) SDs below the mean values.
detecting defects in the central region of the visual field than the HVF 10-2, however, remains to be tested. Despite reasonably good overall agreement between the HRT and the 2 measures of visual function,
there was disagreement for a number of cases. If we consider the 208 sectors that showed agreement between the HVF and the mfVEP, we find discrepancies with the HRT results for 32 (15.4%) of these sectors. If we include the borderline HRT results as abnormal, this only leads to a decrease in the number of sectors showing agreement. Figure 5 shows one example in which the functional tests show a clear defect that is missed on the HRT. Thus, there are clearly cases in which the functional measures and the structural measure disagree.

In summary, a method for comparing mfVEP responses with behaviorally determined visual fields and with structural measures of the optic nerve head has been developed. The preliminary results suggest that, while in general the structural and functional measures agree, there are clear discrepancies that merit further study.

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Table 1. Comparison Between HVF and mfVEP Results for 240 Sectors

<table>
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<th>Normal</th>
<th>Abnormal</th>
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<tbody>
<tr>
<td>Normal</td>
<td>175</td>
<td>12</td>
<td>187</td>
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<tr>
<td>Abnormal</td>
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<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
<td>45</td>
<td>240</td>
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</tbody>
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Abbreviations: HVF, Humphrey visual field; mfVEP, multifocal visual-evoked potential.

*Data are given as number of sectors.

Table 2. Comparison of HRT, HVF, and mfVEP Results for 240 Sectors

<table>
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<th>HRT Results</th>
<th>Agree and Are Normal</th>
<th>Agree and Are Abnormal</th>
<th>Disagree</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>165</td>
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<td>214</td>
</tr>
<tr>
<td>Abnormal</td>
<td>10</td>
<td>11</td>
<td>5</td>
<td>26</td>
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<tr>
<td>Total</td>
<td>175</td>
<td>33</td>
<td>32</td>
<td>240</td>
</tr>
</tbody>
</table>

Abbreviations: HRT, Heidelberg Retina Tomograph; HVF, Humphrey visual field; mfVEP, multifocal visual-evoked potential.

*Data are given as number of sectors.

Table 3. Comparison of HRT, HVF, and mfVEP Results for 240 Sectors, Including Those Classified as Borderline

<table>
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<th>HRT Results</th>
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</table>

Abbreviations: See Table 2.

*Data are given as number of sectors.

REFERENCES


