Comparison of Screening Procedures in Hydroxychloroquine Toxicity

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Objectives: To compare different screening procedures for hydroxychloroquine sulfate (Plaquenil) toxicity at different stages of damage.

Methods: Ten patients were studied using 10-2 automated fields, multifocal electroretinography, spectral domain optical coherence tomography (SD-OCT), and fundus autofluorescence.

Results: All 10 patients used hydroxychloroquine for more than 6 years, and those with severe toxicity had been overdosed. Fundus examination findings were normal except for the patients with severe toxicity. All the patients showed parafoveal field loss, but this was sometimes subtle. Multifocal electroretinography demonstrated parafoveal weakness in the milder cases. The SD-OCT subfield thickness plots showed a ring of parafoveal thinning in all the patients. The SD-OCT cross-sections showed parafoveal loss of the inner segment–outer segment and cone outer segment tip lines at early stages of toxicity, progressing to parafoveal thinning of the outer nuclear layer and eventually to retinal pigment epithelium damage. There was a ring of autofluorescence in most patients.

Conclusions: Overdosage with hydroxychloroquine seemed a significant risk factor for toxicity. Different individuals were more or less sensitive to different tests. Fields can be sensitive but only if read with a low threshold for change. Hydroxychloroquine causes early parafoveal loss of the outer segment lines on SD-OCT, with the first changes often evident in the inferotemporal quadrant. Parafoveal thinning of the outer nuclear layer follows, before retinal pigment epithelium damage is visible. Careful screening with multiple tests can detect toxic damage before prominent loss of the outer nuclear layer.


HYDROXYCHLOROQUINE sulfate (Plaquenil; Sterling Winthrop) toxicity remains a relatively rare disease, with the incidence of toxicity estimated to be approximately 1% after 5 years of use and rising with continued drug use.1 The retinopathy, classically described as a bull’s-eye, is untreatable and tends to progress even after discontinuing use of the drug. Thus, it is important for screening to catch signs of toxicity early before central vision is threatened.

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The recent publication2 of revised screening recommendations for hydroxychloroquine toxicity has raised awareness of objective modalities, such as multifocal electroretinography (mfERG), spectral domain optical coherence tomography (SD-OCT), and fundus autofluorescence (FAF), as adjuncts or successors to the traditional visual field as screening tools. However, data are still limited on the relative sensitivity and specificity of these procedures.

This article describes 10 referred patients with hydroxychloroquine retinopathy seen within 1 year (February 2010 to February 2011) and examined using the same battery of modern tests. The results show earlier changes than previous series and give insight into the relative value of these tests and the sequence of tissue damage as toxicity develops.

Methods

Medical records were reviewed with the approval of the institutional review board of Stanford University Medical Center, Stanford, California, and in compliance with the Declaration of Helsinki. All the patients were receiving hydroxychloroquine for lupus erythematosus or rheumatoid disease. All the patients were diagnosed as having hydroxychloroquine toxic-
ity by evidence of parafoveal or bull’s-eye retinal damage confirmed by at least 3 different tests, including fundus appearance, visual fields, mfERG, SD-OCT, and FAF. Patient demographics are given in Table 1.

Visual field testing was performed using a standard 10-2 white III recording paradigm (Humphrey perimeter; Carl Zeiss Meditec). Multifocal ERG was performed using a VERIS system (Electro-Diagnostic Imaging Inc) according to International Society for Clinical Electrophysiology of Vision guidelines. All the recordings used 103 pixels, spanning approximately 20° on either side of the fixation. The average response density within concentric rings from the center (ring 1) to the periphery (ring 6) was displayed in nanovolts per degree squared. The ratios of centric rings from the center (ring 1) to the periphery (ring 6) were displayed using VERIS software to look for relative parafoveal damage using 4 to 5 different tests and were displayed using VERIS software to look for relative parafoveal signal weakness. Full-field ERGs were recorded using an Espion unit (Diagnosys LLC) in accord with the International Society for Clinical Electrophysiology of Vision standard. The SD-OCT was performed using a Cirrus HD system (Carl Zeiss Meditec), which displays a thickness plot of 9 Early Treatment of Diabetic Retinopathy Study subfields and high-resolution grayscale cross-section images. Cirrus thickness plots correct for age and show extreme deviation from norms in red. Vertical and horizontal sections were examined. The FAF was recorded using a Spectralis unit (Heidelberg Engineering).

### RESULTS

#### DEMOGRAPHICS AND DOSAGE

The demographics in Table 1 show that patients ranged in age from 27 to 77 years and that there was no clear relation between age or sex and the presence or severity of toxicity. Visual acuity was mostly excellent but was modestly subnormal in some of the more affected patients. The daily hydroxychloroquine sulfate dosage was 400 mg for all patients except patient S2 (who took 400 mg for 12 years and 200 mg for 10 years). Relative to the recommended dose² of 6.5 mg/kg or less, patients with early (E) and moderate (M) toxicity all received close to that level. However, patients with severe (S) toxicity were overdosed: patients S1 and S2 were short individuals who were overdosed relative to ideal weight; patient S3 had long-standing renal failure, which reduces excretion of the drug. All data are presented relative to ideal weight, which is estimated on the basis of height (Table 1). Hydroxychloroquine is not distributed in fatty tissues, and real weight gives a false estimate of drug dosage for overweight individuals. None of these patients with toxicity had less than 6 years’ exposure or less than 1000 mg or 19 g/kg cumulative dose except for patient S3.

In the tables and figures, patients have been arranged into categories of severity by grading the damage found on the various screening tests as early, moderate, and severe (Table 2). Although some of the individual test results from patients with early toxicity were inconclusive or even debatable, each patient had evidence of localized parafoveal damage using 4 to 5 different tests and evidence of a complete ring of damage from at least 1 of the tests (Table 3). Patients E1 to E3 had 10 to 25 years of hydroxychloroquine exposure, putting them all at relatively high risk. Thus, in the absence of any clues to other macular disease, it seemed reasonable to judge that these patients were all showing signs of early bull’s-eye damage, indicative of hydroxychloroquine toxicity. In this

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### Table 1. Patient Demographics and Hydroxychloroquine Dosage

<table>
<thead>
<tr>
<th>Patient/VA, OD–OS</th>
<th>Height, ft–in</th>
<th>Ideal Weight, lb A</th>
<th>Daily Dose/kg, mg</th>
<th>Duration, y</th>
<th>Cumulative Dose, g</th>
<th>Cumulative Dose/kg, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1/F/69 20/20–20/20</td>
<td>5–6.5</td>
<td>132.5</td>
<td>6.6</td>
<td>18</td>
<td>2628</td>
<td>43.7</td>
</tr>
<tr>
<td>E2/F/54 20/20–20/20</td>
<td>5–5</td>
<td>135</td>
<td>6.5</td>
<td>10</td>
<td>1460</td>
<td>23.8</td>
</tr>
<tr>
<td>E3/F/49 20/20–20/20</td>
<td>5–5</td>
<td>125</td>
<td>7</td>
<td>25</td>
<td>3650</td>
<td>64.2</td>
</tr>
<tr>
<td>M1/F/65 20/25–20/25</td>
<td>5–9</td>
<td>145</td>
<td>6.1</td>
<td>10</td>
<td>1460</td>
<td>22</td>
</tr>
<tr>
<td>M2/F/41 20/20–20/20</td>
<td>5–7</td>
<td>135</td>
<td>6.5</td>
<td>8</td>
<td>1168</td>
<td>19</td>
</tr>
<tr>
<td>M3/F/75 20/50–20/30</td>
<td>5–5</td>
<td>125</td>
<td>7</td>
<td>10</td>
<td>1460</td>
<td>25</td>
</tr>
<tr>
<td>M4/F/75 20/30–20/30</td>
<td>5–5</td>
<td>125</td>
<td>7</td>
<td>13</td>
<td>1898</td>
<td>33.4</td>
</tr>
<tr>
<td>S1/F/77 20/40–20/25</td>
<td>4–8</td>
<td>80</td>
<td>11</td>
<td>10</td>
<td>1022</td>
<td>26</td>
</tr>
<tr>
<td>S2/F/65 20/60–20/40</td>
<td>4–11</td>
<td>95</td>
<td>9.2/4.6 B</td>
<td>22</td>
<td>2482</td>
<td>57</td>
</tr>
<tr>
<td>S3/F/27 20/25–20/25</td>
<td>5–10</td>
<td>150</td>
<td>5.9</td>
<td>6</td>
<td>876</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Abbreviations: E, early toxicity; M, moderate toxicity; S, severe toxicity; VA, visual acuity.

A Ideal weight is calculated as follows on the basis of height: for women, 100 lb for 5 ft + 5 lb/in; for men, 110 lb for 5 ft + 5 lb/in.

B Kilograms represent ideal weight. All the patients were given 400 mg/d except patient S2, who was given 400 mg/d for 12 years and 200 mg/d for 10 years.

C Patient was in renal failure.

### Table 2. Grading Scale for Severity of Toxicity

<table>
<thead>
<tr>
<th>Score</th>
<th>Fields, SD-OCT, FAF mfERG Full-Field ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal Normal Normal Normal</td>
</tr>
<tr>
<td>1</td>
<td>Patchy damage (1–2 quadrants) Normal amplitude + parafoveal weakness Mild cone loss on</td>
</tr>
<tr>
<td>2</td>
<td>Bull’s-eye damage (&gt;2 quadrants) Subnormal centrally parafoveal weakness Mild rod and cone loss</td>
</tr>
<tr>
<td>3</td>
<td>Bull’s-eye damage + fovea or RPE Center too weak to judge parafovea Amplitudes 25%–75%</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse posterior pole damage Diffuse posterior pole damage Amplitudes &lt;25%</td>
</tr>
</tbody>
</table>

Abbreviations: ERG, electroretinography; FAF, fundus autofluorescence; mfERG, multifocal electroretinography; RPE, retinal pigment epithelium; SD-OCT, spectral domain optical coherence tomography.
study, patients with early toxicity showed mostly patchy areas of paracentral damage, those with moderate toxicity showed more complete bull’s-eye damage, and those with severe toxicity had diffuse macular damage with visible retinal pigment epithelium (RPE) loss. These are not absolute discriminants, as different individuals were more or less sensitive to different responses. However, the categories show a general progression of damage.

**FUNDUS EXAMINATION**

Figure 1 shows the right macula of all the patients. Patients with early and moderate toxicity had generally unremarkable fundus examination findings, whereas those with severe toxicity showed bull’s-eye retinal pigment epithelium atrophy.
All the patients had some degree of parafoveal field loss evident on 10-2 white automated field testing. Representative fields are shown in Figure 2. Patients E1 and E2 demonstrated that field loss can be subtle or even questionable at an early stage of toxicity, although the changes were reproducible in these patients. There were focal parafoveal zones of loss in the pattern deviation plots and more generalized but weak loss of sensitivity in the full displays. The patient with moderate toxicity (patient M3) (pattern 1) shows a distinct partial bull’s-eye scotoma. Patient S1 shows a complete bull’s-eye scotoma with foveal sparing. OD indicates right eye; OS, left eye.

Initial field loss was most consistently, but not always, superonasal. Based on information from all the tests, the inferotemporal retina was most often the site of earliest or most severe damage (Table 4).

### VISUAL FIELDS

Multifocal ERGs were performed on all the patients except patient S3 and showed 4 distinct patterns of damage (Figure 3). (Pattern 1) Signals that are largely within laboratory reference ranges, although individual responses in the area of early damage are diminished (patient E3). Ring response densities are normal and the focal damage can appear subtle, but ring ratios show relative weakness of the parafoveal rings (Table 5). (Pattern 2) Diminished central macular responses, with the greatest severity in the parafovea (patient E1). This is evident in the trace array and the ring ratios (Table 5). (Pattern 3) Central responses reduced to the point that a parafoveal predilection can no longer be recognized (patient M4). (Pattern 4) Signals depressed severely across the posterior pole (patient S1).

Full-field ERGs were performed only in selected patients to evaluate the extent of damage beyond the macula. Patient E3 showed reduced cone amplitude in 1 eye only that was of doubtful significance because the flicker response times to peak were normal and symmetrical. Patients M3 and M4 had normal full-field ERG findings. Patients S1 and S2 had major ERG loss, with rod and cone
response reduced to approximately 20% of normal in S1 and 60% of normal in S2, with marked signal delay.

SPECTRAL DOMAIN OCT

Figure 4 shows SD-OCT data from all the patients, arbitrarily choosing a horizontal scan from the left eye so that both eyes are illustrated among the images shown in the article. The subfield thickness maps (colored boxes at the right of each image) show a consistent ring of parafoveal thinning even when visual fields had only small scotomata or SD-OCT cross-section changes were early and limited.

The SD-OCT cross-section changes in patients with early toxicity were subtle, and at low magnification (or lower-resolution recordings), some of the scans might pass as unremarkable. Patients with moderate toxicity showed a distinct loss of outer retinal substance on both sides of the fovea, giving the outer nuclear layer an appearance that some have likened to a flying saucer or sombrero. Patient S3 also showed this appearance, but the other patients with severe toxicity had such diffuse thinning of the outer retina beyond the fovea that a bull's-eye pattern was unrecognizable. Disruption of the RPE and debris above the RPE were seen only in very small focal areas in patients M3 and M4 but were clearly evident in a bull's-eye pattern in those with severe toxicity.

Figure 5 shows magnified views of 4 scans to illustrate early changes as this toxicity develops. Much of the literature on macular disease stresses damage to the inner segment–outer segment line as a critical early sign of photoreceptor damage and dysfunction, and this line (open arrows) disappeared where there was distinct thinning of the photoreceptor layers (patients E2, M1, and M2). However, in these same eyes, there was even earlier disappearance (closed arrows) of the line above the RPE that had been called the Verhoeff membrane but is better described as the cone outer segment tip line. In the patient with the mildest SD-OCT change (patient E1), there is parafoveal disappearance of the cone outer segment tip line, whereas the inner segment–outer segment line persists.

FUNDUS AUTOFLUORESCENCE

Figure 6 shows FAF views of the right eye for all the patients. No clear change in fluorescence was seen in patient E1, and there was a patch of nasal hyperfluorescence in the right eye only of patient E2. However, patient E3 showed a bull’s-eye pattern. Patients with moderate toxicity all showed hyperfluorescence around the fovea except the right eye of patient M1. There was early RPE breakup temporally in the right eyes of patients M3 and M4, although this was not yet seen in the left eye. Patients with severe toxicity showed more extensive hyperfluorescence, and the dark parafoveal ring in patients S2 and S3 probably represents RPE cell loss. Correlations among screening tests for hydroxychloroquine toxicity have been made for many years, but the

Table 5. Ring Ratio Analysis for Patients E1 and E3

<table>
<thead>
<tr>
<th></th>
<th>Rings 1:2</th>
<th>Rings 1:3</th>
<th>Rings 1:4</th>
<th>Rings 1:5</th>
<th>Rings 1:6</th>
<th>Rings 2:5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical range</td>
<td>1.4-1.7</td>
<td>2.1-2.6</td>
<td>3.0-3.8</td>
<td>3.5-5.5</td>
<td>3.4-5.6</td>
<td>2.3-2.9</td>
</tr>
<tr>
<td>Pattern 1c (patient E3)</td>
<td>1.99d</td>
<td>3.17d</td>
<td>3.64</td>
<td>4.09</td>
<td>4.44</td>
<td>2.05e</td>
</tr>
<tr>
<td>Pattern 2c (patient E1)</td>
<td>1.83d</td>
<td>2.18d</td>
<td>2.62f</td>
<td>2.69f</td>
<td>2.83f</td>
<td>1.47e</td>
</tr>
</tbody>
</table>

aData correspond to the trace arrays shown in Figure 3. Ring 1 is central; ring 6 is most peripheral. bLaboratory values for ratios of response density between the indicated rings. cThe multifocal electroretinography response patterns are described in the “Results” section of the text and are illustrated in Figure 3. dIncreased ratio means that parafoveal rings 2 and 3 are weak relative to foveal ring 1. eDecreased ratio means that parafoveal rings are weak relative to peripheral areas. fDecreased ratio means that the foveal ring is weak relative to peripheral areas.
older literature is not applicable to modern imaging and electrophysiologic testing, with their vastly superior sensitivity. Kellner et al\textsuperscript{10} provided the first good comparative study, and the present series extends that work with more patients, a broader and earlier spectrum of toxicity, and the illustration of more findings.

**DEMOGRAPHICS AND DOSAGE**

Earlier literature has suggested that most toxic cases occur with dosages greater than 6.5 mg/kg or after long-term use of the drug.\textsuperscript{2} The incidence of toxicity increases to greater than 1\% after 5 to 7 years or an approximately 1000-g cumulative dose for patients using 400 mg/d (which is the most typical dose prescribed).\textsuperscript{1} The present series is consistent with these observations except in patient S3, whose renal failure probably exposed her to significantly elevated blood levels. It may seem puzzling that there was no relation between the severity of toxicity and the duration of use or cumulative dose in grams or grams per kilogram, but this is to be expected in a small series given the low incidence of toxicity even after long-term use.

On the other hand, all the patients with severe toxicity had been significantly overdosed, which suggests that this may be a significant risk factor for accelerating toxicity. Patients with the highest daily dosage (patient S1 taking 11 mg/kg and patient S3 in renal failure) had toxicity after only 6 to 7 years.

**FUNDUS EXAMINATION**

All patients with early and moderate toxicity had normal fundus examination findings except for some subtle areas of foveal depigmentation that were recognized only by correlation with FAF images. Thus, a significant amount of retinal damage (as measured by fields, SD-OCT, FAF, and mfERG) can be present without RPE damage on SD-OCT or funduscopically visible bull’s-eye retinopathy. In other words, fundus examination is not a screening test for hydroxychloroquine toxicity because it cannot detect early toxicity (consistent with the results of other studies\textsuperscript{11,12}).
VISUAL FIELDS

Although standard 10-2 field testing can be a sensitive test for early toxicity, its effectiveness depends on patient cooperation and physician sensitivity to early changes. Small focal pattern loss, as seen in patients E1 and E3, needs to be recognized, taken seriously, and retested for confirmation and should initiate the addition of objective tests to confirm or rule out abnormality. Although patchy 10-2 field loss was an early sign in this series, it did not show the full extent of retinal dysfunction. Consistent with previous case reports, bull’s-eye damage was more extensive in mfERG and FAF in some patients and in SD-OCT thickness measurements in all patients.

A study by Easterbrook and Trope in 1989 suggests that use of a red target (which is effectively dimmer and, thus, provides more targets near threshold) might be more sensitive and show scotomata more distinctly (although at a cost of specificity). This remains to be verified. The American Academy of Ophthalmology recommendations advised standard 10-2 white testing primarily because it is routinely available; it did not intend to prohibit red fields but merely to advise that field tests need to be used wisely.

Patients with severe toxicity who have extensive retinal damage and nearly flat foveal mfERG signals still showed fields with foveal sparing. The extent to which retinal sensitivity can be retained, despite significant photoreceptor damage, may explain why fields may not detect the full extent of retinal damage.

MULTIFOCAL ERG

These patients show that the value of mfERG in hydroxychloroquine retinopathy highly depends on the stage of disease. It is most useful in early cases to demonstrate or confirm bull’s-eye damage. However, mfERG takes careful inspection to recognize focal loss (as illustrated in patients E1 and E3) and requires ring ratio analysis (Table 5) to maximize the recognition of ring-shaped loss.

Once parafoveal tissue loss is obvious on SD-OCT (patients with moderate toxicity), mfERG is less useful diagnostically because it only confirms what is evident to the eye. However, it still demonstrates the degree to which foveal function may be compromised. Similarly, it has little diagnostic value in patients with severe toxicity, where macular damage is dramatic on SD-OCT, FAF, and fundus examination. The patterns of mfERG loss seen herein at various stages of toxicity are similar to what has been observed previously. Lai et al suggested that mfERG may show gradual signal loss that correlates with dose and field sensitivity, but it remains to be shown whether such changes can be recognized clinically and prospectively.

SPECTRAL DOMAIN OCT

The SD-OCT subfield thickness diagrams show 360° parafoveal thining at a very early stage of toxicity, even when such widespread damage is hard to recognize in the cross-sections. In using these diagrams, it is important to remember that there can be sex and racial differences in regional thickness. For example, the central and parafoveal thinning at a very early stage of toxicity, even when such widespread damage is hard to recognize in the cross-sections. In using these diagrams, it is important to remember that there can be sex and racial differences in regional thickness.
of the earliest functional and anatomic changes, even before a “flying saucer” sign is evident on SD-OCT. Such changes include subtle field loss, the first stages of mfERG abnormality, beginning disruption of the inner segment–outer segment lines or cone outer segment tip lines, and documentation of parafoveal SD-OCT thinning.

**FUNDUS AUTOFLUORESCENCE**

The FAF images show dramatic changes when toxicity is moderate or severe. In this series, FAF seemed a bit less sensitive than SD-OCT, mfERG, and visual fields, perhaps, in part, for photographic reasons because the fluorescent intensity was not standardized. Only 1 of 3 patients with early toxicity (patient E3) showed a full ring of FAF abnormality. Nevertheless, one cannot discount its value because patient E3 had a ring of FAF dysfunction of greater extent than her fields. The FAF does provide a sensitive indicator of RPE degeneration as toxicity progresses, visible as dark areas or speckles surrounding the fovea.

**IMPLICATIONS FOR SCREENING**

Table 3 demonstrates that fields, mfERG, FAF, and SD-OCT can all show damage at a relatively early stage of hydroxychloroquine toxicity, but it is not predictable as to which test will be most definitive for any given individual. This makes a strong argument for using more than 1 modality routinely or at least for bringing in multiple modalities at the slightest hint of abnormality. Fields are used widely and are sensitive if read with a low threshold for damage. However, in every patient in this series, field loss was mirrored by SD-OCT changes so that it is hard to say which test is most sensitive. The choice will depend on availability, the quality of the records, and the experience of the examiner. It is easy to talk about subtle cone outer segment tip line changes, but the sign is useless if the line is not visible or recognized in the SD-OCT records, and it may not be visible without sufficient image sampling or in patients with media opacity.

The same is true of mfERG changes and FAF.

It is still unclear whether screening can prevent damage, and the goal at present is to catch toxicity early enough so that significant functional loss (especially central vision) will be avoided. The progression of damage long after stopping the drug therapy likely represents decompensation of photoreceptor and RPE cells that were initially injured beyond repair. However, many cases of late progression in the literature were already severe, with visible retinopathy. If hydroxychloroquine toxicity can

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Figure 6. Fundus autofluorescence images from the right eye for all the patients, matching the fundus images in Figure 1. Of patients with early toxicity, only patient E3 shows a clear bull’s-eye (although there is some nasal increase in the right eye only of patient E2). Patients with moderate and severe toxicity show a ring of increased fluorescence and parafoveal dark areas representing retinal pigment epithelium cell loss.
be detected early by the criteria in this article, when anatomic photoreceptor damage is subtle, there may be few cells injured beyond repair or survival and, hopefully, less risk of progression.

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